



Turkish Society of Hematology

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# 5<sup>th</sup> International Congress

on

# Leukemia Lymphoma Myeloma

May 21 - 23 | **İSTANBUL**  
**2015** | **TURKEY**  
WOW Convention Center



Proceedings & Abstract Book

# **5<sup>th</sup> International Congress on Leukemia – Lymphoma – Myeloma**

**May 21 – 23, 2015 • Istanbul, Turkey**

## **Proceedings & Abstract Book**



**Turkish Society of Hematology**

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**Teoman Soysal**

*Istanbul University, Cerrahpaşa School of Medicine, Department of Hematology, Istanbul, Turkey*

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*Trakya University, School of Medicine, Department of Hematology, Edirne, Turkey*

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H. Joachim Deeg

*Fred Hutchinson Cancer Research Center, Seattle, USA*

### **Multipl Myeloma**

Angela Dispenzieri

*Division of Hematology, Mayo Clinic, Minnesota, USA*

### **Chronic Lymphocytic Leukemia**

Peter Dreger

*University of Heidelberg, Hamburg, Germany*

### **Hodgkin Lymphoma**

Andreas Engert

*Department of Internal Medicine, Cochrane Haematological Malignancies Group (CHMG), University Hospital Cologne, Cologne, Germany*

### **Diffuse Large B-Cell Lymphoma**

Christian Gisselbrecht

*Service d'Onco-Hématologie, Hôpital SaintLouis, Paris, France*

### **Acute Lymphoblastic Leukemia**

Nicola Gökbüget

*J.W. Goethe University Hospital, Frankfurt, Germany*

### **Follicular Lymphomas**

Burhan Ferhanoğlu

*Koç University Medical School, İstanbul, Turkey*

### **Chronic Myeloid Leukemia**

Richard A. Larson

*University Of Chicago, Chicago, USA*

### **Aggressive Lymphomas**

Anna Sureda

*Hospital de la Santa Creu i Sant Pau, Barcelona, Spain*

### **Acute Myeloid Leukemia**

Martin Tallman

*Memorial Sloan-Kettering Cancer Center, New York, USA*

### **Chronic Myeloproliferative Disorders**

Ayalew Tefferi

*Mayo Clinic, Mayo Graduate School of Medicine, Division of Hematology. Mayo Clinic, Minnesota, USA*

### **Meeting the Challenge of Emerging Pathogens in Patients with Hematological Malignancies: Rational Approaches to Diagnosis, Treatment, and Prevention**

Thomas J. Walsh

*National Cancer Institute Bethesda, Bethesda, USA*

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*Surnames are in alphabetical order.*

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Dear Colleagues,

It gives us great pleasure to host the 5<sup>th</sup> International Congress on Leukemia, Lymphoma Myeloma (ICLLM 2015) in Istanbul, Turkey. The ICLLM2015 Congress provides a unique forum for scientists and medical professionals gathered from around the world to meet and exchange ideas and information in the fields of hematology and oncology. The scientific program of the ICLLM Congress boasts most of the hematology masters who aim to provide a perfect balance between clinical education and news of the latest scientific developments. There are 12 scientific sessions, 3 satellite symposia and 3 meet the expert sessions with 12 scientific chair with a total 49 chair and speakers. New diagnosis and treatment strategies of the malignant hematological diseases will be discussed with every aspect besides the standard therapies.

Istanbul as the capital of culture in Europe for 2010 started to associate with culture and the arts all over the world. Istanbul will achieve lasting gains in the fields of urban renewal, urban living and environmental and social development. Those who come to Istanbul for cultural and artistic projects will visit the city's cultural riches, mosques, churches, palaces and museums. The cultural program also promises to be special, highlighting Istanbul's proud culture and national heritage. Istanbul has been inhabited since the end of the 4<sup>th</sup> century B.C. Remains from the Hellenic, Roman, Byzantine and Ottoman periods are scattered throughout the city, prominent among them the Hagia Sophia, Basilica Cistern, Blue Mosque, Grand Bazaar, Topkapı Palace and Turkish Baths, making Istanbul a fascinating open air museum. You would have the opportunity to discover Turkish music, art and architecture, enjoy the delicious tastes of Turkish and Ottoman cuisine, and experience the world famous Turkish hospitality. The unique geography of Istanbul gives the opportunity to meet where the two continents meet. The Istanbul Wow Convention Center, located near the airport and easily accessible by public transport, offers excellent facilities, including all the necessary infrastructure and professionalism to successfully host a medical convention of this import.

Participants from 22 countries are registered, and the Congress has been accredited by both the European Hematology Association (EHA) and the Turkish Medical Association. On behalf of the Board of the Turkish Society of Hematology and scientific faculty, I would like to welcome you to the 5th International Congress on Leukemia-Myeloma-Lymphoma. I believe that you will enjoy both the scientific and cultural aspects of the program, and that you also take advantage of the pleasure of the nice Istanbul spring.

**Prof. Dr. Teoman Soysal**

*Congress President*

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Dear Colleagues,

It is great honor for me to invite you to the 5<sup>th</sup> International Congress on Leukemia, Lymphoma, Myeloma (ICLLM 2015). Scientific level of ICLLM congresses has been improving progressively year by year. I believe that this conference will represent the challenge the development of the basic studies as well as clinical researches of Hematology.

The abstracts and the educational lectures have been designated to establish an area suitable for the exchange of ideas during the meeting days. There are 124 abstracts from 13 different countries. The full text content of the educational books of the meetings of International Congress on Leukemia Lymphoma Myeloma (ICLLM), will be available in the website of Turkish Society of Hematology ([www.thd.org.tr](http://www.thd.org.tr)) just after the end of the conference.

During its long history, Istanbul has served as the Capital of the Roman Empire, the East Roman (Byzantine) Empire, the Latin Empire, and the Ottoman Empire. Located in the center of the old world, this exotic city is famous for its historical monuments and scenic views. It is the only city in the world which spreads over two continents: Asia and Europe which are separated by a narrow strait - the Bosphorus.

Even if you spend only a short time in Istanbul, you can see much of this great heritage. Although renowned for its mystery and history, Istanbul is also a rapidly modernising country with a prime location; one foot in Europe and one in the East. Thus Istanbul retains its fascinating differences, and its contradictions: mosques coexist with churches, and remnants of the Roman Empire still stand alongside ancient Hittite and Neolithic sites.

We are so glad to welcome you in this beautiful city.

Best regards.

**Prof. Dr. Ahmet Muzaffer Demir**

*On behalf of Congress Secretariat*

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# **5<sup>th</sup> International Congress on Leukemia – Lymphoma – Myeloma**

**May 21 – 23, 2015 • Istanbul, Turkey**

## **SCIENTIFIC PROGRAM**

# May 21, 2015, Thursday

## HALL A

TIME MEETING

07:15 - 08:15 **MEET THE EXPERT (Meet The Expert Hall)**  
**Providing Best Options for Myeloma Treatment**



Moderator: **G. Hayri Özsan** (*Dokuz Eylül University, Izmir, Turkey*)

Speaker: **Shaji Kumar** (*Mayo Clinic, Rochester, USA*)

08:30 - 10:00 **ACUTE MYELOID LEUKEMIA**

Scientific Chairs: **Martin Tallman** (*Memorial Sloan-Kettering Cancer Center, New York, USA*)  
**Muhit Özcan** (*Ankara University, Ankara, Turkey*)

Speakers:

- Molecular Pathogenesis and Therapy of AML Driven by Mutations and Epigenetic Modifiers: **Mark Levis** (*Johns Hopkins University, Baltimore, USA*)
- Are Novel Therapeutic Strategies Changing the Natural History of AML?: **Martin Tallman** (*Memorial Sloan-Kettering Cancer Center, New York, USA*)
- Who Should Be Transplanted for AML, and How?: **Jacob M. Rowe** (*Shaare Zedek Medical Center, Jerusalem, Israel*)

10:00 - 10:30 COFFEE BREAK

10:30 - 12:00 **MULTIPLE MYELOMA**

Scientific Chairs: **Angela Dispenzieri** (*Mayo Clinic, Rochester, USA*)  
**G. Hayri Özsan** (*Dokuz Eylül University, Izmir, Turkey*)

Speakers:

- Should We Treat SMM?  
Pro: **Angela Dispenzieri** (*Mayo Clinic, Rochester, USA*)  
Con: **Morie A Gertz** (*Mayo Clinic, Rochester, USA*)
- Should Continuous Therapy Be Used in All Patients?  
Pro: **Morie A Gertz** (*Mayo Clinic, Rochester, USA*)  
Con: **Angela Dispenzieri** (*Mayo Clinic, Rochester, USA*)
- Treating Relapsed Disease: **Shaji Kumar** (*Mayo Clinic, Rochester, USA*)

12:00 - 14:00 LUNCH

POSTER DISCUSSION

12:30 - 13:30 **NOVARTIS SATELLITE SYMPOSIUM**



Moderator: **Sema Karakuş** (*Başkent University, Ankara, Turkey*)

- Management of CML; Clinical Monitoring, Decision Points and Strategies: **Richard Clark** (*Royal Liverpool University Hospital, Liverpool, United Kingdom*)

14:00 - 15:30 **DIFFUSE LARGE B-CELL LYMPHOMA**

Scientific Chairs: **Christian Gisselbrecht** (*Hôpital Saint Louis, Paris, France*)  
**Levent Üндar** (*Akdeniz University, Antalya, Turkey*)

Speakers:

- Biopathology of DLBCL in Clinical Practice: **Philippe Gaulard** (*Hôpital Henri Mondor, Creteil, France*)
- What Can We Expect With New Drugs in DLBCL?: **Carol Portlock** (*Memorial Sloan Kettering Cancer Center, New York, USA*)
- How To Optimize Treatment in Various DLBCL. : **Christian Gisselbrecht** (*Hôpital Saint Louis, Paris, France*)

15:30 - 16:00 COFFEE BREAK

16:00 - 17:30 **CUTTING EDGE ADVANCES IN MYELOPROLIFERATIVE NEOPLASMS**

Scientific Chairs: **Ayalew Tefferi** (*Mayo Clinic, Minnesota, USA*)  
**Reyhan Diz Küçükaya** (*Istanbul Bilim University, Istanbul, Turkey*)

Speakers:

- Clonal Origination and Evolution in MPN: **Radek Skoda** (*University Hospital Basel, Basel, Switzerland*)
- Current Prognostication and Treatment of ET, PV and PMF: **Ayalew Tefferi** (*Mayo Clinic, Minnesota, USA*)
- Contemporary Science and Practice in Mast Cell and Eosinophilic Disorders: **Animesh Pardanani** (*Mayo Clinic Rochester, Minnesota, USA*)

17:30 - 19:00 OPENING CEREMONY

**May 22, 2015, Friday**

**HALL A**

**TIME MEETING**

07:15 - 08:15 **MEET THE EXPERT (Meet The Expert Hall)**



Moderator: **Nilgün Sayinalp** (*Hacettepe University, Ankara, Turkey*)

- Integrated Approach to Primary & Secondary Thrombocytopenia with Eltrombopag: **İbrahim C. Haznedaroğlu** (*Hacettepe University, Ankara, Turkey*)

08:30 - 10:00 **ACUTE LYMPHOBLASTIC LEUKEMIA**

Scientific Chairs: **Nicola Gökbuget** (*J.W. Goethe University Hospital, Frankfurt, Germany*)

**Siret Ratip** (*Acibadem Kozyatagi Hospital, Istanbul, Turkey*)

Speakers:

- Molecular Characterization of ALL: New Markers and Subgroups: **Sabina Chiaretti** (*Sapienza University, Roma, Italy*)
- Modern Management of Adult ALL : **Nicola Gökbuget** (*J.W. Goethe University Hospital, Frankfurt, Germany*)
- Optimal Treatment of Ph-positive ALL: Role of TK Inhibitors and Stem Cell Transplantation : **Josep Ribera** (*Institut Català d'Oncologia-Hospital, Badalona, Spain*)

10:00 - 10:30 COFFEE BREAK

10:30 - 12:00 **HODGKIN LYMPHOMA**

Scientific Chairs: **Bastian Von Tresckow** (*University Hospital Cologne, Cologne, Germany*)

**Rauf Haznedar** (*Gazi University, Ankara, Turkey*)

Speakers:

- Controversies in Early-Stage Hodgkin Lymphoma : **Marc Andre** (*UCL de Mont Godinne, Yvoir, Belgium*)
- Controversies in Advanced-Stage Hodgkin Lymphoma : **Bastian Von Tresckow** (*University Hospital Cologne, Cologne, Germany*)
- New Drug for Relapsed Hodgkin Lymphoma : **Bastian Von Tresckow** (*University Hospital Cologne, Cologne, Germany*)

12:00 - 14:00 LUNCH

POSTER DISCUSSION

12:30 - 13:30 **GILEAD SATELLITE SYMPOSIUM**



Moderator : **Önder Arslan** (*Ankara University, Ankara, Turkey*)

Speakers: **Paolo Ghia** (*Universita' Vita-Salute San Raffaele, Milano, Italy*)  
**Önder Arslan** (*Ankara University, Ankara, Turkey*)

14:00 - 15:30 **MEETING THE CHALLENGE OF EMERGING PATHOGENS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: ADVANCES IN DIAGNOSIS, TREATMENT, AND PREVENTION**

Scientific Chairs: **Thomas J Walsh** (*Weill Cornell Medical Center of Cornell University, New York, USA*)

Speakers:

- New Antimicrobial Agents and the Challenges of Multidrug Resistant Bacteria in Patients with Hematological Malignancies: **Thomas J Walsh** (*Weill Cornell Medical Center of Cornell University, New York, USA*)
- Advances in the Epidemiology and Treatment of Invasive Fungal Infections in Patients with Hematological Malignancies: **Maria Gamaletsou** (*National and Kapodistrian University of Athens, Greece*)
- Evolving Challenges and New Treatment Options of Respiratory and Systemic Viral Infections in Patients with Hematological Malignancies: **Nikolaos Sipsas** (*National and Kapodistrian University of Athens, Greece*)

15:30 - 16:00 COFFEE BREAK

16:00 - 18:00 **MYELODYSPLASTIC SYNDROMES**

Scientific Chairs: **H. Joachim Deeg** (*Fred Hutchinson Cancer Research Center, Seattle, USA*)  
**Mustafa Çetiner** (*American Hospital, Istanbul, Turkey*)

Speakers:

- Impact of Gene Mutations and Expression on Clinical Variables and Prognosis in MDS: **Jacqueline Boulwood** (*University of Oxford, London, UK*)
- Treatment of Higher Risk MDS (Excluding Transplant): **Lionel Adès** (*Hopital Saint Louis, Paris, France*)
- Hematopoietic Cell Transplantation for MDS: Success and Challenges: **H. Joachim Deeg** (*Fred Hutchinson Cancer Research Center, Seattle, USA*)

# May 23, 2015, Saturday

## HALL A

TIME MEETING

07:00 - 08:15 **MEET THE EXPERT (Meet The Expert Hall)**



- Welcome & Introductions: **Sevgi Beşişik** (*Istanbul University, Istanbul, Turkey*)
- Treatment of Relapsed/Refractory Hodgkin Lymphoma & Systemic Anaplastic Large Cell Lymphoma: **Anna Sureda** (*Institut Català d'Oncologia - Hospital Duran i Reynals, Barcelona, Spain*)
- R/R HL & sALCL: Two Case Studies From Ankara: **Sevgi Beşişik** (*Istanbul University, Istanbul, Turkey*)
- Discussion of Case Studies: **Anna Sureda** (*Institut Català d'Oncologia - Hospital Duran i Reynals, Barcelona, Spain*)
- Q&A From Audience: **Sevgi Beşişik** (*Istanbul University, Istanbul, Turkey*)

08:30 - 10:00 **FOLLICULAR LYMPHOMA**

Scientific Chairs: **Burhan Ferhanoğlu** (*Koç University, Istanbul, Turkey*)  
**Jane Winter** (*Northwestern University, Chicago, USA*)

Speakers:

- Introduction and Standard Therapy: **Burhan Ferhanoğlu** (*Koç University, Istanbul, Turkey*)
- The Biology of Follicular Lymphoma and How This May Inform Future Treatment: **Daniel Hodson** (*National Cancer Institute, NIH, Bethesda, USA*)
- New Treatments for Follicular Lymphoma - Towards a Chemotherapy Free Future: **Carol Portlock** (*Memorial Sloan Kettering Cancer Center, New York, USA*)

10:00 - 10:30 COFFEE BREAK

10:30 - 12:00 **CHRONIC MYELOID LEUKEMIA**

Scientific Chairs: **Richard A. Larson** (*University Of Chicago, Chicago, USA*)  
**Burhan Turgut** (*Namık Kemal University, Tekirdağ, Turkey*)

Speakers:

- Managing Complications and Pregnancy During CML Treatment : **Akif Selim Yavuz** (*Istanbul University, Istanbul, Turkey*)
- Molecular Monitoring and Treatment-free Remissions : **Ehab Atallah** (*Medical College of Wisconsin and Froedtert Hospital, Milwaukee, USA*)
- Choosing Wisely – Is There a Best TKI for CML? : **Richard A. Larson** (*University Of Chicago, Chicago, USA*)

12:00 - 14:00 LUNCH

POSTER DISCUSSION

12:30 - 13:30 **CELGENE SATELLITE SYMPOSIUM**

**The Challenge of Managing Relapsing Patients in Multiple Myeloma “Myeloma Never Gives Up - So Do We!”**

Moderator: **Mutlu Arat** (*Istanbul Bilim University, Istanbul, Turkey*)

Speaker: **G. Hayri Özsan** (*Dokuz Eylül University, Izmir, Turkey*)



14:00 - 15:30 **CHRONIC LYMPHOCYTIC LEUKEMIA**

Scientific Chairs: **Peter Dreger** (*University of Heidelberg, Hamburg, Germany*)

**Osman İlhan** (*Ankara University, Ankara, Turkey*)

Speakers:

- How To Treat CLL in Elderly Patients : **Emili Montserrat** (*University of Barcelona, Barcelona, Spain*)
- How To Treat CLL in Younger Patients : **Eva Kimby** (*Karolinska Institute Huddinge University, Karolinska, Sweden*)
- How To Treat Refractory CLL : **Peter Dreger** (*University of Heidelberg, Hamburg, Germany*)

15:30 - 16:00 COFFEE BREAK

16:00 - 18:00 **AGGRESSIVE LYMPHOMAS**

Scientific Chairs: **Anna Sureda** (*Institut Català d'Oncologia - Hospital Duran i Reynals, Barcelona, Spain*)

**Mutlu Arat** (*Istanbul Bilim University, Istanbul, Turkey*)

Speakers:

- Treatment of T-cell Lymphomas in the Era of New Drugs : **Norbert Schmitz** (*ASKLEPIOS Klinik St. Georg, Hamburg, Germany*)
- Current Therapy for Primary CNS Lymphoma : **Benjamin Kasenda** (*Universitatsspital Basel, Basel, Switzerland*)
- Treatment Options for transformed Follicular Lymphoma : **Jane Winter** (*Northwestern University, Chicago, USA*)
- New Insights in the Treatment of Mantle Cell Lymphoma : **Olivier Hermine** (*Imagine Institut des Maladies Génétiques, France*)



**5<sup>th</sup> International Congress on  
Leukemia – Lymphoma – Myeloma**

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**PROCEEDINGS**



## CURRICULUM VITAE

### Mark J. Levis

#### Current Appointments

University: Associate Professor of Oncology and Medicine Director, Adult leukemia program  
Kimmel Comprehensive Cancer Center at Johns Hopkins  
Johns Hopkins School of Medicine

Hospital: Active Staff  
The Johns Hopkins Hospital  
Baltimore, Maryland

#### Personal Data

Business Address: Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins  
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Tel. 410 502-3629  
Fax 410 614 7279  
Email: [levisma@jhmi.edu](mailto:levisma@jhmi.edu)

#### Education and Training:

Undergraduate:	1984	A.B. University of California, Berkeley, CA, in Genetics
Doctoral/Graduate:	1992	PhD, University of California, San Francisco School of Medicine, San Francisco, in Biochemistry; Henry Bourne MD thesis advisor
	1994	MD, University of California, San Francisco School of Medicine, San Francisco, in Medicine
Postdoctoral	1994-1997	Residency in Internal Medicine. Osler Medical Service at Johns Hopkins, Baltimore, MD
	1999-2002	Fellowship in Medical Oncology, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD

#### Professional Experience

1984-1985	Staff Research Associate, Department of Genetics, University of California, Berkeley.
1997-1998	Clinical Instructor, Department of Medicine, Union Memorial Hospital, Baltimore, MD
1998-1999	Associate Chief of Service, Osler Medical Service, Johns Hopkins University, Baltimore, MD
2002-2008	Assistant Professor of Oncology, Johns Hopkins University, Baltimore, MD
2008-present	Associate Professor of Oncology and Medicine, Johns Hopkins University, Baltimore, MD
2013-present	Director, Adult Leukemia Program, Sidney Kimmel Comprehensive Cancer Center

#### RESEARCH ACTIVITIES

##### Publications: Peer-reviewed Original Science Research

1. Levis M, Bourne H. Activation of the alpha subunit of Gs in intact cells alters its abundance, rate of degradation, and membrane avidity. *J Cell Biol.* 1992 Dec;119(5):1297-307.
2. Wedegaertner P, Chu D, Wilson P, Levis M, Bourne H. (1993). Palmitoylation is required for signaling functions and membrane attachment of Gq alpha and Gs alpha. *J Biol Chem.* 1993 Nov 25;268(33):25001-8.
3. Levis M, Tse K-F, Smith BD, Garrett E, Small D. A FLT3 tyrosine kinase inhibitor is cytotoxic to AML blasts harboring a FLT3/ITD mutation. *Blood.* 2001 Aug 1;98(3):885-7.
4. Levis M, Allebach J, Tse K-F, Sheng R, Baldwin BR, Smith BD, Jones-Bolin S, Ruggeri B, Dionne C, Small D. A FLT3-targeted tyrosine kinase inhibitor is cytotoxic to leukemia cells in vitro and in vivo. *Blood.* 2002 Jun 1;99(11):3885-91.
5. Tse K-F, Allebach J, Levis M, Small D. A FLT3 tyrosine kinase inhibitor induces growth arrest and apoptosis in BaF3 cells expressing constitutively activating mutant forms of the receptor. *Leukemia.* 16:2027.
6. Smith BD, Levis M, Beran M, Giles F, Brown P, Russell L, Hellriegel E, Murphy K, Dausers T, Allebach J, Small D. Single agent CEP-701, a novel FLT3 inhibitor, shows initial response in patients with refractory acute myeloid leukemia. *Blood.* 2004 May 15;103(10):3669-76. Epub 2004 Jan 15.
7. Murphy KM, Levis M, Hafez MJ, Geiger T, Cooper LC, Smith BD, Small D, Berg KD. Detection of FLT3 internal tandem duplication and D835 mutations by a multiplex polymerase chain reaction and capillary electrophoresis assay. *J Mol Diagn.* 2003 May 3;5(2):96-102. PMC1907323
8. Zheng R, Levis M, Pilotto O, Brown P, Baldwin B, Small D. FLT3 ligand (FL) causes autocrine signaling in AML cells. *Blood.* 2004 Jan 1;103(1):267-74. Epub 2003 Sep 11.
9. Zheng R, Friedman AD, Levis M, Li L, Weir EG, Small D. Internal tandem duplication mutation of FLT3 blocks myeloid differentiation through suppression of C/EBPalpha expression. *Blood.* 2004 Mar 1;103(5):1883-90. Epub 2003 Oct 30.
10. Levis M, Pham R, Smith BD, Small D. In vitro studies of a FLT3 inhibitor combined with chemotherapy: sequence of administration is important in order to achieve synergistic cytotoxic effects. *Blood.* 2004 Aug 15;104:1145-50.
11. Levis M, Murphy KM, Pham R, Kim KT, Stine A, Li L, McNiece I, Smith BD, Small D. Internal tandem duplications of the FLT3 gene are present in leukemia stem cells. *Blood* 2005 Jul 15;106(2):673-80. PMC1895185
12. Brown P, Meshinchi S, Levis M, Alonzo TA, Gerbing R, Lange B, Arceci R, Small D. Pediatric AML Primary Samples with FLT3/ITD Mutations are Preferentially Killed by FLT3 Inhibition. *Blood.* 2004 Sep 15;104(6):1841-9.
13. Brown P, Levis M, Shurtleff S, Campana D, Downing J, Small D. FLT3 inhibition selectively kills child hood acute lymphoblastic leukemia cells with high levels of FLT3 expression. *Blood* 2005;Jan 15;105:812-20
14. Kim KT, Baird K, Ahn JY, Meltzer P, Lilly M, Levis M, and Small D. Pim-1 is upregulated by constitutively activated FLT3 and plays a role in FLT3-mediated cell survival. *Blood.* 2005 Feb 15;105(4):1759-67.
15. Chen P, Levis M, Brown P, Kim KT, Allebach J, Small D. FLT3/ITD mutation signaling includes suppression of SHP-1. FLT3/ITD mutation signaling includes suppression of SHP-1. *J Biol Chem.* 2005 Feb 18; 280(7):5361-9.
16. Pilotto O, Levis M, Huso D, Li Y, Li H, Wang M-N, Bassi R, Balderes P, Ludwig DL, Witte L, Zhu Z, Kicklin DJ, Small D. Inhibitory Anti-FLT3 antibodies are capable of mediating Antibody-dependent cell-mediated cytotoxicity and reducing engraftment of acute myelogenous leukemia blasts in nonobese diabetic/severe combined immunodeficient mice. *Cancer Research* 2005 Feb 15;65(4): 1514-22.
17. Brown P, Levis M, McIntyre E, Griesemer M, Small D. Combinations of the FLT3 inhibitor CEP-701 and chemotherapy synergistically kill infant and childhood MLL-rearranged ALL cells in a sequence-dependent manner. *Leukemia* 2006 Aug;20(8):1368-76. Epub 2006 Jun 8.
18. Levis M, Brown P, Smith BD, Stine A, Pham R, Stone R, DeAngelo D, Galinsky I, Giles F, Estey E, Kantarjian H, Cohen P, Wang Y, Roesel J, Karp J, and Small D. Plasma inhibitory activity (PIA): A pharmacodynamic assay reveals insights into the basis for cytotoxic response to FLT3 inhibitors. *Blood* 2006 Nov 15;108(10):3477. PMC1895426
19. Knapper S, Burnett AK, Littlewood T, Kell WJ, Agrawal S, Chopra R, Clark R, Levis MJ, Small D. A phase 2 trial of the FLT3 inhibitor lestaurtinib (CEP701) as first line treatment for older patients with acute myeloid leukemia not considered fit for intensive chemotherapy. *Blood* 2006 Nov 15;108:3262.
20. Kim KT, Levis M, Small D. Constitutively activated FLT3 phosphorylates BAD partially through Pim-1. *Br J Haematol* 2006 Sep;134(5):500-9.

21. Piloto O, Wright M, Brown P, Kim KT, **Levis M**, Small D. Prolonged exposure to FLT3 inhibitors leads to resistance via activation of parallel signaling pathways. *Blood*. 2007 Feb 15;109(4):1643-52. PMC 1794049
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Savani BN, Fraser JW, Sadrzadeh H, Rajkhowa T, Ito S, Jain NA, Battiwalla M, Fathi AT, <b>Levis MJ</b> , Barrett AJ, Hourigan CS. Expression of putative targets of immunotherapy in acute myeloid leukemia and healthy tissues. <i>Leukemia</i> 2014; 28(5):1167-70. PMID 24472813.	9/1/13-8/31/18	Incorporating FLT3 inhibitors into AML treatment regimens MD Anderson SPORE \$198,913 (per year) PI, 15% 1.8 calendar
67. Ravandi F, Arana Yi C, Cortes JE, <b>Levis M</b> , Faderl S, Garcia-Manero G, Jabbour E, Konopleva M, O'Brien S, Estrov Z, Borthakur G, Thomas D, Pierce S, Brandt M, Pratz K, Luthra R, Andreeff M, Kantarjian H. Final report of phase II study of sorafenib, cytarabine and idarubicin for initial therapy in younger patients with acute myeloid leukemia. <i>Leukemia</i> 2014; 28(7):1543-5. PMID 24487412.	1/1/14 - 6/30/16	Correlative studies relating to in vivo FLT3 inhibition after transplant Novartis \$178,842 PI, 5% 0.60 calendar
68. Yang X, <b>Levis M</b> . Pulsed-high-dose dexamethasone as a treatment for pure red cell aplasia following ABO-incompatible allogeneic stem cell transplantation. <i>Transfus Med</i> . 2014. 24(4):246-8. PMID: 25124073.	1/1/14 - 6/30/15	In vivo FLT3 inhibition in patients treated with ASP2215 Astellas 248,035 PI, 5 % 0.60
69. Chen YB, Li S, Lane AA, Connolly C, Del Rio C, Valles B, Curtis M, Ballen K, Cutler C, Dey BR, El-Jawhri A, Fathi AT, Ho VT, Joyce A, McAfee S, Rudek M, Rajkhowa T, Verselis S, Antin JH, Spitzer TR, <b>Levis M</b> , Soiffer R. Phase I trial of maintenance sorafenib after allogeneic hematopoietic stem cell transplantation for Fms-like tyrosine kinase 3 internal tandem duplication acute myeloid leukemia. <i>Biol Blood Marrow Transplant</i> . 2014. 20(12):2042-8. PMID: 25239228.	<b>Previous:</b> 7/1/03-6/30/08	Targeting FLT3 as a novel, specific therapy for leukemia 1K08 CA95600 NIH/NCI 126,750 (per year) PI, 75% effort, 9 calendar
70. Grunwald MR, Tseng LH, Lin MT, Pratz KW, Eshleman JR, <b>Levis MJ</b> , Gocke CD. Improved FLT3/ITD PCR assay predicts outcome following allogeneic transplant for AML. <i>Biol Blood Marrow Transplant</i> . 2014. 20(12):1989-95. PMID: 25240816.	7/1/03-6/30/06	Targeting FLT3 as a novel, specific therapy or leukemia M031068 Sydney Kimmel Foundation for Cancer Research \$200,000 (per year) PI
71. Kanakry CG, Tsai HL, Bolaños-Meade J, Smith BD, Gojo I, Kanakry JA, Kasamon YL, Gladstone DE, Matsui W, Borrello I, Huff CA, Swinnen LJ, Powell JD, Pratz KW, DeZern AE, Showel MM, McDewitt MA, Brodsky RA, <b>Levis MJ</b> , Ambinder RF, Fuchs EJ, Rosner GL, Jones RJ, Luznik L. Post-transplantation cyclophosphamide as single-agent GVHD prophylaxis after myeloablative conditioning and HLA-matched allografting for acute leukemias and myelodysplastic syndrome. <i>Blood</i> . 2014. 124(25):3817-27. PMID: 25316679.	7/1/03-6/30/06	Targeting FLT3 as a novel, specific therapy or leukemia M030933 ASCO \$170,100 (per year) PI
72. Galanis A, <b>Levis M</b> . Inhibition of c-Kit by tyrosine kinase inhibitors. <i>Haematologica</i> 2014 [Epub ahead of print]. PMID: 25425690.	7/1/03-6/30/06	Targeting FLT3 as a novel, specific therapy or leukemia M030933 ASCO \$170,100 (per year) PI
73. Strati F, Kantarjian H, Ravandi F, Nazha A, Borthakur G, Daver N, Kadia T, Estrov Z, Garcia-Manero G, Konopleva M, Rajkhowa T, Durand M, Andreeff M, <b>Levis M</b> , Cortes J. Phase I/II Trial of the combination of midostaurin (PKC412) and 5-azacytidine for patients with acute myeloid leukemia and myelodysplastic syndrome. <i>Am J Hematol</i> . 2014. [Epub ahead of print]. PMID: 25530214.	4/1/08-2/28/13	Incorporating FLT3 inhibitors into AML treatment regimens R01CA128864 NIH \$207,500 (per year) PI, 35% effort, 4.2 calendar
<b>Reviews:</b>	5/1/08-4/30/13	FLT3: Role in leukemia stem cells P01 CA070970 NIH \$184,111 (per year) Co-Investigator, 15% effort, 1.8 calendar
1. <b>Levis M</b> , Small D. FLT3: IT Does matter in leukemia. <i>Leukemia</i> . 2003 Sep;17(9):1738-52. Review.	7/1/08-6/30/11	Incorporating FLT3 inhibitors into AML treatment regimens American Society of Clinical Research (ASCO) \$139,500 (per year) PI, 10% effort, 1 calendar
2. <b>Levis M</b> , Small D. Novel FLT3 kinase inhibitors. <i>Expert Opin Investig Drugs</i> . 2003 Dec;12(12):1951-62. Review.	7/1/08-6/30/13	Incorporating FLT3 inhibitors into AML treatment regimens Leukemia & Lymphoma Society \$104,762 (per year) PI, 10%, 1.2 calendar
3. <b>Levis M</b> , Small D. Small molecule FLT3 tyrosine kinase inhibitors. <i>Curr Pharm Des</i> . 2004;10(11):1183-93. Review.	9/1/08-8/31/13	Incorporating FLT3 inhibitors into AML treatment regimens MD Anderson \$184,111 (per year) PI, 15% 1.8 calendar
4. <b>Levis M</b> , Small D. Kinase Inhibitors in Leukemia. <i>Advances in Pharmacology</i> 2004;51:1-33.	<b>Research Program Building/Leadership</b>	None
5. <b>Levis M</b> , Small, D. FLT3 Tyrosine Kinase Inhibitors. <i>Int J Hematol</i> . 2005 Aug;82(2):100-7.	<b>EDUCATIONAL ACTIVITIES</b>	
6. <b>Levis M</b> . Recent advances in the development of small-molecule inhibitors for the treatment of acute myeloid leukemia. <i>Curr Opin Hematol</i> . 2005; 12(1):55-61. PMID:15604892.	<b>Educational Publications</b>	
7. Pratz KW, <b>Levis M</b> . Incorporating FLT3 inhibitors into acute myeloid leukemia treatment regimens. <i>Leuk Lymphoma</i> 2008 49(5):852-63. PMID: 3031857.	<b>Invited Editorials</b>	
8. Fathi A, <b>Levis M</b> . Lestaurtinib: a multi-targeted FLT3 inhibitor. <i>Expert Rev Hematol</i> . 2009; 2(1):17-26. PMID: 21082990.	1. <b>Levis M</b> . CXCR-4: homing in on FLT3. <i>Blood</i> . 2004;104: 303-304.	
9. Pratz KW, <b>Levis MJ</b> . Bench to bedside targeting of FLT3 in acute leukemia. <i>Curr Drug Targets</i> . 2010;11(7):781-9. PMID: 3023996	2. <b>Levis M</b> . FLT3 and acute myeloid leukemia: what is the wild type receptor up to? <i>Haematologica</i> . 2005;90(12):1586.	
10. <b>Levis MJ</b> . Will newer tyrosine kinase inhibitors have an impact in AML? <i>Best Pract Res Clin Haematol</i> . 2010;23(4):489-94. PMID: 3049331.	3. <b>Levis M</b> . FLT3: The root of the problem. <i>Blood</i> . 2006;108:2501.	
11. Fathi A, <b>Levis M</b> . FLT3 inhibitors: a story of the old and the new. <i>Curr Opin Hematol</i> . 2011;18(2):71-6. PMID: 3082742.	4. <b>Levis M</b> . Who's dancing with FLT3? <i>Blood</i> . 2008;111:2503.	
12. Grunwald MR, <b>Levis MJ</b> . FLT3 inhibitors for acute myeloid leukemia: a review of their efficacy and mechanisms of resistance. <i>Int J Hematol</i> . 2013; 97(6):683-94. PubMed PMID: 23613268.	5. <b>Levis M</b> . Targeting IDH: the next big thing in AML. <i>Blood</i> . 2013;122(16):2270-1.	
13. Kayser S, <b>Levis MJ</b> . FLT3 tyrosine kinase inhibitors in acute myeloid leukemia: Clinical implications and limitations. <i>Leuk Lymphoma</i> . 2014; 55(2):243-55. PMID: 23631653.	<b>Textbooks:</b>	
14. <b>Levis M</b> . FLT3 mutations in acute myeloid leukemia: what is the best approach in 2013? <i>Hematology Am soc Hematol Educ Program</i> . 2013;2013:220-6. PMID: 24319184.	1. "Selected Topics in Oncology" in The Johns Hopkins Internal Medicine Board Review, 2nd edition, 2007. Mosby Press.	
15. <b>Levis M</b> . Quizartinib in acute myeloid leukemia. <i>Clin Adv Hematol Oncol</i> . 2013; 11(9):586-8. PMID 24518522.	2. "FLT3: A prototype receptor tyrosine kinase target in AML" in <i>Acute Myelogenous Leukemia</i> . 2007. Humana Press.	
16. König H, <b>Levis M</b> . Is targeted therapy feasible in acute myelogenous leukemia? <i>Curr Hematol Malig Rep</i> . 2014; 9(2):118-27. PMID: 24599573.	3. "FLT3: A Receptor Tyrosine Kinase Target In Adult And Pediatric AML" in <i>Innovative Leukemia and Lymphoma Therapy</i> . Informa Healthcare. In Press.	
17. Wander SA, <b>Levis MJ</b> , Fathi AT. The evolving role of FLT3 inhibitors in acute myeloid leukemia: quizartinib and beyond. <i>Ther Adv Hematol</i> . 2014 5(3):65-77 PMID: 24883179.	<b>Teaching</b>	
18. König H, <b>Levis M</b> . Targeting FLT3 to treat leukemia. <i>Expert Opin Ther Targets</i> . 2014 18:1-18. PMID: 25231999.	9/83-5/84	"Laboratory Methods in Cell Biology", University of California, Berkeley, Teaching assistant.
19. <b>Levis M</b> . Quizartinib for the treatment of FLT3/ITD acute myeloid leukemia. 2014; 10(9):1571-9. PMID: 25145428.	7/97-6/98	Clinical Instructor, Department of Medicine, Union Memorial Hospital, Baltimore,
20. König H and <b>Levis M</b> . Targeting FLT3 to treat leukemia. <i>Expert Opin Ther Targets</i> . 2015 19(1):37-54. PMID: 25231999.		
21. Showel MM, <b>Levis M</b> . Advances in treating acute myeloid leukemia. <i>F1000Prime Re</i> . 2014 6:96. PMID: 25374674.		
<b>Inventions, Patents, Copyrights</b>	<b>None</b>	
<b>Extramural Funding:</b>		
<b>Current Grants:</b>		
12/1/95-8/31/17	Bone Marrow Transplantation in Human Disease NCI \$226,341 Co-Investigator, 2% 0.24 calendar	

MD. Full time clinical instruction of medical residents and medical students, inpatient and ambulatory medicine.

1998 Instructor, clinical skills course, The Johns Hopkins University School of Medicine, Clinical preceptor for Johns Hopkins medical students, instruction in physical diagnosis and oral presentations.

7/98-6/99 Instructor (Associate Chief of Service), Department of Medicine, Johns Hopkins University School of Medicine, Clinical instruction of medical residents and medical students.

1999-present Osler Medical Residency Intern Selection Committee

2003-2007 Board Review in Internal Medicine (CME course): "Topics in Medical Oncology"

2004-2007 Clinical instruction: Hematologic malignancies, housestaff lecture series, (biweekly lectures).

2005-present Firm faculty, Janeway Firm, Department of Medicine, Johns Hopkins University.

2005-present Designated as "Key Clinical Faculty," Osler Medical Residency, Johns Hopkins University.

CME Instruction None  
Workshops None

**Mentoring**

2001-2003 Rosalyn Pham, Johns Hopkins University undergraduate, tutorial 2001-2003.  
2004 Michele Armacost, Johns Hopkins University post-Baccalaureate program  
2006, fall Resident research project, Amy DeZern MD  
2008-2009 Omotayo Arowojolu, Johns Hopkins University undergraduate, tutorial 2008-2009

**Thesis committee member**

2003-2006 Oduilio Piloto, graduate student in molecular and cellular biology.  
2004-present Melissa Greidinger, graduate student in molecular and cellular biology.  
2008-present Emily Bailey, graduate student in molecular and cellular biology.

**Laboratory mentor**

2007-2010 Keith Pratz MD, fellow in medical oncology.  
2007-2009 Amir Fathi MD, fellow in medical oncology.

**Graduate student thesis advisor**

2009-2012 Amy Sexauer, Johns Hopkins University MSTP student  
2010-present Allison Galanis, Johns Hopkins University, Cellular and Molecular Medicine

Educational Program Building/Leadership None

Educational Extramural Funding None

**CLINICAL ACTIVITIES**

**Certification**

1997-present Maryland medical license # D0052391  
1997-present DEA # BL5543841  
2002 Board Certified, Medical Oncology, valid through 2012

**Service Responsibilities:**

2002-present Attending physician, Leukemia Service, 8-10 weeks/year  
2002-present Weekly hematologic malignancies clinic, new and follow-up patients.

Clinical Program Building/Leadership None

Clinical Extramural Funding None

**SYSTEM INNOVATION AND QUALITY IMPROVEMENT ACTIVITIES:**

Production of guidelines and/or protocols: Cancer.gov/PDQ Adult treatment guidelines (2004-present)

**ORGANIZATIONAL ACTIVITIES**

**Institutional Administrative Appointments**

**Editorial Activities**

Editorial Boards  
2004-present PDQ Adult Treatment Editorial Board, member (NCI-sponsored cancer literature review board).  
2004-2010 ISDC Signal Transduction Task Force, member (NCI-sponsored working group).  
2009-present Section Editor, *Leukemia*

**Ad hoc manuscript referee** for the following peer-reviewed journals:

Blood  
Leukemia  
Haematologica  
Leukemia Research  
Leukemia and Lymphoma  
Clinical Leukemia  
Cancer Research  
Clinical Cancer Research  
New England Journal of Medicine

**Abstract reviewer:**

American Society of Hematology Annual Meeting 2005  
European Hematology Association Annual Meeting, 2010.

**Grant review**

2011-present Developmental Therapeutics study section, National Cancer Institute- Charter member  
2009-present The ASCO Conquer Cancer Foundation Grants Selection Committee  
2014 The ASCO Conquer Cancer Foundation Grants Selection Committee- president

**Advisory Committees, Review Groups/Study Sections**

**Professional Societies:**

2000-present Member, American Society of Hematology  
2000-present Member, American Society of Clinical Oncology

**Conference Organizer, Session Chair**

**Consultantships**

**RECOGNITION**

**Awards, Honors**

1984 Baccalaureate awarded with High Honors and High Distinction in General Scholarship, University of California, Berkeley  
1984 Beta Kappa  
2005-2007 Medical Scientist Training Program, University of California, San Francisco, awarded 1985.  
1998-1999 Associate Chief of Service, Osler Medical Service, Johns Hopkins University, 1998-1999.  
2002 Daniel Nathans Research Award, (Young Investigator Award, Johns Hopkins University), for work entitled "A FLT3 tyrosine kinase inhibitor is cytotoxic to leukemia cells *in vitro* and *in vivo*".  
2005-2007 Osler housestaff teaching award, 2005; 2007.  
2008 Director's Teaching Award in Clinical Science 2008.  
2008 Clinical Scholar of the Leukemia and Lymphoma Society 2008.  
2008 Advanced Clinical Research Award, American Society of Clinical Oncology, 2008.

**Invited Talks**

11/02 FLT3: A rational therapeutic target in AML; Molecular Therapeutics Symposium; American Society of Clinical Oncology  
6/06 The Promise of FLT3 Inhibitors in AML; 11<sup>th</sup> Congress, Amsterdam; European Hematology Association  
6/06 A randomized, open-label study of lestaurtinib (CEP-701), an oral FLT3 inhibitor, administered in sequence with chemotherapy in patients with relapsed AML harboring FLT3 activating mutations; 11<sup>th</sup> Congress, Amsterdam; European Hematology Association  
10/06 Hematology Grand Rounds; Durham, NC; Duke Comprehensive Cancer Center  
7/07 Hematology Grand Rounds; Charleston, SC; Medical University of South Carolina  
10/07 Invited speaker; Yokohama Japan; Annual Meeting, Japanese Society of Hematology  
6/08 Satellite Symposium; Copenhagen, European Hematology Association  
1/09 Invited speaker; Nagoya, Japan; GCOE first annual symposium  
2/09 Hollings Hematologic Malignancies Symposium, Invited speaker; Medical University of South Carolina  
11/09 International Conference on Differentiation Therapy; Chicago, IL; Northwestern University  
4/10 Acute Leukemia Forum (ALF); San Francisco, CA  
5/10 ASCO/ASH joint symposium; McCormick Place, Chicago IL  
11/10 Hematologic Malignancies; Houston, TX; MD Anderson Cancer Center  
11/10 Hematology Grand Rounds; Bethesda, MD; NHLBI  
11/10 15<sup>th</sup> UC San Diego/Univ. Heidelberg Joint Symposium – Advances in stem cell transplantation  
Heidelberg, Germany; UC San Diego/Univ. Heidelberg  
2/11 Grand Rounds, Bone marrow transplant/Heme malignancies; Boston, MA; Dana Farber Cancer Institute  
5/11 Grand Rounds, Bone marrow transplant/Heme malignancies; Penn State Hershey Medical Center  
9/11 ASH 2011 State-of-The-Art Symposium; Chicago, IL; American Society of Hematology  
10/11 Leukaemia & Lymphoma Research – Acute Myeloblastic Leukaemia in 2011: A Symposium in recognition of the work of Alan Burnett; London, England; LLS UK  
12/12 Meet the Professor, San Diego, CA; ASH  
6/12 Grand Rounds; Houston, TX; MD Anderson Cancer Center  
1/9/13 Grand Rounds, Hematology/Oncology; Boston, MA; Massachusetts General Hospital  
5/31/13 New Drugs in Oncology Seminar; McCormick Place, Chicago IL; FDA/ASCO  
9/18/13 Meet the Professor/Role of FLT3 inhibitors; Houston, TX; MD Anderson/SOHO  
11/6/13 Quizartinib; New York, NY; Chemotherapy Foundation Symposium/NYU  
12/8/13 Chair, Education Session AML, ASH New Orleans, LA: FLT3 mutations in acute myeloid leukemia: what is the best approach in 2013?  
3/28/14 Highlights of ASH, Asia: Acute Myeloid Leukemia. Singapore.  
4/18/14 University of Chicago Phase 2 Symposium keynote speaker. Chicago, IL.

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# Are Novel Therapeutic Strategies Changing the Current Outcomes of AML?

**Martin S. Tallman**

*Leukemia Service*

*Memorial Sloan-Kettering Cancer Center, Weill Cornell Medical College New York, NY*

**C**urrent areas of research in the therapy of acute myeloid leukemia (AML) include new insights into outcomes with established agents or regimens, combinations of agents with known activity in AML, minimal residual disease (MRD), allogeneic hematopoietic cell transplantation (HCT) and a burgeoning number of promising novel agents. In acute promyelocytic leukemia (APL), ATRA and ATO without chemotherapy for low-risk patients (pts) is a new standard of care. The APLO406 trial led by the GIMEMA for low-risk pts was updated at the recent ASH 2014 Meeting and demonstrated an even better OS advantage for the ATRA plus ATO combination without chemotherapy than initially reported. The APLM4 trial from Australia, in which all pts received 4 doses of idarubicin with ATRA and ATO, was updated at ASH 2014 and showed excellent outcome in all risk groups, but age over 70 and risk score influenced outcome. A follow-up analysis of the ECOG E1900 trial which initially demonstrated improved outcome for intensified daunorubicin (dauno) (90 mg/m<sup>2</sup> dose) in AML pts less than age 50 and those with intermediate-risk cytogenetics now revealed a benefit across all cytogenetic risk groups including unfavorable-risk and in FLT3 positive patients, groups not observed to benefit in the initial report. The UK AML17 trial included a randomization in induction between dauno 90 mg/m<sup>2</sup> dose and 60 mg/m<sup>2</sup> and showed no OS benefit in any subgroup in a presentation at ASH 2014, but the study was complicated. A final analysis of the ALFA0701 trial no longer demonstrated an improvement in OS,

but still did show a benefit in the primary endpoint of EFS and RFS for the addition of GO to chemotherapy in pts with AML ages 50-70. Sorafenib improved event-free (EFS) and relapse-free survival, but not OS in a randomized trial with conventional chemotherapy in younger pts with new AML. Its role remains to be determined. Dasatinib as a c-KIT inhibitor may overcome the unfavorable impact of this mutation in core binding factor AML, but randomized trials will need to be carried out before such a strategy can be routinely adopted. MRD has been shown to be a powerful independent prognostic factor in NPM1-pos AML. Furthermore, the DNMT3A mutation status added to prognostication in pts with the double mutation at diagnosis. Allogeneic HCT studies continue to benefit CR1 pts with intermediate- and high-risk cytogenetics, in older adults and in those with secondary AML. An unprecedented number of novel agents with unique mechanism(s) of action are in phase I or II studies in AML and include CSL362 (anti-IL3Ra/anti-CD123), ABT-199 (BH3 mimetic/anti-BCL2), MDM2 antagonist (activates p53 promoting apoptosis), BET inhibitor, IDH inhibitors 1 and 2 (AG221 and AG120), DOT1L inhibitor (against MLL and DNMT3A), Crenolanib (FLT3 inhibitor), SGN33A (humanized anti-CD33 antibody). These agents are promising and it is hoped that 1 or more, either as a single agent or very possibly combined with chemotherapy or other novel agents, will change the current outcomes in AML.

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# Who Should Be Transplanted for AML, and How?

**Jacob M. Rowe**

*Shaare Zedek Medical Center, Jerusalem; Rambam Medical Center and Technion, Haifa, Israel*

## Why

Acute myeloid leukemia (AML) remains a devastating disease. Despite significant progress over the past four decades, less than 40% of adults <60 years are long-term survivors and only 10% of patients over age 60. Disease resistance is the main cause for these data.

Allogeneic hematopoietic cell transplantation (HSCT) provides the most potent anti-leukemia therapy through harnessing of the immune system, known as the graft-versus-leukemia effect (GvL).

The only reason not to offer an allogeneic transplant to every patient with AML is the significant morbidity and mortality, mostly through GvHD, associated with this procedure. Allogeneic ASCT has dramatically altered the outcome of many patients who could not otherwise be cured. Through the vastly increasing availability of unrelated donors and the development of novel conditioning regimens, the procedure can now be offered to older adults as well as those with co-morbidities.

## When

One of the most important questions asked by patients and physicians is the rationale for transplanting patients early in the course of the disease. Specifically, for AML patients, there are adequate data that transplantation in second remission offers a 25-35% probability of long-term survival. Therefore, despite data for the increased efficacy of a transplant in first remission compared to non-transplant regimens, many physicians or patients may prefer to take the risk of an increasing risk of relapse knowing that there is a reasonable chance of achieving a long-term survival and, even cure, in second remission. While the data for transplantation in second remission is correct, such thinking is thoroughly misleading. These data represent patients who have survived the relapse, who have attained a second remission, who have survived the period waiting for a donor and are then fit again

to undergo a second transplant. In reality, the long-term survival from relapse in large studies for young adults with AML is no more than about 10-15%. Thus, it is crucial to understand and interpret the data appropriately when advising patients

## How

Allogeneic transplantation has been around for decades and it is fair to say that in the first 2 decades almost all transplants were from HLA-matched family members, almost always siblings.

The time to find an HLA donor, the paucity of donors in any registries and the excessive toxicity from such, all conspired to make transplantation from alternative donors unrealistic. The availability of a donor in close to 90% of the Caucasian population, through worldwide registries of over 20,000,000 donors, has dramatically increased the donor pool, enabling many more patients to be transplanted. Additionally, alternative donor strategies using genetically haploidentical donors as well as umbilical cord transplantation provide a safe and efficacious alternative to patients who do not have a suitable donor. The average waiting time for a matched unrelated has decrease for about 6 months to 6-10 weeks in most cases. Without doubt, the use of alternative donors has dramatically altered the landscape of transplantation.

## For Whom

Because of the potent graft-versus-leukemia effect, allogeneic transplantation has a superior efficacy for all patients with AML. Nevertheless, the significant toxicity from allogeneic transplants dictate that such a procedure only be performed if risk of relapse without an allogeneic transplant significantly outweighs the non-relapse mortality from the transplant. The European Leukemia Net, has proposed a prognostic model that considers all pre-, peri- and post-transplant prognostic factors in assigning an appropriate model to determine those

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patients who are most appropriately referred for an allogeneic transplant. In essence, for a fit adult patient, this should include all patients in the intermediate or poor-risk groups. The less poor-risk groups (mistakenly referred to as “good-risk”) are those with t(8;21) without a high WBC; inv(16), mutated CEBPA (double allelic); mutated MPM1 (no FLT3-ITD mutation). For such patients, an allogeneic transplant in first remission is generally not recommended, at least not until we learn to significantly reduce or completely overcome the toxicity of the procedure.

For those patients who are not candidates for an allogeneic transplantation either because the risk-benefit ratio is not in favor of a transplant or because a donor is not available, autologous hematopoietic stem cell transplantation offers the most potent anti-leukemia therapy, consistently providing superior data when assessing the rate of relapse or disease-free survival. Early studies from two decades ago were hampered by excessive mortality from this procedure which abrogated the benefit of the potent anti-leukemia strategy. However, currently, autologous transplantation, using peripheral blood

cells as the source of hematopoietic stem cells, has a mortality that is similar to a standard course of consolidation with high-dose cytarabine. Thus, all AML patients, in first remission, who are not candidates for an allogeneic transplant should be offered an autologous transplant in consolidation as the most potent anti-leukemic therapy.

The use of minimal residual disease (MRD) pre-transplantation is becoming an important tool to predict for success, and timing, of a transplant both in first or second complete remission.

For patients who have relapsed and have achieved a second remission, an allogeneic transplant is the preferred choice, irrespective of any pre-transplant genetic risk factors. In general, patients in relapse or refractory state have a poor outcome post-transplantation, mostly due to disease recurrence. For such patients, an allogeneic transplant should only be considered in select cases where the likelihood of achieving a remission without a transplant is remote, and in the presence of a low pre-transplant co-morbidity score.

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# Dr. Gertz's Debates

**Angela Dispenzieri, Morie A. Gertz**

*Mayo Clinic, Rochester, USA*

**Debate 1:** Recently a subset of patients with asymptomatic multiple myeloma have been defined as high risk. These patients are at substantial risk of developing symptomatic multiple myeloma in a twenty-four month follow-up period. Recent trials have begun to look at the role of early intervention in these patients. I will be presenting data to suggest that the standard of care remains observation and those patients with asymptomatic disease, even if high risk should only be considered for therapy in the context of well-designed prospective randomized clinical trials.

**Debate 2:** Maintenance therapy both in the transplant and non-transplant population has increasingly been adopted for improvement in progression free survival, quality of life, and overall survival in multiple myeloma patients. An unsettled issue is to whether patients should be on maintenance therapy after induction, consolidation and or transplantation. I will be arguing that, maintenance therapy optimizes outcomes in patients with multiple myeloma.

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# Management of Relapsed Multiple Myeloma

**Shaji Kumar**

*Mayo Clinic, Rochester, USA*

The treatment paradigms for multiple myeloma has dramatically shifted during the last decade due to availability of several effective drugs. The introduction of new drugs, increased utilization of high dose therapy and increasing awareness of the supportive care issues have all led to significant improvement in overall survival in myeloma. However, the vast majority of patients suffer disease relapse, which eventually becomes refractory to available therapies. The improved survival of myeloma patients have led to a large proportion of patients with relapsed disease in the daily clinical practice and the need to develop new approaches for relapsed disease that are effective. Several uniform definitions have been adopted to well categorize the disease relapse in order to facilitate conduct of clinical trials and also to allow clinicians to make meaningful comparisons across different studies and determine the relative merits of the newer treatment regimens. Biochemical relapse is characterized by a 25% increase in the tumor measurements as defined by the International Myeloma Working Group (IMWG) uniform response criteria. In addition, patients may present with new or worsening end organ damage related to MM without meeting the threshold for tumor measurements, and is considered to have a clinical relapse that requires therapy. This is an important distinction as some of the patients with biochemical relapse can be observed for some time before initiating therapy. Refractoriness to any drug is defined as progression while receiving that therapy or within 60 days of discontinuing it.

There is increasing awareness of the heterogeneity in the outcomes of relapsed patients, and the importance of risk stratification for relapsed disease. Prognostic factors such as the ISS and high-risk FISH abnormalities retain their value during the initial relapses. In addition, development of new abnormalities such as deletion 17p and del 1p also contributes to poor outcomes among the relapsed patients. One of the most important predictors for long-term outcomes has been the duration

of initial response. Patients who relapsed early (within a year) after their initial therapy that may or may not have included high dose therapy have very poor survival from the time of the relapse and novel approaches are needed for these patients. Biological studies in the relapsed patient population have provided important information regarding the clonal evolution in this disease. It is now clear that multiple subclones evolve during the disease progression, with those patients with high-risk disease being at significantly higher risk of developing new clones.

The decision to proceed with treatment of relapsed disease and the choice of therapy should be based on the clinical characteristics, responses to previously used drugs, residual toxicity if any from those treatments and development of new high risk features. Several important questions need to be asked at the time of disease relapse:

Do I really need to treat this patient? Patients who have biochemical progression and no evidence of CRAB features, can be watched closely for early signs of end organ effect and can be initiated on treatment at that time. However, one has to be very vigilant with patients who may have presented with renal failure or with neurological complications.

Does the patient have new “high risk features”? This is an important consideration. Patients who present with newly identified del 17p have a poor outcome and should be managed aggressively with combinations that include a proteasome inhibitor.

What drugs have been used so far? Response to previous treatments: efficacy, duration of response, toxicity? Patients who had good response to previous therapies and had durable remissions off treatment should always be considered for retreatment with the same, especially if they do not have any residual toxicity for the drugs. Patients who have residual toxicity such as neuropathy should be started on drugs with less risk of peripheral neuropathy.

How well is the patient? (PS, marrow reserve) The overall performance status of the patient is clearly important in deciding therapy. Patients with compromised PS should be started on reduced doses of drugs and preferably less intense combinations and once the disease is under better control, consideration can be given to increasing the drug doses.

What are the patient's goals/ preferences? Finally, this is one of the most important aspects of shared decision-making. Given that the disease is incurable, and most of the therapies carry some degree of toxicity, one needs to take into account the patients goals.

There has been an explosion of new drugs currently undergoing evaluation in myeloma in addition to several drugs that have been approved over the past decade. The major classes of drugs currently in use in the clinic include the proteasome inhibitors, immunomodulatory drugs (IMiDs), alkylators, anthracyclines and corticosteroids. Several new

classes of drugs are in various phases of clinical trials and include monoclonal antibodies, cell cycle inhibitors, signal transduction inhibitors, apoptosis modulators among several others. Many of the new and old drugs have been evaluated in combinations, many of which are quite synergistic and result in deep responses. Table 1 provides a summary of drugs currently in the clinic as well as classes of drugs currently being evaluated.

In general, deeper responses translate to longer response duration and patients should be treated to maximum response, balancing toxicity. It is important to understand that even minor responses have clinical value in the relapsed disease. The ideal duration of therapy is not clear, but "drug holidays" can help with toxicity, and QOL. New drugs with different mechanisms of action are clearly needed for continued control of the disease. Myeloma is a heterogeneous disease and the key may be to match the mechanism with the biologic abnormality.

**Table 1**

<b>Proteasome inhibitors</b>	
Bortezomib	First proteasome inhibitor in the clinic, administered i.v. or SQ, once or twice weekly
Carfilzomib	Irreversible inhibitor of the proteasome, administered i.v. two days every week, 3 of 4 weeks
Ixazomib*	First oral proteasome inhibitor, boronate containing similar to bortezomib
<b>Immunomodulatory drugs (IMiDs)</b>	
Thalidomide	First of the immunomodulatory drugs, orally daily, used in combination with dexamethasone
Lenalidomide	Thalidomide derivative, less side effects, orally daily for three weeks followed by one week off, used in combination with dexamethasone
Pomalidomide	Thalidomide derivative, orally daily for three weeks followed by one week off, used in combination with dexamethasone
<b>Alkylators</b>	
Melphalan	Used in combination with prednisone orally, can be used IV, used for conditioning prior to stem cell transplant
Cyclophosphamide	Less myelotoxic than melphalan, used orally in daily or weekly doses, intravenously for high dose therapy
Bendamustine	Administered i.v. two days every 4 weeks
<b>Anthracyclines</b>	
Adriamycin, Pegylated doxorubicin	Used as part of combination regimens, doxil approved in combination with bortezomib
<b>Corticosteroids</b>	
Dexamethasone, prednisone	Part of most combinations used in myeloma, active as single agent
<b>HDAC inhibitors</b>	
Panobinostat	First HDAC inhibitor to be approved for use in myeloma
<b>Monoclonal antibodies</b>	
Daratumumab*	Monoclonal antibody directed against CD38, single agent activity and active in combination with lenalidomide
SAR*	Monoclonal antibody directed against CD38, single agent activity and active in combination with lenalidomide
Elotuzumab*	Monoclonal antibody directed against SLAMF7
<b>Cell cycle inhibitors</b>	
Filanesib*	Spindle protein inhibitor, active as single agent, synergistic with dexamethasone
Dinaciclib*	Cdk5 inhibitor, single agent activity
<b>Signal transduction inhibitors</b>	
Afuresertib*	PI3-K inhibitor with single agent and in combination
<b>Apoptosis modulators</b>	
Venetoclax (ABT199)*	Bcl2 inhibitor with clinical activity in t(11;14) inhibitor

\* In clinical trials



### **Philippe Gaulard**

Philippe Gaulard, M.D., is Professor of Pathology at the Paris-Est University School of Medicine in Créteil (France) and is the director of the research Unit at the INSERM focusing on the oncogenesis of lymphoid malignancies. He maintains an important practice of hematopathology comprising many consultation cases. He is the former medical director of the Pathology Institute of the GELA (Groupe d'Etude des Lymphomes de l'Adulte), a large multicentric consortium of hematologists and pathologists from France and several neighbouring European countries conducting clinical trials for the treatment of adult lymphomas, which, since 2012, is designated the LYSA (Lymphoma Study Association) and is the scientific director of the Institute Carnot CALYM, a national research lymphoma network.

He is a member of the International Lymphoma Study Group (ILSG) and of the WHO 4th and 5th Lymphoma Classification Committees, and, as so, has authored several chapters of the WHO classification of Tumours of Haematopoietic and Lymphoid Tissues. He has been the President of the European Association for Hematopathology (EAHP) from 2010 to 2012.

He has authored more than 300 scientific publications indexed in PubMed. His H index is 64. His current research interest includes mainly the molecular characterization of lymphoma entities including primary mediastinal large B-cell lymphoma and neoplasms derived from mature T and NK cells. In this field, he has described hepatosplenic gd T cell lymphomas (HSTL), now recognized as a distinct clinicopathologic entity, has characterized the molecular signature of HSTL and NK/T-cell lymphomas, nasal-type and identified the normal cell counterpart of angio-immunoblastic T-cell lymphoma.

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# Biopathology of DLBCL in Clinical Practice

**Philippe Gaulard**

*Département de Pathologie & INSERM U955, Hôpital Henri Mondor, Créteil, France*

**D**iffuse large B-cell lymphoma (DLBCL) is the most frequent lymphoma category, representing more than one third of adult non-Hodgkin lymphomas worldwide. This lymphoma category, defined as a diffuse proliferation of neoplastic large B cells, has been recognized from the beginning as heterogeneous with respect to clinical presentation, morphology, immunophenotype, genetic and molecular features and clinical evolution, with an increasing number of clinico-pathologic subtypes or entities included in the latest WHO classification. Among these DLBCL subtypes, the most prevalent is Primary Mediastinal large B-cell Lymphoma (PMBL), a DLBCL entity thought to arise from thymic B cells, which presents as an anterior mediastinal mass in young adults, associates with constitutive activation of the JAK-STAT signaling pathway (STAT6), as well as recurrent CIITA translocations resulting in downregulation of surface HLA class II expression. Such features are shared by classical Hodgkin lymphoma (cHL) of nodular sclerosing type and may explain the occurrence of cases which show pathological and clinical overlapping features between PMBL and cHL, referred as “B-cell lymphoma, with features intermediate between DLBCL and cHL”, a provisional category of the 2008 WHO classification.

DLBCL usually affects adults with a median age at presentation in the 6-7th decades, but also occurs in children and young adults. Up to one third of the cases present in extranodal sites. DLBCL may occur de novo or as a transformation of an underlying small B-cell lymphoma. On histopathology, DLBCL include centroblastic, immunoblastic or anaplastic variants, the immunoblastic variant being reported to associate with a poorer prognosis. In clinical practice, the diagnosis of DLBCL is most often easy but it is important to recognize the rare cases which lack the typical morphological and phenotypic features of DLBCL. Such examples are given by “Histiocytes/T-cell rich large cell lymphoma” which may mimic Hodgkin lymphoma on morphological grounds with scattered neoplastic B cells on a background of T cells and histiocytes, or DLBCL lacking CD20 and/or other B-cell markers

(including PAX5 or CD79a) because they are composed of plasmablasts (with a terminal CD20-, PAX5-, CD79a+/-, CD138+profile-cell immunophenotypic profile) in the usually EBV-associated “plasmablastic lymphoma” DLBCL subtype and in the HHV8-associated “Primary Effusion lymphoma” entity. A difficult situation occurs also when the material submitted for diagnosis is limited or inadequate. At a time when core biopsies tend to expend, this situation may be responsible for a number of difficult differential diagnoses with other reactive or neoplastic conditions and has critical therapeutic implications. Currently, the differential diagnosis raised by Burkitt lymphoma (BL) is usually solved using an approach combining morphology, immunophenotype (BL are CD10+, BCL6+, BCL2-, with virtually all cells expressing the Ki67 cell cycle antigen and CMYC protein) and cytogenetics (karyotype or FISH analysis) demonstrating an IG-MYC rearrangement. However, a small proportion of “aggressive B-cell lymphomas” remains difficult to classify as DLBCL or BL, referred as “lymphomas with borderline features between DLBCL and BL”. Although ill-defined, this provisional category of the WHO classification will comprise most cases showing an accumulation of genetic alterations in BCL2 and c-MYC (and/or BCL6) genes, designated as “double or triple--hit” lymphomas.

DLBCLs have clonally rearranged IG genes with somatic mutations in the variable regions, and hence are thought to derive from antigen-exposed B cells that have migrated to or passed through the germinal center (GC). Reflecting their heterogeneity, no single genetic aberration typifies DLBCL. Recurrent translocations involving the BCL6, BCL2 and c-MYC genes occur in about 50% of cases, inducing deregulated expression of these oncogenes. Somatic hypermutation activity is common, not only affecting “physiological” target genes such as the IG variable regions, but may also aberrantly targets multiple other oncogenes, including c-MYC, PIM1, PAX5 and RhoH/TTF. Recently, application of next generation sequencing (NGS) technology has led to the identification of novel recurrent genetic alterations such as EZH2 and CREBBP mutations,

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or mutations in genes of the BCR signalling and the NFkB pathways in DLBCL (see below).

Gene expression profiling (GEP) studies have recognized at least 3 different molecular DLBCL subgroups, namely germinal-center B-cell-like (GCB), activated B-cell-like (ABC) and PMBL, which are associated with a peculiar molecular signature, distinct genetic pathways and also different outcomes. Patients with GCB-DLBCL have better outcomes than those with ABC-DLBCL. The t(14;18) translocation involving BCL2 and chromosomal amplifications of the c-REL locus associate with GCB-DLBCLs, while BCL6 translocations, BCL2 amplification and deletions affecting the INK4a/ARF locus are much more common in ABC-DLBCLs. GCB-DLBCLs also show recurrent abnormalities affecting the tumor suppressor PTEN leading to constitutive activation of the PI3K/AKT signaling pathway. Using a different clustering approach, other DLBCL comprehensive clusters have been identified which are related to different biological pathways (oxidative/phosphorylation, B-cell receptor/proliferation, host response) which might have important impact for the development of specific therapies.

The recent application of high throughput deep-sequencing technologies has also provided novel insights in the landscape of somatic mutations involving DLBCLs. Interestingly, they tend to cluster in common oncogenic pathways, such as mutations in genes of the B-cell receptor (BCR) signalling and the NFkB pathways in DLBCL of ABC subtype. Remarkably, ABC-DLBCL display a chronic active BCR signalling with recurrent mutations in genes of the BCR signalling pathway - CD79A or CD79B, CARD11, or biallelic mutations/deletions of TNFAIP3 encoding A20 (about 30% of the cases) – as well as mutations of MYD88, an adapter for Toll-like receptor in 30%. These alterations result in activation of the NFkB signalling pathway, which is the pathogenic hallmark of the ABC-DLBCL, and interference with this pathway selectively kills ABC-DLBCL tumor cells. Bi-allelic mutations of the plasma cell master regulator BLIMP1 is another frequent alteration observed in 30% of ABC-DLBCL. It is also noteworthy that mutations of genes involving DNA methylation and chromatin remodelling (CREBBP, EP30021, EZH2, MLL2, , ..) have been reported with a higher prevalence in GCB than in ABC subtypes, implying the importance of epigenetic changes in lymphomagenesis. Indeed, somatic mutations of the histone methyltransferase EZH2 are found in around 20% of GCB-DLBCLs whereas aberrations affecting CREBBP and EP300, two related histone and non-histone methyltransferases that act as transcriptional coactivators of several

pathways, have been identified in about one third of GCB-DLBCL cases and a lesser proportion of ABC tumors. Therefore, the GCB and ABC DLBCL appear to differ both in their oncogenic mechanisms and clinical outcome, validating the notion of two distinct diseases.

In clinical practice, the findings issued from GEP and NGS have important potential clinical implications. Although it still drives the diagnostic procedure, histology is no more the only characteristic to define DLBCL as a molecular precise definition of DLBCL tumours is needed for the clinical management and therapeutic decision of patients at diagnosis and, above all, in refractory or relapsed patients. DLBCL are aggressive tumors, which are potentially curable with anthracyclin-based chemotherapy regimens with a demonstrated benefit of Rituximab association. However, DLBCL remains a fatal disease in around one third of patients. Currently, the therapeutic strategy essentially relies on the use of the International Prognostic Index (I.P.I.) which integrates several clinical variables but remains an imperfect approach in identifying risk group patients. It is therefore a priority to incorporate biologically informative prognostic and predictive biomarkers along with clinical factors. As an example, in addition to GCG/ABC profiles, several studies have highlighted the poor prognosis of MYC-rearranged DLBCL patients and especially those with MYC-IG translocation as detected by FISH, and also of concurrent MYC and BCL2 translocations, as well as of concurrent high expression levels of BCL2 and cMYC by immunohistochemistry. The choice of the most robust technique(s) in the clinical setting, ie in routinely fixed tumor samples is therefore a critical issue. Additionally, it becomes more and more important to identify predictive biomarkers as potential therapeutic targets are being discovered such as BCL6, EZH2, PI3K/AKT in GCB-DLBCL, IRAK4, JAK-STAT and BCR in ABC-DLBCL and JAK-STAT, PD1 in PMBL. Detection of biomarkers related to interaction between lymphoma cells and non malignant cells of the microenvironment as well as motility and dissemination may also become another important goal, as expression of lymph node signature predicts outcome in R-CHOP DLBCL patients and serum PDL1 level also has prognostic impact. In this respect, especially at a time when targeting of immune checkpoint may be promising in several lymphoma subtypes.

However, the translation of these molecular data into daily practice has been hampered by several limitations, including the need to identify robust biomarkers with clinical impact and the difficulty in applying several of these sophisticated methods

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on routinely formalin-fixed paraffin-embedded (FFPE) specimen and the need to assess their interlaboratory reproducibility. In view of its clinical and biological impact, determination of the GC-ABC certainly is the best illustration. The ABC and GCB molecular signatures are reflected at the protein level by the immunohistochemical expression – applicable on FFPE samples - of antigens normally related to physiological B-cell differentiation, including GC markers such as CD10 and BCL6 and post-GC markers such as MUM1/IRF4. Different algorithms have been developed that variably correlate with the gene expression profiling classification, but the concordance is imperfect and more robust tests are needed. Analysis of the expression of a limited number of genes by real time RT-PCR can also predict outcome in DLBCL and may be more amenable to routine diagnostic use. Recently, a multiple assay capture system based on the expression of 20 GCB/ABC-related genes referred as “Nanostring nCounter system” has been designed for FFPE samples. This assay has proved to be accurate, robust and reproducible, retains the prognostic power of GCB/ABC segregation and therefore seems promising.

Altogether, the progress in the understanding of DLBCL pathogenesis has implications for the treatment of the patients, providing information for the development of targeted and usually less toxic therapies. The segregation between GCB and ABC DLBCL subtypes is critical as a first step. For

example, the therapy of GCB-DLBCL may take advantage of BCL2 and EZH2 inhibitors that are already available, whereas specific compounds inhibiting the key BCR and NFκB signaling pathways activated in ABC-DLBCL - such as Ibrutinib, an anti-BTK inhibitor – or Bortezomib, an inhibitor of the proteasome, should have an effect on ABC-DLBCL, as already demonstrated in other B-cell malignancies. A number of targeted therapies are being designed especially in the context of clinical trials for refractory or relapsed DLBCL patients. In addition to the targeting of lymphoma cells, strategies to modulate cell-cell interactions between lymphoma cells and immune cells of the environment – such as PD1 blockade with Nivolumab – may become attractive.

In conclusion, we have been moving within the last years, into an era when immunophenotypic features and molecular characteristics are taking an important value to the diagnosis and for the clinical management and therapeutic decision. If the diagnosis of DLBCL is easy based on an immunomorphological approach, there is a need to better identify high risk DLBCL patients at diagnosis as those patients will require a more adapted/precise therapy in addition to immune-chemotherapy. In addition to IPI, the determination of the GCB/ABC profile and of additional predictive biomarkers for new therapies through robust standardized molecular tests applicable in routine practice is needed in these patients as well as in refractory/relapsed DLBCL patients.

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# What Can We Expect With New Drugs in DLBCL?

**Carol S. Portlock**

*Memorial Sloan Kettering Cancer Center, New York, USA*

**D**LBCL is an aggressive B cell lymphoma with curative potential, utilizing doxorubicin-based R-chemotherapy regimens. The many new agents on the horizon are expected to improve this curative potential, although this has not been conclusively demonstrated to date.

DLBCL subset analysis can help provide a rational selection of agent, such as: Lineage by immunohistochemistry or genetic studies (Germinal center B cell, GCB; Activated B cell, ABC); mutation status (CD 79B, MYD-88, CARD 11) for example.

Immune checkpoint inhibitors have become standard in melanoma. Most recently, genetic analysis of the melanoma has permitted identification of a four amino acid residue predictor (tetrapeptide somatic neoepitopes) of excellent outcome with CTLA-4 blockade. It will be of interest to apply this technology to lymphoma and correlate such genetic markers with outcome.

Current investigations at MSK include: Upfront studies: ABC-DLBCL, R-CHOP with/without ibrutinib; R-CHOP vs G (obinutuzumab)-CHOP with GDC (ABT)-199 for all DLBCL. For relapse/refractory disease and transplant eligible: Ibrutinib plus R-ICE prior to autotransplant; and for less than complete response to R-ICE only, consolidative CAR (chimeric antigen receptor) T-cells after autotransplant. Phase I/II studies are generally monotherapy, including: B-cell immune conjugates (shigella toxin; MMAF or maytansanoid-DM1, tubule toxin); B cell metabolic inhibitors (BET-Bromodomain; PI3Kinase/HDAC, CUDC; Syk/JAK); immune checkpoint inhibitors (anti-PDL-1; anti-CTLA-4 plus anti-PD-1). Also, there are phase I/II immune modulatory agents combined with B cell antibodies: Urelumab, an agonistic anti-137 is being combined with Rituximab, and an anti-PDL-1, MPDL 33280A combined with Obinutuzumab.

New agents will move forward to standard curative management only when it is clear that the toxicity profile is acceptable and that efficacy is clearly sustained. It would be of particular interest if a new drug had CNS penetration (ibrutinib is being studied in this regard); if a genetic mutation or marker could identify a susceptible DLBCL subset; and ideally, that a treatment regimen could be of reasonably short duration (as are the chemotherapy regimens) and without prolonged maintenance.

DLBCL new drugs have certainly contributed to clinical palliation. Perhaps new combinations with synergy or unique sequencing can result in durable remissions without the need for standard chemotherapy. In particular, immune checkpoint agents and CAR T cell technology may provide that opportunity in the future.

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### **Christian Gisselbrecht**

Professor of Hematology  
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Hôpital Saint Louis  
Paris, France

Christian Gisselbrecht, MD is a professor of hematology at Paris Diderot University VII in the Hemato-Oncology Department at the Hôpital Saint Louis in Paris, France. He received his MD from the University of Paris. He completed a residency in hematology and served as chief resident. Dr Gisselbrecht also earned a molecular biology certificate from the University of Sciences Paris VII and an oncology certificate from Saint-Louis-Paris VII University. He is co-founder of the Groupe d'Etude des Lymphomes de l'Adulte (GELA), a French-Belgian cooperative group which has organized numerous randomized trials in lymphoma since 1984. He has been president of the group until 2002. GELA has recently extended its activities and changed its name to LYSA (Lymphoma Study Association). Dr Gisselbrecht and his research group are currently undertaking prospective trials to investigate the combination of dose-intensive chemotherapy and monoclonal antibodies in lymphoma.

Dr Gisselbrecht has been lead investigator of several major phase II and phase III clinical trials, which investigated the place of autologous stem cell transplantation in the treatment of lymphoma. Currently, he is chair of the international CORAL study on relapsed diffuse large B-cell lymphoma. His research interests include clinico-pathologic correlative studies in lymphoma, the pharmacology of novel antineoplastic agents, and stem cell transplantation.

Dr Gisselbrecht is an active member of several European and American scientific societies and is an expert with the European Medical Agency (EMA) as well as several cancer research agencies. He has published over 300 peer-reviewed papers and several book chapters and is on the editorial board of a number of highly respected journals.

**BIOGRAPHICAL SKETCH**

Provide the following information for the key personnel in the order listed for Form Page 2.  
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

<b>NAME</b> <b>Skoda, Radek C.</b>		<b>POSITION TITLE</b> Professor of Molecular Medicine and Chair	
<b>EDUCATION/TRAINING</b> ( <i>Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.</i> )			
<b>INSTITUTION AND LOCATION</b>	<b>DEGREE</b> (if applicable)	<b>YEAR(s)</b>	<b>FIELD OF STUDY</b>
University of Zürich Medical School, Zürich, Switzerland	Medical Diploma	1981	Medicine
University of Zürich Medical School, Zürich, Switzerland	M.D.	1983	Cell biology
Swiss Board Examination for Internal Medicine	FMH Internal Medicine	1999	Internal Medicine

**A. Positions and Employment**

1981-83 MD thesis, Department of Biochemistry, University of Zürich, Switzerland  
 1983-86 Postdoctoral Fellow, Biozentrum, University of Basel, Switzerland  
 1986-89 Resident in Internal Medicine, University Hospital Basel, Basel, Switzerland  
 1989-93 Postdoctoral Fellow, Department of Genetics, Harvard Medical School, Boston, MA  
 1993-2000 SNF-SCORE and Group leader, Biozentrum, University of Basel, Basel, Switzerland  
 2000-2002 Head, Molecular Hematology-Oncology, German Cancer Research Center (DKFZ)  
 2002-present Professor of Molecular Medicine and Chair, Department of Biomedicine, University of Basel and University Hospital Basel, Switzerland

**Other Experience and Professional Memberships**

1994-present: Member, American Society of Hematology  
 1995-present: Member, Swiss Society of Hematology  
 2001-2004: Member of the editorial board of the Journal of Molecular Medicine  
 2003: Co-chair of the Educational and Scientific Committee, 9<sup>th</sup> Meeting of the European Hematology Association  
 2004-present: Co-chair of ESH-Conferences on Myeloproliferative Disorders, Cascais, Portugal (2004), Madeira, Portugal (2006), and Athens, Greece (2008), Albufeira, Portugal (2010), Vienna (2012)  
 2004-present: Member of the Scientific Committee of the European School of Hematology  
 2008: Chair of the Scientific Program Committee, 14<sup>th</sup> Meeting of the EHA  
 2006-2012: Councilor to the Board of the European Hematology Association, 2009-2012 member of the EHA Executive Board  
 2011-present Member of the Scientific Subcommittee on Myeloid Malignancies of the American Society of Hematology (ASH)  
 2013: Elected member of the Swiss Academy of Medical Sciences (SAMW)

**Honors**

1997: Prize of the Swiss Society of Hematology  
 1999: Ellermann-Prize for Hematology  
 2004: Research Prize of the Cloetta-Foundation  
 2007: Ham-Wasserman Lecture Award, American Society of Hematology, Atlanta  
 2008: Hematological Malignancies Award, Bristol-Myers Squibb and Swiss Society of Hematology  
 2014: Gateway/RT F-CCR/SAKK Cancer Research Grant Award 2014

**B. Selected peer-reviewed publications (in reversed chronological order).**

- Grisouard, J., Shimizu, T., Duek, A., Kubovcakova, L., Hao-Shen, H., Dirnhofer, S., **Skoda, R.C.**, Deletion of Stat3 in hematopoietic cells enhances thrombocytosis and shortens survival in a JAK2-V617F mouse model of MPN, (2015) *Blood*, [Epub]
- Lundberg, P., Takizawa, H., Kubovcakova, L., Guo, G., Hao-Shen, H., Dirnhofer, S., Orkin, S.H., Manz, M.G., **Skoda, R.C.**, Myeloproliferative neoplasms can be initiated from a single hematopoietic stem cell expressing JAK2-V617F as the sole gene mutation. (2014) *J. Exp. Med.* 211: 2213-30
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1. **Skoda R.C.**, Less Jak2 makes more platelets. (2014) *Blood* 124: 2168-9
2. **Skoda R.C.**, Schwaller J. HiJAKing the methylome in myeloproliferative disorders. (2011) *Cancer Cell*. 19:161-3.
3. Tefferi, A., **Skoda, R.**, Vardiman, J.W. Myeloproliferative neoplasms: contemporary diagnosis using histology and genetics. *Nat Rev Clin Oncol*. 2009 6:627-37
4. **Skoda R.C.**. Thrombocytosis. *Hematology Am Soc Hematol Educ Program*. 2009:159-67
5. **Skoda, R.C.** The genetic basis of myeloproliferative disorders. (2007) *Hematology. Am Soc Hematol Educ Program*. 2007:1-10
6. **Skoda, R.** Prochal, J.T. Lessons from familial myeloproliferative disorders. (2005) *Seminars in Hematology* 42: 266-73
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10. **Skoda, R.C.** Some signals are more equal than others. (2001) *Blood* 98: 3504
11. Cazzola, M., **Skoda, R.C.** Translational pathophysiology: a novel molecular mechanism of human disease. (2000) *Blood*, 95: 3280-3288

#### C. Research Support

##### Ongoing Research Support

- 2013-2016 "Genetic analysis of myeloproliferative disorders". Grant from the Swiss National Science Foundation
- 2012-2015 "The pathogenesis of myeloproliferative disorders". Grant from the Swiss Cancer League/ Oncosuisse (Role: PI)
- 2010-2013 Mutations in the JAK2 Tyrosine Kinase in Children with Down Syndrome Acute Lymphoblastic Leukemia Grant from the Botnar-Stiftung (Role: PI)
- 2011-2013 „Genetic alterations in myeloproliferative disorders – tools to study the clonal evolution of disease“ Grant from the Else Kröner-Fresenius-Stiftung (Role: PI)
- 2015-2018 "A systems approach to hematopoietic stem cell diseases" SystemsXR MRD grant (Role: PI)

##### Completed Research Support

- 2008-2013 "Genetic analysis of myeloproliferative disorders". Grant from the Swiss National Science Foundation (Role: PI)
- 2009-2012 "The pathogenesis of myeloproliferative disorders". Grant from the Swiss Cancer League/ Oncosuisse (Role: PI)
- 2010-2012 „Genetic screening for disease-causing mutations in familial polycythemia using next generation DNA sequencing“. Grant from the Gebert Rüt Foundation (Role: PI)
- 2007 Analysis of stem cell origin of the MPD clone in patients with essential thrombocytosis and idiopathic myelofibrosis. Grant from the Novartis Stiftung, vormals Ciba - Geigy - Jubiläums - Stiftung
- "The pathogenesis of myeloproliferative disorders". Grant from the Swiss Cancer League/ Oncosuisse
- 2006 Search for a primary mutation in myeloproliferative disorders. Grant from the Krebsliga Beider Basel
- 2005-2007 "The Role of Smad4-Dependent Signaling in Anemia". Grant from the RoFAR Foundation (Role: PI)
- 2005-2008 "Genetic analysis of hematopoietic stem cell disorders". Grant from the Swiss National Science Foundation (Role: PI)
- "The pathogenesis of myeloproliferative disorders". Grant from the Swiss Cancer League/ Oncosuisse (Role: PI)
- 2002-2005 The role of extracellular signals in the control of normal and aberrant hematopoiesis". Grant from the Swiss National Science Foundation (Role: PI)
- 2002-2004 "The pathogenesis of myeloproliferative disorders". Grant from the Swiss Cancer League/ Oncosuisse (Role: PI)
- 1998-2000 "Extracellular signals in the regulation of hematopoiesis". Grant from the Swiss National Science Foundation (Role: PI)
- 1998-2000 "The pathogenesis of essential thrombocythemia". Grant from the Swiss Cancer League (Role: PI)
- 1996-1998 "Extracellular signals in the regulation of hematopoiesis". Grant from the Swiss National Science Foundation (Role: PI)
- 1996-1998 "The pathogenesis of essential thrombocythemia". Grant from the Swiss Cancer League (Role: PI)
- 1993-1996 "The role of mpl in hematopoiesis". Grant from the Swiss National Science Foundation (Role: PI)

# Should Continuous Therapy Be Used in All Patients?:

Radek Skoda

University Hospital Basel, Experimental Hematology, Department of Biomedicine, Basel, Switzerland

The discoveries of somatic mutations in *JAK2*, *MPL*, and *CALR* advanced our understanding of the molecular pathogenesis of MPN. High throughput sequencing provides additional insights into the mutational landscape of MPN. With today's sequencing strategies, only about 2% of patients with polycythemia vera (PV), and about 10% of patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF) do not display any detectable somatic mutation. Mutations can be classified as "drivers" and "passengers". The mutations in *JAK2*, *MPL*, and *CALR* are considered "phenotypic drivers", because they are associated with specific MPN phenotypes. Other frequently mutated genes exert roles in epigenetic regulation (*TET2*, *ASXL1*, *DNMT3a*, *EZH2*), signaling (*CBL*, *LNK*, *NRAS*, *NF1*) or genome stability (*TP53*). These gene mutations are also found in many other forms of cancer and alone do not induce a MPN phenotype, but rather collaborate with the phenotypic driver mutations in disease initiation and/or progression. In MPN patients that carry several somatic mutations, the majority of mutations are already present at diagnosis, and very few new mutations appear to occur during follow-up.

## Learning objectives

- To understand the increasingly complex mutational landscape in MPN and categorize the mutations according to their roles in the pathogenesis
- To learn about the differences in phenotypes linked to individual driver mutations in *JAK2*, *CALR* and *MPL*
- To understand the potential impact of the clonal architecture on prognosis and outcome of MPN

It is now 10 years since the discovery of the *JAK2*-V617F mutations in MPN,<sup>1-4</sup> and we have since then gained an increasingly complete picture of the genes and mutations that make up the diversity of MPN phenotypes.<sup>5,6</sup> A major gap was filled with the identification of mutations in the calreticulin gene (*CALR*)<sup>7,8</sup>. In a cohort of 200 MPN patients that were screened for mutations in 110 genes with known or

suspected to role in MPN using capture based next generation sequencing, only approximately 10% of all MPN patients did not carry any detectable somatic mutation (Figure 1).<sup>9</sup> Whole exome sequencing in 151 MPN patients revealed only 1 patient with no candidate mutation.<sup>8</sup> The median number of mutations per patient was 6.5 in patients with PV and ET and 13 in patients with PMF. The majority of the mutations were non-recurrent and since non-hematopoietic DNA was not available for all patients analyzed, some of the candidate mutations could represent rare germline variants.<sup>8</sup> Overall, both studies concluded that the mutation rate in MPN was lower than that reported for some solid tumors. Thus, we are still missing some of the disease causing mutations in MPN, in particular in ET and PMF. These "triple negative" MPN cases are particularly interesting, as they also appear to have an unfavorable prognosis.<sup>10</sup>

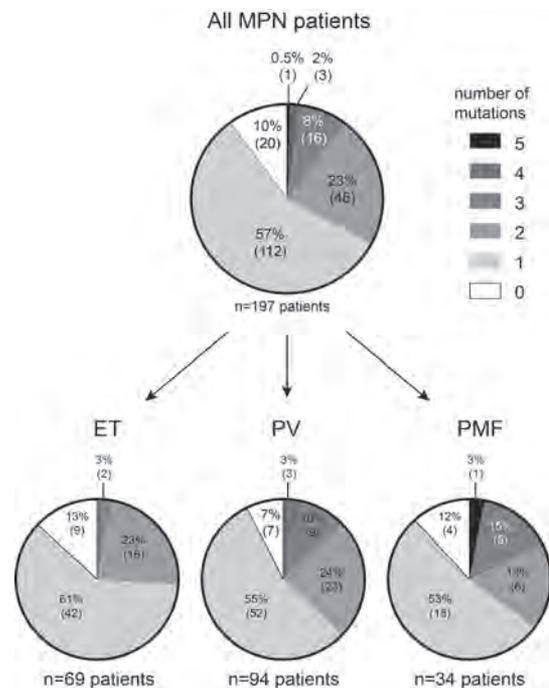
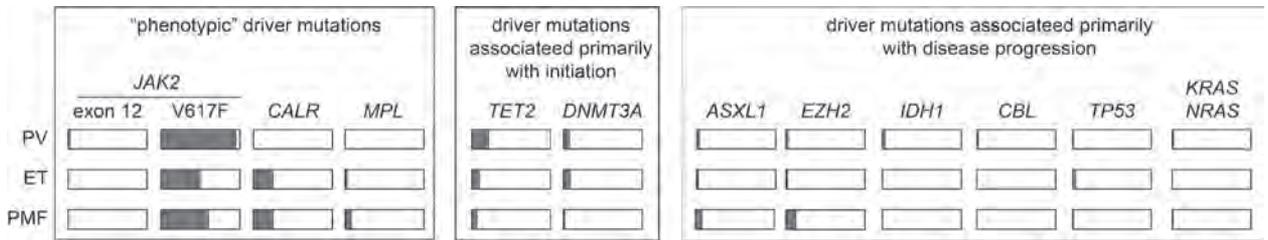


Figure 1. Frequencies of somatic mutations among the 197 MPN patients according to phenotype. The shades of gray indicate the number of somatic mutations per patient. (From Lundberg et al, ref. 9)



**Figure 2.** Distribution and frequencies of somatic mutations in MPN. The width of the red boxes is proportional to the percentages of patients who carry the corresponding mutation.

Genes that exert a role in epigenetic regulation (*TET2*, *ASXL1*, *DNMT3a*, *EZH2*) are frequently mutated in MPN.<sup>5</sup> Other recurrent mutations affect genes encoding signaling molecules, such as *CBL*, *NRAS* and *NF1*. A particularly poor prognosis is associated with the presence of mutations in *TP53*.<sup>9,11</sup> Some of the individual gene mutations are of low frequency (Figure 2), but as a sum they are likely to be relevant. The availability of high throughput sequencing will make it possible to screen patients for all known mutations at the time of MPN diagnosis and the mutational profiles will likely become additional factors in determining the individual risk and influencing therapeutic decisions. Indeed, the numbers of mutations in patients with MPN had a negative prognostic effect on the likelihood of survival.<sup>9</sup>

It is now becoming apparent that the phenotypes and outcomes of the MPN subtypes, i.e. PV, ET, and PMF, are to some degree dependent on the type of the "phenotypic driver mutation". While PV is almost exclusively caused by *JAK2*-V617F and to a much lesser degree by *JAK2* exon 12 mutations, ET and PMF can be caused by mutations in *JAK2*, *CALR* or *MPL* (Figure 2). ET and PMF patients with *CALR* mutation are on average younger, and more likely to be males, have lower risk of thrombosis and longer overall survival than patients with the *JAK2*-V617F mutation.<sup>10,12,13</sup> Here is a short summary of the pathogenetic aspects of the two most common "phenotypic driver mutations", *JAK2*-V617F and *CALR*.

### MPN caused by the *JAK2*-V617F mutation

*JAK2*-V617F is one of the most common somatic mutations occurring in a single genomic hot spot.<sup>1-4</sup> The reason why this G>T transversion in exon 14 of the *JAK2* gene is so highly recurrent remains unknown. Although most commonly associated with PV, *JAK2*-V617F can also be found in more than half of patients with ET and PMF. PV patients have in most cases detectable subclones that underwent mitotic recombination resulting in uniparental

disomy that can be detected as homozygous *JAK2*-V617F by analysis of single colonies grown in methylcellulose.<sup>14</sup> In contrast, patients with ET in most cases display colonies that remained heterozygous for *JAK2*-V617F, although on more in depth analysis some ET patients can also carry a minor subclone homozygous for *JAK2*-V617F.<sup>15</sup> A somewhat reminiscent correlation between PV phenotype and higher expression of *JAK2*-V617F was described in a *JAK2*-V617F transgenic model.<sup>16</sup> However, our understanding of the genotype-phenotype correlation between *JAK2*-V617F and PV or ET phenotype still remains incomplete. Increased activity of Stat1, probably driven by interferon-gamma favors the ET phenotype.<sup>17,18</sup> The role of *JAK2*-V617F in the pathogenesis of MPN was confirmed in retroviral, transgenic and knockin mouse models (reviewed in refs.<sup>19,20</sup>).

Ruxolitinib (Jakafi/Jakavi), a *JAK1/2* inhibitor was approved for the treatment of intermediate and high-risk myelofibrosis.<sup>21-23</sup> Patients treated with ruxolitinib show a rapid decrease in spleen size and a rapid improvement in symptoms, but has little effect on the *JAK2*-V617F mutant allele burden. Interestingly, Ruxolitinib is also effective in patients with wild type *JAK2*, thus showing that the same pathways are hyper-activated also in these patients. Preliminary results indicate that ruxolitinib is also effective in patients with *CALR* mutations.

Clonal analysis in patients with MPN revealed that mutations other than *JAK2*-V617F, such as *del20q*, *TET2* and *DNMT3A* are frequently acquired before *JAK2*-V617F.<sup>24-27</sup> Furthermore, the *JAK2*-V617F mutation was detected in blood cells from healthy controls/non-MPN cases, suggesting that *JAK2*-V617F may not be sufficient.<sup>28-30</sup> These data support the fertile ground hypothesis, which predicts that acquisition of *JAK2*-V617F does not invariably result in MPN, but needs such "early" mutations upon which the *JAK2*-V617F mutated cells can more easily propagate.<sup>31</sup>

The effect of *JAK2-V617F* on HSC function has recently been debated. Other hyperactive tyrosine kinases, such as *FLT3* and *BCR-ABL* have been shown to impair HSC function. The reports on *JAK2-V617F* are not unanimous, and while one mouse model observed a decreased function of *JAK2-V617F* expressing HSCs,<sup>32</sup> other mouse models have found the opposite results.<sup>33-35</sup> In a model where *JAK2-V617F* provides a disadvantage to HSCs, one can hypothesize that pre-*JAK2* mutations with a beneficial effect on HSC function have to be present in the patient to counteract the disadvantage provided by *JAK2-V617F*. Recently, it was demonstrated that *JAK2-V617F* provides a competitive advantage for the HSCs and alone can initiate MPN from a single stem cell.<sup>36</sup> Bone marrow cells that express *JAK2-V617F* also have a dramatic effect on the stem cell niche, in that the Nestin-positive mesenchymal stem cells, which are innervated by sympathetic nerve fibers are rapidly reduced.<sup>37</sup> This effect appears to be caused by early glial and sympathetic nerve damage and MSC apoptosis triggered by the mutant HSCs. This effect can be reversed by treating the mice with an adrenergic agonist.<sup>37</sup>

### MPN caused by mutations in *CALR*

*CALR* mutations always occur in exon 9 of the *CALR* gene, but there is no mutational hotspot, rather, all mutations reported so far are insertions or deletions leading to a shift into an alternative reading frame.<sup>7,8</sup> There are two known functional consequences of this change in reading frame. First, the C-terminal negatively charged amino acids responsible for Ca<sup>2+</sup> binding in the wild type protein are replaced with positively charged or neutral amino acids, thus the Ca<sup>2+</sup> binding capacity of the protein is reduced. Second, the last amino acids in the wild type reading frame of the *CALR* protein contain an endoplasmic reticulum retention signal, and in the mutated version of *CALR* this signal peptide is absent. Subsequently, an altered subcellular localization of mutated *CALR* was reported, which could affect the chaperone function of *CALR*.<sup>7</sup> It is currently unknown which of these two functional consequences of *CALR* mutations that are relevant for the development of MPN. Clinically, patients carrying *CALR* mutations have a more favorable prognosis compared to patients positive for *JAK2-V617F*.<sup>10,12,13</sup> In contrast to *JAK2-V617F*, mutations in *CALR* have so far been reported to be early events in MPN pathogenesis.<sup>7-9</sup>

### Outlook

We are rapidly expanding our knowledge of the somatic mutations that occur in patients with MPN and it can be expected that we will soon have a near complete catalogue. The new sequencing technologies will allow to determine the complete mutational profiles of individual patients at diagnosis and we will determine their impact on prognosis. Furthermore, some of the highly recurrent mutations, such as *JAK2-V617F* and *CALR* represent attractive targets for new therapeutic approaches.

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# Contemporary Science and Practice in Mast Cell and Eosinophilic Disorders

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**S**ystemic mastocytosis (SM) results from a clonal proliferation of mast cells (MC). The major criterion is presence of multifocal clusters of morphologically abnormal MC in the bone marrow. Minor diagnostic criteria include elevated serum tryptase level, abnormal MC expression of CD25 and/or CD2, and presence of KITD816V. The 2008 World Health Organization (WHO) classification of SM has been shown to be prognostically relevant. Classification of SM patients into indolent (SM), aggressive SM (ASM), SM associated with a clonal non-MC lineage disease (SM-AHNMD) and mast cell leukemia (MCL) subgroups is a useful first step in establishing prognosis. SM treatment is generally palliative. ISM patients have a normal life expectancy and receive symptom-directed therapy; infrequently, cytoreductive therapy may be indicated for refractory symptoms. ASM patients have disease-related organ dysfunction; interferon- $\alpha$  (+/- corticosteroids) can alleviate symptoms, but is hampered by poor tolerability. Similarly, cladribine has broad therapeutic activity, with particular utility when rapid MC debulking is indicated; the main toxicity is myelosuppression. Imatinib has a therapeutic role in the presence of an imatinib-sensitive KIT mutation or in KITD816-unmutated patients. Treatment of SM-AHNMD is governed primarily by the non-MC neoplasm; hydroxyurea has modest utility in this setting; there is a role for allogeneic stem cell transplantation in select cases.

Acquired eosinophilia is operationally categorized into secondary, clonal, and idiopathic types. Causes of secondary eosinophilia include parasite infections, allergic or vasculitis conditions, drugs, and lymphoma. Clonal eosinophilia is distinguished from idiopathic eosinophilia by the presence of histologic, cytogenetic, or molecular evidence of an underlying myeloid malignancy. The WHO system recognizes 3 distinct subcategories of clonal eosinophilia: chronic eosinophilic leukemia-not otherwise specified, myeloid neoplasms with associated eosinophilia, and eosinophilia with mutations involving PDGFR or FGFR1 genes. Hypereosinophilic syndrome is defined by the presence of a peripheral blood eosinophil count of  $1.5 \times 10^9/L$  or greater for at least 6 months (a shorter duration is acceptable in the presence of symptoms that require eosinophil-lowering therapy), exclusion of both secondary and clonal eosinophilia, evidence of organ involvement, and absence of phenotypically abnormal and/or clonal T lymphocytes. The presence of the latter defines lymphocytic variant hyper eosinophilia.

This presentation will provide updated information regarding diagnostic criteria, classification, prognostication and treatment of systemic mastocytosis and eosinophilic disorders, with a focus on recent advances in molecular pathophysiology and development of novel therapeutics.

## Curriculum Vitae

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	Listening	Reading	Spoken interaction	Spoken production	
European level (*)	C2	C2	C2	C2	C2

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Language (\*) Common European Framework of Reference for Languages

**Additional information** Include here any other information that may be relevant, for example contact persons, references, etc.

### Receiving

**Annexes** List any items attached. (see below)

## Scientific Publication

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# Molecular Characterization of ALL: New Markers and Subgroups

**Sabina Chiaretti**

*Hematology, Dept of Cellular Biotechnologies and Hematology, Sapienza University of Rome*

**A**cute lymphoblastic leukemia (ALL) is a heterogeneous disease that comprises several molecular subgroups. The molecular characterization of ALL has drastically changed over the years, mostly due to the introduction of novel technologies. In fact, beside the known aberrations, namely BCR/ABL, ETV6/RUNX1, E2A/PBX1 and MLL rearrangements, which are recurrent, are differently distributed among children and adults and harbor a well-established prognostic significance, the use of gene expression profiling (GEP), SNP array analysis, and more recently next generation sequencing (NGS) and whole exome sequencing (WES), have eventually allowed to better define the molecular scenario of ALL.

Among the groups that have been recognized it is worth mentioning the so called BCR/ABL-like and the ETP subgroups, both characterized by a distinctive transcriptional profile and a dismal prognosis, mostly when coupled to a persistent MRD persistence.

The genetic constellation of BCR/ABL-like cases is wide and might be patient-specific: on the other

hand, all cases could benefit by the combination of a standard chemotherapeutic backbone with tyrosine kinase inhibitors. Efforts are ongoing to promptly identify these cases by simpler methods, such as Q-PCR approaches, rather than GEP.

In ETP it was possible to show an involvement of the RAS pathway (NRAS, KRAS and FLT3), as well as of several genes usually detected in myeloid disorders such as IDH1, IDH2, DNMT3A, as well as of transcripts involved in chromatin remodeling.

Other groups that have been identified are represented by the iAMP21 subgroup and the hypodiploid cases: also in these subsets, the prognosis is poor and their recognition may permit a better patients' stratification,

Finally, a constellation of molecular aberrations affecting genes and/or pathways, the most prevalent being again NRAS, KRAS, FLT3 and JAK2 have been identified in cases lacking other specific features, and being differently distributed among children and adults. These topics will be discussed during this talk.



## CURRICULUM VITAE (simplified)

### Jose-Maria Ribera

#### Present Position:

Head of the Clinical Hematology Department and the Stem Cell Transplantation Unit of the Catalan Institute of Oncology-Hospital Germans Trias i Pujol, Barcelona, Spain

**Date of Birth:** 02-11-1956

**Place of Birth:** Balsareny, Barcelona, Spain

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#### Biography

Dr Josep-Maria Ribera was born in Balsareny (Barcelona, Spain). He achieved the speciality in Hematology in the Hospital Clinic of Barcelona, Spain, where he worked as a staff member of the Clinical Hematology Department and achieved the PhD. Since 2003 he is the Chief of the Clinical Hematology Department and the Stem Cell Transplantation Unit of the Catalan Institute of Oncology-Hospital Universitari Germans Trias i Pujol, in Badalona, Barcelona, Spain. Since 2007 is Professor of Medicine-Hematology in the Universitat Autònoma de Barcelona, Spain. Author or co-author of 98 chapters of books of Hematology, Oncology and Internal Medicine, and 490 articles published in peer-reviewed journals, as well as reviewer of several journals of Hematology and Oncology and several research agencies. Member of the Steering Committee of European LeukemiaNet, Workpackage 6 (acute lymphoblastic leukemia) and member of the EWALL (European Working Group for Adult Acute Lymphoblastic Leukemia). He is also the Chairman of the Acute Lymphoblastic Leukemia division of the Spanish co-operative group PETHEMA (Programa Español de Tratamiento en Hematología) from the Spanish Society of Hematology, as well as President of the Board of the PETHEMA Foundation. His main fields of interest are acute lymphoblastic leukemia and lymphomas arising in HIV-infected patients.

#### Education

1979 MD Universidad de Barcelona, Barcelona, Spain  
1980-1984 Medicine Residency Program. Speciality of Hematology-Hematotherapy. Hospital Clinic, Barcelona, Spain  
1987 PhD, Medicine. Universidad de Barcelona, Barcelona, Spain

#### Positions and Employments

1985-1989 Staff member. Hematology Department. Hospital Clínic, Barcelona, Spain  
1989-2003 Head of Clinical Hematology Section. Hematology Department. Hospital Germans Trias i Pujol, Badalona, Spain  
1999-Present Director of Stem Cell Transplantation Unit. Hospital Universitari Germans Trias i Pujol. Badalona. Spain  
2003-Present Head of Clinical Hematology Department. ICO-Hospital Germans Trias i Pujol. Badalona. Spain  
Teaching activity  
1996-2002 Past President of Postgraduate Teaching Committee of *Hospital Universitari Germans Trias i Pujol de Badalona*  
2003 Past President of Research Committee of Hospital Universitari Germans Trias i Pujol de Badalona  
2000-2003 Associate professor, Faculty of Medicine, Universidad Autónoma de Barcelona  
2007-present Professor of Medicine-Hematology. *Universidad Autónoma de Barcelona*  
Professor of University Post-graduate courses. Universities of *Barcelona, Autónoma de Barcelona, Internacional Menéndez y Pelayo*, and *Escuela Nacional de Sanidad*.

#### Research Activity

Doctoral theses directed: 6  
Reserch projects as Principal investigator: 12

#### Editorial Activity and other aspect of interest

- Author or co-author of 94 chapters of books of Hematology, Oncology and Internal Medicine.
- Author or co-author of 490 papers, international journals and Spanish journals.
- Researcher of the following agencies: *Fondo de Investigaciones Sanitarias (FIS)*, *Comissió Interdepartamental de Recerca i Innovació Tecnològica (CIRIT)*, *Fundació La Marató de TV* and *Instituto de Salud Carlos III*
- Member of the Steering Committee of European *LeukemiaNet*, Workpackage: acute lymphoblastic leukemia, supported by a Grant of the European Union.
- Member of the EWALL (European Working Groups for Adult Acute Lymphoblastic Leukemia), a working group from the European Hematology Association
- Executive Secretary of the journal *Medicina Clínica* (Included in Science Citation Index)

- Reviewer of the following journals: Lancet, Lancet Oncology, Blood, Leukemia, Haematologica, American Journal of Hematology, Bone Marrow Transplantation, International Journal of Hematology, Annals of Hematology, International Journal of Cancer, Cancer Treatment Reviews, Drugs, Journal of Clinical Immunology, Expert Opinion on Pharmacotherapy, Hematological Oncology, Future Oncology, PLoS ONE, Cochrane Reviews, Journal of Hematological Malignancies, AIDS, British Journal of Medicine and Medical Research, Medical Oncology, Case Reports in the Oncological Medicine, Journal of Leukemia, International Archives of Medicine, Biomedical Journals, Medicina Clínica Enfermedades Infecciosas Microbiología Clínica.
- Head of the Acute Lymphoblastic Leukemia (ALL) division of the co-operative group PETHEMA (*Programa Español de Tratamiento en Hematología*) Spanish Society of Hematology
- President of the Board of the PETHEMA Foundation
- President of ALL sub-Committee of the GETH (*Grupo Español de Trasplante Hematopoyético*)
- President of the Catalan Society of Hematology
- Member of the following scientific societies:  
Catalan Society of Haematology- Hemotherapy, Spanish Society of Haematology- Hemotherapy, ISH, EHA, and ASH.

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# Optimal Treatment of Ph+ ALL. Role of TK Inhibitors and Stem Cell Transplantation

**Josep-Maria Ribera**

*Institut Català d'Oncologia-Hospital, Badalona, Spain*

The first attempt to treat Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) in adult patients in the tyrosine kinase inhibitors (TKI) era consisted in the simple addition of imatinib to the previous standard chemotherapy regimens in relatively small non-randomized studies. It became rapidly evident that the prognosis improved compared with historical chemotherapy-based studies in the pre-imatinib era. Complete remission (CR) rates improved from 60-70% to over 90% and short and long-term outcomes were better, with survival reaching approximately 50% as compared to 20% or less in the pre-imatinib era, making combined imatinib/chemotherapy the standard treatment of Ph+ ALL. Usually TKI therapy is initiated front-line together with the first chemotherapy cycle.

Allogeneic stem cell transplantation (SCT) in first CR was considered as the only curative option for Ph+ ALL patients in the pre-imatinib era. The improved results with imatinib and chemotherapy questioned the need of SCT for all patients. Although no formal trial addressing this question has been conducted, a recent prospective trial from the GRAALL intergroup confirmed that myeloablative allogeneic SCT is still associated with a longer relapse-free survival in younger Ph+ ALL patients. The role of reduced-intensity conditioning (RIC) SCT remains to be confirmed. The results of SCT are better in patients with low minimal residual disease (MRD) level (evaluated by residual BCR-ABL1 transcript levels) at the time of SCT. Autologous SCT followed by TKI and conventional maintenance chemotherapy could be an option in patients in molecular remission achieved with TKI and chemotherapy and who have no allogeneic donor, or in those not capable to tolerate allogeneic SCT. The definition of the subset of patients at a lower risk of relapse who might be treated with continuous imatinib/chemotherapy and not receive allogeneic SCT in first CR, if they

exist, is still under evaluation. A randomized study from the GMALL group suggests that prophylactic post-SCT imatinib maintenance is a better option than pre-emptive imatinib reintroduction based on a careful BCR-ABL1 MRD monitoring in terms of maintenance of the MRD-negative status after SCT.

The studies conducted in older Ph+ ALL patients, who are usually not candidates for allogeneic SCT due to their worse SCT tolerance, showed that hematologic CR can be achieved with TKI and low or very low dose chemotherapy, although the rate of molecular remissions is low and the frequency of relapses is high. The low toxicity of these combinations allows to perform RIC SCT in a proportion of these patients. Poor BCR-ABL1 MRD response is associated with a worse outcome.

The best TKI/chemotherapy combination as early treatment in all patients is not defined and some studies have shown that the reduction of the amount of chemotherapy does not have impact on outcome, as has been randomly demonstrated by the GRAALL group. To date, there is no comparative study evaluating second-generation TKI (nilotinib, dasatinib) versus imatinib as first-line treatment. There are some promising early data on the efficacy of third generation TKI (ponatinib) upfront combined with chemotherapy, but there are also concerns on toxicity.

In patients with persistent BCR-ABL1 MRD detection or progressing after a front TKI/chemotherapy ± SCT, the recommendation is to switch for another TKI while screening for TKI resistance mutations, and then to adapt TKI choice according to the resistance profile. Third-generation TKI ponatinib is currently the only option in patients progressing with the T315I mutation. These patients could also be candidates for targeted antibody (immunoconjugated, bispecific) treatments or cell therapies (chimeric antigen receptor T-cells).



### **Marc André**

Professor Marc André is the current Head of the department of Hematology at the Centre Hospitalier Universitaire Dinant Godinne, Yvoir, Belgium. He is the Vice-President of the Lymphoma Study Association (LYSA), member of the scientific board of LYSA and President of the Hodgkin Lymphoma Committee of LYSA. Professor André is President of the JACIE committee at the Centre Hospitalier Universitaire Dinant Godinne, Yvoir, Belgium and member of the board at the Belgian Hematology Association. He is also Professor of Clinical Trial Management at the Université de Namur, Namur, Belgium.

He received his medical degree from the Université Catholique de Louvain in 1989, a professional training at the Hôpital Saint-Louis in Paris, France (1994-95) and became a board certified Hematologist. Prof. André has a broad range of translational scientific and clinical interests, spanning non-Hodgkin lymphoma, Hodgkin lymphomas and late toxicities, design and conduction of clinical trials, and clinical management. Currently, he is a principal investigator of the international H10 and BREACH studies. He has published over 50 peer-reviewed papers and several book chapters.

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# Controversies in the Management of Early Stage Hodgkin Lymphoma

**Marc André**

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## Introduction

Classical Hodgkin lymphoma (HL) is a highly curable disease and considered as one of the most successful stories in hematology. More than 90% of the patients are alive 5 years after diagnosis and the progression-free survival is 85-93% for localized disease. After a first relapse, the disease remains curable in nearly half of the cases when high-dose chemotherapy (HDCT) with autologous stem cell transplantation (ASCT) is feasible. As HL is one of the most common malignancies in young adults, most patients will have very long survival. However, during their follow-up a significant fraction of patients experiences serious long-term toxicities such as second primary malignancies, cardio-vascular diseases and fertility issues. Most of these late toxicities have been related to the treatments for HL. To reduce long term treatment related toxicities, optimization of the balance between the risks and benefits of the different treatment strategies is actually a matter of controversies and a main goal of most clinical trials.

## Recent trials

Two European risk models (proposed by GHSG and EORTC/LYSA) are commonly used and were established to separate HL in three different risk categories: early favorable, early unfavorable or intermediate, and advanced disease. Using these prognostic categories European lymphoma groups have been major contributors in clinical trials that established ABVD (adriamycin, bleomycin, vinblastine and dacarbazine) as a reference chemotherapy in for HL. Alteration of ABVD by omitting any drug including bleomycin was recently reported as inferior in term of disease control (1).

Radiotherapy is a major contributor to the late toxicities such as secondary cancers and cardio-vascular diseases. Several trials provided an important contribution to the reduction of radiotherapy for HL treatment from extended to involved-fields. Moreover, these trials have also demonstrated that beside the field, the dose of radiotherapy can be

safely reduced without compromising treatment efficacy. The dose of radiotherapy in the more favorable group of localized patients has been reduced to 20 cGy.

More recently, European trials have also tested the possibility of omitting radiotherapy in selected patients. As it has been shown that patients with a fluorodeoxyglucose positron emission tomography (PET) negativity after 2 cycles of ABVD have an excellent outcome, it was suggested that those PET negative patients could receive less intense therapy without any radiotherapy (2). This PET-driven treatment adaptation is still restricted to clinical trials and whether this selected population of patients can be safely treated without radiotherapy remains controversial and a matter of debate.

Of interest, for the first time since the 1970s and the introduction of adriamycin, new drugs (CD30-antibody drug-conjugate and PD-1-blocking antibodies) are nowadays becoming available in the field of HL and are suggested as treatments with a safe toxicity profile.

The definition of the place of already developed new drugs in our armamentarium and individualized therapy strategies is one of the main goals of the next generation of clinical trials.

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## **Bastian von Tresckow**

Bastian von Tresckow is doctor of internal medicine at Cologne University Hospital and study physician of the German Hodgkin Study Group (GHSG, head: Professor Andreas Engert).

In 2001 he joined Professor Andreas Engert's laboratory as a medical student and started to perform basic research in Hodgkin Lymphoma. He received his licence to practice medicine and his Doctorate (M.D.) in medicine in 2005, both from the University of Cologne, Germany. Since 2005 he has worked as a clinical physician with a focus on hematologic malignancies. He joined the GHSG in 2005 and specialises in early unfavourable stages, advanced stages and relapsed disease. Between 2005 and 2007 he received a grant from the "Köln Fortune"-programme and performed scientific work in Professor Andreas Engert's group, Laboratory for Immunotherapy: Investigation of small molecules for the treatment of Hodgkin Lymphoma. Dr von Tresckow received board certification in 2011 as a specialist in internal medicine and haematology and oncology. Since 2012 he is Editor of the Cochrane Haematological Malignancies Group (CHMG). In 2013 he was appointed scientific secretary of the 9th International Symposium on Hodgkin Lymphoma (ISHL-9) in Cologne. His current position is Medical Head of the Clinical Trial Unit Department I of Internal Medicine, University Hospital of Cologne.

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# Summary of the Presentation “New Drugs for Relapsed Hodgkin Lymphoma”

**Bastian von Tresckow**

*University Hospital Cologne, Cologne, Germany*

**M**ost Hodgkin lymphoma (HL) patients are cured with modern first-line treatments. Even ~ 50% of patients with relapsed/refractory HL can be cured with high-dose chemotherapy (HDCT) and autologous stem cell transplantation. However, chemotherapy and radiotherapy cause significant acute and long-term side effects and patients relapsing after HDCT have a dismal prognosis. New drugs are therefore needed to reduce the toxicity of first-line treatments and to increase the efficacy of relapse treatments. Moreover, new drugs are needed for the treatment of older patients with HL because results with current treatments are disappointing.

This presentation discusses promising new drugs for the treatment of classical HL that have been

evaluated in the last years. There is a focus on the antibody drug conjugate brentuximab vedotin and its potential for the future treatment of HL. Moreover, data on the histone deacetylase inhibitors panobinostat and mocetinostat, the mammalian target of rapamycin inhibitor everolimus, the Janus kinase 2 inhibitor pacrinostat and the immunomodulatory agent lenalidomide are summarized. Additionally, the most recent data on the checkpoint-inhibitors nivolumab and pembrolizumab will be reviewed.

Besides discussing data on new drugs as single agents, current combination approaches of conventional chemotherapy and new drugs will be presented.

ICLLM 2015

# ***Meeting the Challenge of Emerging Pathogens in Patients with Hematological Malignancies: Advances in Diagnosis, Treatment, and Prevention***

**Chairman:** Thomas Walsh

Thomas Walsh

## **Introduction**

Thomas J. Walsh

**New Antimicrobial Agents and the Challenges of Multidrug Resistant Bacteria in Patients with Hematological Malignancies**

Maria N. Gamaletsou

**Advances in the Epidemiology and Treatment of Invasive Fungal Infections in Patients with Hematological Malignancies**

Nikolas V. Sipsas

**Evolving Challenges and New Treatment Options of Respiratory and Systemic Viral Infections in Patients with Hematological Malignancies**

## **Faculty**

**Thomas J. Walsh, MD, PhD (hon), FCCP, FAAM, FIDSA**

Director, Transplantation-Oncology Infectious Diseases Program  
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## **Symposium Abstract**

During the past decade, major shifts have occurred in the pathogens that cause infectious diseases in our immunocompromised patients with haematological malignancies. These pathogens have emerged in the context of rapidly changing immunosuppressive therapies, new antimicrobial selective pressures, emergence of resistant organisms, and more advanced malignancies being treated with an expanding antineoplastic armamentarium. At the same time, the armamentarium of effective antimicrobial agents is diminishing against a rising tide of resistant bacterial, fungal, and viral pathogens. This symposium will review these microbial threats and discuss new approaches to meeting the challenges to our standards of infectious diseases supportive care in hematological malignancies.

## Biographical Outline

### Thomas J. Walsh

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### EDUCATION & TRAINING

1974 B.A., Biology/Chemistry, Summa cum laude, Valedictorian, Assumption College, Worcester, Massachusetts  
1978 M.D., Alpha Omega Alpha, Phi Beta Kappa, The Johns Hopkins University School of Medicine, Baltimore, Maryland  
1978 Intern, Internal Medicine. Michael Reese Hospital and Medical Center, Chicago, IL  
1979 Resident and Fellow, Pathology, The Johns Hopkins Hospital and The Johns Hopkins University School of Medicine, Baltimore, MD  
1980 Resident, Internal Medicine. Michael Reese Hospital and Medical Center, Chicago, IL  
1982 Fellow, Infectious Diseases, University of Maryland Hospital and School of Medicine; University of Maryland Cancer Center; Baltimore Veterans Administration Medical Center, Baltimore, MD  
1984 National Foundation for Infectious Diseases - Janssen Medical Mycology Fellow, University of Maryland School of Medicine, Baltimore Veterans Administration Medical Center, Loyola College, and Johns Hopkins Medical Institutions, Baltimore, MD  
1984 Postdoctoral Course (National Foundation for Infectious Diseases (NFID) Fellowship Training Program): Basic Mycology, Loyola College, Baltimore, MD  
1985 Postdoctoral Course (National Foundation for Infectious Diseases (NFID) Fellowship Training Program): Medical Mycology, The Johns Hopkins University School of Medicine, Baltimore, MD  
1985 Clinical Associate, Medical Oncology, University of Maryland Cancer Center, Baltimore, MD  
1986 Research Fellow, Infectious Disease Section, Pediatric Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD  
1987 Senior Staff Fellow, Infectious Disease Section, Pediatric Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD  
2000 Clinical Research, NIH Clinical Center, Bethesda, MD  
2001 DNA Sequence Analysis, Genecodes Corp., NIH Clinical Center, Bethesda, MD

### POSITIONS

1987-2009: Attending Physician, National Institutes of Health Clinical Center, Bethesda, MD  
1988-1991: Medical Officer, Infectious Disease Section, Pediatric Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland  
1991-1996: Senior Investigator (Tenured), Infectious Disease Section, Pediatric Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland  
1991-1996: Head, Mycology Unit, Infectious Disease Section, Pediatric Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD  
1996-2009: Chief, Immunocompromised Host Section, Pediatric Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD  
1998-present: Adjunct Professor, Division of Infectious Diseases, University of Maryland School of Medicine, Baltimore, Maryland  
2004-present: Adjunct Professor of Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD  
2010-present: Professor of Medicine, Pediatrics, and Microbiology & Immunology; Founding Director, Transplantation-Oncology Infectious Diseases Program, Weill Cornell Medical Center of Cornell University and New York Presbyterian Hospital, New York City, NY  
2011-present: Attending Physician, Medical Staff, New York-Presbyterian Hospital, New York, NY  
2012-present: Attending Physician, Medical Staff, Hospital for Special Surgery, New York City, NY

### BOARD CERTIFICATION:

Diplomate, National Board of Medical Examiners, 206223, July 2, 1979  
American Board of Internal Medicine:  
Internal Medicine, 085642, September 15, 1982  
Infectious Diseases, 085642, November 13, 1984  
Medical Oncology, 085642, November 10, 1987

### National Committees and Offices (selected)

1993-2009 Chair, Institutional Animal Care & Use Committee, National Cancer Institute  
1998-2000 Councilor-East, Medical Mycological Society of the Americas  
2002-2005 Board of Directors, Medical Representative, Commissioned Officers Association  
2002-2006 Councilor, International Immunocompromised Host Society  
2003-2005 President, Medical Mycological Society of the Americas  
2010-2011 Chair, Division F (Medical Mycology)  
2011-2012 Councilor, Division F (Medical Mycology), American Society for Microbiology  
1993-2013 Clinical & Laboratory Standards Institute (CLSI)

### Honors and Awards (selected)

1978 Phi Beta Kappa, The Johns Hopkins University School of Medicine  
1978 Alpha Omega Alpha, The Johns Hopkins University School of Medicine  
1978 Henry Strong Denison Award for Medical Research, The Johns Hopkins University School of Medicine  
1984 Medical Mycology Fellowship Award of the National Foundation for Infectious Diseases (first recipient),  
1985 Interscience Conference on Antimicrobials and Chemotherapy (ICAAC) Young Investigator Award

- 1992 Ian Murray Memorial Lectureship, British Society for Mycopathology, 1992
- 1993 2001 U.S. Public Health Service Commendation Medals
- 1996 Billy H. Cooper Memorial Meridian Award, Medical Mycology Society of the Americas
- 1996 U.S. Public Health Service Outstanding Service Medal
- 1997 Maxwell-Littman Award, Medical Mycology Society of New York
- 1999 Malcolm Grow Memorial Lectureship and Award, Society of Air Force Physicians, United States Air Force
- 2001 Distinguished Clinical Teacher Award, NIH Clinical Center
- 2001, 2002 U.S. Public Health Service Outstanding Unit Citations
- 2002 American Society for Microbiology (ASM) Foundation Lecturer,
- 2002 U.S. Public Health Service Citation
- 2003 U.S. Public Health Service Meritorious Service Medal
- 2003 Physician Researcher of the Year Award, Physicians Professional Advisory Committee to the Surgeon General, USPHS
- 2004 NIH Merit Award for "outstanding achievement in advancing the understanding of host defenses, treatment, and prevention of invasive candidiasis in immunocompromised pediatric patients."
- 2005 U.S. Public Health Service Unit Commendation
- 2006 Visiting Professor Lecture, Center for Drug Evaluation and Research, Food and Drug Administration
- 2007 A.I. duPont Hospital for Children Visiting Professor in Pediatric Oncology and Infectious Diseases
- 2008 Rhoda Benham Award, Medical Mycology Society of the Americas (MMSA)
- 2009 Lucille K. Georg Medal, International Society for Human and Animal Mycology (ISHAM)
- 2009 Edouard Drouhet Lectureship, European Confederation of Medical Mycology (ECMM)
- 2010 Gilbert B. Forbes Visiting Scholar, Strong Children's Research Center, University of Rochester Medical Center
- 2010 Sanofi-Aventis ICAAC Award, American Society for Microbiology
- 2011 Honorary Doctoral Degree (Doctor Honoris Causa), Assumption College
- 2012 Thomas J. Walsh MD Young Investigator Award of the Medical Mycological Society of the Americas
- 2012 Henry Schueler Foundation Scholar in Mucormycosis
- 2013 100 Highly Influential Biomedical Researchers, 1996–2011 (European Journal of Clinical Investigation)
- 2013 Sharp Family Foundation Scholar in Pediatric Infectious Diseases, 2013
- 2013 Honorary Doctor of Philosophy (PhD), National and Kapodistrian University of Athens, Athens, Greece,
- 2014 Excellence in Teaching Award, Weill Cornell Medical College, Cornell University
- 2014-2003 Excellence in Teaching Award in Host Defenses and Infectious Diseases, University of Maryland School of Medicine
- 2015 David C. White Award for Interdisciplinary Research and Mentoring, American Academy of Microbiology and American Society for Microbiology
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**C. Selected Publications (from 905 publications) Publications most relevant to symposium:**

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# New Antimicrobial Agents and the Challenges of Multidrug Resistant Bacteria in Patients with Hematological Malignancies

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## Global Emergence of Multi-Drug Resistant Bacteria and Its Implications for Patients with Hematological Malignancies

The emergence of infectious diseases caused by multidrug-resistant (MDR) bacteria poses a major public health threat to immunocompromised patients, especially those with hematologic malignancies. The causes for this emergence of resistance is multifactorial and includes deficient infection control practices, limited antimicrobial stewardship, the intrinsic ability of bacteria to develop and transmit resistance-conferring mutations, and complicated hosts needing intensive antimicrobial management. This paper reviews the challenges of multidrug resistant bacterial infections with a focus on multidrug resistant bacteria and the recent advances in antimicrobial therapy to address this challenge.

## Global Emergence of Carbapenem-resistant Enterobacteriaceae

Enterobacteriaceae cause approximately one-fourth of all healthcare-associated infections. These organisms include *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp. Historically Enterobacteriaceae were reliably susceptible to carbapenems, even when resistant to other antimicrobial classes.

During the past decade, there has been a global emergence of carbapenem-resistant Enterobacteriaceae (CRE) [1-4]. These carbapenemases confer resistance to all cephalosporins such as cefepime and ceftazidime, to all extended spectrum penicillins, such as piperacillin-tazobactam, and to all carbapenems, such as meropenem and imipenem.

*Klebsiella pneumoniae* carbapenemase (KPC) producing bacteria were initially described in hospitals of New York City and Israel [2]. Subsequent reports now described these organisms in multiple cities throughout the developed nations. There are now

reports of CRE infection in more than 35 states in the United States and more than 30 countries reporting these infections. The Center for Disease Control and Prevention in 2006-2007 reported that 21% of *Klebsiella pneumoniae* isolates from NYC were carbapenem-resistant. Carbapenem resistance among Enterobacteriaceae in the USA is most commonly caused by KPC [1, 2]. These organisms express the plasmid-based gene *bla*<sub>KPC</sub>, which encodes a broad spectrum carbapenemase.

Further adding to this public threat of multidrug resistance has been the emergence of New Delhi Metallo-beta-lactamase-1 (NDM-1) in Enterobacteriaceae as a new antibiotic resistance mechanism in India, Pakistan, Iran, and the United Kingdom [5, 6]. From an historical perspective, clones of CRE historically have resided in hospitals or long-term care facilities. However, the NDM-1 containing Enterobacteriaceae now also now have the capability of thriving in the community and quickly spreading across countries and continents in relation to accessible, rapid global travel. Among the conditions favoring these organisms are profigate antibiotic use and poor infection control procedures. The rapid global transmission of NDM-1 from New Delhi demonstrates a local problem of resistance can rapidly become a worldwide health crisis.

## Clinical Manifestations and Consequences of CRE Infections.

Patel and colleagues reported from Sinai Hospital in New York City the demographic and clinical characteristics of patients with carbapenem-resistant *Klebsiella pneumoniae* infection [7]. Among 99 infections caused by KPC, the mean age of the population was 61 years with a wide SD of  $\pm 15\%$  with 59% males. There were 56 bacteremias and 34 intra-abdominal infections, 15 of which had secondary bacteremia. Other infections included

urosepsis, ventriculitis, osteomyelitis, empyema, and deep sinus infection.

Using a two-matched case-control analysis in these patients, KPC infection was independently associated with recent organ or hematopoietic stem-cell transplantation ( $P = .008$ ), receipt of mechanical ventilation ( $P = .04$ ), longer length of stay before infection ( $P = .01$ ), and exposure to cephalosporins ( $P = .02$ ) and carbapenems ( $P < .001$ ) [7]. KPC infection also was independently associated with increased death during hospitalization (48% vs 20%;  $P < .001$ ) and increased death from infection (38% vs 12%;  $P < .001$ ). Mechanical removal of the focus of infection, such as debridement and drainage, was independently associated with patient survival ( $P = .002$ ).

Notably, administration of antibiotics with *in vitro* activity against KPC was not associated with patient survival. This finding may be related to a delay in diagnosis of life threatening infections, such that by the time KPC was identified, the hemodynamic consequences of untreated KPC infection were irreversible.

### **Emergence of Carbapenem-resistant Enterobacteriaceae as a Cause of Bloodstream Infections in Patients with Hematologic Malignancies.**

Satlin and colleagues at Weill Cornell Medical Center in New York hypothesized that expansion of KPC and other CRE organisms into patients with hematologic malignancies was a serious threat to survival and would have serious implications for empirical antimicrobial therapy [8]. As Enterobacteriaceae are the most common causes of Gram-negative blood stream infections in this patient population, a CRE phenotype would have potentially lethal consequences. All of the recommended empirical antimicrobial agents for the initial management of fever and neutropenia in patients with hematologic malignancies have no *in vitro* or *in vivo* activity against CRE. Such patients would be expected to have a high risk of mortality as they would not be receiving effective therapy for 24-48 hours while the CRE was being identified.

These investigators, therefore, studied the emergence of CRE in patients with hematologic malignancies in a large, oncology-hematopoietic stem cell transplant (HSCT) center located in an endemic area (2007-2010) in New York City. Eighteen patients with hematologic malignancies developed bloodstream infections (BSIs) caused by CRE during the study period. Fourteen of these BSIs

were caused by *Klebsiella pneumoniae*, three by *Enterobacter cloacae*, and one was polymicrobial. Initial empirical antimicrobial therapy was active in only two patients (11%). Moreover, a median of 55h elapsed between culture collection and receipt of an active agent. Ten (56%) of the 18 patients died. Nine (69%) of the 13 neutropenic patients also died. Accounting for this strikingly elevated mortality, a median of 4 days elapsed between time of culture collection and death.

Among the CRE isolates that were analyzed for carbapenemase production,  $\beta$ -lactamase genes, and outer membrane porin deletions, carbapenem resistance mechanisms included  $bla_{KPC}$  in most cases, while CTX-M-15 production with an absent outer membrane porin protein was found in one isolate. Among the isolates that were further characterized by multilocus sequence typing and pulsed-field gel electrophoresis (PFGE), no isolate had  $\geq 95\%$  homology on PFGE, indicating a heterogeneous, non-outbreak population of isolates. Such a heterogeneous group of CRE BSI isolates suggests that infection control measures alone will not be sufficient to curtail this spread into the population of hematological malignancies.

These findings indicate that CRE infections are emerging in patients with hematological malignancies and are associated with ineffective initial empirical therapy, long delays in administration of active antimicrobials, and high mortality rates. The mortality rate of 69% in neutropenic patients with CRE is consistent with earlier data in the 1960's and 1970's when monotherapy with gentamicin was being used for treatment of febrile neutropenic hosts. The mortality of the CRE bacteremic neutropenic patients were also compatible with earlier studies three decades ago that in the absence of immediate, effective, broad-spectrum, empirical antimicrobial therapy, approximately 50% of neutropenic patients with Gram-negative bacteremia died within 3 days of presentation.

### ***Pseudomonas aeruginosa***

Treatment of *Pseudomonas aeruginosa* infections has driven the recommended empirical antibiotic choices for fever in neutropenic patients with hematologic malignancies. This organism is the third most common cause of Gram-negative bacteremia in this population and *P. aeruginosa* bacteremia is associated with high mortality rates. Early studies conducted before development of empirical antibacterial therapy for fever demonstrated that approximately 50% of neutropenic patients with *P. aeruginosa* bacteremia died within three days and 70%

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died within seven days. *Pseudomonas aeruginosa* is especially challenging because of its intrinsic resistance to many classes of antibiotics and its ability to develop resistance during therapy through multiple mechanisms. Antimicrobial options for MDR *P. aeruginosa* infections have historically been limited to polymyxins and/or aminoglycosides.

### **Developing Solutions for Diagnosis, Treatment, and Prevention of CRE and *P. aeruginosa* Infections in Patients with Hematological Malignancies.**

In predicting risk of CRE bacteremia in patients with hematological malignancies, prior exposure to carbapenems is not a reliable determinant. Satlin and colleagues demonstrated that the absence of recent carbapenem exposure does not preclude the development of CRE BSI in patients with hematologic malignancies. The majority of CRE BSIs that occurred during neutropenia were not “breakthrough” infections. These infections principally occurred as the initial BSI during neutropenia. Indeed, the most common setting for CRE bacteremia in these patients was the new onset of fever and neutropenia.

The two most common possible risk factors in patients with hematological malignancies predicting CRE bacteremia are (1) exposure to a non-oncology unit, such as a surgical unit or an ICU, elsewhere in the hospital that is known to have CRE infection, and (2) previous or ongoing exposure to a fluoroquinolone or cephalosporin. That patients with hematological malignancies presented with CRE bacteremia is consistent with studies in non-oncology patients where exposure to fluoroquinolones and cephalosporins were important risk factors. These broad spectrum compounds most likely eliminate or reduce the competing endogenous microbiome in the alimentary tract to permit acquisition, colonization, and ultimately infection with CRE pathogens.

Given the continued expansion of CRE into the highly vulnerable population of patients with hematological malignancies, new diagnostic, therapeutic, and preventive strategies are critically needed [9-11]. Surveillance cultures of mucosal surfaces may provide early warning of patients with hematological malignancies who would be colonized with CRE. Among the possible strategies currently being studied for surveillance of mucosal surfaces of high risk patients with hematological malignancies are selective chromophore agar-based media for CRE, rapid PCR systems for the *bla*<sub>KPC</sub> gene, and mass spectroscopic systems, such as Matrix Assisted Laser Desorption Ionization Time of Flight (MALD-TOF). Once patients are found to be colonized, they

are placed on isolation and specific plans are implemented for including antimicrobial agents active against CRE if they become febrile.

There are limited options for specific treatment of CRE bacteremias. Amikacin, tigecycline, and polymyxin B were active against the majority but not all of the CRE isolates from the study of Satlin et al. Of course, these agents are not commonly used for empirical therapy of fever and neutropenia in patients with hematologic malignancies. The rates of tigecycline and polymyxin B susceptibility to CRE isolates are globally declining. Recent foreboding reports from Greece and China of increasing polymyxin and tigecycline resistance among CRE isolates augurs for diminished utility of these antimicrobial agents. Aminoglycosides are also not consistently active *in vitro* against CRE. Some centers describe susceptibility of CRE to aminoglycosides to be as low as 10%.

Once potentially active agents, such as polymyxin B or colistin with tigecycline or an aminoglycoside are initiated against documented CRE bacteremia, several limitations ensue. First, emergence of resistance may occur during therapy to one or both agents. Second, these agents are not potentially microbicidal against CRE. As bactericidal agents are critical for successful therapy of neutropenic patients, breakthrough bacteremias may develop when the plasma concentrations of the agents reach trough in the dosing interval. Third, as nephrotoxic agents, polymyxin B, colistin, and aminoglycosides may not be well tolerated in patients with hematological malignancies who may have underlying renal dysfunction and who may be receiving concomitant nephrotoxic agents, such as foscarnet or amphotericin B.

The development of CRE is occurring in the context of multiple micro-evolutionary events that presage increasing antimicrobial resistance. Among Enterobacteriaceae development of plasmid-based genes encoding extended spectrum beta-lactamases (ESBLs) has created widespread use of carbapenems to treat infections caused by these organisms.

Ceftolozane-tazobactam (CXA-201) has potent antimicrobial activity against strains of multidrug resistant *P. aeruginosa* that express resistance through several mechanisms, including porin mutations, efflux pumps, and beta-lactamase production. Currently licensed for treatment of complicated urinary tract infections and intraabdominal infections, ceftolozane-tazobactam has the potential for targeted therapy against multidrug resistant *P. aeruginosa* in patients with hematological

malignancies. Ceftazidime-avibactam (NXL-104) has potent activity in the inhibition of KPC-encoded beta-lactamases, including those associated with NDM-1-resistant Enterobacteriaceae. This compound also serves a critical unmet need in expanding the antimicrobial armamentarium against multidrug-resistant Gram-negative bacterial infections.

*Acinetobacter baumannii*, which is frequently pan-resistant due to multiple integron-resistant regulated genes encoding proteins mediating high level resistance to most classes of antimicrobial agents. The problems also of resistant Gram-positive bacteria, such as MRSA and VRE, pose additional challenges to patients with hematological malignancies. While there is a small armamentarium of antimicrobial agents for resistant Gram-positive bacteria, there is a serious dearth of potent agents for the multidrug resistant Gram-negative bacteria.

Despite portentous global trends of increasing multidrug resistant pathogens, there is a paucity of new antimicrobial agents being developed. A recent review discusses recent developments in targeting beta-lactamases and beta-lactam combinations [12]. Among the new  $\beta$ -lactam- $\beta$ -lactamase inhibitor combinations in late-stage (phase II and beyond) clinical trials are ceftolozane-tazobactam, ceftazidime-avibactam, ceftaroline-avibactam, and imipenem-cilastatin-MK-7655. Ceftolozane is an antipseudomonal cephalosporin and tazobactam is designed to protect it against ESBLs. Avibactam and MK-7655 are non- $\beta$ -lactam diazabicyclooctane inhibitors, which inhibit class A carbapenemases and class C enzymes.

There is a crisis in the development of new antimicrobial agents that threatens the public health of all patients, especially those with hematological malignancies, where infections are treated through well-established algorithms. Although new agents are being developed that show activity against CRE, other multidrug resistant bacteria will continue to emerge with different mechanisms of resistance. There needs to be a global effort directed to improved surveillance, infection control measures, and new antimicrobial agents, in order to better protect our patients with hematological malignancies.

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Residency: Internal Medicine Resident, Department of Internal Medicine, Laikon General Hospital, Athens, Greece,  
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### FOREIGN LANGUAGES

2007: French: Certificat DELF B1  
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### CREDENTIALS

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- 1998: Ranked 1st “Summa Cum Laude” and selected as Valedictorian in the 1998 graduating class, Athens Technological Educational Institute (TEI), Athens, Greece
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- 2003: Ranked 7th “Summa Cum Laude” for M.D., and selected as Valedictorian in the 2003 graduating class, Medical School, National and Kapodistrian University of Athens, Greece
- 2003: Aristeio (certificate of excellence) awarded by the President of the Medical School of the National and Kapodistrian University of Athens
- 2004: Aristeio (certificate of excellence) awarded by the Board of the Medical School of the National and Kapodistrian University of Athens
- 2011: Award presented at the 4th Congress of the Hellenic Association of Medical Mycology for the oral presentation of the paper “Prospective multicenter study of candidemia in adult hematology malignancy patients”, by Gamaletsou MN, et al.

- 2013: Attendance Grant ESCMID, "Hot topics in Infections of critically ill patients", Athens, Greece, May 31st – Jun 1st
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#### SCIENTIFIC MEDICAL SOCIETIES MEMBERSHIP

1. Hellenic Medical Council
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#### RESEARCH EXPERIENCE

I have participated in several clinical trials as an investigator. I have been formally trained in Good Clinical Practice (GCP) procedures.

#### RESEARCH INTERESTS

Invasive fungal infections in the immunocompromised host

#### CURRENT STATUS OF CAREER

- Scientific Associate, Athens University Medical School, Athens, Greece

#### INVITED SPEAKER IN NATIONAL AND INTERNATIONAL CONFERENCES

- Continuing Antifungal Research & Education (CARE), "Critical issues in systemic candidosis: can we deal with Candida?", November 4-5, 2011, London, United Kingdom  
Topic: Presentation and discussion of an interesting case on candidemia in a neutropenic patient with hematological malignancy. Invited by the Continuing Antifungal Research & Education (CARE)-Gilead
- 4th International Congress on Leukemia Lymphoma Myeloma May 22-25, 2013, Istanbul, Turkey  
Topic: Invasive fungal infections in patients with hematological malignancies. Invited by the Turkish Society of Hematology
- 5th Congress of the Hellenic Society of Medical Mycology, November 22-24, 2013, Athens, Greece  
Topic: Presentation and discussion of an interesting case of a child with hematological malignancy and Candida endocarditis-interactive presentation. Invited by the Hellenic Society of Medical Mycology
- 25th Hellenic HIV Congress, November 29-December 1, 2013, Athens, Greece  
Topic: Workshop for Medical Students, Diagnosis of HIV infection. Invited by the Hellenic Society of Study and Control of AIDS
- Educational program of the Hellenic Society of Rheumatology, April 28, 2014, Athens, Greece  
Topic: Prophylaxis against opportunistic infections among patients with autoimmune rheumatic diseases. Invited by the Hellenic Society of Rheumatology
- Educational Seminars of the Hellenic Society of Medical Mycology, June 2, 2014, Athens, Greece  
Topic: Presentation and discussion of a case of a patient with Aspergilloma. Invited by the Hellenic Society of Study and Control of AIDS
- Educational Course of the Hellenic AIDS Society, September 28, 2014, Athens, Greece  
Topic: Presentation and discussion of an interesting case of a patient with refractory HIV-related cryptococcal meningitis-interactive presentation. Invited by the Hellenic Society of Study and Control of AIDS
- 26th Hellenic HIV Congress, November 28-30, 2014, Athens, Greece  
Topic: Presentation and discussion of an interesting case of a patient infected with a multidrug-resistant strain of HIV-interactive presentation. Invited by the Hellenic Society of Study and Control of AIDS

#### TEACHING PORTOFOLIO

- Clinical training of 6th year medical students in Internal Medicine, (Subject: Internal Medicine, official curriculum of the Athens University Medical School)
- Lectures in Pathophysiology of Infectious Diseases to the 3rd year medical students (Subject: Pathophysiology, official curriculum of the Athens University Medical School)
- "Fever of Unknown Origin" (at the Program of Pre-graduate lectures) at Pathophysiology Department, University of Athens, Medical School, Oct 2010, Sept 22, 2014
- "Coagulation-transfusion and Infectious Diseases" (at the Program of Postgraduate studies "Transfusion Medicine"), University of Athens, Medical School March 8, 2012 and November 2014
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of Surgery), University of Athens, Medical School, March 13, 2012, and April 15, 2014

- Antibiotics-Vaccination (Program of Postgraduate studies "Dental hygiene"), National and Kapodistrian University of Athens, Dental School, July 9, 2014

#### REVIEWER IN «PEER-REVIEWED» JOURNALS

- The Journal of Rheumatology, 2012
- Mycoses, 2013
- BMC Research Notes, 2014
- Journal of Infection, 2014

#### PUBLICATIONS IN «PEER-REVIEWED» JOURNALS

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## ATTENDANCES IN CONGRESSES/ SEMINARS/ EDUCATIONAL COURSES 107 (84 national, 25 international) in a period of 14 years

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# Advances in the Epidemiology and Treatment of Invasive Fungal Infections in Patients with Hematological Malignancies

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Invasive fungal infections (IFIs) are important causes of morbidity and mortality in patients with hematological malignancies. The risk for IFIs in patients with hematological malignancies varies as a function of underlying neoplastic process and degree of immunosuppression. Recent therapeutic advances in antifungal therapy expand the antifungal armamentarium against these life-threatening infections.

## Risk factors for development of IFIs in patients with hematological malignancies

Neutropenia is a key risk factor for the development of invasive fungal infections. Neutropenia may develop as the result of chemotherapy, radiation, bone marrow failure (myelodysplasia and aplastic anemia), and by replacement of hematopoietic cells in the bone marrow by malignant cells. In the classic description of the inverse relation between risk of infection and degree of neutropenia, Bodey *et al.* underscored the role of profound neutropenia (ANC<100) in leukemia patients for increasing the risk for infection. In a classic study of patients receiving treatment for acute leukemia Gerson *et al.* later demonstrated that the risk of invasive aspergillosis is directly related to the duration of neutropenia in patients with acute leukemia. After 14 days of neutropenia, the risk of aspergillosis increased in direct relation to the duration of neutropenia. Neutropenia is also a surrogate marker for other risk factors for IFIs. For example, mucositis associated with intensive chemotherapy increases the risk for translocation of *Candida* spp. across the alimentary tract.

Lymphocytopenia in hematological malignancies increases the risk for fungal infections associated with impaired cell mediate immunity (CMI). Fludarabine, which is a lymphotoxic compound primarily affecting CD4+ lymphocytes and corticosteroids markedly increase the risk of infection caused by *Pneumocystis jirovecii* and *Cryptococcus neoformans*.

In addition to their effects on CMI, corticosteroids markedly alter the distribution, trafficking and functions of neutrophils, monocytes, and lymphocytes. Corticosteroids also impair oxidative function and hyphal damage capacity of neutrophils and impair phagocytosis of macrophages. The risk of infections caused by invasive filamentous fungi, such *Aspergillus* spp. and the *Mucorales*, is significantly increased in these patients receiving a prednisone equivalent of 0.5 mg/kg for longer than 30 days. Among allogeneic HSCT recipients, corticosteroid therapy for GVHD is a major risk factor for invasive Aspergillosis and filamentous fungal infections.

Among the humanized immunosuppressive biological agents, alemtuzumab (Campath-1H; anti-CD52 humanized monoclonal antibody that targets normal and most malignant T-lymphocytes) is associated with severe lymphopenia and an increased risk for opportunistic infections, including *Pneumocystis jirovecii* pneumonia. TNF-alpha inhibiting agents, including infliximab, etanercept, and adalimumab, increase the risk for infections caused by intracellular pathogens, such as *Histoplasma capsulatum*.

## Candidiasis

*Candida* species are a component of the endogenous microbiome that invade the bloodstream through disruptions in anatomical barriers. The alimentary tract is the principal portal of entry in patients with acute leukemia.

*Oropharyngeal and esophageal candidiasis.* Invasive candidiasis in patients with hematological malignancies may present initially as oropharyngeal and esophageal candidiasis. Therapy for oropharyngeal candidiasis includes clotrimazole troches or oral fluconazole. Esophageal candidiasis typically presents as odynophagia. The differential diagnosis include herpes simplex virus, cytomegalovirus (principally in HSCT recipients), and

bacteria. Fluconazole is used as initial therapy for esophageal candidiasis, while an echinocandin is used in refractory cases. *Candida glabrata* and *Candida krusei* may emerge resistant to fluconazole and cause recurrent symptoms of odynophagia.

**Candidemia.** *Candida albicans* historically was the most common *Candida* species isolated from blood. With the advent of triazole and echinocandin prophylaxis and therapy, there has been a major shift in the causes of candidemia toward non-*albicans* *Candida* spp.

A recent prospective, multicenter study specifically designed to investigate the epidemiology, risk factors, and outcome of candidemia among hospitalized patients with hematological malignancies found that most infections (87.5%) were caused by non-*Candida albicans* species, with *C. parapsilosis*, being most common. Independent risk factors for the development of candidemia were the presence of CVC, hypogammaglobulinemia, and high APACHE II score. Twenty-eight-day crude mortality was 45%. Patients with candidemia had significantly lower survival than those without candidemia. Among patients with candidemia, an elevated APACHE II score was an independent risk factor for death, while recovery from neutropenia was independently associated with improved survival.

Among the different *Candida* species, *Candida krusei* is always resistant to fluconazole. *Candida glabrata* may emerge as breakthrough infection with resistance to all triazoles. *Candida tropicalis* bloodstream infection often has a severe course with cutaneous dissemination, myalgias, renal failure, and hemodynamic collapse. *Candida parapsilosis* is mostly associated with vascular catheters and may emerge during the course of echinocandin therapy.

Recent studies indicate that removal of central vascular catheters in patients with hematological malignancies does not improve outcome. This finding suggests that the likely portal of entry is the alimentary tract. If a multi-lumen catheter is not immediately removed, antifungal therapy should be administered parenterally through all lumens. As candidemia in neutropenic patients may be complicated by chronic disseminated candidiasis of liver, spleen, and kidney, and eyes, ophthalmologic examination and CT scan of the abdomen is recommended upon recovery from neutropenia.

**Chronic disseminated candidiasis.** Chronic disseminated candidiasis (hepatosplenic candidiasis) may persist with new fever after recovery from neutropenia. Following resolution of neutropenia, elevated alkaline phosphatase and development of

numerous target lesions in the liver and spleen develop. An open liver biopsy is advisable but may not be feasible. Antifungal therapy with fluconazole or echinocandin is initiated with anticipation of treatment for several months until resolution of lesions. The presence of persistent lesions does not preclude further chemotherapy.

**Treatment of Invasive Candidiasis.** As most patients with hematological malignancies are receiving fluconazole prophylaxis, an echinocandin (anidulafungin, caspofungin, or micafungin) is recommended as the initial therapy of invasive candidiasis in neutropenic patients with hematologic malignancies. For non-neutropenic stable patients with uncomplicated candidemia an initial course of echinocandin followed by fluconazole is reasonable if the organism proves to be *C. albicans*.

## Aspergillosis

The sino-pulmonary tract is the most common portal of entry of *Aspergillus* spp. and other filamentous fungi. Profound and persistent neutropenia, repeated cycles of prolonged neutropenia, concomitant corticosteroid therapy, and graft *versus* host disease (GVHD) increase the risk of development of invasive sino-pulmonary aspergillosis (ISPA). Other risk factors in HSCT recipients include lymphopenia, GVHD, CMV disease, and respiratory viral infections.

ISPA may initially only manifest as fever. More advanced infection presents sinus pain or congestion, cough, pleuritic chest pain, and hemoptysis. Invasive pulmonary aspergillosis (IPA) includes nodules, halo sign, bronchopneumonia, lobar consolidation, wedge-shaped segmental pneumonia, and cavitary lesions. CNS aspergillosis may present as focal neurological deficits. Early diagnosis of aspergillosis is important for improved outcome. Recovery of organism from bronchoalveolar lavage, percutaneous needle aspirate, and biopsies, in sino-pulmonary lesions is advised but may have limited sensitivity. Serum galactomannan detected by double sandwich ELISA improves early detection of aspergillosis and complements CT scans. Serial quantitation of galactomannan antigenemia also predicts response to antifungal therapy. Serum (1 $\rightarrow$ 3)- $\beta$ -D-glucan may also detect invasive aspergillosis and other invasive mold infections. PCR-based detection of *Aspergillus* DNA in BAL fluid may be useful for the diagnosis of IPA. *Aspergillus fumigatus* followed by *Aspergillus flavus* are the most common species causing invasive aspergillosis. *Aspergillus terreus* is observed with increasing frequency at several hematological malignancies

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centers, and is notable for being resistant to amphotericin B.

*Treatment of invasive aspergillosis.* Voriconazole or isavuconazole are the preferred agents for initial therapy of ISPA and disseminated aspergillosis. Isavuconazole has been recently shown in laboratory animal studies and in clinical trials to be comparable voriconazole in antifungal activity against IPA. Isavuconazole, however, has fewer adverse events, particularly reduced CNS toxicity, cutaneous reactions, and hepatotoxicity. Isavuconazole also demonstrates linear dose-proportional plasma pharmacokinetics with once daily dosing and fewer adverse drug interactions. For patients for whom voriconazole and isavuconazole is contraindicated, liposomal amphotericin B (LAmB) is used instead. Posaconazole is approved for use as prevention of invasive aspergillosis in patients with acute leukemia and in HSCT recipients.

The combined data from laboratory animal studies, case controlled studies, and a recently completed prospective randomized controlled trial support the use of voriconazole and anidulafungin in the treatment of invasive aspergillosis. The rationale is that echinocandins target a unique site of cell wall biosynthesis while triazoles target synthesis of the fungal cell membrane. Patients who recover from an episode of ISPA are at risk for relapse of infection during subsequent immunosuppression. Secondary prophylaxis is indicated in those patients who undergo additional cycles of cytotoxic chemotherapy or HSCT.

## Mucormycosis

The agents of mucormycosis (zygomycosis) include the following members of the order Mucorales: *Rhizopus* spp., *Mucor* spp., and *Lichtheimia* (formerly *Absidia*) *corymbifera*. Risk factors for mucormycosis among patients with hematological malignancies include prolonged neutropenia, corticosteroids, diabetic mellitus, iron overload, and GVHD. Mucormycosis in patients with hematological malignancies typically manifests as pulmonary, sinus, sino-orbital, rhino-cerebral, or cutaneous disease. Patients with pulmonary mucormycosis may present with cough, hemoptysis, pleuritic pain, and single or multiple pulmonary nodules, which also may demonstrate a reverse halo sign. In rhino-cerebral disease, fever, facial pain and headache are common symptoms. Contiguous extension may lead to orbital involvement with proptosis and extraocular muscle paresis, involvement of hard palate, and extension into the brain. Invasion of the veins draining the ethmoid sinuses

and orbits may lead to cavernous sinus thrombosis. An eschar over the palate or nasal turbinates is suggestive of mucormycosis, but other filamentous fungi can produce similar findings. Occasionally, isolated primary cutaneous disease may follow minor trauma.

*Treatment of mucormycosis.* There are three cornerstones of therapy for mucormycosis: lipid formulation of amphotericin B or conventional deoxycholate amphotericin B; early and aggressive surgical debridement; and reversal of immunosuppression, as well as correction of hyperglycemia in diabetic patients. Isavuconazole is an emerging alternative for primary therapy for treatment of mucormycosis. Laboratory animal data and the results of a prospective non-randomized trial for primary treatment of mucormycosis demonstrate favorable activity of isavuconazole. The role of isavuconazole in relation to lipid formulations of amphotericin B remains to be defined through future studies. There is no advantage to combination therapy of isavuconazole and amphotericin B.

## Fusarium infections

*Fusarium* species in patients with hematological malignancies cause sino-pulmonary and disseminated infection. Prolonged neutropenia is the most common risk factor. The portal of entry is most frequently respiratory but may also be from peri-unguinal and soft tissue infection. Fungemia with positive blood cultures occurs in approximately one-half of cases during neutropenia. Multiple hematogenously disseminated cutaneous lesions are common and usually reveal the organism in biopsy. Other sites of infection in the process of dissemination include CNS, bone, joints, eyes, and liver. Initial localized manifestations include onychomycosis, paronychia, and cellulitis. Early identification of localized skin disease and debridement may be life-saving.

*Treatment of fusariosis.* *Fusarium* species, which include *Fusarium solani* species complex and *Fusarium oxysporum* species complex, have variable *in vitro* susceptibility to amphotericin B and to voriconazole. Initial therapy consists of both amphotericin B and voriconazole for spectrum (not synergy) while awaiting susceptibility results. Although interpretive breakpoints have not been established, readings of >4µg/ml usually signify lack of response ("resistance") to the antifungal agent. Survival from disseminated fusariosis is critically dependent on resolution of neutropenia. Granulocyte transfusions have been life-saving in selected patients until recovery from neutropenia.

## Scedosporium infections

*Scedosporium apiospermum* and *Scedosporium prolificans* are the principal pathogenic species. In neutropenic patients, *S. apiospermum* causes sinopulmonary disease, and dissemination to the central nervous system infection. *S. apiospermum* is clinically and histologically indistinguishable from aspergillosis. Voriconazole is the preferred first line antifungal agent against this organism.

**Treatment of *Scedosporium* infections.** As *S. apiospermum* is often resistant to amphotericin B but susceptible to voriconazole and posaconazole, establishing a microbiological diagnosis is important. *Scedosporium prolificans*, by comparison, causes a similar spectrum of disease as *Aspergillus* but is resistant to all systemically available antifungal agents. Reversal of immunosuppression and surgical resection are the keys to the management of infections caused by *S. prolificans*.

## Dematiaceous moulds

These organisms are distinguished as dark-walled molds contain melanin in their cell walls that confers a black, brown, or olive-green pigment in culture. Infections caused by these dematiaceous moulds are sometimes termed phaeohyphomycosis. Among patients with hematological malignancies, sinusitis, pneumonia, central nervous system infection, fungemia, soft tissue infection, and disseminated disease may develop. As a group, these organisms have a strong predilection to cause central nervous system infection. Among the most common organisms are *Alternaria* spp., *Bipolaris* spp., *Ochroconis gallopava*, *Cladophialophora* (*Xylohypha* or *Cladosporium*) *bantiana*, and *Exophiala* (*Wangiella*) *dermatitidis*, and *Exserohilum rostratum*. The recent outbreak of fungal meningitis caused by *Exserohilum rostratum* in the United States in association with exposure to contaminated methylprednisolone solution demonstrates the morbidity cause by these organisms.

**Treatment of infections caused by dematiaceous moulds.** Treatment consists of systemic antifungal therapy and surgical excision of localized disease when feasible. Based upon susceptibility profiles and clinical reports, voriconazole is the primary agent for therapy. Amphotericin B and posaconazole may be alternatives. However, as antifungal susceptibility profiles vary according to species, guidance by an expert infectious diseases and mycologist is advisable.

## Cryptococcosis

As host defense against cryptococcal infection is principally dependent on cell-mediated immunity, patients with isolated neutropenia are rarely infected with *C. neoformans*. Instead, patients receiving corticosteroids, those with lymphopenia, and those suffering from GVHD are at greatest risk. As the respiratory tract is the principal portal of entry, patients may present initially with pneumonia or may have concomitant CNS infection. Although meningitis is the most common presentation of cryptococcal infection, other manifestations include primary pneumonia, fungemia, and cutaneous and visceral dissemination.

Risk factors for poor outcome include elevated cerebrospinal fluid opening pressure, a low glucose level, less than 20 leukocytes/ml, a positive India ink preparation, culture of cryptococci from extraneural sites, and high titers of cryptococcal antigen in serum and cerebrospinal fluid. Central nervous system complications include development of a mass lesion, obstructive hydrocephalus requiring shunting, and visual loss, especially cortical blindness related to elevated intracranial pressure.

**Treatment of cryptococcosis.** Initial therapy consists of deoxycholate amphotericin B (0.7 mg/kg daily) plus 5-flucytosine (100 mg/kg daily) for the first 2 weeks, followed by maintenance fluconazole therapy (400 mg daily). Liposomal amphotericin 5 mg/kg/d may also be used in lieu of deoxycholate amphotericin B. As fluconazole is well tolerated, continuing therapy with this agent through immunosuppressive therapy is reasonable.

## Pneumocystis pneumonia (PCP)

Patients defective CMI and T-cell immunity are at risk for PCP. Corticosteroid therapy is the most common predisposing factor in patients with hematological malignancies. PCP can have a more fulminant course with more rapid progression to respiratory failure. Patients treated with corticosteroids may develop initial clinical manifestations of PCP during steroid taper. Although diffuse bilateral interstitial pulmonary infiltrates are the most common manifestation of PCP, unilateral, segmental or patchy infiltrates may also develop. Extrapulmonary infection is uncommon in patients with PCP and hematological malignancies.

Bronchoalveolar lavage is indicated in patients with hematological malignancies who present with these findings. Diagnosis of PCP relies on microscopic visualization of the organism. Immunofluorescent

staining using monoclonal antibodies is more sensitive than cell wall staining methods, such as silver staining or Wright-Giemsa. Where available, PCR on BAL fluid may also facilitate diagnosis. Serum (1→3)-β-D-glucan recently has been shown to be a sensitive circulating biomarker for PCP.

**Treatment of PCP.** The treatment of choice for PCP is trimethoprim/sulfamethoxazole (trimethoprim: 15 mg/kg daily in 3 divided doses) (TMP/SMX). For patients intolerant of TMP/SMX, intravenous pentamidine, primaquine-clindamycin, and dapsone-trimethoprim are acceptable alternatives. Although response rates are significantly lower than with that of TMP/SMX, atovaquone may be used for treatment of mild to moderate PCP. For patients with moderate to severe PCP ( $\text{PaO}_2 \leq 75$  mmHg), corticosteroids should be administered. As TMP/SMX is highly effective as prophylaxis against PCP, it should be administered to patients at risk using any one of several oral regimens. Among those patients found to benefit are children with acute lymphoblastic leukemia, adult and pediatric patients with allogeneic HSCT, patients with CNS tumors receiving high-dose corticosteroid therapy, those receiving Fludarabine, and patients receiving combination corticosteroid therapy.

### **Mycoses caused by other fungal pathogens.**

**Trichosporonosis.** *Trichosporon* species may emerge as breakthrough infections in neutropenic patients receiving amphotericin B. Trichosporonosis in profoundly neutropenic patients typically manifests with refractory fungemia, funguria, cutaneous lesions, renal failure, pulmonary lesions, and chorioretinitis. Disseminated trichosporonosis may yield a false positive cryptococcal latex antigen test because of cross-reactivity with the polysaccharide capsule of *C. neoformans*. *In vitro* and experimental infections indicate that most *Trichosporon* species are inhibited, but not killed, by achievable serum levels of conventional amphotericin B. Fluconazole and voriconazole have superior activity in experimental infections and are the preferred antifungal agents.

**Malassezia infections.** *Malassezia furfur* fungemia is often associated with lipid-containing parenteral nutrition administered through a central venous catheter in immunocompromised patients or premature infants. Clinical manifestations include persistent fungemia and pulmonary infiltrates. Blood culture recovery is enhanced by addition of olive oil or other long chain fatty acids to the culture plates. Discontinuation of lipid infusions and removal of the central catheter are essential. As *Malassezia furfur* is resistant to amphotericin B

therapy, fluconazole therapy is the drug of choice. Among neutropenic patients and patients treated with corticosteroids, a folliculitis resembling disseminated candidiasis may occur. This localized process does not imply disseminated infection.

**Endemic dimorphic fungi.** These organisms include *Histoplasma capsulatum*, *Coccidioides* spp., *Blastomyces dermatitidis*, and *Penicillium marneffei*. *Histoplasma capsulatum* is found in central US, and in areas of northern Mexico. *Coccidioides* is endemic in the southwestern U.S. *Penicillium marneffei* (*Talaromyces marneffe*) is endemic in Southeast Asia. Endemic dimorphic fungi are so named because of their characteristic geographic distribution. These fungi are dimorphic, existing in nature in the mycelial stage and converting to the yeast stage at body temperature. Impaired cell-mediated immunity and a geographic exposure are the key risk factors. Endemic mycoses in the central U.S. include histoplasmosis and blastomycosis. For histoplasmosis fever, pulmonary infiltrates, and hypoxia with dissemination to liver, spleen, lymph nodes, bone marrow, adrenal glands, mucocutaneous tissues, gastrointestinal tract, and central nervous system may occur. The chest radiograph may show a miliary reticulonodular pattern that is suggestive of disseminated tuberculosis. A rapid diagnosis of histoplasmosis can be made by Giemsa staining of a peripheral blood smear or bone marrow aspirate demonstrating characteristic intracellular yeast forms. Blood cultures may be positive for small yeast-like cells. Severe pulmonary or disseminated histoplasmosis should be treated with an amphotericin B formulation. Prolonged therapy with itraconazole may be initiated after stabilization of disease.

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## Nikolaos V. Sipsas

FIDSA received his medical degree from the National and Kapodistrian University of Athens in Greece. He did his post-doctoral thesis, in History of Medicine at the Medical School of the Zurich University in Switzerland, and in Infectious Diseases at the Athens University Medical School, Greece. Then he was trained in Internal Medicine at the Athens Naval Hospital and the Athens University Laikon Hospital. He was subsequently trained as a research fellow in Infectious Diseases at Massachusetts General Hospital in Boston. He is currently attending physician at the Department of Medicine, head of the Infectious Disease Unit at the Laikon General Hospital, in Athens, Greece and Associate Professor at the Athens University Medical School. He is a reviewer in peer-reviewed journals in Infectious Diseases and Internal Medicine and he has authored more than 80 peer-reviewed manuscripts.

### PERSONAL DATA

Name: Nikolaos V. Sipsas, M.D., FIDSA

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### GRADUATE MEDICAL EDUCATION

M.D. Degree: Athens University Medical School, Greece; 1985

Doctorates in Science: [1] Zurich University Medical School, Switzerland; 1990

[2] Athens University Medical School, Greece; 1997

### FOREIGN LANGUAGES PROFICIENCY

- Greek (native language)
- English (Certificate of Proficiency in English, University of Cambridge, TOEFL, Educational Testing Service, U.S.A.)
- French (Diplome d' Etudes Francaises (2e Degre), Universite de Paris – Sorbonne (Paris IV))

### POSTDOCTORAL TRAINING:

Internship: Department of Medicine, Athens Naval Hospital, 2/87-2/88

Residency: Department of Internal Medicine, Athens Laikon General Hospital and Athens University Medical School, 7/90-7/94

Fellowship: Infectious Disease Unit, Massachusetts General Hospital and Harvard Medical School, Boston, USA, 10/94- 4/97

Sabbatical: M.D. Anderson Cancer Center, University of Texas, Houston, Texas, USA, 3/2007 – 6/2007

### BOARD CERTIFICATION

Diplomate, Educational Commission for Foreign Medical Graduates, (ECFMG), USA, 1990

Diplomate, Hellenic Board of Internal Medicine, September 1993

Diplomate, Hellenic Board of Infectious Diseases, June 2002

### ACADEMIC APPOINTMENTS

Lecturer, Athens University Medical School, (2003-2008)

Assistant Professor, Athens University Medical School, (2008 -2013)

Associate Professor, Athens University Medical School, (2013- currently)

### MAJOR HOSPITAL APPOINTMENTS

Attending Physician, Internal Medicine Department and Infectious Disease Unit, Athens Laikon Hospital

Visiting consultant: 2nd Department of Orthopedics, Athens Medical School, KAT Hospital

Visiting consultant: Bone Marrow Transplantation Unit, Children's Hospital, Athens

### COMMITTEES AND OTHER OFFICES

European Union of Medical Specialists (UEMS) - Section of Infectious Diseases  
2012 – 2014 Delegate

#### Hellenic Ministry of Health

2004 – Present Committee for the selection of Fellows for the Infectious Diseases Training Programs

#### Hellenic Center for Disease Control & Prevention (HCDCP)

2007: Committee of anti-retroviral drugs (member)

#### Athens University Medical School

2008 – 2013: Elected member of the General Assembly

#### Consultant for Appointment, Promotion and Tenure Committees:

Athens University Medical School, 2009-2014

Athens Laikon General Hospital

2000 – Present Committee of Infection Control and Hospital Epidemiology (member)

2003 – 2007 Pharmacy and Therapeutics Committee (member)

2002 – 2003 Internal Review Board (IRB) (member)

2005 Elected member of the Hospital Scientific Committee

### MEDICAL SOCIETIES

- Hellenic Medical Society (1985 - Present)
- Athens Medical Society (1985- Present)
- Massachusetts Medical Society (1994 -1997)
- American Medical Association (1994-1997)
- Infectious Disease Society of America (1997- Present)
- o Advancement to Fellow of IDSA - 2012
- HIV Medicine Association (2000- Present)
- ESCMID (2012 - Present)
- Hellenic Society of Medical Mycology (2012 – Present)
- Hellenic Society of Infectious Diseases, 1997 – Present
- o Elected member of the Board of Directors, Treasurer (2011-2014)

### HONORS AND AWARDS

- Hellenic Scholarships Foundation, (1994)
- International Research Fellow Award, (1995)
- Fogarty International Center, National Institutes of Health, USA
- Fellow Award (1997)
- The Fourth National Conference on Human Retroviruses and Related Infections. Washington D.C., January 22-26, 1997
- Second place winner, research poster competition (1998)
- 10th Hellenic Conference on AIDS, Athens, Greece
- First place winner, research poster competition (2000)
- 12th Hellenic Conference on AIDS, Athens, Greece
- L. Tsaldaris – S & E Malamos Award, (2003)
- National and Kapodistrian University of Athens
- Second Place winner (Mentor) for Oral Presentation competition (2012)

4th Conference of the Hellenic Society of Medical Mycology  
 • “G. L. Daikos” Award (2012) for the best Thesis on Infectious Diseases  
 (Mentor)  
 Hellenic Society of Infectious Diseases

#### EDITORIAL BOARD AND REVIEWER ACTIVITIES

##### Reviewer

1. Lancet
2. Clinical Infectious Diseases
3. Clinical Microbiology and Infection
4. European Journal of Clinical Microbiology and Infectious Diseases
5. Eurosurveillance
6. Journal of Infection
7. BMC Infectious Diseases
8. Medical Mycology
9. American Journal of Medicine
10. Clinical and Diagnostic Laboratory Immunology
11. Clinical Experimental Immunology
12. Journal of Medical Case Reports
13. Lung
14. Neuroimmunomodulation

##### Editorial Board

1. BMC Research Notes (Associate Editor)
2. Journal of Invasive Fungal Infections

#### RESEARCH EXPERIENCE

I have participated in several clinical trials as an investigator. I have been formally trained in Good Clinical Practice (GCP) procedures.

#### RESEARCH FOCUS

- HIV infection: Worked on markers of immune activation, cytotoxic T-cell responses, autoimmune phenomena, anemia, and metabolic complications
- Infections in the immunocompromised host: Candidemia in patients with hematological malignancies, neutropenic patient, autoimmune rheumatic disease patients
- Zoonoses: work on brucella infections, especially spondylodiskitis, leishmaniasis in immunocompromised patients, Q-fever
- CNS infections: endocrine complications of meningitis, treatment of cryptococcal meningitis
- Nutrition and hospital-acquired infections
- Animal models for experimental osteomyelitis
- Bacterial infections

#### BIBLIOGRAPHY

Author or co-author in 81 peer-reviewed papers (see attached list)

Bibliography (n=81)

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2. Mavrogenis AF, Triantafyllopoulos GK, Kokkinis K, Stefanos A, Sipsas NV, Pneumatikos SG. Continuous I3 spondylitis caused by an infected endovascular aortic graft. *Surg Infect (Larchmt).* 2014;15:861-2.
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5. Triantafyllopoulos G, Kokkinis K, Stefanos A, Sipsas N, Pneumatikos S. Lumbar vertebral osteomyelitis caused by Staphylococcus epidermidis infection of an endovascular stent graft of the abdominal aorta. *Surgical Infections (Larchmt).* 2014; 15:861-2.
6. Gamaletsou MN, Walsh TJ, Sipsas NV. Epidemiology of Fungal Osteomyelitis. *Current Fungal Infection Reports* 2014; 8: 262-270
7. Gamaletsou MN, Daikos GL, Walsh TJ, Perlin DS, Ortigosa CJ, Psaroulaki A, Pagoni M, Argyropoulou A, Nepka M, Perivolioti E, Kotsopoulou M, Perloretzou S, Marangos M, Kofteridis D, Grammatikou M, Goukos D, Petrikos G, Sipsas NV. Breakthrough Candidemia Caused by Phenotypically Susceptible Candida spp. in Patients with Hematological Malignancies Does Not Correlate with Established Interpretive Breakpoints. *International Journal of Antimicrobial Agents* 2014; 44:248-55
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10. Papadopoulou D, Sipsas NV. Comparison of National Clinical Practice Guidelines and Recommendations on Vaccination of Adult Patients with Autoimmune Rheumatic Diseases. *Rheumatology International* 2014; 34:151-63
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27. Tsiakalos A, Loukeris D, Georgiadou SP, Sipsas NV. A Yeast in the Blood is Not Always Candida: a Case of Invasive Trichosporonosis in a Splenectomized Patient. *Journal of Invasive Fungal Infections* 2011; 5:83-86.
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31. Tsiakalos A, Georgiadou SP, Anastasiou I, Sipsas NV, Tzioufas A. Bilateral dilation of the urinary tract due to iliopsoas pyomyositis: a case report. *J Med Case Reports.* 2011;5:195.
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# Evolving Challenges and New Treatment Options of Respiratory and Systemic Viral Infections in Patients with Hematological Malignancies

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## Introduction

Over the past several decades, there has been substantial progress in the treatment of hematological malignancies. Research has provided a new array of chemotherapeutic agents, while modern treatment modalities, including hematopoietic stem cell transplantation (HSCT), have been successfully introduced into the clinical practice. Unfortunately, the majority of these treatment options cause a profound suppression of innate and/or acquired immunity. Neutropenia in particular, remains as the most prominent chemotherapy-induced immune defect, making the patients vulnerable to infections. Infections are an important complication of hematological malignancies, especially after chemotherapy or during HSCT procedures and contribute significantly to morbidity and mortality. Besides the risk of developing bacterial and fungal infections, there is a substantial risk of primary viral infection and reactivation.

Respiratory viruses can cause severe pneumonia after chemotherapy and/or HSCT, with high morbidity and mortality. Historically, their clinical significance in patients with hematological malignancies has been underestimated, but nowadays they are increasingly recognized as common causes of upper respiratory infection (URI), lower respiratory tract infection (LRTI), and are frequently associated with bacterial or fungal co-infections. Common respiratory viruses affecting patients with hematological malignancies include respiratory syncytial virus (RSV), influenza and parainfluenza viruses, adenoviruses, rhinoviruses, and coronaviruses. More recently, newly identified viruses such as human metapneumoviruses (HMPV), new strains of coronaviruses, and bocavirus have also been detected in symptomatic HSCT recipients. Respiratory viruses can be acquired in the community or during hospitalization.

Heavy immunosuppression of patients with hematological malignancies can lead to reactivation of latent viruses, including herpes viruses (varicella-zoster virus [VZV], herpes simplex virus [HSV], human herpes virus 6 [HHV-6], Epstein Barr virus [EBV] cytomegalovirus [CMV]), polyomaviruses, and adenovirus. These systemic viral infections occur usually in HSCT recipients, after conditioning or later, due to the administration of immune-suppressive agents.

In this review we address the problem of viral infections that are associated with high morbidity and mortality in the immunocompromised patient with hematological malignancy, and we provide a practical guideline for their treatment.

## Respiratory viral infections in patients with hematological malignancies

Respiratory viruses cause acute illness in the general population and are responsible for hospitalizations of elderly patients and patients with underlying medical conditions. These viruses are also a common cause of severe respiratory disease in patients with hematological malignancies and/or HSCT. Community-acquired respiratory viruses have a significant impact on the morbidity and mortality of the hematological patient, causing a variety of diseases ranging from self-limited upper respiratory tract illnesses to life-threatening lower respiratory tract infection and occasionally disseminated disease. Disease manifestations are dependent on the specific virus, the type of chemotherapy, immunosuppressive therapy, and transplant, as well as the net state of immunosuppression. Pneumonia following infection with these viruses may be primarily viral, bacterial, fungal, or mixed in origin.

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Nosocomial transmission of respiratory viruses is common, and widespread outbreaks have occurred in hematology units with sometimes devastating consequences. Prevention of transmission of respiratory viruses in a hospital setting, especially in units caring for patients with hematological malignancies is considered a basic standard of care for the hospitals. Strict enforcement of infection control measures is mandatory to prevent spread within a hospital ward; such measures include respiratory isolation of infected patients, handwashing before and after contact with patients, and educational efforts targeting healthcare workers and family members.

With regard to diagnosis, prompt and accurate identification of the respiratory viral pathogen is of paramount importance for the management of infection, because it allows for timely implementation of virus-specific infection control measures, the initiation of appropriate antiviral therapy, and for potential modifications of immunosuppressive therapy or rescheduling of HSCT. Proper collection of specimens is critically important for accurate identification of viruses in clinical samples. Different diagnostic methods have been used, but during recent years, the use of multiplex PCR technique is becoming the standard of care, it may detect multiple respiratory viruses from a single, readily obtained specimen.

Management of respiratory viral infections has been controversial. With the exception of influenza infections for which neuramidase inhibitors have been shown to be effective, there are no established treatments. There is a paucity of well-designed, randomized, controlled, clinical studies for the treatment of respiratory viral infections among patients with hematological malignancies. There are only a few studies, mostly retrospective and from single centers, and “expert opinions” that guide physicians on the therapy of these serious and sometimes fatal infections in patients with hematological malignancies and/or HSCT.

#### *Respiratory Syncytial Virus (RSV) Infection*

Respiratory syncytial virus is a paramyxovirus causing respiratory infection in patients with hematological malignancies, especially those who have undergone HSCT. Infection with RSV affects approximately 2% to 17% of HSCT recipients but usually is not a severe illness. However, outbreaks of fatal infections have been reported in transplant recipients. RSV infection is seasonal, occurring in the fall, winter, and spring, with an attack rate up to 10% during winter time. Risk factors for RSV infection among patients with hematological

malignancies include HSCT (especially allogeneic HSCT with mismatched/unrelated transplant), male sex, advanced age, cytomegalovirus seropositivity, and pre-engraftment status.

RSV is detected in clinical specimens, including nasal washes, nasopharyngeal swabs, and bronchoalveolar lavages. The “gold standard” for diagnosis of RSV infection is viral culture (conventional or shell vial), but this technique is time consuming with low sensitivity. Other methods include detection of viral antigens via direct immunofluorescence and molecular detection of viral RNA. HSCT recipients with symptoms of common cold (ie, fever, nasal congestion / rhinorrhea sore throat / cough) during winter should be tested for RSV and other respiratory viruses.

Patients with RSV usually present with symptoms of an upper respiratory tract infection (eg, rhinorrhea, nasal/sinus congestion, sore throat, cough, and otitis media), which progress rapidly to lower respiratory tract infection (tracheobronchitis, pneumonia), and finally to respiratory failure, necessitating admission to an intensive care unit and mechanical ventilation. RSV infection among HSCT recipients is associated with significant morbidity, and mortality ranging from 7% to 83% in patients with LRTI.

#### Treatment of RSV infection

The targets of the treatment of RSV infection are viral replication, coinfections, lung inflammation and respiratory failure. Available therapies that have been used for treatment of RSV infections are limited to ribavirin, intravenous polyclonal (IVIG) and RSV-specific immunoglobulin (RSV-IVIG), and palivizumab (PVZ). Ribavirin is a guanosine analog that is active against RNA and DNA viruses. It is available in aerosol, intravenous, and oral forms. PVZ is an RSV-specific monoclonal antibody derived from murine antibodies and directed against the F glycoprotein of RSV. Early initiation of ribavirin therapy, preferably in the aerosolized form, before development of RSV advanced LRI is necessary in high risk patients with hematological malignancies and/or HSCT. In patients with established RSV LRI, initiation of combination therapy with aerosolized ribavirin and IVIG or PVZ) before the onset of respiratory failure and need for mechanical ventilation may reduce mortality, but this remains controversial.

#### Prevention of RSV

Chemoprophylaxis in susceptible patients may be considered, especially in outbreak situations when horizontal transmission is occurring. Polyclonal

or monoclonal immunoglobulins and palivizumab can be used for the prevention of RSV infection. Currently, there is no vaccine that can prevent RSV infection.

#### *Influenza and parainfluenza viruses*

Transmission of parainfluenza and influenza viruses is by direct droplet spread or aerosolized respiratory secretions. During community outbreaks, influenza, especially type A, and parainfluenza viruses have been reported as a frequent cause of severe and fatal pneumonia in HSCT recipients. Neuraminidase inhibitors, including oseltamivir and zanamivir are effective for the treatment of influenza, when they are used early. Peramivir is a recently licensed and intravenously administered neuraminidase inhibitor that is intended for critically ill patients with influenza. Ribavirin has antiviral effects against parainfluenza virus *in vitro* and has been used for the treatment of lower respiratory tract disease in immunocompromised hosts. Studies have reported decreased viral load and clinical improvement in immunocompromised children with severe parainfluenza virus infection after treatment with aerosolized ribavirin.

#### *Human metapneumovirus infections*

Human metapneumovirus (hMPV) belongs to the *Paramyxoviridae* family. It has been discovered in 2001, but it causes respiratory tract infections in humans for at least 60 years with a worldwide distribution. hMPV should always be considered as a potential cause of respiratory illness in immunocompromised patients. hMPV is transmitted by direct or close contact with contaminated secretions, with an incubation period of three to five days. The virus can cause upper and lower respiratory tract infection in patients of all age groups, but symptomatic disease most often occurs in young children or older adults. hMPV usually causes mild, self-limited infections in children and adults. However, among immunocompromised patients hMPV infections may be more severe and the course more prolonged due to poor clearance of virus. Clinical manifestations in this situation can range from bronchiolitis to severe pneumonia and acute respiratory distress syndrome. Immunocompromised hosts appear to acquire infection at the same frequency as immunocompetent individuals. In studies of upper and lower respiratory tract infection in patients with hematologic malignancies, 3% - 9% percent of episodes were associated with hMPV infection. Reverse transcriptase PCR on nasopharyngeal specimens is the most sensitive method for diagnosis of hMPV infection.

Ribavirin is active against hMPV *in vitro* and reduces viral replication in animal models. However, there are no clinical data on the treatment of hMPV; therefore, treatment is supportive and varies with the clinical manifestations.

#### *Human rhinoviruses*

Human rhinoviruses (HRVs) are a common cause of upper respiratory infection (URI) in hematopoietic stem cell transplant (HSCT) recipients; yet, their role in lower respiratory infection (LRI) has not been well understood. Jacobs and colleagues found that among a cohort of 36 HSCT recipients with HRV URI, 27 (43%) had proven or possible HRV pneumonia. In multivariate analysis, there was a significant association with documented bacterial, fungal, and other viral LRI respiratory co-pathogen(s) and HRV infection. Fever (60%), cough (92%), sputum production (61%), and dyspnea (60%) were common symptoms. Computed tomography scans showed peribronchiolar, patchy, ground glass infiltrates in HRV LRI.

#### **Other respiratory viruses**

Infections from human bocavirus, human coronavirus, and other newly identified viruses such as WU/KI viruses are less likely to cause severe problems in patients with hematological malignancies and/or HSCT, compared with the well-described viral pathogens above.

### **Systemic Viral Infections in Patients with Hematological Malignancies**

#### *Adenovirus*

*Adenovirus*, belonging to the *Adenoviridae* family of DNA viruses, is a common cause of infections in the general population. In patients with hematological malignancies and/or HSCT *Adenovirus* disease may be life threatening. Reactivation within the recipient and horizontal transmission are most common routes of acquisition of the virus. *Adenovirus* infections affect mainly (20%-25%) pediatric populations undergoing HSCT than adults (9%). Risk factors include low numbers of CD3(+) T cells, graft-versus-host disease and the associated use of immunosuppressive agents (cyclosporine-A, methotrexate, steroids, mycophenolate mofetil), and use of serotherapy in conditioning regimens, including agents such as antithymocyte globulin or alemtuzumab.

*Adenovirus* can cause severe respiratory disease, hepatitis, and colitis in patients with hematological malignancies and/or HSCT, with a reported

mortality of 8%-26%. Other manifestations of the infection include hemorrhagic cystitis and keratoconjunctivitis. Disseminated disease with multi-organ failure can also occur. Regarding diagnosis, adenoviral load detection by PCR should be performed weekly for optimal timing of preemptive treatment and careful monitoring of the response to treatment. Ribavirin and cidofovir are agents used in the treatment of *Adenovirus*. Most evidence for efficacy against *Adenovirus*, however, is present for cidofovir. Cidofovir *in vitro* has been shown to be active against all *Adenovirus* subtypes. Probenecid and hyperhydration should be started concurrently to limit cidofovir nephrotoxicity. Ribavirin has *in vitro* activity against some, but not all, *Adenovirus* subtypes. There are case reports suggesting therapeutic efficacy of ribavirin in patients with *Adenovirus* infection refractory to other antivirals. Administration of *Adenovirus*-specific cytotoxic T-cells should be considered for patients who do not adequately respond to cidofovir and/or alternative antivirals.

Preemptive treatment with cidofovir [1 mg/kg 3 times per week (alternatively 5 mg weekly)] can be started when the adenoviral load exceeds a certain critical level, depending on the risk group. Treatment starts when the load exceeds 100 copies/mL (in low-risk patients), or 1000 cp/mL (intermediate-risk patients). Treatment with cidofovir should always be initiated with a viral load > 10,000 copies/mL, or when there are symptoms and signs of disease irrespective of the load. Moreover, immunosuppressive therapy should be tapered as soon as possible. Discontinuation of therapy should be considered when adenoviral load has been < 400 copies/mL for 2 consecutive weeks and immunosuppression has been reversed.

### *Herpes simplex virus*

Most HSV infections in patients with hematological malignancies and/or HSCT are caused by viral reactivation in seropositive patients. The rate of reactivation is as high as 70%, and it is equal after autologous or allogeneic transplantation. The median time to onset of HSV disease is 2-3 weeks.

Clinical manifestations of HSV-1 infections include primarily severe mucositis and infrequently esophagitis. Occasionally, HSCT recipients develop HSV-1 viremia, and subsequent viral infection of other organs including: the trachea (tracheobronchitis), lungs (pneumonitis), liver, CNS, adrenal glands, or gastrointestinal tract. Reactivation of HSV-2 can cause lesions in the genital or perineal area and accounts for 10% - 15% of all HSV infections in HSCT recipients.

Prophylaxis with acyclovir is a standard of care in transplant recipients and has strikingly reduced the incidence of all herpetic infections in this vulnerable patient population. In seropositive HSCT recipients intravenous (5 mg/kg IV twice daily) or oral acyclovir (800 mg twice daily) from marrow infusion until engraftment, prevents reactivation of HSV.

### *Cytomegalovirus*

CMV infections in patients with hematological malignancies and/or HSCT are due to viral reactivation. The risk of reactivation of CMV is 70 - 80 % for seropositive allogeneic HSCT recipients and only 40 % for seropositive autologous or syngeneic HSCT recipients. The risk of seronegative HSCT recipients to acquire CMV infection from blood transfusion or seropositive bone marrow is 40%. Clinical manifestations of CMV infections in patients with hematological malignancies and/or HSCT include protracted fever not responding to antibiotics, interstitial pneumonitis, enteritis, esophagitis, hepatitis, retinitis, and encephalitis. During the last decades, the widespread use of prophylaxis or preemptive antiviral therapy reduced the frequency and severity of CMV disease, but mortality remained as high as 18.3% in seronegative of HSCT recipients from a CMV-seropositive donor.

Various antiviral agents have been used for CMV prophylaxis including ganciclovir, foscarnet, acyclovir, and valacyclovir. Intravenous ganciclovir has been the most effective, but its use is limited by bone marrow toxicity. A more widely accepted approach to minimize toxicity of antiviral prophylaxis is preemptive therapy, based upon active screening for CMV with quantitative PCR assays or the antigenemia assay. Ganciclovir (5 mg/kg IV twice daily) and foscarnet (90 mg/kg IV twice daily) are equally effective as preemptive therapy of CMV infection in allogeneic HSCT recipients.

The use of available antiviral agents for the prevention of CMV disease is limited by frequent toxic effects and the emergence of resistance. CMX001 is an orally bioavailable lipid acyclic nucleoside phosphonate that is absorbed in the small intestine and transported throughout the body as a phospholipid. CMX001 is converted intracellularly to cidofovir diphosphate after cleavage of its lipid moiety and phosphorylation by intracellular kinases. Unlike cidofovir, CMX001 is unlikely to have renal toxicity. A recent study evaluated the safety and anti-CMV activity of CMX001 in patients who had undergone allogeneic hematopoietic-cell transplantation. Treatment with oral CMX001 at a dose of 100 mg twice weekly significantly reduced the incidence of

CMV events while diarrhea was dose-limiting at a dose of 200 mg twice weekly.

Letermovir is a new, highly potent anti-CMV agent with a novel mechanism of action targeting the viral terminase subunit pUL56, a component of the terminase complex involved in viral DNA cleavage and packaging that has no equivalent target enzyme in the human body. In a recent study Letermovir, as compared with placebo, was effective in reducing the incidence of CMV infection in recipients of allogeneic hematopoietic-cell transplants, with an acceptable safety profile.

#### *Varicella zoster virus*

VZV reactivation is common among patients with hematological malignancies, especially among HSCT recipients, with an incidence up to 20-40%. The risk factors for VZV reactivation include recent VZV infection, VZV seropositivity, GvHD, absolute lymphopenia, and intensive conditioning with agents such as anti-thymocyte globulin. VZV infection affects mainly pediatric patients (up to 90 % during the first post-transplant year) and may be complicated by disseminated cutaneous lesions (25 %), post-herpetic neuralgia (25 %), scarring (20 %), and bacterial superinfection (15 %). Dissemination complicates mainly allogeneic HSCT (45%) involving the lungs, liver, and CNS, and is associated with a death rate of at least 5 %. Acyclovir at a dose of 800 mg twice daily, administered for a period of 2-12 months after allogeneic HSCT reduces the risk of VZV infection.

#### *Epstein-Barr virus*

The spectrum of EBV infection among patients with hematological malignancies and/or HSCT includes oropharyngeal viral excretion, fever and neutropenia, oral hairy leukoplakia, aplastic anemia, meningoencephalitis, and posttransplant lymphoproliferative disorder (PTLD). PTLT is due to the lack of EBV-specific T lymphocytes resulting in a polyclonal or monoclonal B cell proliferation, usually of donor origin. Risk factors for PTLT include receipt of allogeneic matched unrelated, mismatched, or T-cell depleted transplants, treatment with anti-thymocyte globulin, chronic GvHD, and T cell depletion. The incidence of PTLT varies from <1 % among matched related allogeneic HCT recipients to up to 18 % among high-risk patients. Clinical manifestations of PTLT range from an indolent infectious mononucleosis-like syndrome to fulminant disseminated disease, including gastrointestinal tract and CNS involvement.

The prevention of PTLT largely relies upon reduction in immunosuppression, when this is feasible.

Quantitative EBV viral load surveillance should be incorporated into the routine evaluation of patients at high risk for PTLT. Preemptive treatment with a single infusion of rituximab given when EBV viral load exceeds 1000 copies/mL has been advocated as a strategy for prevention of PTLT and clearance of EBV-DNA from the peripheral blood; however, the appropriate threshold value of EBV-DNA copy number for this intervention has not been well studied.

Management of PTLT includes reduction of immunosuppression, immunotherapy with rituximab, chemotherapy, radiation therapy, or a combination of these. In patients with refractory disease adoptive immunotherapy with EBV-specific cytotoxic T cells, is an option in some centers.

#### *Human herpesvirus-6*

Reactivation of human herpesvirus-6 (HHV-6) has been documented in 40% - 60 % of HSCT recipients, usually within the first month after transplantation. Risk factors associated with HHV-6 viremia are cord blood transplantation, conditioning regimen, administration of anti-CD3 monoclonal antibodies, and acute GvHD. Clinical syndromes associated with HHV-6 reactivation include rash, fever, interstitial pneumonitis, encephalitis, and myelosuppression. In particular, HHV-6 encephalitis appears to be significant, life-threatening complication. Most cases of HHV-6 encephalitis develop in patients receiving transplant from an unrelated donor, particularly cord blood, typically around the time of engraftment. Symptoms are characterized by short-term memory loss and seizures. Magnetic resonance imaging typically shows limbic encephalitis. Prognosis for HHV-6 encephalitis is poor. There is no known form of prophylaxis for HHV-6 infection.

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## Professor Jacqueline Boulwood

### Biography

I obtained my PhD from the Department of Pathology, University of Wales College of Medicine in Cardiff, UK in 1988. I am now a Professor of Molecular Haematology and Director of the LLR Molecular Haematology Unit at the Nuffield Division of Clinical Laboratory Sciences, John Radcliffe Hospital, Oxford. My research studies have primarily concerned the investigation of the molecular pathogenesis of the myelodysplastic syndromes (MDS). My work has made a significant contribution to the determination of the molecular basis of several subtypes of MDS including the 5q- syndrome, alpha-thalassaemia MDS and refractory anaemia with ring sideroblasts (RARS). Using next generation sequencing technology we have recently further illuminated the molecular landscape of MDS. Our study of the MDS transcriptome has yielded valuable insights into the pathophysiology and molecular pathogenesis of MDS, and has identified new prognostic markers for this disorder. Most recently we have performed a comprehensive analysis to study the relationships between gene mutations, gene expression profiles and diagnostic clinical variables as well as outcome in MDS patients.

### Qualifications and where obtained:

1980 – 1983 Bsc (Hons) Zoology (2:1) University of Swansea  
1983 – 1987 PhD: "Detection of differentiation specific and oncogene mRNA's in human tumour cells and tissues" CRC Thyroid Tumour Biology Research Group, Department of Pathology, University of Wales College of Medicine, Cardiff.

### Present Position

January 2013 – present Professor of Molecular Haematology, Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine, University of Oxford.  
April 2003- April 2010 Co-director of LRF Molecular Haematology Unit at the University of Oxford.  
April 2010- to date Director of LLR Molecular Haematology Unit at the University of Oxford.

### Posts held with dates

November 1987- November 1988 Member of Scientific Staff. LRF Leukaemogenesis Laboratory, MRC Radiology Unit, Chilton, Didcot, Oxon.  
November 1988- March 1995 Postdoctoral Research Assistant LRF Molecular Haematology Unit at the University of Oxford.  
March 1995-1998 Senior Postdoctoral Research Assistant LRF Molecular Haematology Unit at the University of Oxford.  
July 1995 – April 2003 Deputy Director of LRF Molecular Haematology Unit at the University of Oxford.  
January 1998- October 2004 University Research Lecturer Nuffield Department of Clinical Laboratory Sciences, LRF Molecular Haematology Unit at the University of Oxford.  
October 2004 – December 2012 University Reader in Molecular Haematology, Nuffield Department of Clinical Laboratory Sciences, LRF Molecular Haematology Unit at the University of Oxford.

### Selected publications (from total of 125)

1. Gerstung M, Pellagatti A, Giagounidis A, Malcovati L, Della Porta MG, Jädersten M, Dolatshad H, Verma A, Cross NCP, Vyas P, Killick S, Hellström-Lindberg E, Cazzola M, Papaemmanuil E, Campbell PJ, Boulwood J. Combining gene mutation with gene expression data improves outcome prediction in myelodysplastic syndromes. *Nat Commun* 2014 (In press)
2. Dolatshad H, Pellagatti A, Fernandez-Mercado M, Yip BH, Przychodzen B, Attwood M, Malcovati L, Papaemmanuil E, Sahgal N, Kanapin A, Lockstone H, Valcarcel J, Vandenbergh P, Killick S, Smith CJ, Campbell PJ, Ogawa S, Maciejewski JP, Cazzola M, Savage K, I. and Boulwood J. Disruption of SF3B1 results in deregulated expression and splicing of key genes and pathways in myelodysplastic syndromes. *Leukemia*; 2014 Nov 27. doi: 10.1038/leu.2014.331. [Epub ahead of print]
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7. Pellagatti A, Fernandez-Mercado M, Di Genua C, Larrayoz MJ, Killick S, Dolatshad H, Burns A, Calasanz MJ, Schuh A, Boulwood J. Whole-exome sequencing in del(5q) myelodysplastic syndromes in transformation to acute myeloid leukemia. *Leukemia* 28(5):1148-51, 2014.
8. Boulwood J, Dolatshad H, Varanasi SS, Yip BH, Pellagatti A. The role of splicing factor mutations in the pathogenesis of the myelodysplastic syndromes. *Adv Biol Regul* 54C:153-161, 2014. Review.
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# Interconnections Among Mutations, Gene Expression, Clinical Variables and Patient Outcome in Myelodysplastic Syndromes

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## Introduction

The myelodysplastic syndromes (MDS) represent a heterogeneous group of clonal hematopoietic stem cell (HSC) malignancies that are characterized by ineffective hematopoiesis resulting in peripheral cytopenias, and patients typically have a hypercellular bone marrow.<sup>1,2</sup> Recent studies have greatly illuminated the molecular landscape of MDS, and the pace of discovery is accelerating. The most common mutations found in MDS occur in genes that are epigenetic modifiers (*TET2*, *ASXL1*, *DNMT3A*, *EZH2*, *IDH1* and *IDH2*) or regulators of RNA splicing (*SF3B1*, *SRSF2*, *U2AF1* and *ZRSR2*), providing an important link between genetic and epigenetic alterations in this disease. Several regulators of signal transduction (*NRAS*, *JAK2*), transcription factors (*RUNX1*, *TP53*, *CUX1*) and components of the cohesion complex (*STAG2*, *SMC3*) are also frequently mutated in MDS.<sup>3,4</sup> Mutation of *CSNK1A1*, a serine/threonine kinase mapping to the commonly deleted region of the 5q- syndrome,<sup>5</sup> has recently been described in a subset of patients with del(5q) MDS.<sup>6,7</sup> Only four to six genes are consistently mutated in  $\geq 10\%$  MDS patients, while a long tail of approximately 50 genes are mutated less frequently. About 90% of MDS patients carry  $\geq 1$  oncogenic mutations, and at presentation, most patients typically have two or three driver oncogenic mutations.<sup>3,4</sup>

The MDS are pre-leukemic conditions showing frequent progression (approximately 40% of patients) to acute myeloid leukemia (AML). It has been shown that progression to AML is characterized by the persistence of the founding myelodysplastic clone and the emergence of  $\geq 1$  subclone(s) harboring new somatic mutations. Thus, a secondary AML developing from MDS is not truly monoclonal but is instead typically a mosaic of several clones with different sets of somatic mutations.<sup>8</sup>

In MDS it is clear that the common gene mutations impact both the pathophysiology and prognosis of this disease, with important implications for disease classification, risk-stratification and patient management.

## Impact of gene mutations on clinical features

The recently identified splicing pathway mutations are highly specific to MDS and closely related malignancies, and to some extent define distinct clinical phenotypes. Mutation of the splicing factor gene *SF3B1* is most frequent in low-risk MDS and is found in approximately 80% of patients whose disease is characterised by ringed sideroblasts, including both RARS and RCMD-RS.<sup>9,10</sup> The close association between *SF3B1* mutation and ring sideroblasts is consistent with a causal relationship, and makes this the first gene to be strongly associated with a specific morphological feature of MDS. Haploinsufficiency of *Sf3b1* leads to compromised stem cell function but not to myelodysplasia in mice,<sup>11</sup> with some studies reporting the presence of ring sideroblasts.<sup>12</sup> A recent study showed that mutation or deletion of the splicing factor gene *PRPF8* is associated with the presence of ring sideroblasts in advanced MDS and AML, without mutation of *SF3B1*. These studies suggest that mutation of either *SF3B1* or *PRPF8* may lead to the ring sideroblast phenotype. *SRSF2* mutations are strongly associated with CMML,<sup>13</sup> and are commonly found in more advanced MDS (RAEB 1 and 2 subtypes).<sup>14</sup> Thus, whilst *SF3B1* and *SRSF2* are involved in the same splicing pathway they show strikingly different phenotypic associations when mutated. The differing phenotypes observed are likely to be related to distinct sets of target genes, but also to the very different combinations of co-mutated genes associated with each splicing factor.<sup>4</sup> Many genes significantly differentially expressed at the transcript and/or exon level have been identified in bone marrow mononuclear cells/CD34+ cells of *SF3B1*, *SRSF2* or *U2AF1* mutant MDS or AML cases, using RNA-sequencing analysis.<sup>12,15-17</sup> Whilst based on small numbers of patients these data do nonetheless suggest that splicing factor gene mutations plays a critical role in myeloid malignancies by affecting the expression and splicing of genes involved in specific cellular processes/pathways, including RNA processing and the cell cycle.

The splicing factor gene mutations frequently co-occur with mutations in specific epigenetic regulators, or oncogenes.<sup>3,4,18</sup> For example, *SF3B1* mutations are associated with mutation of *DNMT3A*, *SRSF2* mutations with *TET2* mutations and *U2AF1* mutations with *ASXL1* mutations. Emerging data suggest a hypothesis of genetic “predestination,” in which early driver mutations, predominately affecting genes involved in RNA splicing, shape the future trajectories of clonal evolution through constraints on the repertoire of co-operating mutations, resulting in distinct clinical phenotypes.<sup>4</sup>

An association between mutation of *ASXL1* and *NRAS* has also been reported in MDS<sup>4</sup> and interestingly a mouse model combining loss of *Asxl1* expression with the *NRas* mutation results in a MDS phenotype with myeloproliferative features, showing a more aggressive disease than either abnormality alone.<sup>19</sup> These networks of interacting genes provide us with important clues to better understand the biology of MDS and the disease mechanisms underlying this disorder.

Several studies have demonstrated that the presence of somatic mutations in specific genes are associated with distinct effects on cytopenias and blast proportion in MDS. Mutations of *ASXL1*, *RUNX1*, and *EZH2* have been significantly associated with reduced hemoglobin levels, for example.<sup>20</sup> Mutation of *RUNX1*, *TP53*, *NRAS*, *ASXL1* and *U2AF1* have been associated with thrombocytopenia,<sup>20,21</sup> whilst *SF3B1* mutations have been associated with a normal or increased platelet count.<sup>9,20</sup> Mutations in *ASXL1*, *SRSF2*, *CBL*, *RUNX1*, *WT1*, *IDH2*, *STAG2*, and *NRAS* have all been shown to correlate with an increased percentage of bone marrow blasts.<sup>3,4,20,22</sup> Intriguingly, MDS patients with *TP53* mutations may represent a distinct molecular subclass of MDS, typically with intermediate-2 or high risk MDS, thrombocytopenia, an elevated blast proportion, a complex karyotype, and poor survival.<sup>21</sup>

A recent large study, aiming to define genotype/phenotype relationships of clinical relevance, investigated 308 patients with MDS, MDS/MPN, or secondary AML using unsupervised statistical analysis, including the WHO classification criteria and gene mutations.<sup>23</sup> It was demonstrated that MDS associated with *SF3B1* mutation is a distinct nosologic entity irrespective of current morphologic classification criteria, whilst MDS with ring sideroblasts with non-mutated *SF3B1* segregated in different clusters with other MDS subtypes. Mutations of genes involved in DNA methylation, splicing factors other than *SF3B1*, and genes of the RAS pathway and cohesin complex were independently associated with multilineage dysplasia

and identified a distinct subset of patients (20.8% cases). Irrespective of driver somatic mutations, a threshold of 5% bone marrow blasts retained a significant discriminant value for identifying cases with clonal evolution. Co-occurrence of *TET2* and *SRSF2* mutations was strongly predictive of a myeloid malignancy characterized by myelodysplasia and monocytosis, including chronic myelomonocytic leukemia. These results indicate that a molecular classification of myeloid malignancy is feasible.

Intriguingly, it was recently demonstrated that the blood cells of more than 2% of individuals (5-6% of people older than 70 years) contain gene mutations that may represent premalignant events that cause clonal hematopoietic expansion.<sup>24</sup>

### Impact of gene mutations on prognosis

The MDS are clinically heterogeneous and the accurate prediction of prognosis is an essential component of patient management and is critical to the selection of the most appropriate therapy. The International Prognostic Scoring System (IPSS)<sup>25</sup> and revised IPSS (IPSS-R)<sup>26</sup> are able to classify patients into risk groups with different survival rates. The clinical impact of the common gene mutations on patient survival in MDS has been widely studied in an attempt to identify other, more objective, molecular prognostic parameters for the stratification of MDS patients into different risk groups. Several of the recurrently mutated genes found in MDS have been shown to influence patient survival and outcome, though mostly in univariate analyses only.<sup>27</sup> An important study of 18 recurrent gene mutations in 439 MDS patients by Bejar *et al*, showed, in a multivariable analysis that included clinical features and other mutations, that mutations in five genes (*TP53*, *EZH2*, *ETV6*, *RUNX1*, and *ASXL1*) were independently associated with decreased overall survival.<sup>21</sup> In a subsequent study, Bejar *et al* showed that combining the lower-risk-IPSS and *EZH2* mutation status identifies 29% of patients with lower-risk MDS with a worse-than-expected prognosis, identifying a patient group that may benefit from more aggressive therapy.<sup>20</sup>

Papaemmanuil *et al* sequenced 111 genes across 738 patients with MDS or closely related neoplasms<sup>4</sup> and showed that leukemia-free survival negatively correlated with the combined number of oncogenic mutations and cytogenetic lesions. This remained true if only oncogenic gene mutations were considered and remained significant independent of *TP53* or *SF3B1* mutation status. The leukemia-free survival deteriorated steadily as numbers

of driver mutations increased. Importantly, it was shown that driver mutations had equivalent prognostic significance, whether clonal or subclonal.<sup>4</sup> In a similar study Haferlach and colleagues investigated the mutation status of 14 genes combined with conventional risk factors (including the parameters used in the IPSS-R) in a large group of MDS patients, and revealed a novel prognostic model ('Model-1') separating patients into four risk groups with significantly differing 3-year survival.<sup>3</sup>

In the near future it is probable that mutational data correlating with survival will contribute to a refined risk classification of MDS. Indeed this is the goal of the International Working Group for Prognosis in MDS (IWG-PM).

### Impact of gene expression on prognosis

Gene expression profiling (GEP) is a powerful tool that has the potential to enhance current prognostic systems for MDS by providing objective, standardized gene signatures. A classification model has been developed to distinguish MDS from both leukemia and nonleukemia profiles by an international research consortium using GEP analysis of total bone marrow mononuclear cells from patients with leukemia and MDS.<sup>28</sup> While this model could accurately predict leukemia in 93% of AML samples, only 50% of the 174 MDS samples were correctly classified, highlighting the marked heterogeneity of MDS, even within defined subsets of this disorder.<sup>28</sup> A prognostic classification model that predicts the time-dependent probability of leukemic transformation in MDS has been generated using GEP analysis of total bone marrow mononuclear cells. The prognostic classification model accurately discriminated patients with a rapid transformation to AML within 18 months from those with more indolent disease.<sup>29</sup>

Recently, we have used GEP data on CD34+ cells from a group of 125 MDS patients to investigate the relationship between gene expression levels and prognosis. We identified several genes, the expression of which was significantly associated with survival of patients with MDS, including *LEF1*, *CDH1*, *WT1*, and *MN1*. A gene expression signature, based on expression data on 20 genes, was identified that outperformed other predictors including one which additionally used clinical information.<sup>30</sup> Moreover, the gene signature based on CD34+ cells significantly identified a separation of MDS patients with a good or bad prognosis in an independent GEP dataset generated from unsorted bone marrow mononuclear cells, enhancing the likely clinical applicability of the prognostic signature in routine

practice.<sup>30</sup> GEP-based signatures correlating with survival may contribute to a refined risk classification of MDS.

### Comprehensive analysis of gene mutations, gene expression, clinical variables and patient outcome

We have recently performed a comprehensive analysis to study the relationships between mutations in genes frequently mutated in MDS, common cytogenetic aberrations, microarray-based gene expression profiles from bone marrow CD34+ cells, and diagnostic clinical variables as well as outcome in 124 MDS patients.<sup>31</sup> We used linear models to deconvolute the expression of genes into contributions stemming from 16 genetic and cytogenetic abnormalities, providing deep insights into how driver mutations affect the transcriptome, and ultimately clinical features and outcome. The MDS transcriptome was globally perturbed by genetic and cytogenetic driver alterations, with expression levels of ~20% of genes (present on the microarray) significantly associated with at least one of the driver mutations investigated. Per genetic lesion, the number of target genes whose expression correlates with the mutation was variable, ranging from 11 differentially expressed genes for *DNMT3A* mutations to 605 for *SF3B1* mutations. The observed associations of genotype and expression changes reflect the biological function of the mutated genes, for example, mutations in the polycomb group proteins *ASXL1* and *EZH2* resulted in a derepression of certain Polycomb group target loci leading to an increased expression in mutated cases. Distinct differentially expressed genes were associated with the most common splicing gene mutations (*SF3B1*, *SRSF2*, *U2AF1* and *ZRSR2*) in MDS, suggesting that different phenotypes associated with these mutations<sup>3</sup> may be driven by different effects on gene expression and that the target genes are different.<sup>31</sup>

In predicting survival, genomic, transcriptomic and diagnostic clinical variables all had utility in our integrative study, with a large contribution from the transcriptome.<sup>31</sup> When combining all available data types in a multivariable survival model, prognostic accuracy was found to be greater than that based on individual datasets. This indicates that the accuracy of prognostic models for MDS can benefit from incorporating multiple data types, including gene expression data.<sup>31</sup> However, the prognostic information contained in each category was found to be partially redundant. Thus, causality flows from genome through the transcriptome to clinical variables and, ultimately, outcome: each partially predicts downstream effects, and can partially proxy for upstream sources.

Our study provides important insights into the impact of the common gene mutations and cytogenetic alterations on the transcriptome in MDS, and highlights the mechanisms underlying this disorder. The approach used is readily applicable to other cancers. As we move towards integrating genomic and transcriptional screens into the clinical management of patients with cancer, these interconnected streams of data will require careful modeling to ensure optimal predictive performance.

## Conclusions and future developments

A wealth of recurrent gene mutations has been identified in MDS and to move the field forward, a new classification and risk-stratification of MDS that incorporates genetic information should be achieved. It is now possible for hematologists to detect a broad range of such mutations in bone marrow or peripheral blood samples using sensitive genotyping methods, with the potential to improve diagnosis, prognostication and the evaluation of disease progression in this disorder. The central problem with MDS is its heterogeneity. A better understanding of the genetic and biological basis for this heterogeneity is emerging, and this in turn will lead to improved therapies.

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# Treatment of Higher Risk MDS

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## Background

Treatment strategy of MDS remains challenging and remains largely based on the IPSS. In patients Higher risk MDS (Int-2 or High according to the IPSS classification) with a median survival of only 15 months in the absence of treatment, treatment should aim at modifying the disease course, ie avoiding progression to AML, and improving survival.

Allogeneic stem cell transplantation remains, with few exceptions, the only curative treatment of higher risk MDS, with prolonged disease free survival in 35-50% of the patients. However, it can generally be offered only to younger patients for myeloablative allogeneic SCT and elderly patients up to 70 years for reduced-intensity SCT with an HLA identical donor (familial or unrelated) ie to a small minority of MDS. Nevertheless, MDS patients often carry comorbidities impaired functional status, as potential causes for poor treatment outcome<sup>1</sup>. It has also been shown that an increased bone marrow blast percentage at the time of transplant (especially if > 10%) is associated with a higher risk of relapse, suggesting that a cytoreductive regimen (with chemotherapy or perhaps hypomethylating agents) in patients with an excess of marrow blasts might be useful, although there are no prospective studies to support this attitude.

## The role of Chemotherapy

During the past decade, hypomethylating agents have become the first line treatment in most higher risk MDS, after clinical trials establishing a clear efficacy of azacitidine (and, to a lower extent, Decitabine) in MDS over other treatment options<sup>2,3</sup>. Nevertheless, high dose chemotherapy might remain a valuable option in some higher risk MDS. It mainly uses anthracycline-AraC combinations, as in AML<sup>4,6</sup>, but it yields only 40 to 60% complete remission (CR) rates in MDS (or AML post MDS), and shorter CR duration (median less than 12 months) than in de novo AML<sup>4,5,6</sup>, very few patients experiencing prolonged CR. In contrast to hypomethylating agents, intensive chemotherapy might lead to a

higher CR rate even if this does not translate into a survival advantage. This option might nevertheless be useful, as a bridge to transplant. Patients with unfavorable karyotype have fewer CR and shorter CR duration<sup>4</sup>. No drug combination (including fludarabine, topotecan, Gemtuzumab ozogamicin, with AraC and with or without G-CSF) has demonstrated any survival advantage over classical anthracycline-Ara C combinations<sup>5,7-9</sup>. Thus, intensive chemotherapy has limited indication in higher risk MDS and is generally offered to younger patients (< 65 years) with favorable cytogenetics, particularly as a bridge to allogeneic SCT.

Low dose Ara-C (Ara-C 20mg/m<sup>2</sup>/day, 14 to 21 days every month) yields CR and PR rates of 15-20% and 15-20%, respectively in higher risk MDS, but no proven survival advantage<sup>10,11</sup>. Response is only seen in patients without unfavourable karyotype<sup>3,11</sup>. In a randomized study, LD araC gave significantly fewer responses, shorter responses and lower survival than azacitidine, irrespective of WHO classification and karyotype, while being more myelosuppressive<sup>12</sup>.

## Hypomethylating agent

### *Azacitidine*

In a first randomized phase III study conducted by the CALGB evaluating azacitidine versus best supportive care (BSC) in MDS patients, the overall response rate was significantly higher in patients treated with AZA (60% vs 5%,  $P < 0.0001$ ), but the median survival was similar in the two groups, presumably due to a cross over in the design of the study<sup>2</sup>. Nevertheless, when the analysis was restricted to patients with an excess of marrow blasts, a survival advantage was observed. This trial conducted to the approval of the drug in the US but not in Europe. Thus, the non significant overall survival benefit with AZA in that trial lead to a Phase III, international randomized, trial (AZA001 trial) that compared azacitidine and conventional care (CCR) treatments (including BSC, low dose cytarabine (LDAC) or intensive chemotherapy, based on the investigator's choice pre randomization) in

Higher-risk MDS patients<sup>3</sup>. Despite a low CR rate (17%), AZA showed a significant survival advantage (24.4 months vs 15 months), a significantly lower risk of progression to AML, and led significantly more frequently to transfusion independence. The survival advantage with AZA was seen irrespective of age, percentage of bone marrow blasts and karyotype<sup>3</sup>. Importantly, response to azacitidine were often delayed, with a median time to response of 2 to 3 months, many patients responding after only 6 cycles<sup>13</sup>, suggesting that patients should not be considered resistant before administration of at least 6 cycles, unless documented progression of the disease. Side effects were scarce and early deaths irrelevant (68). Following those results, AZA has been approved in the treatment of higher risk in Europe.

### Decitabine

Following Phase I of Decitabine, a phase II trial was conducted based on a continuous IV infusion of decitabine (45 mg/m<sup>2</sup> for three times /day) during 3 days, the cycles being repeated every 6 weeks. This schedule allowed to reach a CR rate higher than in similar trials with AZA (about 30%), but was associated with higher hematologic toxicity, leading to a treatment-related mortality of 7 %<sup>14</sup>. Subsequently, a phase III trial has been conducted on 170 MDS patients, treated with a rather similar schedule (DAC 15 mg /m<sup>2</sup>/for 4 hrs 3 times a day for 3 days) repeated every 6 weeks, versus best supportive care. The overall response rate was 29%. Although a delay in transformation to AML in the arm DAC has been observed among patients with higher risk MDS, no significant benefit in overall survival was noted. This absence of benefice was probably due to low number of cycles received by the patients before response assessment (3 cycles), but also due to a rather high treatment related mortality related to myelosuppression (10%). The results of the phase III trial conducted by the EORTC and the German MDS study group, with the same schedule, confirmed these results in terms of achievement of CR/PR, but could not demonstrated any survival advantage of decitabine treated versus BSC treated patients<sup>15</sup>. For this reason, at present decitabine has not been approved by EMA for MDS patients. However, as already mentioned, it appears from a historical comparison that decitabine might anyway confer a survival advantage over intensive chemotherapy in high-risk MDS<sup>16</sup>.

### Conclusions

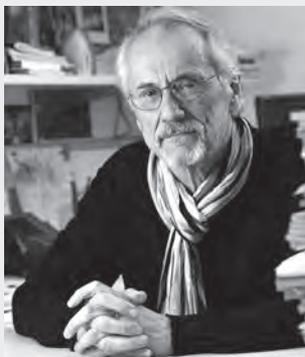
Azacitidine is the first drug to significantly improve survival in higher risk MDS, although it is not

curative. This survival improvement generally does not result from achievement of CR, but rather from disease stabilization or even return of the disease to an earlier phase of MDS, with less cytopenias and delayed progression to AML. The survival improvement obtained with azacitidine must be the starting point for combination studies, and for utilization of this drug in other situations ( before allo SCT, or after chemotherapy or allo SCT as maintenance treatment).

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# Hematopoietic Cell Transplantation for MDS: Success and Challenges

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## Background

High dose chemotherapy may cure a small subset of patient with MDS. Allogeneic hematopoietic cell transplantation (HCT) has been shown to be curative for as many as 30%-40% of patients with high risk and 70%-40% of patients with low risk MDS. Clearly, while there has been progress, there is considerable room for improvement.

HCT is associated with several risks including treatment-related toxicity and mortality, relapse of MDS, and graft-versus-host disease (GVHD). Disease classification and risk assessment schemes such as the WPSS, the IPSS-R and the HCT-CI score both disease and patient risk factors that impact the outcome of transplantation. These tools allow to assess the prognosis of patients and assist in the decision-making process for or against HCT.

In general, the emphasis in allogeneic HCT has shifted from high-dose therapy, aimed at maximum tumor cell kill, to low or reduced intensity conditioning (RIC), relying on immune effects mediated by donor cells (graft versus tumor [GVT] effects) to eradicate the disease. Unfortunately the GVT effects are most prominent in patients who also develop the undesired complication of GVHD, in particular chronic GVHD.

## Success

The ideal regimen for HCT has not been determined yet. However, the development of RIC regimens has substantially reduced the up-front toxicity and mortality (to less than 5% by day 100) and has permitted to apply HCT to older patients. The trade-off is a higher relapse rate.

Encouraging results have been achieved recently with regimens including treosulfan, which are associated with low toxicity and excellent efficacy. In a trial conducted at our Center 60 patients with MDS or AML were prepared with a regimen of Fludarabine (30 mg/m<sup>2</sup> × 5) and treosulfan (12g or 14 g/m<sup>2</sup> × 3) for HCT from HLA-matched related or unrelated donors. Two-year non-relapse mortality

was less than 10%, and relapse-free survival for patients with standard or intermediate risk cytogenetics was 80%. Patients with high risk karyotype, in contrast, showed long-term relapse-free survival of only 35%-40%.

## Challenges

Two major challenges remain, GVHD and, in patients with high risk cytogenetics, relapse of MDS. Relapse is clearly related to disease state at HCT, minimal residual disease, and high risk cytogenetics, including monosomal karyotype. Our most recent trial, using the above treosulfan regimen with the addition of 2 Gy of TBI, suggests that this combination is able to improve results for all-comers with MDS, and improve relapse-free survival in patients with high risk cytogenetics to about 65%. These data remain to be confirmed.

Both acute and chronic GVHD occur in as many as 40% to 60% of patients, and particularly with unrelated HCT, involvement of the intestinal tract prove life-threatening. First line therapy with steroids, while effective in a proportion of patients, is often poorly tolerated, especially in older individuals. Metabolic abnormalities, infections and long-term effects on muscles and skeleton can severely impact quality of life.

## Outlook

Various strategies of post-HCT therapy, for example with hypomethylating agents or cellular therapy with NK cells or genetically modified T cells (directed for example at WT1) are currently being explored. The use of post-HCT administration of cyclophosphamide, in the hands of several investigators, has been effective in preventing GVHD. Such a strategy may also allow additional cellular interventions without concern about GVHD.

Clearly, the rapidly expanding understanding of the impact of various mutations in clonal cells will impact disease risk classification and may also lead to novel anti-relapse strategies aimed at molecular targets.



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# The Biology of Indolent Lymphomas and How This May Inform Future Therapy

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**T**he indolent lymphomas comprise a heterogeneous collection of tumours that includes follicular lymphoma, marginal zone lymphoma, small lymphocytic lymphoma and hairy cell leukaemia. Recent technological advances such as next generation sequencing have greatly increased our understanding of the underlying biology of these tumours. This has implicated new genes and signalling pathways, many of which may be targets for pharmacological manipulation; however it has also revealed new complexities and challenges.

## Normal B cell development

Follicular lymphoma (FL) is the commonest of the indolent lymphomas. Its pathogenesis is a multi-step process involving both early and late stages of B cell maturation. As such a brief summary of normal B cell development is helpful. The earliest B cells are found in the bone marrow where they negotiate the process of VDJ recombination. Double stranded breaks, introduced by the RAG enzymes allow the immunoglobulin (Ig) genes to be “cut and pasted” leaving each B cell with a unique surface receptor (BCR). Those cells that generate a functional BCR exit the bone marrow as mature, naive B cells. Subsequent encounter with antigen, in the context of T cell help, then initiates the formation of microanatomical structures termed the germinal centres (GC). These form within within the B cell follicles of lymph node and spleen through a process co-ordinated at the gene expression level by the transcriptional regulator BCL6. The GC reaction is associated with intense B cell proliferation, class switch recombination (CSR) to other Ig isotypes and somatic hypermutation (SHM) of the Ig variable region. Higher affinity variants are selected to survive as long-lived memory B cells or plasma cells, whilst those with lower antigen binding ability undergo apoptosis. This default to apoptosis is in part related to the absent expression of BCL2 in normal germinal centre B cells. SHM and CSR in the GC require a further period of deliberately induced DNA damage, this time mediated by the enzyme AID. Whilst the DNA damaging activity of RAG and AID is targeted primarily to the Ig genes, unintended oncogenic lesions are the

inevitable cost and contribute to the initiation of B cell malignancy.

## Follicular Lymphoma and the t(14:18) translocation

Follicular lymphoma is considered a germinal centre derived tumour<sup>1</sup>. Its immunophenotype and gene expression profile resembles normal GC cells including high expression of the germinal centre genes CD10, BCL6 and AID. However, unlike germinal centre B cells it expresses high levels of the antiapoptotic factor BCL2. This results from the t(14:18) translocation, which is seen in the majority of cases of FL and brings the BCL2 gene under the control of the Ig heavy chain enhancer region. It seems clear that this translocation occurs as an error of RAG mediated VDJ recombination in early stages of bone marrow B cell development but probably has little effect prior to the GC stage as BCL2 expression is a normal feature of immature and naive B cells. Although considered a hallmark feature of follicular lymphoma t(14:18) can not by itself be the cause of FL. BCL2 transgenic mice do not develop FL<sup>2</sup> and in fact a small population of t(14:18) translocated B cells can be detected in a sizable majority of the healthy human population without posing an obvious elevated risk of FL<sup>3</sup>. Surprisingly, these t(14:18) cells are not naive B cells but instead resemble post-germinal centre memory B cells, evidenced by somatic hypermutation of the variable regions and class-switching on the non-productive IgH allele. This excludes the initial assumption that the act of entering the GC is itself the transforming event for a t(14:18) cell. In fact, the entity of Follicular Lymphoma In Situ (FLIS) may represent the coincidental capture of an innocuous t(14:18) naive B cell on its way through the germinal centre reaction, rather than an early stage of true FL. Therefore it seems clear that in addition to the t(14:18) translocation one or more secondary oncogenic genetic hits are required for the development of FL. The likely perpetrator of these secondary hits is “off-target” somatic mutations introduced by AID during the germinal centre reaction. Whilst BCL2 is itself a logical target for pharmacological manipulation clarifying the

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identity of these subsequent oncogenic events is clearly a critical step in the development of effective targeted therapy for FL.

### Additional Genetic Defects

Recurrent chromosomal aberrations provide clues as to what these subsequent genetic hits might be. A recent systematic analysis of genes in the deleted region of chromosome 6q identified the gene EPHA7 as a negative regulator of oncogenic signalling pathways in follicular lymphoma<sup>4</sup>. Tissue microarray showed its expression to be absent in over 70% of FL whereas it was robustly expressed in normal germinal centres. Importantly an exogenously administered soluble form of EPHA7 suppressed xenograft models of FL suggesting that targeting this pathway is a potential therapeutic strategy in human FL. Also frequently deleted is chromosome 1p36 – deleted in 67% of FL<sup>5, 6</sup>. The 12kb minimum deleted region is home to the TNFRSF14 gene, which is further inactivated by point mutation in 44% of FL. TNFRSF14 is reported to be involved in fas-mediated apoptosis however its full function in FL currently remains a subject of current research.

Cancer genetics is currently being transformed by the application of next generation sequencing (NGS)<sup>7</sup>. The first human genome took a massive collaborative effort and more than a decade to sequence. Current technology, in theory, allows the coding region (exome) to be sequenced in a day for a price similar to that paid for an MRI scan. Although NGS has so far only been applied to small cohorts of FL it has already begun to reveal which aberrations that may be required for the development of follicular lymphoma. Surprisingly, a class of genes involved in the modification of histone proteins have emerged as the most frequent mutations in FL. Inactivating mutations of the histone methyl transferase MLL2 were found in nearly 90% of FL tumours sequenced<sup>8</sup>. This remarkably high frequency is equivalent to that of the “hallmark” t(14:18) translocation suggesting a major role for MLL2 in FL. Another histone methyltransferase, EZH2, showed recurrent activating mutations<sup>9-11</sup>. The same mutations are found in GCB type DLBCL and are consistent with increased EZH2 activity in the normal germinal centre, where it appears to promote a programme of gene expression that favours proliferation and survival, and blocks exit from the GC. The histone acetyltransferases CREBBP MEF2B and EP300 were also mutated in 41% of FL tumours<sup>8, 12</sup>. In addition to their effects on chromatin they also acetylate BCL6 and p53 and their mutation leads

to enhanced BCL6 and suppressed p53 activity<sup>12</sup>. MEF2B is also able to bind the BCL6 promoter and activate its expression, an activity that is enhanced by its mutation. Although there is still much that is not understood about the way these mutations contribute to the pathogenesis of FL it is clear from the mutation frequency alone that pathways regulating histone modification are of vital importance to the development of FL and as such represent important potential opportunities for therapeutic targeting.

It is likely that as larger cohorts of FL tumours are subjected to next generation sequencing in the near future our understanding of FL genetics will continue to expand further. It is pertinent to point out that next generation sequencing has also recently identified unexpected pathways for therapeutic targeting in the other indolent lymphomas. BRAF mutation is found in 100% hairy cell leukaemia<sup>13</sup>, MYD88 mutation in 100% Waldenström's Macroglobulinaemia<sup>14</sup> and NOTCH or NFKB mutation in over half of splenic marginal zone lymphomas<sup>15, 16</sup>. Importantly many of these mutations are in pathways for which targeted therapies are already approved for use in other indications.

### The Importance of the Lymph Node Microenvironment

FL cells do not exist in isolation. The tumour is generally localised to lymph node and bone marrow. FL cells fail to grow when put into culture *ex vivo* and there are no cell lines that represent the untransformed stage of the disease. This is because the survival of FL cells is dependent upon signals received from the microenvironment. The histology of FL recapitulates the cellular architecture of normal B cell follicles and germinal centres including the close interaction with follicular dendritic cells (FDCs) and T follicular helper cells (TFH). This interaction is promoted by the receptors CXCR4 and CXCR5 on FL cells with the chemo-attractants CXCL12 and CXCL13 secreted by FDCs and TFHs<sup>17</sup>. Blocking this interaction, for instance with a CXCR4 agonist, might render FL cells more susceptible to therapy by releasing them from their supportive environment

Another crucial signal is delivered through the B cell receptor (BCR). An important feature of normal B cells and many B cell derived tumours is their dependence upon a continued signal through the BCR. The evidence suggests that FL is also dependent upon the continued expression of a functional BCR. Despite ongoing mutation of the BCR there is a strong selective pressure against mutations that

render the BCR non-functional<sup>18</sup>. Further evidence for the importance of BCR signalling in FL comes from a recent sequencing study that identified recurrent mutation of components of the BCR signalling pathway<sup>11</sup>. Interestingly there appears to be a preference for the persistence of the IgM isotype and although switching to IgG is seen on the non-productive allele the majority of FL retains IgM on the expressed allele<sup>19</sup>. Retention of IgM expression seems to be a common feature of many GC derived lymphomas and transmits a qualitatively different BCR signal from IgG. In some B cell malignancies, such as CLL, the immunoglobulin repertoire is biased toward the use of specific variable gene families suggesting antigen or autoantigen is a source of the BCR signal. Such biased VH gene usage does not appear to be a feature of FL. Although BCR autoreactivity has been detected in some cases of FL<sup>20</sup> it appears that the source of BCR signalling in the majority of cases of FL is probably antigen independent. Fascinatingly, in more than 80% of FL the BCR is modified by the addition of glycans to the Ig variable regions, a feature not seen in normal B cells<sup>18</sup>. This results from the introduction of specific acceptor motifs for glycan addition as a consequence of somatic hypermutation of the Ig V regions. The result of this glycosylation is that it may allow the BCR to interact with mannose-binding lectins on stromal cells, thus generating an antigen independent BCR signal that may promote the survival and proliferation of FL cells. The implication of this finding is that antibodies to lymphoma specific glycans might specifically deprive lymphoma cells of their essential BCR survival signal. An alternative way to block BCR survival signals would be to target one or more of the many downstream kinases such as SYK, BTK, PI3K and mTOR using drugs that are already in clinical use for other indications.

As well as being dependent upon signals from the microenvironment FL seems able to suppress the activity of infiltrating immune cells that might otherwise mount an anti-tumour immune response. FL cells appear capable of biasing the differentiation of CD4 T cells into regulatory T cells (Tregs), which suppress the activity of effector T cells, and total numbers of Treg cells are increased in FL lymph node<sup>21</sup>. Effector cells infiltrating the tumour are dysfunctional in their ability to respond to cytokine stimulation<sup>22</sup>. This appears to be related to high expression of the inhibitory molecule PD1 on FL infiltrating T cells. The implication of these findings is that PD1 neutralising antibodies may be able to restore the anti-tumour immune response as has been shown in a number of other malignancies.

## Individual tumours consist of multiple competing subclones

A growing appreciation common to many types of malignancy is that one tumour in a single patient may actually consist of dynamic mix of distinct and competing subclones. Whilst there is often one dominant clone, post-treatment relapses and transformation to high-grade lymphoma (tFL) may not result from the direct evolution of this dominant clone but rather from the expansion of a pre-existing dormant clone or may represent divergent evolution from a common progenitor clone (CPC). A comparison of SHM patterns in paired biopsies before and after high-grade transformation was able to compute genealogical trees reflecting the clonal evolution of tumours<sup>23</sup>. This was able to infer that in two thirds of cases tFL arose through evolution from a common progenitor cell. In only one third of cases did the tFL appear to arise directly from the primary clone. Similarly, analysis of a father / son donor / recipient pair who developed FL and tFL 3 and 10 years respectively after bone marrow transplant showed both shared and unique mutations in each tumour. This suggests that both tumours arose from a common progenitor transferred at the time of BMT that must have existed many years before development of disease<sup>23</sup>. The exact identity of this CPC is unclear although shared Ig variable region somatic mutations suggested a GC experienced cell. However, there is mounting evidence that the earliest steps towards tumour development may occur even before commitment to the B cell lineage. Mice transplanted with haematopoietic stem cells from humans with CLL develop oligoclonal CD5+ B cells<sup>24</sup>. Separate VDJ gene usage confirms that this is not a result of contamination of the graft with CLL cells but rather an intrinsic property of the haematopoietic stem cell compartment. Exome sequencing analysis of a separate donor / recipient pair who both developed FL 7 years after BMT / DLI again showed both shared and unique mutations, suggesting a common progenitor cell<sup>25</sup>. As expected the BCL2 translocation was retrospectively detected in the CD19+ donor lymphocyte infusion but not in CD34+ purified cells. However, fascinatingly, mutation of three genes including EP300, which was identified in both donor and recipient tumours, was also detectable in the CD34+ purified cells. The implication of this finding is that the t(14:18) may not be the first genetic hit in FL but may in fact be a facilitating event in a cell that has already taken the first step to lymphomagenesis. Determining the order of these genetic lesions in FL is not just academic. Mutation targeted treatment will only be effective if it is aimed at pathways that are both key drivers of the tumour and occur in every tumour cell.

Otherwise relapse through outgrowth of subclones is inevitable. Current mutation screening usually describes the presence or absence of a mutation and has not until recently attempted to quantify the proportion of cells that possess the mutation as our ability to sequence more deeply continue to improve this will surely become a feature of future sequencing projects. Indeed a recent exome sequencing analysis of FL has attempted to do this and to determine which mutations are key early drivers and which are later subclonal accelerators<sup>26</sup>. One key finding of this analysis was that the histone acetyltransferase CREBBP was identified as a key early driver. Combined with the high percentage of FL cases that show mutation of CREBBP (or one of its partners MEF2B or EP300)<sup>8</sup> and the previously described presence of EP300 mutation in the stem cell compartment of the BMT transferred FL<sup>25</sup>, this suggests that histone acetyltransferase inactivation represents a major component in the pathogenesis of FL and must therefore represent an important pathway for potential target therapy.

## Summary

Our understanding of the biology of indolent lymphomas and the rate of discovery has increased dramatically over the last few years. This is largely due to the development of next generation sequencing. Encouragingly, many of the most promising pathways currently established may be amenable to targeting with agents that are already in use or in development for other indications.

## Abbreviations used

BCR	B Cell Receptor
FL	Follicular Lymphoma
GC	Germinal Centre
Ig	Immunoglobulin
NGS	Next Generation Sequencing
SHM	Somatic Hypermutation
TFh	T Follicular Helper Cell
tFL	Transformed Follicular Lymphoma
Treg	Regulatory T cell

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# New Treatments for Follicular Lymphoma - Toward a Chemotherapy-Free Future

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**F**ollicular lymphoma is an indolent lymphoma which is highly manageable, but not curable with standard management. Systemic disease is the rule and patients are generally older than 40. The only potentially curative approach is allotransplant, which applies to the minority. Since survival with FL may be measured in many years, cumulative toxicity is a significant concern.

The first targeted agent for FL was rituximab, and it has proven to be a successful monotherapy as well as utilized with chemotherapy. Since its introduction, survivorship of FL has improved incrementally. A chemotherapy-free future should also keep in mind that many patients do not need treatment when a diagnosis is first made. Approximately 60% of FL patients in the US can be monitored initially, and need for therapy may be several years thereafter. In addition, some FL patients may have a sustained lymphoma response with cure of associated Hepatitis C or other infection such as *H pylori*.

We are now on the threshold of many new targeted agents, and these will be discussed. It is hoped that they will similarly improve outcomes and transition our patients to a chemotherapy-free future. Important considerations in this transition include: What is the aim of therapy? If palliation remains the aim, then should there be more formal criteria for when to initiate therapy? Will these agents be additive or synergistic together or with Rituximab? Since many of these agents are to be utilized chronically, what is their toxicity profile, as well as their psychological and financial impact? How do we select rationally among the wealth of options going forward?

It is helpful to understand the new targeted categories: Antibodies targeting cell surface antigens (sometimes conjugated to a drug or other toxin); Inhibitors of B-cell metabolism; and Immune modulating agents, including checkpoint inhibitors and cellular therapy with CAR (chimeric antigen receptor) T cells.

Clinical trials will be essential to clarify the sequence of future management. Expectant monitoring when first diagnosed remains the first option > Antibody alone (Rituximab for now) or with a new drug (phase II studies combining ibrutinib or idelalisib are ongoing; phase III R-lenalidomide vs R, reported). Current phase I/II agents of all classes will be increasingly available. Immune modulating agents will likely be studied most in transformed lymphoma, particularly double-hit DLBCL, and preliminary data does suggest that checkpoint inhibitors have good activity in FL. In transformed aggressive lymphoma, R-chemotherapy plus new agents are likely to be of critical importance.

Thus, the future is bright for a “chemo-free” FL life. A slow and steady approach to patient management will set the stage for that opportunity, while remaining mindful of the risk of histologic transformation.

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# Managing Pregnancy in Chronic Myelogenous Leukemia (CML)

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The incidence of CML is about 1–2 / 100,000 cases per year constituting about 15% of adult leukemias with a median onset in the sixth decade. Traditionally, therapeutic options for CML included bone marrow transplantation, interferon alpha (INF- $\alpha$ ) and chemotherapy. However, in recent years the treatment of the disease has evolved dramatically with the introduction of tyrosine kinase inhibitors (imatinib, nilotinib, dasatinib, bosutinib, ponatinib) and the expected survival for properly treated CML patients today is probably similar to that of the general population. About ten percent of CML cases occur in women in childbearing age. Fertility, family planning of CML patients and treatment of pregnant women with CML present specific management and therapeutic challenges for the patients and the hematologists.

## Male patients

To date, in the pregnancies reported in the partners of men receiving imatinib there has been no complications occurring with conception, pregnancy, delivery or any increase in congenital abnormalities of the baby. Men with CML who wish to have a child can safely remain on imatinib without treatment interruption. The data on the effects of second and third generation TKIs are limited or lacking, although there is the possibility of equal safety of these drugs like imatinib. Semen cryopreservation can be discussed with the patient because of these limitations and even the requirement of allogeneic stem cell transplantation during the course of CML.

## Female patients

The results are less favourable for children born to women exposed to imatinib during pregnancy. Spontaneous abortions can occur in patients receiving imatinib. The congenital malformations

observed in babies born to mothers receiving imatinib in the first trimester were craniosynostosis, hypoplastic lungs, duplex kidney, absent kidney, shoulder anomaly, exomphalos, hemivertebrae and scoliosis. With dasatinib spontaneous abortions, foetal hydrops and normal deliveries were reported. Single case reports described healthy babies exposed to nilotinib in the first trimester. But foetal malformations (exomphalos and congenital transposition of the great vessels) were reported, too.

Planning pregnancy in CML should be considered if the patient has deep and durable responses. Ideally, the patient should have molecular negativity of bcr-abl assessed with sufficient sensitivity to detect 4.5-5 logs reductions in the tumour load sustained for at least two years like in the cessation studies of imatinib. As this situation is not frequent, patients in major molecular remission for at least two years can be advised to stop the treatment temporarily and become pregnant. During the pregnancy close monitoring of bcr-abl levels are recommended.

Sometimes after discontinuation of TKI some forms of treatment might be necessary during the pregnancy.

In the first trimester leukapheresis to keep WBC count  $<100 \times 10^9/L$  and platelets  $<500 \times 10^9/L$  can be used. Aspirin and low molecular weight heparins (LMWH) can be given, if platelet counts are  $>500 \times 10^9/L$ . INF- $\alpha$ , hydroxycarbamide and TKIs should be avoided.

In the second and third trimester leukapheresis and INF- $\alpha$  may be considered. Aspirin and LMWH can be used.

During the breastfeeding hydroxycarbamide and TKIs are contraindicated. INF- $\alpha$  is not recommended. Pegylated IFN- $\alpha$  is contraindicated throughout pregnancy due to the harmful effects of polyethylene glycol.



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Clinical Leukemia  
Cancer  
American Journal of Hematology  
Biology of Blood and Marrow Transplantation  
Leukemia Research

## INVITED LECTURES/WORKSHOPS/PRESENTATIONS/SITE VISITS:

### National

- 12/2007 - Grand Rounds Fairview Hospital, "Are we curing Leukemia yet"  
9/23/2009 - Grand rounds: Chicago Medical School at Rosalind Franklin, The Department of Medicine, "Leukemia: Have We Made Progress?"  
6/1/2011 - Grand rounds: Chicago Medical School at Rosalind Franklin, The Department of Medicine, "Myeloproliferative neoplasms"  
3/24/2010- Grand Rounds: An Update on CML-Cincinnati VA Medical Center, Cincinnati, OH  
4/30/2010- Grand Rounds: An Update on CML-Aleda E Lutz VA Medical Center, Saginaw, MI  
5/12/2010- Grand Rounds: An Update on CML-VA Western New York Health Care System-Buffalo, NY  
5/18/2010- Grand Rounds: An Update on CML-VA Pittsburgh Healthcare System, Pittsburgh, PA  
7/7/2010- Grand Rounds: An Update on CML-Louis A Johnston VA Medical Center, Clarksburg, WV  
5/4/2012-AML: The case for 7 + 3. New Advances in Oncology. Marquette General Hospital  
5/4/2012-CLL: New Advances in Oncology. Marquette General Hospital  
8/18/12: COMFORT-I Publication Steering Committee  
10/6/2012: Myelodysplastic syndrome: Have we made any progress. Advances in Hematology and oncology-Green Bay symposium  
4/4/2014: The Confusing Treatment Landscape in CML: A Welcome Development. Robert H. Lurie Comprehensive Cancer Center of Northwestern University's Grand Rounds Lecture Series  
5/16/14: Acute Promyelocytic Leukemia. The 42nd Annual Dameshek Symposium, Wichita, Kansas

### Regional and Local

- Hematology Update Meeting, 2009 – 2011, Course Director  
3/4/2011: Grand Rounds: Froedtert Memorial Lutheran Hospital, The Department of Medicine.  
Leukemia: Have we made progress  
3/24/2012: First Annual Controversies in Hematological Malignancies, Milwaukee, WI.

### Course Director

- 1/29/13: AML. Fond du Lac Cancer center CME lecture  
5/21/13: CML. Elmbrook hospital CME program  
3/09/2013: Second Annual Controversies in Hematological Malignancies, Milwaukee, WI, Co-program Director  
9/24/13: Leukemia for primary care. Columbia St. Marys grand rounds.  
10/4/2013: CML, diagnosis, prognosis and follow up. Northeastern Wisconsin Oncology Nursing Society Chapter Fall conference, Greenbay, WI.  
3/8/14: Third Annual Controversies in Hematological Malignancies, Milwaukee, WI

### Co-program Director

- 2/21/15: Inaugural Leukemia Survivorship conference, Oshkosh, WI. Program Director.

## PEER REVIEWED WORKSHOPS/PRESENTATIONS:

### (Oral presentation):

- Atallah E, Kantarjian H, O'Brien S, Jones D, Borthakur G, Nicaise C, Cortes J. Dasatinib (SPRYCEL®) is safe and effective in patients with previously untreated chronic myelogenous leukemia in chronic phase. American Society of Clinical Oncology Meeting, Chicago, 6/2007
- Wael Saber, Corey Cutler, Ryotaro Nakamura, Mei-Jie Zhang, Ehab Atallah, J. Douglas Rizzo, Richard T. Maziarz, Jorge E. Cortes, Matt Kalaycio, Mary M. Horowitz. Comparison of Outcomes After Related and Unrelated Hematopoietic Cell Transplantation in Adults with Myelodysplastic Syndromes: A Report From the Center for International Blood and Marrow Transplant Research (CIBMTR). ASH 2012
- Guillermo Garcia-Manero, MD1, Pierre Fenaux, MD, PhD2, Aref Al-Kali, MD3, Maria R. Baer, MD4, Mikkael A. Sekeres, MD, MS5, Gail J. Roboz, MD6, Gianluca Gaidano, MD, PhD7, Bart L. Scott, MD8, Peter L. Greenberg, MD, PhD9, Uwe Platzbecker, MD10, David P. Steensma, MD11, Suman Kambhampati, MD12\*, Karl-Anton Kreuzer, MD13\*, Lucy A Godley, MD, PhD14, Robert Collins15, Ehab Atallah, MD16, Francois Wilhelm, MD, PhD.17, Isabelle E. Wilhelm, MD17, Nozar Azarnia, PhD17\*, Manoj Maniar, PhD17\* and Lewis R. Silverman, MD. Overall Survival and Subgroup Analysis from a Randomized Phase III Study of Intravenous Rigosertib Versus Best Supportive Care (BSC) in Patients with Higher-Risk Myelodysplastic Syndrome (HR-MDS) after Failure of Hypomethylating Agents (HMAs). ASH 2014
- Mikkael A. Sekeres, MD, MS1, Megan Othus, PhD2\*, Alan F. List, MD3, Olatoyosi Odenike, MD4, Richard M. Stone, MD5, Steven D. Gore, MD6,7, Mark R. Litow, MD8, Rena Buckstein, MD9, Mario R. Velasco Jr., M.D.10\*, Rakesh Gaur, MD11, Ehab Atallah, MD12, Eyal Attar, MD13, Frederick R. Appelbaum, MD14 and Harry P Erba, MD, PhD. A Randomized Phase II Study of Azacitidine Combined with Lenalidomide or with Vorinostat vs. Azacitidine Monotherapy in Higher-Risk Myelodysplastic Syndromes (MDS) and Chronic Myelomonocytic Leukemia (CMML): North American Intergroup Study SWOG S1117. Late breaking abstract ASH 2014

- Lewis R. Silverman, MD1, Pierre Fenaux, MD2, Aref Al-Kali, MD3, Maria R. Baer, MD4, Mikkael Sekeres, MD5, Gail Roboz, MD6, Gianluca Gaidano, MD7, Bart Scott, MD8, Peter Greenberg, MD9, Uwe Platzbecker, MD10, David P. Steensma, MD11, Suman Kambhampati, MD12, Karl-Anton Kreuzer, MD13, Lucy Godley, MD14, Robert Collins, Jr, MD15, Ehab Atallah, MD16, Shyamala C. Navada, MD1, Nozar Azarnia, PhD17, Guillermo Garcia-Manero, MD18. RANDOMIZED PHASE III STUDY OF IV RIGOSERTIB VERSUS BEST SUPPORTIVE CARE (BSC) IN PATIENTS WITH HIGHER-RISK MDS (HR-MDS) AFTER FAILURE OF HYPOMETHYLATING AGENTS (HMAs). International Symposium on Myelodysplastic Syndromes, April 29 – May 2, 2015. Washington, D.C.

### (Poster Presentation):

- Atallah E, Durand J, Kantarjian H, Jabbour E, O'Brien S, Rios M, Cortes J. Congestive Heart Failure is a Rare Event in Patients Receiving Imatinib Therapy. Blood (ASH Annual Meeting Abstracts), Nov 2006; 108: 2146.
- Atallah E, Verstovsek S. Clinical Features and Prognosis of Patients with Myelodysplastic/Myeloproliferative Syndrome-Unclassified (MDS/MPD-U): Refractory Anemia with Ringed Sideroblasts with Thrombocytosis (RA-RST) is a Favorable Prognostic Subgroup. Blood (ASH Annual Meeting Abstracts), Nov 2006; 108: 2612.
- Verstovsek S, Atallah E, Thomas D, Cortes J, Ravandi-Kashani F, Nicaise C, Sams I, Giles F, Kantarjian H. Dasatinib Therapy for Patients with Philadelphia negative Myeloproliferative Disorders Including Systemic Mastocytosis. ASCO 2007.
- Jabbour E, Kantarjian H, Atallah E, Borthakur G, Wierda W, Faderl S, Kornblau S, Cortes J. Impact of Imatinib Mesylate Dose Escalation on Resistance and Sub-Optimal Responses to Standard-Dose Therapy in Patients (pts) with Chronic Myeloid Leukemia (CML). Blood (ASH Annual Meeting Abstracts), Nov 2007; 110: 1035
- Kirschbaum M, Gojo I, Goldberg S, Kujawski L, Atallah E, Marks P, Garcia-Vargas J, Rizvi S, Di Gravio D, Ahern J and Issa JP. Phase I Study of Vorinostat in Combination with Decitabine in Patients with Relapsed or Newly Diagnosed Acute Myelogenous Leukemia or Myelodysplastic Syndrome. Blood (ASH Annual Meeting Abstracts), Dec 2008; 112: 3651
- Olatoyosi Odenike, Emily Curran, Neil Iyengar, Leslie Popplewell, Mark Kirschbaum, Harry P Erba, Margaret Green, Xavier Poiré, Pamela Ihonor, Ernesto Diaz-Flores, Kevin Shannon, Ehab Atallah, James L Wade, III, Maria Perdekamp, Sreenivasa Nattam, Sachdev P. Thomas, Scott E. Smith, L. Austin Doyle, Elizabeth Shima Rich, Richard A. Larson, and Wendy Stock Phase II Study of the Oral MEK Inhibitor AZD6244 in Advanced Acute Myeloid Leukemia (AML). Blood (ASH Annual Meeting Abstracts), Nov 2009; 114: 2081.
- Mark Kirschbaum, Ivana Gojo, Stuart L. Goldberg, Lisa Kujawski, Ehab Atallah, Peter Marks, Donna Di Gravio, Lisa Pyle, Syed Rizvi, and Jean-Pierre J Issa. Vorinostat in Combination with Decitabine for the Treatment of Relapsed or Newly Diagnosed Acute Myelogenous Leukemia (AML) or Myelodysplastic Syndrome (MDS): A Phase I, Dose-Escalation Study. Blood (ASH Annual Meeting Abstracts), Nov 2009; 114: 2089.
- T.A. Giever, K.M. Adwan, Y. Cheng, E.L. Atallah; Medical College of Wisconsin, Milwaukee, WI; Clement J. Zablocki VA. Medical Center, Milwaukee, WI. Comparison of venous thromboemboli (VTE) incidence in cancer patients who are on statin therapy versus those not on statin therapy. J Clin Oncol 28:15s, 2010 (suppl; abstr 9033)
- Nitin Jain, Emily Curran, Neil M. Iyengar, Ernesto Diaz-Flores, Rangesh Kumnavakkam, Leslie Popplewell, Mark H. Kirschbaum, Harry Paul Erba, Margaret Green, Xavier Poiré, Greg Koval, Kevin Shannon, Ehab L. Atallah, Philip Dy, Scott E. Smith, L. Austin Doyle, Richard A. Larson, Wendy Stock, Olatoyosi Odenike Phase II study of the oral MEK inhibitor selumetinib (AZD6244) in advanced acute myeloid leukemia (AML). J Clin Oncol 30, 2012 (suppl; abstr 6582)
- Ehab Atallah, Tanya L. Pedersen, Erica D. Warlick, Anne Dircks, Daniel Weisdorf, Mary M. Horowitz, J. Douglas Rizzo. The Outcome of Hematopoietic Cell Transplantation (HCT) for Myelodysplastic Syndrome (MDS) in Adults ≥ 65 Years of Age: First Report of the Coverage with Evidence Development (CED) in Medicare Beneficiaries. (ASH 2012)
- Ruben A. Mesa, Srdan Verstovsek, Vikas Gupta, John Mascarenhas, Ehab Atallah, William Sun, Victor A. Sandor, and Jason Gotlib. Improvement in Weight and Total Cholesterol and Their Association with Survival in Ruxolitinib-Treated Patients with Myelofibrosis From COMFORT-I. (ASH 2012)
- Ruben A. Mesa, Srdan Verstovsek, Ehab Atallah, Vikas Gupta, John Mascarenhas, William Sun, Nicholas J. Sarlis, Victor Sandor, Richard S. Levy, Hagop Kantarjian, Jason Gotlib. Relationship Between Ruxolitinib Dose and Improvements in Spleen Volume and Symptoms in Patients with Myelofibrosis: Results from COMFORT-I. (Hematologic Malignancies conference 2012, Houston, TX)
- Thomas A. Giever, Emily L. Richter, Kristine M. Broge, Patrick C. Foy, Linda S. Blust, Jeanne M. Palmer, Timothy S. Fenske, William S. Rilling, Parameswaran Hari, Ehab L. Atallah. Incidence of venous thromboembolism in patients with hematologic malignancies related to upper extremity peripherally inserted central venous catheters. J Clin Oncol 31, 2013 (suppl; abstr 6586)
- Lisa M. Baumann Kreuziger, MD, MS; Emily Richter; Lucy Gomez-Timana; Thomas Giever; Ehab Atallah. Duration of anticoagulation treatment for catheter related thrombosis in hematologic malignancy. 60th Scientific and Standardization Committee meeting of the International Society on Thrombosis and Haemostasis. 2014
- Namrata Pswani. Timothy Fenske, Parameswaran Hari, Karen S. Carlson, Laura Michaelis, Christopher Chitambar, Lisa Baumann-Kreuziger, Ehab Atallah. D-DIMER KINETICS, CHEMOTHERAPY AND THE RISK OF BLEEDING IN PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA. Congress on Controversies in Thrombosis and Hemostasis (CITH). Berlin, Germany, October 30-November 1, 2014
- Sarang Patel, Amara S. Hussain, Rahul Nanchal, Ehab Atallah. Acquired Angioedema in a Patient with a History of Chronic Lymphocytic Leukemia. ACP Wisconsin Chapter meeting, September 2014
- Guillermo Garcia-Manero, MD1, Ehab Atallah, MD2, Olatoyosi Odenike, MD3, Bruno

- C Medeiros<sup>4</sup>, Jorge Cortes, MD<sup>5</sup>, Vanessa Esqueibel<sup>6\*</sup>, Steven Cha, MD<sup>7\*</sup> and Samer K Khaled. Pracinostat in Combination with Azacitidine Produces a High Rate and Rapid Onset of Disease Remission in Patients with Previously Untreated Acute Myeloid Leukemia (AML). *ASH 2014*
18. Namrata Peswani, MD, MBBS<sup>1\*</sup>, Baumann Lisa, MD<sup>2\*</sup>, Karen Carlson, MD, PhD<sup>1\*</sup>, Laura C Michaelis, MD<sup>1</sup>, Christopher R. Chitambar, MD<sup>1</sup>, Timothy S. Fenske, MD<sup>1</sup>, Parameswaran Hari, MBBS, MD, MRCP<sup>1</sup> and Ehab Atallah, D-Dimer Kinetics, Chemotherapy and Risk of Bleeding in Acute Promyelocytic Leukemia. *ASH 2014*
  19. Ahmad Hanif, MD<sup>1\*</sup>, Parameswaran N. Hari, MD, MRCP, MS<sup>2</sup>, Ehab Atallah, MD<sup>3</sup>, Karen-Sue B. Carlson, MD, PhD<sup>4</sup>, Marcelo C. Pasquini, MD<sup>4</sup> and Laura C Michaelis. Ruxolitinib Prior to Allogeneic Stem Cell Transplantation Does Not Adversely Affect Post-Transplant Outcomes. *ASH 2014*
  20. Lewis R. Silverman, MD<sup>1\*</sup>, Pierre Fenaux, MD, PhD<sup>2</sup>, Aref Al-Kali, MD<sup>3</sup>, Maria R. Baer, MD<sup>4</sup>, Mikkael A. Sekeres, MD, MS<sup>5</sup>, Gail J. Roboz, MD<sup>6</sup>, Gianluca Gaidano, MD, PhD<sup>7</sup>, Bart L. Scott, MD<sup>8</sup>, Peter L. Greenberg, MD, PhD<sup>9</sup>, Uwe Platzbecker, MD<sup>10</sup>, David P. Steensma, MD<sup>11</sup>, Suman Kambhampati, MD<sup>12\*</sup>, Karl-Anton Kreuzer, MD<sup>13\*</sup>, Lucy A Godley, MD, PhD<sup>14</sup>, Robert Collins<sup>15</sup>, Ehab Atallah, MD<sup>16</sup>, Shyamala C. Navada, MD<sup>1</sup>, Francois Wilhelm, MD, PhD<sup>17</sup>, Nozar Azarnia, PhD<sup>17\*</sup> and Guillermo Garcia-Manero. Relationship of Bone Marrow Blast (BMBL) Response to Overall Survival (OS) in Patients with Higher-Risk Myelodysplastic Syndrome (HR-MDS) Treated with Rigosertib after Failure of Hypomethylating Agents (HMAs)

#### COMMITTEE SERVICES:

##### Medical College Committees

- 2009-2013: Committee of Intern Selection, Medical College of Wisconsin  
 2009-2014: Member, Fellowship committee, Medical College of Wisconsin  
 2010-Present: Oncology PNT Subcommittee  
 2011-2012: Antibiotic PNT Subcommittee  
 2012-2013: Cancer Center Working Group  
 2012: Leukemia Business Planning Committee  
 2013: MCW Data Safety Monitoring Committee member co-chair  
 2014: Blood Management Committee FMLH

Data Safety Monitoring Committee Chair: A Phase 2, Open-Label, Prospective Study of PRM-151 In Subjects With Primary Myelofibrosis (PMF), Post-Polycythemia Vera MF (post-PV MF), Or Post-Essential Thrombocythemia MF (post-ET MF). (Promedior, Inc.)

#### MEDICAL COLLEGE OF WISCONSIN TEACHING ACTIVITIES:

##### Medical Student Education

- 2008 – Present - Clinical preceptorship: Ambulatory immersion rotation for medical students  
 2010 - Instructor in the "General Principles" Module of the M1 Curriculum, "Neoplasia and Carcinogenesis"  
 2012- Instructor in the "General Principles" Module of the M2 Curriculum, "Neoplasia and Carcinogenesis"  
 2014-- Instructor in the "Heme/Lymph" Module of the M2 Discovery Curriculum  
 2011: Preceptor for CER History & Physical for M2 students  
 2013-2015: Clinical Apprenticeship Preceptor

##### Resident & Fellow Education

- Resident Clinical preceptorship: Ambulatory clinical rotations for residents  
 Resident Core curriculum lectures 2009-2014  
 Fellows Core curriculum lecture 2009-2014  
 Fellows Clinical preceptorship -Jenny Petkova, Leena Varkey and Sunita Sukumaran

##### American College of Physicians/Wisconsin Chapter

AML with blast negative CNS involvement. Shannon Schmidt, MD and Ehab Atallah, MD

##### Nursing Education

- 3/2009: Leukemia for Nurses  
 1/8/2013: Leukemia for Nurses. What you need to know  
 11/2014: Therapy related AML

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1. Atallah E, Talpaz M, O'Brien S, Rios MB, Guo JQ, Arlinghaus R, Fernandes-Reese S, Kantarjian H. Chronic Myelogenous Leukemia In T Cell Lymphoid Blastic Phase Achieving Durable Complete Cytogenetic And Molecular Remission With Imatinib Mesylate (STI571; Gleevec) Therapy. *Cancer* 2002; 94(11):2996-2999.
2. Atallah E, Durand J, Kantarjian H, Cortes J. Congestive Heart Failure is a Rare Event in Patients Receiving Imatinib Therapy. *Blood*. 2007 Aug 15;110(4):1233-7
3. Atallah E, Cortes J, O'Brien S, Pierce S, Rios MB, Estey E, Markman M, Keating M, Freireich EJ, Kantarjian H. Establishment of Baseline Toxicity Expectations with Standard Frontline Chemotherapy in Acute Myelogenous Leukemia. *Blood*. 2007 Nov 15;110(10):3547-51.
4. Nand, J, Bryce C, Divgi A, Kroft S, Bredeson C, Atallah E. Myeloproliferative Disorder with Eosinophilia and ETV6-ABL Gene Rearrangement: Efficacy of Second Generation Tyrosine Kinase Inhibitors. *Leukemia Research*. 2009 Aug;33(8):1144-6. Epub 2009 Apr 25.
5. Bilgi N, Bell K, Ananthakrishnan AN, Atallah E. *Ann Pharmacother*. Imatinib and Panax ginseng: a potential interaction resulting in liver toxicity. 2010 May;44(5):926-8. Epub 2010 Mar 23.

6. Oltanu H, Schur BC, Bredeson C, Atallah E, Kroft SH. Expression of natural killer receptors in T- and NK-cells: comparison of healthy individuals, patients with prior stem cell transplant, and patients undergoing chemotherapy. *Leuk Lymphoma*. 2010 Mar;51
7. Atallah E., Abrams, J., Ayash, L., Bentley, G., Abidi, M., Ratanatharathorn, V., et al. (2010). Long term follow-up of allogeneic stem cell transplantation in patients with myelodysplastic syndromes using busulfan, cytosine arabinoside, and cyclophosphamide. *American Journal of Hematology*, 85(8): 579-83.
8. Angel, J. R., Parker, S., Sells, R.E., & Atallah, E. (2010). Recurrent deep vein thrombosis and pulmonary embolism in a young man with klinefelter's syndrome and heterozygous mutation of MTHFR-677C>T and 1298>C. *Blood Coagulation & Fibrinolysis: An international Journal in Haemostasis and Thrombosis*, 21(4), 372-375.
9. Randhawa J, Wong S, Atallah E, Olasz E. Clinical regression of squamous cell carcinoma and keratoacanthomas in a patient treated with a hypomethylating agent. *Int J Dermatol*. 2012 Sep 24[Epub ahead of print]
10. 1: Saber W, Cutler CS, Nakamura R, Zhang MJ, Atallah E, Rizzo JD, Maziarz RT, Cortes J, Kalaycio ME, Horowitz MM. Impact of donor source on hematopoietic cell transplantation outcomes for patients with myelodysplastic syndromes (MDS). *Blood*. 2013 Jul 11. [Epub ahead of print].
11. Verstovsek S, Gottlieb J, Gupta V, Atallah E, Mascarenhas J, Quintas-Cardama A, Sun W, Sarlis NJ, Sandor V, Levy RS, Kantarjian HM, Mesa RA. Management of cytopenias in patients with myelofibrosis treated with ruxolitinib and effect of dose modifications on efficacy outcomes. *Oncol Targets Ther*. 2013 Dec 17;7:13-21.
12. Jain N, Curran E, Iyengar NM, Diaz-Flores E, Kunnavakkam R, Popplewell L, Kirschbaum MH, Karrison T, Erba HP, Green M, Poire X, Koval G, Shannon K, Reddy PL, Joseph L, Atallah EL, Dy P, Thomas SP, Smith SE, Doyle LA, Stadler WM, Larson RA, Stock W, Odenike O. Phase II study of the oral MEK inhibitor selumetinib in advanced acute myelogenous leukemia: a University of Chicago phase II consortium trial. *Clin Cancer Res*. 2014 Jan 15;20(2):490-8.
13. Yan M, Geyer H, Mesa R, Atallah E, Callum J, Bartoszko J, Yee K, Maganti M, Wong F, Gupta V. Clinical Features of Patients With Philadelphia-Negative Myeloproliferative Neoplasms Complicated by Portal Hypertension. *Clin Lymphoma Myeloma Leuk*. 2014 Jun 11.
14. Kataria A, Cohen E, Saad E, Atallah E, Bresnahan B. Large Granular Lymphocytic Leukemia Presenting Late After Solid Organ Transplantation: A Case Series of Four Patients and Review of the Literature. *Transplant Proc*. 2014 Sep 18.
15. Mesa RA, Verstovsek S, Gupta V, Mascarenhas JO, Atallah E, Burn T, Sun W, Sandor V, Gottlieb J. Effects of Ruxolitinib Treatment on Metabolic and Nutritional Parameters in Patients With Myelofibrosis From COMFORT-I. *Clin Lymphoma MyelomaLeuk*. 2014 Dec 27.

#### BOOKS, CHAPTERS & REVIEWS

1. Atallah E, Flaherty L. Treatment Of Metastatic Malignant Melanoma. *Current Treatment Options in Oncology* 2005; 6(3):185-193.
2. Atallah E, Garcia-Manero G. New Treatment Strategies in the Treatment of Myelodysplastic Syndrome. *Cancer Invest*. 2008 Mar;26(2):208-16
3. Atallah E, Garcia-Manero G. Use of hypomethylating agents in myelodysplastic syndromes. *Clinical Advances in Hematology and Oncology* 2007 Jul;5(7):544-52.
4. Atallah E, Schiffer CA. Granulocyte Transfusion. *Current Opinion in Hematology* 2006; 13(1):45-49.
5. Atallah E, Garcia-Manero G. The Role of Decitabine in the Treatment of Myelodysplastic Syndromes. *Expert Opinion On Pharmacotherapy*. *Expert Opinion in Pharmacotherapy* 2007 Jan;8(1):65-73.
6. Atallah E, Cortes J. Optimal Initial Therapy for Patients With Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase. *Current Opinion in Hematology* 2007 Mar;14(2):138-44.
7. Atallah E, Cortes J. Emerging Safety Issues with Abl Kinase Inhibitors. *Clinical Lymphoma Myeloma*. 2007 Mar;7 Suppl 3:S105-12
8. Atallah E. CML. Initial Therapy in Previously Untreated Patients with Chronic Myelogenous Leukemia in Chronic Phase. *Clinical Leukemia*, Vol. 2, No. 4, 248-251, 2008
9. Atallah E, Verstovsek S. The Prospect of JAK2 Inhibitor Therapy in Myeloproliferative Neoplasms. *Expert review of anticancer therapy*. 2009 May;9(5):663-70.
10. Atallah E, O'Dwyer. Practical considerations for the management of patients in the tyrosine kinase inhibitor era. *Seminars in Hematology* April 2009 (Vol. 46, Pages S16-S21)
11. Atallah E, Garcia-Manero G. Hypomethylating agents Chapter in "Epigenetics in Biology and Medicine". Editor Dr. Manel Esteller
12. Kim J, Haddad RY, Atallah E. Myeloproliferative neoplasms. *Disease a Month*. 2012 Apr;58(4):177-94.
13. Torgerson SR, Haddad RY, Atallah E. Chronic myelogenous leukemia for primary care physicians. *Disease a Month*. 2012 Apr;58(4):168-76.
14. Randhawa J, Ostojic A, Vrhovac R, Atallah E, Verstovsek S. Splenomegaly in myelofibrosis--new options for therapy and the therapeutic potential of Janus kinase 2 inhibitors. *Hematol Oncol*. 2012 Aug 1;5(1):43. [Epub ahead of print]
15. Atallah E, Verstovsek S. Emerging drugs for myelofibrosis. *Expert Opin Emerg Drugs*. 2012 Dec;17(4):555-70.
16. Kremyanskaya M, Atallah EL, Hoffman R, Mascarenhas JO. Clarifying the use of ruxolitinib in patients with myelofibrosis. *Oncology (Williston Park)*. 2013 Jul;27(7):706-14.
17. Troy JD, Atallah E, Geyer JT, Saber W. Myelodysplastic syndromes in the United States: an update for clinicians. *Ann Med*. 2014 Apr 10. [Epub ahead of print]
18. Atallah E, Bylow K, Troy J, Saber W. Treatment of older patients with high-risk myelodysplastic syndromes (MDS): the emerging role of allogeneic hematopoietic

stem cell transplantation (Allo HSCT). *Curr Hematol Malig Rep*. 2014

19. Randhawa J, Atallah E. Myelodysplastic syndrome. *Hospital Physician. Hematology Board Review Manual*. Volume 9, Part 3. August 2014

#### EDITORIALS, LETTERS TO EDITOR, OTHER

1. Atallah E, Nussenzweig R, Yin CC, Bueso-Ramos C, Tam C, Manshour T, Pierce S, Kantarjian H, Verstovsek S. JAK-2 mutation in patients with myelodysplastic/myeloproliferative disorders unclassifiable and refractory anemia with ringed sideroblasts and thrombocytosis (RARS-T) is associated with good prognosis. *Leukemia*. 2008 Jun;22(6):1295-8.
2. Atallah E, Kantarjian H, Cortes J. In reply to 'Cardiotoxicity of the cancer therapeutic agent imatinib mesylate'. *Nature Medicine* 2007 Jan;13(1):14.
3. Atallah E, Schiffer CA. Agent Orange, Prostate Cancer Irradiation And Acute Promyelocytic Leukemia (APL): Is There A Link? *Leukemia Research* 2006.
4. Vernon M, Pearson L, Atallah E. Resolution of rheumatoid arthritis symptoms with imatinib mesylate. *Journal of clinical Rheumatology*. 15(5):267, August 2009
5. Atallah E. Nilotinib cardiac toxicity: Should we still be concerned? *Leuk Res*. 2011 Feb 16

#### NON-REFEREED JOURNAL PUBLICATIONS/ORIGINAL PAPERS

Atallah E, O'Dwyer. Practical considerations for the management of patients in the tyrosine kinase inhibitor era. *Semin Hematol*. 2009 Apr;46(2 Suppl 3):S16-21

#### ABSTRACTS

1. Atallah E, Uberti J, Ayash L, Bentley G, VanDahm J, Ratanatharathorn V. Fifteen-Year Follow up of Allogeneic Stem Cell Transplantation in Patients with Myelodysplastic Syndromes Using Busulfan-Based Regimens. *ASH Annual Meeting Abstracts* 2005; 106(11):5461.
2. Atallah E, Salomon M, Schiffer CA, El-Rayes B. The role of venography in the evaluation of malfunctioning central venous access ports (CVAPs). *ASCO Annual Meeting Proceedings Part 1, Vol 24, No. 18S (June 20 Supplement)*, 2006: 18535
3. Atallah E, Kantarjian H, De Lima M, Borthakur G, Wierda W, Champlin R, Cortes J. The Role of Dasatinib in Patients with Philadelphia (Ph) Positive Acute Lymphocytic Leukemia (ALL) and Chronic Myeloid Leukemia (CML) Relapsing after Stem Cell Transplantation. *Blood (ASH Annual Meeting Abstracts)*, Nov 2006; 108: 4520.
4. Atallah E, O'Brien S, Kaled S, Lynn A, Cortes J, Kantarjian H, Thomas D Traumatic Lumbar Puncture (TLP) Is Not Associated with Worse Outcome in Adults with Acute Lymphocytic Leukemia (ALL) or Burkitt-Type Leukemia/Lymphoma (BLL) Receiving Hyper-CVAD Regimens. *Blood (ASH Annual Meeting Abstracts)*, Nov 2007; 110: 4346.
5. Atallah E, O'Brien S, Kaled S, Lynn A, Cortes J, Kantarjian H, Thomas D Safety of Lumbar Puncture (LP) and Instillation of Intrathecal (IT) Chemotherapy during Thrombocytopenia in Adults with Acute Lymphocytic Leukemia (ALL). *Blood (ASH Annual Meeting Abstracts)*, Nov 2007; 110: 4347.
6. Jasleen K Randhawa, MD, Christopher Bredeson, MD, Timothy S. Fenske, MD, Ayman Saad, MD, Jeanne Palmer, MD, Parameswaran N Hari, MD, Horatiu Olteanu, and Ehab Atallah, MD. Hypomethylating Agent Therapy for Acute Myelogenous Leukemia (AML) Can Induce Sustained Responses with Low Induction Mortality. *Blood (ASH Annual Meeting Abstracts)*, Nov 2009; 114: 4157.
7. Thompson J, Sweet K, Zhang L, Randhawa JK, Chitambar CR, Altman JK, Pinilla J, and Atallah E. Poor Response to Imatinib in Patients With Secondary Chronic Myelogenous Leukemia. *Blood* 2013 122:5170 ASH annual meeting abstracts
8. Short time to ATRA administration and reduction of early mortality in patients with APL. Namrata I. Peswani, Karen-Sue Carlson, Christopher R. Chitambar, Timothy S. Fenske, Parameswaran Hari, Laura Christian Michaelis, Linda S. Blust, Ehab L. Atallah; Medical College of Wisconsin, Milwaukee, WI; CIBMTR, Milwaukee, WI. *J Clin Oncol* 32, 2014 (suppl); abstr e18020

#### PEER REVIEWED EDUCATIONAL PRODUCTS:

Atallah E, Tam C, O'Brien S. CLL the cutting edge. CME educational activity  
Atallah E. A new asparaginase option for ALL. *Community Oncology* 2012;9:145.

#### RESEARCH GRANTS/AWARDS/CONTRACTS/PROJECTS:

Investigators Name: Ehab Atallah

Title of project: The Life After Stopping Tyrosine Kinase Inhibitors (The LAST study)

Role in Project: PI

Funding agency: NIH grant 1R01CA184798

Total Costs: \$3,357,155

Funding period: 09/19/2014 – 08/31/2019

Investigator's Name: Ehab Atallah, MD

Title of project: Assessment of allogeneic hematopoietic stem cell transplantation in Medicare Beneficiaries with myelodysplastic syndrome and related disorders.

Role in project: PI

Funding Agency/ID# CIBMTR

Investigator's Name: Ehab Atallah, MD

Title of project: Phase I of modified VXLD plus MLN9708 in adults with relapsed or refractory acute lymphoblastic leukemia.

Role in project: Investigator sponsored by a research grant from Millenium

Funding Agency/ID#: Millenium

Total costs: \$80,000

Investigator's Name: Ehab Atallah, MD

Title of project: Phase I of Standard Maintenance plus MLN9708 Maintenance

Therapy in adults with Acute Lymphoblastic Leukemia in Complete Remission (accepted for funding)

Role in project: PI

Funding Agency/ID#: Investigator sponsored by a research grant from Millenium

Total costs: 180,000 \$

Investigator's Name: Ehab Atallah, MD

Title of project: A Phase 2 Study Investigating Clofarabine, Cyclophosphamide and Etoposide for Children, Adolescents, and Young Adults (AYA) with Acute Leukemia and Minimal Residual Disease

Role in project: Study Vice Chair

Investigator's Name: Ehab Atallah, MD

Title of project: Phase IIB, Multicenter, Randomized, open label Trial of CPX-351 (cytarabine:Daunorubicin) Liposome injection versus cytarabine and Daunorubicin in patients with untreated AML 60-75 years of age.

Role in project (if not PI): Institutional PI

Funding Agency/ID#: Celator Pharm/CLTR0308-204

Funding Period (start/end dates): 7/16/09 – 7/15/13

Total Direct Costs for Current Year: \$7280 per patient

Investigator's Name: Ehab Atallah, MD

Title of project: A Randomized Double-Blind, Placebo-controlled Study of the JAK inhibitor INCB018424 tablets administered orally to Subjects with Primary Myelofibrosis (DMF), post polycythemia vera-Myelofibrosis (PPVOMF) or Post Essential Thrombocythemia (COMFORT I trial)

Role in project (if not PI): Institutional PI

Funding Agency/ID#: PPD Development/INCB 18424-351

Funding Period (start/end dates): 10/29/2009 – 10/28/2012

Total Direct Costs for Current Year: \$19,232.50 per patient

Investigator's Name: Ehab Atallah, MD

Title of project: A Phase II Study to Evaluate the Efficacy, Safety and Tolerability of Tosedostat (CHR-2797) in elderly subjects with treatment Refractory or Relapsed Acute Myeloid Leukemia

Role in project (if not PI): Institutional PI

Funding Agency/ID#: Quintiles/CHR – 2797-038

Funding Period (start/end dates): 1/27/10 – 1/26/13

Total Direct Costs for Current Year: \$19,513 per patient

Investigator's Name: Ehab Atallah, MD

Title of project: Clavis\_CP4055-306: A Randomised Phase III Study of Elacetytarabine vs. Investigator's Choice in Patients with Late Stage Acute Myeloid Leukaemia

Role in project: Institutional PI

Funding Agency/ID#: Clavis\_CP4055-306

Funding Period (start/end dates): 7/29/2010 – till now

Total Direct Costs for Current Year: \$7,000 per patient

Investigator's Name: Ehab Atallah, MD

Title of project: Protocol CA180399: An Open Label, Randomized Phase 2B Study Of Dasatinib Vs. Imatinib In Patients With Chronic Phase Chronic Myeloid leukemia Who Have Not Achieved An Optimal Response To 3 Months Of Therapy With 400 Mg Imatinib.

Role in project: Institutional PI

Funding Agency/ID#: BMS

Funding Period (start/end dates): 5/24/13 – till now

Total Direct Costs for Current Year: \$12,440.00 per patient

Investigator's Name: Ehab Atallah, MD

Title of project: A Phase III Randomized Study of Oral Sapacitabine in Elderly Patients with Newly Diagnosed Acute Myeloid Leukemia

Role in project: Institutional PI

Funding Agency/ID#: Cyclacel

Funding Period (start/end dates): 10/13/2011 – till now

Total Direct Costs for Current Year: \$7,375.00 per patient

Investigator's Name: Ehab Atallah, MD

Title of project: A Phase II Randomized Trial of Carboplatin and Topotecan; Flavopiridol, Mitoxantrone and Cytosine Arabinoside; and Sirolimus, Mitoxantrone, Etoposide and Cytosine Arabinoside for the Treatment of Adults with Primary Refractory or Initial Relapse of Acute Myelogenous Leukemia (AML)

Role in project: Institutional PI  
Funding Agency/ID#: ECOG  
Funding Period (start/end dates): 9/10/2010 – till now

Investigator's Name: Ehab Atallah, MD  
Title of project: Phase III Randomized Trial of Clofarabine as Induction and Post-Remission Therapy vs. Standard Daunorubicin & Cytarabine Induction and Intermediate Dose Cytarabine Post-Remission Therapy, Followed by Decitabine Maintenance vs. Observation in Newly-Diagnosed Acute Myeloid Leukemia in Older Adults (Age ≥ 60 Years)  
Role in project: Institutional PI  
Funding Agency/ID#: ECOG  
Funding Period (start/end dates): 4/21/2011 – till now

Investigator's Name: Ehab Atallah, MD  
Title of project: A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study Evaluating the Efficacy and Safety of GS-1101 (CAL-101) in Combination with Bendamustine and Rituximab for Previously Treated Chronic Lymphocytic Leukemia  
Role in project: Institutional PI  
Funding Agency/ID#: Gilead sciences  
Funding Period (start/end dates): 11/27/2012 – till now  
Total Direct Costs for Current Year: \$27,778.75 per patient

Investigator's Name: Ehab Atallah, MD  
Title of project: A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study Evaluating the Efficacy and Safety of Idelalisib (GS-1101) in Combination with Rituximab for Previously Treated Chronic Lymphocytic Leukemia  
Role in project: Institutional PI  
Funding Agency/ID#: Gilead sciences  
Funding Period (start/end dates): 11/27/12 – till now  
Total Direct Costs for Current Year: \$22,250.00 per patient

Investigator's Name: Ehab Atallah, MD  
Title of project: A Phase 3, Double-Blind Extension Study Evaluating the Efficacy and Safety of Two Different Dose Levels of Single-Agent Idelalisib (GS-1101) as Therapy for Patients with Previously Treated Chronic Lymphocytic Leukemia  
Role in project: Institutional PI  
Funding Agency/ID#: Gilead Sciences  
Funding Period (start/end dates): 11/27/12 – till now  
Total Direct Costs for Current Year: \$18,975.00 per patient

Investigator's Name: Ehab Atallah, MD  
Title of project: A Three-part Study of Eltrombopag in Thrombocytopenic Subjects with Myelodysplastic Syndromes or Acute Myeloid Leukemia (Part 1: open-label, Part 2: randomized, double-blind, Part 3: extension).  
Role in project: Institutional PI  
Funding Agency/ID#: GSK  
Funding Period (start/end dates): 1/10/2013 – till now  
Total Direct Costs for Current Year: \$14,691.00 per patient

Investigator's Name: Ehab Atallah, MD  
Title of project: Polycythemia Vera Symptom Study Evaluating Ruxolitinib Versus Hydroxyurea in a Randomized, Multicenter, Double-Blind, Double-Dummy, Phase 3 Efficacy and Safety Study of Patient Reported Outcomes  
Role in project: Institutional PI  
Funding Agency/ID#: Incyte  
Funding Period (start/end dates): 12/13/12 – till now  
Total Direct Costs for Current Year: \$18,317.00 per patient

Investigator's Name: Ehab Atallah, MD  
Title of project: Protocol I3X-MC-JHTB(a) A Phase 2 Study of LY2784544 in Patients with Myeloproliferative Neoplasms  
Role in project: Institutional PI  
Funding Agency/ID#: Lilly  
Funding Period (start/end dates): 1/10/2013 – till now  
Total Direct Costs for Current Year: \$23,168.00 per patient

Investigator's Name: Ehab Atallah, MD  
Title of project: Protocol I3X-MC-JHTC(a) A Phase 1 Study of LY2784544 Testing Alternative Dosing Regimens in Patients with Myeloproliferative Neoplasms  
Role in project: Institutional PI  
Funding Agency/ID#: Lilly  
Funding Period (start/end dates): 5/6/2013 – till now  
Total Direct Costs for Current Year: \$27,557.00 per patient

Investigator's Name: Ehab Atallah, MD  
Title of project: A Phase III, Multi-Center, Randomized, Controlled Study to Assess the Efficacy and Safety of ON 01910.Na Administered as a 72-Hour Continuous Intravenous Infusion Every Other Week in Myelodysplastic Syndrome Patients with Excess Blasts Relapsing After, or Refractory to, or Intolerant to Azacitidine or Decitabine  
Role in project: Institutional PI  
Funding Agency/ID#: Onconova Therapeutics Inc  
Funding Period (start/end dates): 8/24/2011 – till now  
Total Direct Costs for Current Year: \$23,569.00 per patient

Investigator's Name: Ehab Atallah, MD  
Title of project: Phase II two-stage dose finding run-in study of SAR3419, an anti-CD19 antibody-maytansine conjugate, administered as a single agent by intravenous infusion in patients with relapsed or Refractory Acute Lymphoblastic Leukemia (ALL)  
Role in project: Institutional PI  
Funding Agency/ID#: Sanofi  
Funding Period (start/end dates): 6/10/2013 – till now  
Total Direct Costs for Current Year: \$25,485.00 per patient

Investigator's Name: Ehab Atallah, MD  
Title of project: A Randomized Phase II Study of Azacitidine in Combination with Lenalidomide (NSC-703813) vs. Azacitidine Alone vs. Azacitidine in Combination with Vorinostat (NSC-701852) for Higher-Risk Myelodysplastic Syndromes (MDS) and Chronic Myelomonocytic Leukemia (CMML)  
Role in project: Institutional PI  
Funding Agency/ID#: ECOG  
Funding Period (start/end dates): 8/2/2012 – till now

# Molecular monitoring and Treatment-Free Remission in Patients with Chronic Phase Chronic Myelogenous Leukemia

Ehab Atallah

CML is caused by the BCR-ABL tyrosine kinase, the product of the t(9;22) translocation visible as the Philadelphia chromosome (Ph). This fusion gene confers a proliferative advantage to the cells acquiring this translocation. Prior to the introduction of imatinib (Gleevec®) in 2001, the only cure for CML was hematopoietic stem cell transplantation, and the median survival of all patients was 4-5 years<sup>1</sup>. Since the introduction of imatinib, about 87% of patients are expected to be alive at 8 years of follow up<sup>2</sup>. Several other TKIs were subsequently developed and FDA-approved for the treatment of CML patients, including nilotinib<sup>3</sup> and dasatinib<sup>4</sup>. Response to TKIs is described at three different levels. First complete hematological response whereby patients achieve normalization of blood counts and spleen size. The second level of response is cytogenetic response as assessed by karyotyping. Complete cytogenetic response is characterized by the disappearance of Ph-positive metaphases. Deeper responses at the molecular level are measured by reductions of the BCR-ABL transcripts in the blood or marrow to very low or undetectable levels using real-time quantitative polymerase chain reaction (RQ-PCR)<sup>5,6</sup>. Molecular response (MR) is reported as % BCR-ABL/control gene, with ABL1 being the most commonly used control gene. For BCR-ABL to be considered undetectable, adequate amplification of a quality control gene is needed to ensure that the measurement is sufficiently sensitive. MR 3.0

is a 3.0 log reduction in BCR-ABL transcripts and is equivalent to BCR-ABL ≤ 0.1%. MR 4.0 is BCR-ABL ≤ 0.01%, MR 4.5 is BCR-ABL ≤ 0.0032%, and MR 5.0 is BCR-ABL ≤ 0.001%. Based on the depth of response at 3, 6, and 12 month from the start of treatment, response is considered optimal or non-optimal. Both the European LeukemiaNet and the National Comprehensive Cancer network have published clear guidelines on response to TKI therapy in patients with CML. Following these guidelines is prudent in optimal management of patients with CML.

Current recommendations are to continue therapy indefinitely, sometimes despite significant side effects. Moreover, the costs of TKI therapy are substantial and place a financial burden on the health care systems. Several, single-armed studies from Europe and Australia suggest that some patients with CML in a TKI-induced complete molecular response (MR 4.5) maintain this response after discontinuation of TKIs. Currently there are several ongoing US and international studies evaluating the safety and efficacy of stopping TKIs. Examples of such studies include the EURO-SKI study (European Leukemia Net Stop TKI study)<sup>7</sup>, the LAST study (Life After Stopping Tyrosine Kinase Inhibitors) and the DESTINY (De-Escalation and Stopping Treatment of Imatinib, Nilotinib or spry-cel in Chronic Myeloid Leukaemia)<sup>8</sup> study. In this presentation we will discuss the role of molecular

Table 1. Previously reported stopping TKI studies

	#	TKI	Prior IFN N (%)	Median F/U (months)	Definition of Relapse	Relapse N (%)
STIM1 <sup>9</sup>	100	I	51 (51)	50	detectable <i>BCR-ABL</i> on 2 consecutive tests with at least 1 log increase between the 2 or loss of MMR once	61 (61)
STIM2 <sup>10*</sup>	124	I	0	12		48 (39)
TWISTER <sup>11</sup>	40	I	21 (52)	42	detectable <i>BCR-ABL</i> on 2 consecutive tests at any level or loss of MMR once	22 (52)
KIDS <sup>12** ^</sup>	78	I	NR	14	loss of MMR on 2 consecutive tests	16 (21)
A-STIM <sup>13 ^</sup>	80	I	40 (52)	31	Loss of MMR once	29 (36)
2G-TKI <sup>14 ^</sup>	34	D or N	25 (74)	14	Loss of MMR once	15 (44)

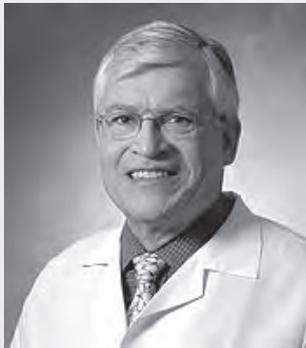
\*No prior therapy with IFN, \*\*21 patients had prior HCT, ^Restarting with loss of MMR only, \*7 months, NR: Not reported, I: Imatinib, D: dasatinib, N: nilotinib, NR: Not reported, MMR: Major molecular response, F/U: Follow up, 2G-TKI: Second generation TKI, IFN: Interferon

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monitoring and review the currently available data on treatment discontinuation.

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## Richard A. Larson

Dr. Richard A. Larson is a Professor of Medicine in the Section of Hematology/Oncology and Director of the Hematologic Malignancies Clinical Research Program at the University of Chicago. He received his medical degree from the Stanford University School of Medicine in 1977, and completed his postdoctoral training in internal medicine, hematology, and oncology at the University of Chicago. He was a Fellow of the Leukemia Society of America and was granted a Clinical Oncology Career Development Award from the American Cancer Society. He has been a member of the faculty in the Section of Hematology/Oncology and the Cancer Research Center at the University of Chicago since 1983. He was Director of the Leukemia Clinical Research Program at the University of Chicago from 1983 to 2000, during which time he established the adult allogeneic bone marrow transplant program at the University of Chicago Medical Center in 1986. He is board certified in internal medicine, hematology, and medical oncology, and has served as a member of the Hematology Subspecialty Board of the American Board of Internal Medicine.

In 1997, Dr. Larson was appointed chair of the Leukemia Committee of the Cancer and Leukemia Group B, where he directed a large portfolio of clinical trials and ancillary laboratory studies in acute and chronic leukemias and myeloma. He is a former councilor on the Executive Committee of the American Society of Hematology and served as the Treasurer of ASH between 2011-2014. He currently represents ASH as a member of the National Cancer Policy Forum of the Institute of Medicine. He is a member of the American Society of Clinical Oncology and the American Association for Cancer Research. He is a member of the European LeukemiaNet working groups on CML and AML. Dr. Larson has published more than 400 papers, reviews, and book chapters on clinical and laboratory studies in human leukemias and, in addition, has served on the editorial boards of *Blood*, the *Journal of Clinical Oncology*, and *Leukemia*. He is a *Leukemia* Section Editor and one of three Editors-in-chief for Hematology for *UpToDate*.

Dr. Larson maintains an active clinical practice at the University of Chicago Medical Center, and his expertise is widely sought for consultations regarding leukemia and the myelodysplastic syndromes. He participates actively in the training of 22 fellows in hematology/oncology within the Department of Medicine. His current research interests include clinical trials in acute and chronic leukemias and stem-cell transplantation, experimental therapeutics, the determinants of response to therapy in leukemia and myelodysplastic syndromes, and the etiology of therapy-related leukemias.

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# Is there a Best TKI for Chronic Phase CML?

**Richard A. Larson**

*Department of Medicine and Comprehensive Cancer Center, The University of Chicago, Chicago Illinois, USA*

A 58 year old man presented with asymptomatic leukocytosis. His past medical history included hypertension, adequately controlled with metoprolol, and diabetes for which he took metformin. His physical exam was notable only for an enlarged spleen palpable 5 cm below the left costal margin. His white blood cell count was 128,000/ul with a predominance of neutrophilic cells, 2% blasts, 3% eosinophils, and 2% basophils. His hemoglobin was 13 g/dl and platelet count 640,000/ul. Qualitative RT-PCR for BCR/ABL1 on blood was positive for the p210 transcript. A bone marrow exam confirmed the diagnosis of chronic phase CML. His karyotype was 100% 46 XY,t(9;22). His QT interval on electrocardiogram was within normal limits. His clinical risk score was intermediate (Sokal and Hasford) or low (EUTOS).

Is there a best TKI for this patient with newly diagnosed chronic myeloid leukemia (CML) in chronic phase? The development of tyrosine kinase inhibitors (TKIs) over the past 16 years has dramatically altered the management as well as the outcomes for patients with every stage of Philadelphia chromosome-positive (Ph+), BCR-ABL1+, CML. Over a relatively short period of time, treatment recommendations have evolved from allogeneic hematopoietic cell transplantation (allo-HCT) early in the disease course or recombinant interferon-alfa (rIFN $\alpha$ ), to the availability of 5 oral, generally well-tolerated and highly effective TKIs. Three (imatinib, dasatinib, and nilotinib) are approved for front-line use. Two others (bosutinib and ponatinib) are approved for intolerance or failure of prior TKI therapy. How should these agents be used for an individual patient to ensure the best possible duration and quality of life, to avoid treatment-related complications, and potentially to achieve a cure at an affordable cost? Evidence-based care requires an understanding of the optimal use of these drugs, their specific early and late toxicities, the prognostic significance of achieving treatment milestones, and the critical importance of monitoring.

Because CML patients may need to continue TKI therapy indefinitely, the long-term safety

of each treatment option must be considered. Comprehensive data on both safety and efficacy are now available for imatinib after more than 10 years use as initial therapy and after 5-6 years for dasatinib and nilotinib. Long-term TKI therapy can lead to the development of adverse events (AEs) that differ from those seen soon after initiating therapy. In addition, as patients age, concurrent illnesses may develop or preexisting conditions may progress and become clinically apparent. Thus, physicians are called upon to choose among various treatment options in order to recommend the optimal therapy for each individual patient. Such decisions are informed by a detailed understanding of the distinct benefits and risks of each agent, along with careful consideration of patient-specific factors such as age, comorbidities, and personal preferences.

With each of the TKIs, a rapid response has been shown to correlate with more favorable longer term clinical outcomes, both in frontline use and also after imatinib failures. There has been increasing interest in validating early molecular assessments as predictors of long-term outcomes. Data from multiple frontline trials indicate that a rapid decline of the BCR/ABL1 transcript level in peripheral blood cells at 3 (<10%IS) or 6 months (<1%IS) is correlated with higher rates of subsequent major molecular responses (MMR) and with better overall survival. Other measurements, such as a half-log reduction of baseline BCR-ABL1 transcript levels or a halving time  $\leq$ 76 days, emphasize the dynamic nature of this process. However, it is not yet clear whether altering therapy based on the lack of an early molecular response (EMR) leads to better outcomes, although this seems like a reasonable strategy to consider.

Although there was initial concern that responses to TKIs would be quickly followed by the emergence of drug resistance in CML and subsequent progression to accelerated phase or blast crisis (AP/BC), in fact, progression events have been relatively uncommon among subjects who were closely monitored on prospective studies. For most patients, molecular responses become deeper as treatment continues. This may be due in part to repeated

emphasis from treating physicians and nurses that patients maintain adherence to daily dosing. Missing as little as 10% of one's imatinib doses has led to markedly reduced rates of MMR. Similar data are not available for second generation TKIs.

Most progression events have occurred during the first 2-3 years after initiating TKI therapy. In the ENESTnd trial, the incidence of such events after 6 years was 0.7% for those who remained on nilotinib 300 mg BID (2/282) compared to 4.2% for those on imatinib (12/283) [p=0.006]. Including those patients who had discontinued their initial protocol therapy but remained in follow-up on study, progression occurred in 3.9% of those who had been randomized to the nilotinib 300 mg BID arm (11/282) compared with 7.4% of those on the imatinib arm (21/283) [p=0.07].

Overall, mutations acquired in the ABL1 kinase binding domain account for most cases of loss of response to TKI therapy, although progression may occur in the absence of detectable new mutations. Patients presenting in chronic phase rarely, if ever, have detectable baseline mutations. Nilotinib was more effective in reducing the development of mutations compared to imatinib in frontline use. After a minimum follow-up of 2 years on the ENESTnd trial, twice as many patients had mutations detected while on imatinib (20; 7.1%) than on nilotinib 300 mg BID (10; 3.5%); the majority of these mutations occurred in patients with intermediate or high Sokal scores. Most mutations (13; 65%) emerging on imatinib were known to be imatinib-resistant but still nilotinib-sensitive compared with only 2 patients (20%) on nilotinib 300 mg BID. The T315I mutation was rarely observed on either nilotinib or imatinib frontline treatment and mostly occurred in patients with high Sokal risk.

Side effects from TKI therapy are rarely severe, and perhaps for that reason, they tend to be minimized by clinicians. However, low grade toxicities from a therapy that patients may need indefinitely can impact adherence and thus overall outcomes. Compared to patient-reported outcomes, physicians tend to underestimate symptom severity and overestimate the overall health status of CML patients. In a large Italian study, symptom severity was most often underestimated for fatigue (51%), muscle cramps (49%), and musculoskeletal pain (42%).

Initial treatment with any of these potent BCR/ABL1 kinase inhibitors frequently leads to pancytopenia since at diagnosis blood cells from all 3 lineages are predominantly derived from the Ph<sup>+</sup> leukemia stem cell. After the disease recedes and normal

hematopoiesis recovers, these TKIs rarely cause clinically significant myelosuppression. Whether TKI therapy should be transiently suspended during early pancytopenia is uncertain, and some experts prefer to continue the TKI therapy uninterrupted and to support patients with transfusions and filgrastim until normal blood counts recover.

The spectrum of early drug-related adverse effects varies between the available TKIs. Pre-existing co-morbidities such as gastritis, gastrointestinal syndromes, hyperglycemia, fluid retention, or liver dysfunction may be made worse by some agents but less so by others. Physician judgement and ancillary supportive care are required to meet the needs of individual patients.

In the near future, the choice of initial TKI is likely to be driven by two facts – one clinical (survivals appear equivalent regardless of which TKI is started initially), and the other financial (the price of generic imatinib is likely to fall to 20-30% of the branded medication). Equally important determinants for which drug to use for an individual patient include tolerance (because it influences adherence), co-morbidities and thus potential late complications, calculated risk status at diagnosis, and the achievement of EMR. Eventually, gene expression profiling may give us a better way to identify which patients require a more potent second generation TKI from the outset. For now, appropriate monitoring and the use of guidelines regarding when to switch is the key to optimizing outcomes.

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# Therapies for The Older Patient with Chronic Lymphocytic Leukemia

**Emili Montserrat**

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**C**hronic lymphocytic leukemia (CLL) mainly affects older individuals. The median age of patients at diagnosis is 72 years, and the incidence of the disease increases with age, from 5/100,000 in the whole CLL population to over 20/100,000 in persons older than 70 years. While important progress has been made in the treatment of younger patients with CLL, the management of older subjects remains difficult because of the lack of truly effective therapies for this age group. Now, a number of new, non-cytotoxic agents notably active in the treatment of CLL (i.e. Bruton's tyrosine-kinase [BTK] inhibitors and phosphatidylinositol-3-kinases [PIK] inhibitors) are changing the treatment possibilities in CLL, including in elderly subjects. A number of questions are worth to be considered when dealing with CLL therapy in the elderly.

Who is an older person? Although defining who is an old person is difficult and subject to permanent revision, 65-70 years of age is usually the cut-off used to separate "younger" from "older" people. Of note, CLL is a disease which primarily affects subjects older than 70 years (> 50% of patients). It is often emphasized that age in itself is not a criterion to decide type of therapy, with "biological" rather than "chronological" being more important to advice therapy. To that end, performance status, comorbidity, and frailty need to be taken into account. Although a specific system for fitness evaluation in patients with CLL does not exist, a CIRS score > 6 is used in some studies to identify patients in whom intensive treatments are inappropriate. Likewise, a poor renal function (i.e. creatinine clearance < 70 ml./min.) conveys unacceptable toxicity to standard fludarabine-based treatment regimens. As a result, only a small proportion of subjects older than 65-70 years can safely receive cytotoxic therapy; this is a fact worth to be taken into account because of the steadily increasing proportion of persons > 65 years-old, which is currently of 20% both in Europe and the U.S.

What are the goals of therapy? Treatment aims are to improve quality of life and to prolong survival; treatment goals should be kept separated from

treatment endpoints as used in trials (e.g. response rate and its quality). There is however evidence that achieving response with no detectable residual disease (e.g. MRD-negative CR) results in a longer progression-free and overall survival.

Is there any role for chlorambucil? Chlorambucil yields a small proportion of complete responses (around 5%) and although it improves symptoms, survival is only slightly affected. Because of this, chlorambucil is usually given to patients not able to tolerate more effective therapies. The dose of chlorambucil given ranges from 40 to 70 mg/m<sup>2</sup> per month and is given intermittently. In studies comparing chlorambucil vs. fludarabine or bendamustine, the response rate and PFS was found to be better with the newer agents; however no differences in overall survival were detected. Based on a recently finalized trial in which fludarabine, cyclophosphamide, and rituximab (FCR) was compared to bendamustine + rituximab (BR), BR might be useful in older but fit patients. The addition of rituximab to chlorambucil results in better responses and longer PFS than chlorambucil alone. However, it is unclear whether survival is improved. Adding novel anti-CD20 monoclonal antibodies (obinutuzumab or ofatumumab) to chlorambucil results in an even greater improvement in response rates and PFS, and initial results suggest that OS might be improved with obinutuzumab + chlorambucil.

Is there any role for combination chemotherapy? Even in "fit" older patients standard chemotherapy (or chemoimmunotherapy) conveys important toxicity, and many patients cannot receive all planned therapy. "Low-dose" therapy could be a reasonable alternative to treat unfit patients, but unfortunately there are very few studies investigating this approach. However, it has been shown that the so-called FCR-lite regimen may yield a substantial proportion of overall responses, including CRs. Low-dose therapy does warrant further investigation.

What are the results with targeted, non-cytotoxic therapies? In the US, but not in Europe, rituximab is frequently given as single agent to treat

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patients who are not good candidates for chemoimmunotherapy. Likewise, lenalidomide (an immunomodulatory agent not approved by international agencies for CLL therapy) either alone or combined with rituximab, has been reported to produce good treatment results.

The better understanding of the biology of CLL has facilitated the development of agents which target CLL physiopathologic pathways: the delta isoform of phosphatidylinositol-3-kinase (PI3K), which is inhibited by idelalisib; and the Bruton's tyrosine kinase (BTK), which is irreversibly inhibited by ibrutinib. A feature of inhibiting these signals is the re-distribution of CLL cells from the tissue to the blood upon therapy. This is revealed by a very rapid reduction in nodal disease and an increase in peripheral blood lymphocytes which can take several months to resolve. In contrast to what happens with cytotoxic agents, the proportion of CRs with novel therapies is low (10%-20%). However, the PFS rate is high (60%-90% at 1-3 years). This observation, that in some way challenges the paradigm that a MRD-negative response is necessary to prolong survival, needs to be put in the context of both ibrutinib and idelalisib the treatment schedule (i.e. continuous therapy). Treatment discontinuation results in disease progression that in some instances can be extremely difficult to control. In some cases of progression upon ibrutinib therapy, BTK mutations, as well as BTK downstream mutations (i.e. PLCy2) are observed.

In spite of these caveats, these agents are effective, even in high-risk disease. The reported response rates in patients with relapsed/refractory CLL (including the presence of del(17p)/TP53 mutations) are good. The ORR for ibrutinib and idelalisib, are 48%-71% and 39%, respectively, if used as monotherapy in relapsed/refractory patients. If "partial remissions with persistent lymphocytosis" are considered, the response rate to ibrutinib is over 90%. Similarly, ibrutinib monotherapy resulted in significantly better ORR, PFS and OS when randomized against ofatumumab in a prospective trial enrolling 391 patients with relapsed/refractory CLL. Although ibrutinib is effective in patients with del(17p)/TP53 mutations, all parameters measuring treatment effectiveness (i.e. CR, PFS, OS) are inferior in these patients as compared to those with no high-risk genetics. Ibrutinib is an agent approved by both the Food and Drug Administration (FDA) and the European Medicine Agency (EMA) as second-line therapy and also as first-line treatment in patients with del(17p)/TP53 mutations.

The ORR is further increased by combining these agents with anti-CD20 antibodies. A randomized

trial involving 220 patients with relapsed CLL including 44% with del(17p)/TP53 mutations compared idelalisib + rituximab to placebo + rituximab. The ORR rates (81% vs. 13%) and 6-month PFS (93% vs. 46%) strongly favored the idelalisib + rituximab arm. Based on these results the FDA and the EMA approved the combination of idelalisib + rituximab as treatment for CLL in relapse.

Although these agents have a relatively favorable toxicity profile, adverse events can be observed. The most common negative effects of ibrutinib are fatigue, neutropenia, infections, hypertension, bleeding, diarrhea, and atrial fibrillation. Idelalisib may induce gastrointestinal disorders (diarrhea, colitis, intestinal perforation), hepatotoxicity (which in some cases is extremely severe), infections, and pneumonitis.

Where from now? The availability of new agents, particularly BTK-inhibitors (ibrutinib) and PI3K-inhibitors (idelalisib), is rapidly changing the CLL treatment landscape. Because of their relatively favorable toxicity, these compounds can be safely given to elderly or unfit patients. The importance of these new therapies cannot be overrated, particularly because the majority of patients with CLL are old or fragile. The effectiveness of ibrutinib and idelalisib is increased by combining these agents with anti-CD20 MoAbs, and there are also preliminary data suggesting that they can act synergistically when given together; there is also proof that their effectiveness can be potentiated by several agents, for example the XPO1 inhibitor Selixenor. More trials with long follow up are needed to better ascertain the long-term effectiveness, adverse events, clinical behavior of the disease upon progression, and efficacy of salvage treatments.

As the biology of CLL is and key physiopathological pathways discovered, it is plausible that in the near future "biological-only-therapies" will gain increasing importance in the management of all patients with CLL. In this context, treatment cost is an important issue that should be urgently addressed by health-systems authorities and pharma companies. Finally, it is important to remember that, independently of the treatment modality, the goals of therapy are to improve general status and to prolong survival. Furthermore, whenever possible patients should be included in well-designed and large clinical studies to continue making progress in the treatment of this still incurable disease.

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## **Eva Kimby**

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Dr Kimby studied medicine at Karolinska Institutet in Stockholm, where she obtained her M.D., Ph.D. and Professor degrees. Dr Kimby is supervisor for Ph.D. students and lecturer at the Karolinska Institute and in many international meetings on CLL and lymphomas. Her main research interests are indolent lymphoma, CLL and the applications of immunotherapy. She is actively involved as a key investigator in several haematology trial groups and the principal investigator for two large Nordic rituximab +/- interferon trials and co-chair with Prof Zucca in a SAKK-NLG collaborative trial rituximab +/- lenalidomide.

Dr Kimby was elected chairman of the group for indolent lymphomas within the Swedish and Nordic Lymphoma Group in 1997 and was the first chairman of the Swedish CLLL group. Dr Kimby is also a member of several international scientific societies and vice president in ERIC (European Research Initiative on CLL), working member of the EBMT CLL/lymphoma subcommittees and of the International Workshop on Waldenström's macroglobulinemia and has an Advisory role on the Board of European Network Mantle cell lymphoma. She has published more than 140 papers and several reviews in peer-reviewed international journals.



## **Peter Dreger**

Peter Dreger started his scientific career doing basic research in experimental bone marrow transplantation. After joining the Second Department of Medicine at the University of Kiel, he established a scientific program of experimental and clinical blood stem cell transplantation together with Norbert Schmitz. Significant contributions were made in the fields of allogeneic peripheral blood stem cell transplantation and allogeneic and autologous transplantation for lymphoma and CLL.

In 2005 he accepted the position of a Professor and Head of the Division of Stem Cell Transplantation at the University of Heidelberg. Peter Dreger is founding member of the German CLL Study Group (Responsibility: Transplant studies). He has worked with the EBMT for many years and served as chairman of the CLL subcommittee of the EBMT Chronic Leukemia WP from 2005-2010. Since March 2010, he is chairman of the EBMT Lymphoma Working Party. His current scientific focus is on integrating targeting drugs into the treatment algorithm for high-risk CLL and lymphoma.



## Anna Sureda

Anna Sureda, (MD, PhD) graduated with a degree in Medicine from the Autonomous University of Madrid in 1986 and completed her residency in Haematology at the Hospital Ramón y Cajal of Madrid in 1990. In January/1991 she started working in the Clinical Haematology Division of the Department of Haematology at Hospital de la Santa Creu i Sant Pau in Barcelona where she was appointed Head of the Outpatient Department in 2002. In January 2011, Anna Sureda moved to Cambridge University Hospital in Cambridge, UK, Addenbrookes Hospital, where she was appointed Senior Consultant in Lymphomas and Stem Cell Transplantation. In January 2013, she moved back to Barcelona where she is working as Senior Consultant in the Haematology Department of Institut Català d'Oncologia – Hospital Duran I Reynals. She was a Visiting Physician at the University of Heidelberg, Germany in 1990 for four months and at the Fred Hutchinson Cancer Research Center of Seattle in 1993 for another period of four months.

Anna Sureda has focused her career on clinical investigations into the treatment of Hodgkin's lymphoma, non-Hodgkin's lymphoma and multiple myeloma patients evaluating novel therapies such as immunotherapy combined with stem-cell transplantation. Throughout the course of her investigations she has participated in many phase II and III clinical trials for lymphoma patients. As a result of part of her clinical investigations, she achieved her PhD with the work entitled "Autologous Stem Cell Transplantation in Patients with Hodgkin's Lymphoma" in June/2008. Dr. Sureda has been an active member of the Spanish Cooperative Group of Lymphomas and Haematopoietic Stem Cell Transplantation (GELTAMO) since 1993 and during her stay in UK she was elected active member of the NCRN Lymphoma Study Group. In April/2004 she was appointed Chairperson of the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation (EBMT). Since then, her main focus of interest has been the analysis of the results and prognostic factors of autologous and allogeneic stem cell transplantation in lymphoid malignancies. After stepping down as chairperson of the Lymphoma Working Party Anna Sureda was elected Secretary of the EBMT, her current position in this organization right now.

Anna Sureda is a regular reviewer for the journals Blood, Annals of Oncology, Bone Marrow Transplantation, The Hematology Journal, The European Journal of Hematology and Annals of Hematology and has been co-authored more than 250 peer-reviewed journal articles.



## Norbert Schmitz

Norbert Schmitz is Professor of Medicine at Christian-Albrechts-University of Kiel, Kiel, Germany. Dr. Schmitz serves as Chairman of the German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL) and as Chairman of the T-cell subcommittee of the European Group for Blood and Marrow Transplantation (EBMT) Lymphoma Working Party. He was President of the Annual Meeting of EBMT 2006 and President of the Annual Meeting of the German, Austrian and Swiss Societies of Hematology and Oncology 2014, both in Hamburg, Germany. He is a member of the German Society of Hematology and Oncology, the European Haematology Association, and the American Society of Hematology.

His main scientific interest is transplantation of hematopoietic stem cells and lymphoma. Dr. Schmitz has authored numerous scientific abstracts and book chapters and has published more than 300 scientific papers in national and international peer-reviewed journals, including *The Lancet*, *The Lancet Oncology*, *Journal of Clinical Oncology*, *Blood*, *Journal of Immunology*, *British Journal of Haematology*, *Haematologica*, and *Leukemia*. He is a member of the Editorial Board of *Annals of Oncology*. Dr. Schmitz received his medical degree from the University of Giessen, Giessen, Germany. He completed his Ph.D. at the Christian-Albrechts-University in Kiel, Kiel, Germany. Dr. Schmitz is board certified in Internal Medicine and Hematology. He is the director of the Department of Hematology, Oncology, and Stem Cell Transplantation at Asklepios Hospital, Hamburg, Germany.

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# Treatment of T-Cell Lymphoma in The Era of New Drugs

**Norbert Schmitz**

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Survival after conventional first-line therapy with CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) sometimes combined with etoposide (CHOEP) is not satisfactory in most patients with peripheral T-cell lymphoma (pTCL). Other chemotherapy regimens including gemcitabine, bendamustine, or methotrexate have been used; convincing evidence that any of these drugs or combinations thereof improve survival is not available. High-dose therapy followed by autologous stem cell transplantation (HDT/ autoSCT) or allogeneic transplantation of hematopoietic stem cells (alloSCT) gave promising results in selected patients with relapsed/ refractory pTCL. Randomized studies demonstrating the superiority of HDT/ autoSCT over conventional therapy when used as consolidation of CR/ PR achieved with first-line therapy in chemosensitive patients do not exist. The role of allogeneic transplantation as part of first-line therapy remains unclear because both prospective, randomized studies comparing HDT/ autoSCT with alloSCT were inconclusive (Corradini et al., Leukemia 2014, and Schmitz et al., 2015 submitted). All first-line studies reported so far revealed one major problem: up to one third of patients progressed while under

treatment or relapsed shortly thereafter. This problem needs to be addressed in order to improve treatment results for patients with T-cell lymphoma. Recently, a number of new drugs have been used to increase response rates and improve survival of T-cell lymphoma patients. Among others, pralatrexate, romidepsin, belinostat, and alisertib showed response rates between 25 and 50 % in relapsed and refractory patients with pTCL. These data led to FDA approval of pralatrexate, romidepsin and recently also of belinostat in the US; none of these drugs is licensed in the EU. Single agent activity of these drugs in the first-line setting is unknown, a study combining romidepsin with CHOP for primary treatment of pTCL is ongoing. Brentuximab-vedotin (BV) is a CD 30-targeted antibody-drug conjugate with remarkable activity (ORR 84 %) in CD 30-positive anaplastic large cell lymphomas (ALCL, ALK-pos. and ALK-neg.). This molecule can successfully be used to bridge relapsing patients to autologous and allogeneic transplantation. If BV improves outcome of patients with pTCL other than ALCL, can be integrated into first-line therapy of pTCL, or can be used to maintain remission after autoSCT or alloSCT needs further study.



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# Current Therapy for Primary CNS Lymphoma

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**P**rimarily central nervous system lymphoma (PCNSL) is an aggressive Non-Hodgkin Lymphoma (NHL) mostly of B-cell origin, which exclusively invades the CNS compartment. It accounts for 3% to 4% of all primary brain tumors and 4% to 6% of extra-nodal lymphomas. The incidence of PCNSL in immunocompetent patients has been steadily increasing over the last 30 years. However, despite treatment improvement over the last decades, the outcome of PCNSL patients is still poor compared to systemic NHL. Well-established prognostic factors are age and clinical performance status. Further prognostic factors are serum LDH, involvement of deep brain structures, elevated protein in the cerebrospinal fluid. High-dose methotrexate (HD-MTX) in combination with HD-cytarabine (HD-AraC) is the backbone of current treatment. A recent randomized controlled trial investigated the role of whole brain radiotherapy (WBRT) as consolidation compared to no consolidation therapy, suggesting that WBRT does not prolong survival but enhances disease control. To circumvent WBRT, which is associated with significant neurotoxicity,

high-dose chemotherapy with autologous stem cell support (HCT-ASCT) has been investigated in several single arm studies for first-line and salvage therapy. This approach can lead to high remission rates and very good long-term outcome in eligible patients. However, whether HCT-ASCT is superior to optimized conventional chemotherapy in the first-line setting is unclear; a current randomized trial investigates this question. Elderly patients 65 years of age or older constitute about 50% of all PCNSL cases. Most of them cannot be treated with HCT-ASCT, however, HD-MTX based chemotherapy plus an oral alkylating agent (e.g. procarbazine) should always be considered if possible. A recent randomized trial suggested that HD-MTX in combination with procarbazine, vincristine, and cytarabine (MPV-A) might be superior to HD-MTX plus temozolomide in PCNSL patients  $\geq 60$  years; however, statistically significant differences could not be shown in this phase II trial. Further prospective clinical trials are urgently needed to improve outcome and define standard treatments according to age and clinical performance status.



## Jane N. Winter

Dr. Jane N. Winter is Professor of Medicine in the Division of Hematology/Oncology, Feinberg School of Medicine, Northwestern University and a member of the Robert H. Lurie Comprehensive Cancer Center. She is a graduate of Bryn Mawr College and the University of Pennsylvania School of Medicine. Following internship and residency in Internal Medicine at the University of Chicago, she completed fellowships in hematology/oncology at Columbia-Presbyterian Hospital in New York, and Northwestern University in Chicago. She is board certified in both Hematology and Medical Oncology. Dr. Winter has been a member of the Northwestern University faculty since completing fellowship.

Dr. Winter's clinical and research interests focus on the malignant lymphomas (both Hodgkin and non-Hodgkin), particularly the development of novel therapies, including immunotherapy, new targeted agents, and radioimmunotherapy, and the investigation of clinical and biologic correlates of prognosis. Most recently, she developed a new enhanced prognostic scoring system for DLBCL, the NCCN-IPI. She has extensive experience as a clinical investigator, translational researcher and educator/mentor. She has chaired cooperative group clinical trials, institutional trials and multi-institutional collaborations, including phase I and phase II trials. For more than a decade, she chaired the funded correlative science projects related to the US Intergroup trials in diffuse, large B-cell lymphoma, focusing on prognostic indicators. Dr. Winter is a long-standing member of the Lymphoma Steering Committee for the Eastern Cooperative Oncology Group and previously served as its co-chair and as chairman of its Laboratory Subcommittee.

In her role as an educator, Dr. Winter has mentored numerous fellows, residents and students, and served as a faculty member of the American Society of Hematology's (ASH) Clinical Research Training Institute and the ASH/European Hematology Association's Translational Research Training in Hematology. In addition, she has co-chaired the annual ASH Educational Program, the Highlights of ASH program, and the Best of ASH and now serves as Chairman for the Educational Affairs Committee. She served a six year term as one of the nine members of the Hematology Board of the American Board of Internal Medicine responsible for writing the certifying examination in Hematology and continues as a member of the Self-Evaluation Program. She has served as a member of the editorial board of the Journal of Clinical Oncology and serves as a reviewer for numerous journals including the New England Journal of Medicine, BLOOD, Clinical Cancer Research and others. She is a member of the NHLBI's Data and Safety Monitoring Board for the Bone Marrow Transplant-Clinical Trials Network, and has served as a member of the National Comprehensive Cancer Network Hodgkin Lymphoma Guidelines Committee since its inception. Dr. Winter is a trustee of the Leukemia/Lymphoma Society of Illinois.

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# Treatment Of Transformed Follicular Lymphoma

**Jane N. Winter**

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A significant percentage of patients with follicular lymphoma (FL) will experience transformation to a high-grade aggressive non-Hodgkin Lymphoma (NHL), most often diffuse large B-cell lymphoma (DLBCL). The biologic basis of transformation is the subject of current investigation and serves as a model for lymphomagenesis. Genetic analysis suggests that in many cases of transformation, the aggressive clone derives from a common progenitor rather than from the indolent clone, and that this process requires multiple molecular events.

A MYC rearrangement has been linked to transformation. When arising in the 85% of patients with FL and a t(14 ;18), they are by definition « double hit lymphomas ». As many as a third of double hit lymphomas may arise from a previous FL, and have an especially poor prognosis. It is important to identify this subset of patients for prospective clinical trials.

The rate of transformation has been estimated to be 2-3%/year in most studies. It is likely that some of the variation among series derive from differences in pathologic criteria for transformation as well as requirements for diagnosis (biopsy versus clinical features). Most studies suggest that this risk continues over the course of the disease, while a few suggest that the risk plateaus. Most but not all studies show that early treatment with rituximab and/or anthracycline does not alter this risk. Multiple phase three randomized trials have shown that rituximab improves the overall survival of previously untreated patients with FL when combined with chemotherapy, but its true impact on the rate of transformation may not be known for more than another decade or more. Limited followup of the “Watch and Wait versus rituximab” trial has not shown a difference in the occurrence of transformation.

Clinical outcomes for patients with transformed follicular lymphoma have improved in the rituximab era from 1–2 years to 4–5 years. In at least one study of R-CHOP therapy, outcomes for transformed FL patients were similar to those of

patients with *de novo* DLBCL treated similarly. Overall, patients who are chemotherapy naïve at the time of transformation do better than those previously treated. The role of consolidative autologous stem cell transplant (ASCT) in the treatment of transformed FL is unclear. In the pre-rituximab era, clinical trials with limited numbers of patients and retrospective analyses appeared to show benefit to ASCT. Outcomes from more recent trials of ASCT in the rituximab era show improvements in progression-free survival and overall survival compared to earlier studies. Outcomes however, for rituximab-naïve patients or previously untreated patients are quite good with chemo-immunotherapy (e.g. R-CHOP) alone. It appears likely that outcomes for this population may be the same with or without consolidative ASCT. A recent report from the CIBMTR shows a decline in ASCT and a rise in allografting for transformed FL, but overall the numbers are very small suggesting that very few centers are routinely transplanting these patients.

Our approach to transformed FL is personalized. FISH is performed for MYC and BCL2 to identify the double hit lymphomas. These patients are treated with DA-EPOCH-R unless previously treated with an anthracycline-containing regimen. Patients who do not have double hit lymphomas and are chemotherapy naïve are treated with R-CHOP. If they achieve a complete remission, they are often treated with maintenance rituximab for two years, although there is no evidence to support maintenance in this setting. Those who have been previously treated with chemotherapy will receive secondline R-chemotherapy and an autoPSCT. In selected cases, an allogeneic transplant is preferred (extensive marrow involvement, young age, available donor).

Many new targeted agents are under study for the non-Hodgkin lymphomas. Unfortunately relatively few early phase studies have enrolled sufficient numbers of patients with transformed FL to draw conclusions regarding efficacy.

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### **Recommended Reading**

Casulo C, Burack WR, and Friedberg JW: Transformed follicular non-Hodgkin lymphoma; *BLOOD* 2015, 125:40-47.

Pasqualucci L, Khiabani H, Fangazio M, et al. Genetics of follicular lymphoma transformation. *Cell Reports* 2014; 6: 130-140.

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**5<sup>th</sup> International Congress on  
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**ABSTRACTS**



## Acute Lymphoblastic Leukemia

PS-001

Abstract:0102

### A RELAPSE / REFRACTORY ALL CASE WHO WAS TREATED WITH BLINATUMOMAB

Umit Yavuz Malkan<sup>1</sup>, Gürsel Güneş<sup>1</sup>, Sezgin Etgül<sup>1</sup>, Tuncay Aslan<sup>1</sup>, Seda Balaban<sup>1</sup>, Denizcan Güven<sup>2</sup>, Yusuf Şener<sup>2</sup>, İbrahim Celalettin Haznedaroğlu<sup>1</sup>  
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**Introduction:** 80% of the de novo acute lymphocytic leukemia (ALL) patients who were given induction chemotherapy, achieve complete remission. After consolidation and maintenance therapy most of the cases have relapse. Also, 20% of cases have refractory disease. Blinatumomab is a monoclonal antibody that targets CD3 and CD19. Herein, we aimed to report an ALL case who had refractory disease after bone marrow transplantation (BMT) and was treated with Blinatumomab.

**Case Report:** 22 year old woman had diagnosed as ALL in May 2012. She was given BFM 95 chemotherapy. Bone marrow investigation in January 2013 revealed complete remission. She had gone bone marrow transplantation from her brother in March 2013. After this, relapse was detected in her control bone marrow investigation. Because of numbness in the lips cranial MR was performed that revealed central nervous system involvement of the disease. Thereupon spinal MR was performed which revealed vertebral involvement. Also, renal involvement was detected in abdominal tomography which was performed because of the investigation for pelvic pain. In June 2014, she was given Hyper-CVAD chemotherapy. Bone marrow investigation in November 2014 was reported as diffuse blastic infiltration, therefore blinatumomab treatment was planned. Blinatumomab was started in 27 November 2014 which were given for 29 days by continuous intravenous infusion. For the first 8 days blinatumomab was given 9 mcg/day and for the 9-29th days it was given as 28 mcg/day. Myalgia and sub febrile fever were observed. In the two weeks period of without treatment, our patient had sepsis and her general condition was impaired. So, we was transferred to intensive care unit where she was treated right now.

**Discussion:** Blinatumomab is administrated by continuously and intravenously. In the first week it is started with low dose as 9 mcg/day. After 9th day, dose is increased to 28 mcg/day till day 29th. Treatment is given as cycles in which there is two week of no treatment period. If patient has response after two cycles, third cycle is started. In literature the most serious side effects was reported as cytokine release syndrome and some neurologic toxicities. In our case we have experienced myalgia and sub febrile fever. In one study, 69% of relapsed / refractory ALL cases had remission and 52% had undergone BMT after blinatumomab treatment. In the same study, response without minimal residual disease were calculated as 72% for the first cycle, 84% for the second cycle and 88% for the third cycle of blinatumomab treatment. To conclude, our patient did not responded to first cycle of the blinatumomab treatment and could not continue to second cycle because of the sepsis.

**Keywords:** Blinatumomab, acute lymphoblastic leukemia

PS-002

Abstract:0176

### SERUM LEVELS OF SELECTED CYTOKINES AND CYTOKINE RECEPTORS IN PATIENTS WITH NEWLY DIAGNOSED ACUTE LYMPHOBLASTIC LEUKEMIA

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**Background:** Cytokines have been studied as markers of immune system activation in various diseases including hematological malignancies. Alterations in this interacting functional network may have direct effect on the malignant cells or have indirect effect on leukemogenesis through altered functions of bone marrow stromal elements. The knowledge gained from multi-analytical determination of cytokines could allow better diagnosis and management of hematological malignancies, since cytokines or their receptors may also represent a target for specific anticancer therapy at the molecular level. Recently, some studies reported the possible diagnostic and prognostic use of cytokine levels in newly diagnosed acute leukemias.

**Objectives:** The aim of our study was to evaluate serum levels of selected cytokines and cytokine receptors in patients with newly diagnosed acute lymphoblastic leukemia (ALL) and in healthy subjects using the innovative biochip array technology. This approach allows simultaneous detection of multiple cytokines from a single sample.

**Methods:** Serum samples of 21 newly diagnosed ALL patients (median age 46, range 24 - 75 years, 17 males and 4 females, 20 B-ALL, 1 T-ALL) and 15 healthy subjects (median age 41, range 25 - 58 years, 11 males and 4 females) were analyzed. We evaluated serum levels of the following analytes: interleukin-5 (IL-5), interleukin-15 (IL-15), granulocyte macrophage colony stimulating factor (GM-CSF), macrophage inflammatory protein-1 alpha (MIP-1 alpha), soluble IL-2 receptor alpha (sIL-2R alpha), soluble IL-6 receptor (sIL-6R), soluble tumour necrosis factor receptor I (sTNFR-I), soluble tumour necrosis factor receptor II (sTNFR-II), matrix metalloproteinase-9 (MMP-9). All analytes were measured by biochip array technology using chemiluminescent sandwich immunoassays applied to the Evidence Investigator analyzer (Randox). Probability values (p) < 0.01 were considered statistically significant.

**Results:** In newly diagnosed ALL patients, we found significant increase in serum IL-15 (1.74 ± 0.97 ng/L vs. 0.81 ± 0.16 ng/L; p = 0.0008), MIP-1 alpha (6.36 ± 3.26 ng/L vs. 2.68 ± 1.47 ng/L; p = 0.0003), sIL-6R (2.29 ± 1.80 mcg/L vs. 0.89 ± 0.39 mcg/L; p = 0.006), sTNFR-I (0.96 ± 0.50 mcg/L vs. 0.25 ± 0.07 mcg/L; p = 0.000005), sTNFR-II (0.73 ± 0.51 mcg/L vs. 0.29 ± 0.14 mcg/L; p = 0.003). Serum levels of other evaluated analytes were without significant differences.

Conclusion: Our results indicate that serum levels of some cytokines and cytokine receptors (IL-15, MIP-1 alpha, sIL-6R, sTNFR-I, sTNFR-II) are significantly altered in patients with newly diagnosed ALL, reflecting activity of the disease. Further investigation is needed to establish if the alterations observed in the levels of these molecules could be used as a prognostic indicator for ALL.

The work was supported by a long-term organization development plan 1011 (FMHS).

**Keywords:** Cytokines, ALL

PS-003

Abstract:0191

### CASE OF CMV RETINITIS IN A PATIENT OF ACUTE LYMPHOBLASTIC LEUKEMIA ON MAINTENANCE THERAPY

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Introduction: Cytomegalovirus (CMV) is a DNA virus of the viral family known as Herpesviridae. It usually presents with an asymptomatic but may also present with esophagitis, pneumonitis, retinitis, hepatitis, encephalitis. It mostly is reported as HIV related. Other than this, it can present itself secondary to chemotherapy or in immunocompromised hosts, patients who had solid organ transplantation or bone marrow transplantation. In this study, we aim to present the case of CMV retinitis (CMVR) in a case of ALL on maintenance therapy.

Case: 51-year-old male patient with Pre-B ALL who was given 8 courses of Hyper-CVAD chemotherapy and 8 doses of intrathecal methotrexate was confirmed to be in remission with bone marrow biopsy, aspiration and flow cytometer analysis. The patient was started on 15 mg/week methotrexate (MTX) and 75 mg/day mercaptopurine(Mp) orally(po). In 7th month of the MTX and Mp treatment, patient presented to our clinics with increasing vision loss on left side and intermittent black-outs which started 10 days ago. Laboratory test were as follows: hemoglobin 14 gr/dl, leukocyte 2530 mm<sup>3</sup>, neutrophil 1070 mm<sup>3</sup>, platelets 139000 mm<sup>3</sup>. Bilateral retinal hemorrhage which suggests infiltration of leukemic cells most severe in the left eye and a viral retinitis with paving stone degeneration were observed in ophthalmic examination. No pathologies to suggest leukemic involvement or explain vision loss were observed in cranial and orbital MRI. Bone marrow aspiration and biopsy were performed for assessment of remission. According to viral serology; anti-HSV IgM, anti-Chlamydia IgM, anti-EBV IgM, anti-HIV were negative while CMV IgM: 15 COI (0-0.7), CMV IgG: 294 IU/mL (0-0.5). CMV IgG Avidity assay with medium sensitivity was positive and CMV DNA: 299 copy/mL peripheral blood. Patient was diagnosed as CMVR. Intravenous(iv) ganciclovir 5 mg/kg 2x1 (900 mg/d) was started. On 21st day of ganciclovir treatment, ophthalmologic examination revealed visual field was the same and retinal hemorrhage decreased partially. In the follow-up tests; CMV IgM: 23 COI (0-0.7), CMV IgG: 470 IU/mL, CMV IgG Avidity assay with high sensitivity and CMV DNA copy number was found 40/mL. Patient was started valganciclovir 5 mg/kg po 2x1

to be continued for 6 months. The patient is now being followed up while receiving valganciclovir treatment for one month.

Discussion: Ophthalmologic symptoms in leukemic patients are divided into primary and secondary. Primary symptoms are caused by lymphoblastic infiltrations. Secondary symptoms are caused by retinal hemorrhage, retinal detachment, retinitis, and conjunctivitis. CMVR is a rare complication in patients with ALL. CMVR may cause viral necrotizing retinitis and It may progress to blindness. Iv ganciclovir, iv foscarnet and oral valganciclovir are used for the treatment. Along with cranial involvement, CMV retinitis should be considered when ALL patients apply with vision loss or eye pain.

**Keywords:** CMV Retinitis, Acute Lymphoblastic Leukemia

PS-004

Abstract:0199

### TARGETING HRGR FOR THE DIAGNOSIS AND TREATMENT OF T-CELL MALIGNANCIES

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Objective: Acute lymphoblastic leukemia (ALL) is the most common cancer diagnosed in childhood with poor prognosis that appears approximately 25% of cancer diagnoses among children younger than 15 years (1). The oncogene Rgr (ral GDS related) which induces tumorigenesis in rabbit and the human orthologue of it termed hrgr (human ralGDS related) has been shown before (2). The dtr-hRgr (diseased truncated hRgr) protein produced by the abnormal transcript of hRgr, which is only observed in T-cell neoplasms, can induce cellular transformation through the activation of Ras and Ral GTPases (3,4). In this study the purpose was to initially determine eventual differences between hRgr and dtr-hrgr expression levels in subtypes of T cell malignancies. Thus we generate perspectives on inhibiting dtr-hrgr as a future treatment in T-cell ALL. As a future aspect; we aim to design a dtr-hRgr specific antibody, which may help diagnose T-ALL rapidly by immunohistochemical methods.

Materials and Method: Peripheral blood samples were obtained from the patients with T-ALL (n=4), B-ALL (n=2). Human T and B neoplastic cell lines were used as controls. Total RNA was extracted from peripheral blood samples by using trizol methods as described in the manufacturer's protocol. Gene specific oligonucleotides were used for the abnormal hRgr and human GAPDH. Relative fold changes were calculated using the 2<sup>-ΔΔCt</sup> method.

Results: qRT-PCR analysis was performed and over-expression of the abnormal hRgr transcript was only observed in T-ALL derived cell lines -CEM, Jurkat- (Figure 1a) and in human tissues with T-cell malignancies (Figure 1b).

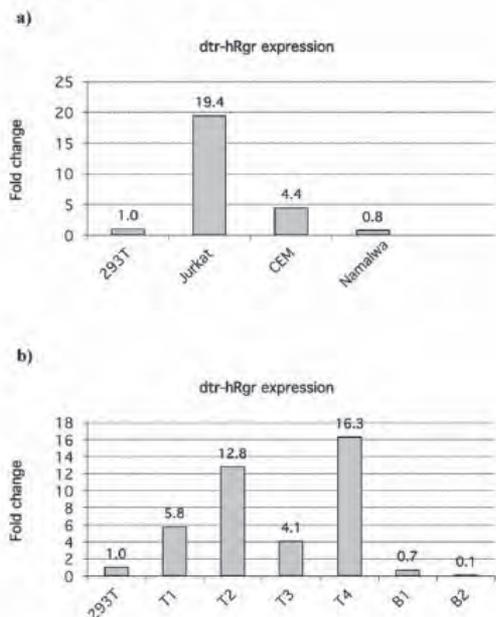
Conclusion: With aggressive treatment the prognosis for relapse in childhood T-cell ALL remains poor. In this study we demonstrate the abnormal expression of dtr-hRgr in T-cell ALL. In ALL treatment, molecular tests are important for risk classification at initial

diagnosis. dtr-hRgr may be a potential target for therapeutic approaches in future treatment in T-cell ALL in both adults and children.

Our project is supported by the TUBITAK BİDEB-2232 grant. Project No:113C035

**Keywords:** hRgr, dtr-hRgr

**Figure 1**



**Abbreviations:** 293T, Jurkat, CEM and Namalwa represent different cell lines. T1, T2, T3 and T4 represent different patients with T-ALL. B1 and B2 represent different patients with B-ALL.

PS-005

Abstract:0248

**DOWN REGULATION OF SNON/SKIL GENE IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA**

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T-cell acute lymphoblastic leukemia (T-ALL), is a severe disease that occurs in the malignant transformation of T-cells in the thymus. Here we performed whole genome expression analysis in 31 T-ALL childhood patients. In addition to some well-known targets a new target has been identified in the patient group SnoN/SKIL gene, known as a regulator of TGF-β pathway, was down regulated when compared to thymocyte controls.

Quantitative Real Time PCR and analysis of proteins by proximity ligation assay (PLA) also confirmed this down regulation in a larger patient group (n=72). In the thought of the reasons of this downregulation, we searched the possible miRNAs that target SnoN/SKIL gene via online databases. Among these miRNAs we select hsa-miR-223, which is also known to be deregulated in hematologic malignancies, and by Stemloop RT-PCR we found out that all the samples with low SnoN/SKIL expression show high hsa-miR-223 expression levels. When we block hsa-miR-223 in Molt4 cell line, by anti-mir223 LNA in vitro, SnoN/SKIL expression was leveled up. In the same group when we checked the apoptosis levels by Annexin V flow cytometry assay we found out that when we block hsa-miR-223, cells were lead to apoptosis almost 10 fold higher than untreated cells. Our study is still ongoing to validate the direct relation between SnoN/SKIL and hsa-miR-223, but these preliminary results show that SnoN/SKIL gene, which is known to have dual role in different malignancies like being oncogene or tumor suppressor, might be acting as a tumor suppressor gene in T-ALL.

This work was supported by TUBITAK Project No: 113S484

**Keywords:** T-ALL, SnoN/SKIL

PS-006

Abstract:0257

**ACUTE LYMPHOBLASTIC LEUKEMIA IN A PATIENT WITH WERNER SYNDROME**

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**Introduction:** Werner syndrome (WS) is an autosomal recessive genetic instability associated with an elevated risk of cancer. We presented a case of acute lymphoid leukemia and WS co-existence. **Case:** 54-year-old male patient was referred to our hospital with complaints of high fever, fatigue and anorexia. Physical examination revealed premature aging, short stature, gray and sparse hair, high pitch edvoice, cachectic appearance, calcification in the external ear, sclerodermic dermis, dystrophy of the nails, feet deformation and bilateral, chronic (maintained for 5years), infected achilles ulcer (Figure 1). Bone mineral density indicated osteoporosis. He was operated due to cataracts 10 years ago. In background story revealed that his brother has the same morphologic findings. The current physical examination and family history were consistent with Werner Syndrome. We confirmed the WS diagnosis using Standard diagnostic criteria suggested by International Registry of Werner Syndrome. The peripheral blood findings showed pancytopenia. Bone marrow aspiration and biopsy showed blastic infiltration. His bone marrow flow cytometry revealed pro-B lymphoblastic leukemia. His cytogenetic findings were 40-45, XY(4)/46, XY(3); t(7;10)(q11.2;q26.1). The patient was treated with HyperCVAD regimen. The patient good tolerated this regimen and complete hematologic response was obtained.

**Discussion:** Patients with WS encounter premature aging beginning in the second decade of life, including bilateral cataracts, graying and loss of hair, sclerodermal like skin changes, diabetes mellitus and osteoporosis. WS

patients are also at elevated risk for common, clinically important age dependent diseases such as cancer and atherosclerotic cardiovascular disease which are the most common causes of death at a median age of 54 years. The most frequent cancers in patients with WS are thyroid neoplasm, malignant melanoma, meningioma, soft tissue sarcomas, myeloblastic leukemia/ myelodysplastic syndromes and osteosarcoma. Among hematologic malignancies; acute megakaryoblastic leukemia, erythroleukemia, myelofibrosis and WS co-existence is mentioned. In the present case, WS and acute lymphoblastic leukemia co-existence is present. This association has not been found yet in the literature. In addition, WS is a disorder of DNA repair, thus these patients are under the risk for severe chemotherapy induced toxicity. Our case well tolerated the HCVAD regimen.

**Keywords:** Acute lymphoblastic leukemia, werner's syndrome



cytometry showed CD2, CD5, CD7, CD 45 and CD 34 positivity. He was diagnosed as T-ALL. Hoelzer induction therapy was started. L-Asparaginase (6000 mg/m<sup>2</sup>/day) administered for fourteen days and three days after the therapy he complained head ache, dizziness and confusion. Cranial MRI demonstrated SVT and associated hemorrhagic infarction (Figure 1). Low molecular weight heparin (LMWH) therapy was initiated with treatment dose and in follow up remarkable recovery was achieved in the neurological status of the patient.

**Discussion:** L-Asparaginase may lead to occur both thrombotic and hemorrhagic complications by reducing the synthesis of coagulation factors and also inhibitors. Use of dexamethasone instead of prednisolone was reported to be associated with a lower incidence of thrombotic events. LMWH's are usually convenient and safe treatment choices for thrombotic complications. In ALL patients treated with L-asparaginase, it should be kept in mind that neurologic complications may develop due to SVT. Early treatment with LMWH may improve clinical status.

**Keywords:** L-Asparaginase, sinus venous thrombosis

PS-007

Abstract:0260

**CEREBRAL SINUS VENOUS THROMBOSIS DUE TO L-ASPARAGINASE THERAPY IN A PATIENT WITH T-ALL**

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Cenk Eraslan<sup>2</sup>, Mahmut Töbü<sup>1</sup>

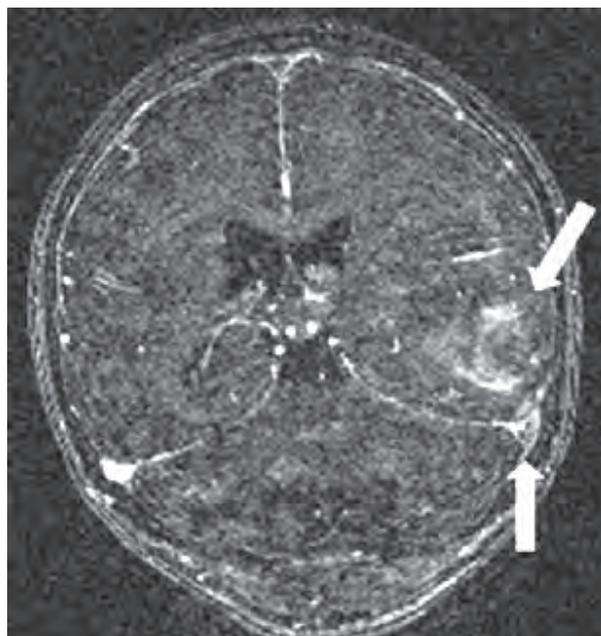
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**Introduction:** Thrombotic complications are frequently seen in the course of hematological malignancies and it may also be associated with specific treatment modalities. Although venous thrombosis is a rare complication in acute lymphoblastic leukemia (ALL) patients, concomitant use of L-Asparaginase and prednisolone in induction therapy significantly increases the risk of thrombosis. In literature, the incidence of cerebral sinus venous thrombosis (SVT) due to L-Asparaginase therapy is reported as 1-3 %. Here we report a case with T-ALL who developed SVT during L-asparaginase therapy.

**Case:** 19 years old male was admitted to hospital with fatigue and palpitation. In his physical examination he was pale but had any lymphadenopathy, hepatosplenomegaly and neurological symptoms. In laboratory WBC: 3240/mm<sup>3</sup>,Hb: 7,1 gr/dL, PLT: 125.000/mm<sup>3</sup>, PT: 13 s (INR: 1.1), APTT: 30.4 s (normal), ESR: 28 mm/h, liver and renal function tests were found normal. Bone marrow aspiration and biopsy demonstrated hypercellularity with lymphoblastic infiltration, the ratio of 95 %. Flow

Cranial CT



White arrow: Hypointense thrombus in left transvers sinus, Yellow arrow: Hypointense hemorrhagic material in infarction site surrounded by hyperintense edema zone in temporal lobe of left cerebral hemisphere

## Chronic Lymphocytic Leukemia

PS-008

Abstract:0088

### STANDARDIZATION AND SCORING OF THE BODY SURFACE AREA (BSA) FORMULAS FOR CALCULATION OF THE DOSES OF ANTICANCER AGENTS FOR CANCER PATIENTS FROM THE NORTH-WESTERN NIGERIA

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**Background:** Allometric scaling is an empirical examination of the relationship between the pharmacokinetic parameters and body size. Because of the importance of body surface area formulas for calculation of anticancer agents, there is need to standardize and score all the existing body surface area formulas with a view to obtaining the best and most efficient formula that can be adopted for use by our hospitals.

**Methods:** A total of 33 (10.3%) out of 319 presented to the Haematology Department of Usmanu Danfodiyo University Sokoto, Nigeria were diagnosed of Leukaemia and lymphoma. Cyclophosphamide, daunorubicin, vincristine, adriamycin and chlorambucil were used for the treatments. Eleven (11) of the affected 33 patients were randomly selected for calculation of their body surface areas using the formulas of DuBois, Boyd, Gehan and George, Haycock et al., Monstaller, Wang et al., Takashira and Fujimoto. The mean of the results obtained from each formula and for each individual was calculated and compared with all the results provided by the respective formulas and all the formulas scored.

**Results:** Wang et al. recorded the highest score 21 (21.2%) followed by DuBois 20(20.2%), this paper 18 (18.2%), Monstaller 18 (18.2%), Haycock et al. 14 (14.2%), Boyd 13 (13.1%), Gehan and George 13(13.1%), Takashira 4 (4.0%) and Fujimoto 3 (3.0%).

**Conclusion:** Wang et al. gives moderate effective anticancer doses and it is therefore recommended for the patients. It provides moderate doses of anticancer agents that may neither cause increased toxicity signs nor high risk of cancer remission.

**Keywords:** Anticancer agents, leukaemia

PS-009

Abstract:0093

### PLEURAL EFFUSION IN THE CLINICAL COURSE OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Pleural effusion in patients with chronic lymphocytic leukemia (CLL) has rarely been reported (1-3). We report a case of pleural effusion in a 57-year-old male whom

was diagnosed as stage II B-cell CLL in November of 2013. At that time, his peripheral blood was consistent with CLL, and a flow cytometric analysis of peripheral blood revealed positivity for CD79, CD45, CD5, CD19, CD20, CD22, CD23 and showed negativity for CD3, CD10, CD14. Cervical screening with ultrasonography (USG) noted bilateral multiple hypoechoic lymph nodes with a maximum diameter of 30 x 10 mm (left-sided). An abdomen USG revealed a moderate splenomegaly (147 mm). Deletion of 17(p13) was negative with fluorescent in situ hybridisation. He was under follow-up without medical treatment until December of 2014. At this time, he applied with complaints including shortness of breath and perspiration. His complete blood count was as follows: Hb: 12.1 g/dL, WBC: 127.2 x 10<sup>3</sup>/mm<sup>3</sup>, lymphocytes: 94.4 x 10<sup>3</sup>/mm<sup>3</sup>, PLT: 227 x 10<sup>3</sup>/mm<sup>3</sup>. His biochemistry and serologic tests were normal except the given parameters: sedimentation rate: 50 mm/h (0-20), lactate dehydrogenase: 576 u/L (< 248), alkaline phosphatase: 152 U/L (30-120), and C-reactive protein: 16 mg/L (0-5). A postero-anterior chest radiography showed a right-sided pleural effusion. This fluid was Rivalta: ++, and was accepted as exsudative. Cytologic and flow cytometric analyses of the fluid were consistent with pleural involvement of CLL. A combination chemotherapy including fludarabine, cyclophosphamide, and rituximab (FCR) was planned for the next medical treatment of the patient. Awareness of this occurrence is essential for appropriate therapy in similar patients with CLL.

**Keywords:** Chronic lymphocytic leukemia, pleural effusion

PS-010

Abstract:0099

### CHRONIC LYMPHOCYTIC LYMPHOMA AND CONCOMITANT RENAL CELL CARCINOMA (CLEAR CELL TYPE)

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**Objective:** The incidence of chronic lymphocytic leukemia (CLL) and renal cell carcinoma (RCC) occurring in the same patient is higher than that expected in the general population. Various explicative hypothesis of this concurrence include treatment-related development of a second malignancy, immunomodulatory mechanisms, viral aetiology, cytokine (IL-6) release from a tumor, and common genetic mutations.

**Patient and Methods:** A 73 years-old male was diagnosed as CLL in December 2009 and was treated with 6 cycles of cyclophosphamide, vincristine and prednisone (CVP). On follow-up, he received 6 cycles of fludarabine because of relapsed disease. He applied to our center with fever, weight loss, and night sweats in December 2012. His physical examination was unremarkable except bilateral axillary and left posterior servical lymphadenopathies. His blood count parameters were as follows: hemoglobin: 14.5 g/dL, leukocyte: 20.08 x 10<sup>3</sup>/mm<sup>3</sup>, lymphocyte: 14.19 x 10<sup>3</sup>/mm<sup>3</sup>, platelet count: 168 x 10<sup>3</sup>/mm<sup>3</sup>. All other laboratory tests were normal. Six cycles of fludarabine and cyclophosphamide (FC) were applied from December 11, 2012 to April 30, 2013, resulting in relief from symptoms and achieving a partial remission in lymphadenopathies. Eritropoietin level was

2030 mIU/mL (normal range: 4.3-20). C-reactive protein and erythrocyte sedimentation rate were 221 mg/L (normal range: 0-5) and 136 mm/hour (normal range: 0-20), respectively, which should not be explained by a significant cause such as an infection or a rheumatological disease. Bone marrow biopsy revealed a hypercellular (%60) and otherwise normal marrow morphology. Direct and indirect antiglobulins tests (initial and repeated) were negative. Reticulocyte correction index was calculated as 0.04. An abdomen USG showed a hypoechoic mass lesion measuring 7.3 x 5 cm including cystic areas in the lower pole of the left kidney. Computed tomography (CT) of the abdomen revealed moderate splenomegaly (13.3 cm) without hepatomegaly, and a heterogen solid mass in 7 x 7 x 8 cm diameter bearing cystic areas in the medial lower pole of the left kidney. Tru-cut biopsy of the renal mass showed vimentin (+), CD10 (+), Ki-67: 10-20%, EMA (+), LMWK (+) cells which were consistent with clear cell type RCC. Torax CT to rule out a probable metastasis of RCC was found to be negative. Surgical removal of the aforementioned lesion was planned. Unfortunately, on follow up, he developed tonic-clonic seizures for two times every other day. Electroencephalography and brain magnetic resonance imaging were unremarkable. Intravenous administration of sodium valproate was initiated following improvement in seizures. However, the general condition of the patient worsened gradually and he expired on December 2014 with active RCC.

**Conclusion:** We suggest caution in treating patients with CLL, particularly those treated with intense courses of chemotherapy, until the relation among CLL and RCC is better understood.

**Keywords:** Chronic lymphocytic leukemia, renal cell carcinoma

PS-011

Abstract:0130

**CLL CASE WITH SKIN INVOLVEMENT**

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Chronic lymphocytic leukemia is one of the chronic lymphoproliferative disorders. CLL is characterized by monoclonal functionally-inefficient lymphocytes accumulation. Skin is the most commonly infiltrated non-lymphoid organ at the diagnosis. Our case is Chronic Lymphocytic Leukemia, but the case is presented with skin involvement. These lesions are often seen in the face and they are presented as macula, papules, nodules, ulcers or blisters. The diagnosis is made with biopsy of the infiltrated skin. Leukemia cutis is observed in less than 5% of the cases and it does not significantly affect the survival if incidence of Richter's transformation does not happen. Exaggerated insect bite reaction, particularly for mosquitoes, was reported and it should suggest the diagnosis of CLL. 50-year-old male patient admitted to the clinic with lymphadenopathy (LAP), fever, night sweats, widespread rash in the body, and lesions in the face, and lesions with maculopapular erythematous (Figure 1). 8 cm painless bilateral in the neck, bilateral 5x4 cm in the axilla, 4x5 cm LAP in inguinal section, 10 cm in the liver, and 6 cm palpable. Because lymphocytosis with 80% was observed in peripheral blood smear, flowcytometry was

applied in the peripheral blood for immunophenotyping. CD5+CD19 69%, CD20 86%, CD22 78%, CD23 83%, CD25 81%, CD19 antikappa were assessed as positive 78%. FISH t(11; 14) required for differential diagnosis of CLL mantle cell leukemia was reported as negative. Cervical LAP excisional biopsy of the patient was CD20, CD23 positive, cyclin D1 negative. CLL involvement was reported. At the bone marrow aspiration biopsy, hypercellular, narrow cytoplasm and round nuclei and these cells with cytoplasmic strong common CD 20, strong staining with CD23 and CD5 and cyclin D1 negative were observed. At the biopsy of skin lesions of the patient, CD20, CD23, CD43, bcl-2 is positive, weak expression with CD 5, and CD3, CD4, CD8, CD10, CD30, cyclin D1 was negative, and these cells were reported as perivascular periadnexial localized lymphoid infiltration and in conclusion CLL involvement was assessed. The patient was diagnosed with RAI stage III treatment was started. At the interim evaluation of the patient after three cycles, partial response was reported and skin lesions completely disappeared after treatment.

CLL is more common in men. Female and male ratio of CLL was approximately 1.7: 1. Based on data in the literature, although the most frequently non-lymphoid organ infiltrated by CLL is skin it is observed in less than about 5% of the patients. As the skin involvement is rarely seen practically and it can be overlooked accordingly, the diagnosis can be late because invasive procedures such as skin biopsy are required for diagnosis. There is no definitive information about the effect of the diagnosis on the overall survival. Skin involvement in CLL was evaluated in this case report.

**Keywords:** Chronic lymphocytic leukemia, skin involvement

Maculopapular erythematous skin lesions in the patient's back



PS-012

Abstract:0135

### A RARE SECONDARY CANCER DURING CHRONIC LYMPHOCYTIC LEUKEMIA: MALE BREAST CANCER

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Breast cancer is the most common cancer of female but approximately 1% of cases are male. In Western countries chronic lymphocytic leukemia (CLL) accounts for 25–30% of all leukemias and 1% of all the newly diagnosed malignancies. An increased incidence of various malignant neoplasms has been reported in patients with CLL and is attributed to disease or therapy related immunosuppression. An 83 years old male with a right breast lump was consulted before biopsy due to high leukocyte level persisted over 2 years. He was diagnosed as Rai stage I CLL and periodic follow-up planned. His breast mass biopsy revealed invasive ductal carcinoma that stained as HER2 (3+) by immunohistochemistry and treated with TCH (Docetaxel, Carboplatin and Trastuzumab). We want to present a rare secondary cancer during chronic lymphocytic leukemia; male breast cancer and the challenges in diagnosis, staging and treatment of the disease.

**Keywords:** chronic lymphocytic leukemia, male breast cancer

PS-013

Abstract:0144

### TRANSFORMATION OF CRONIC LYMPHOCYTIC LEUCHEMIA TO WALDENSTRÖM MACROGLOBULINEMIA

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B-cell chronic lymphocytic leukemia (B-CLL) and waldenström macroglobulinemia are both mature B-cell neoplasms. There are some case reports associated with transformation from CLL to multiple myelomas. But according to our knowledge; there are a few cases with CLL who had waldenström macroglobulinemia (WM) at last. Here, we described a patient with prior B-CLL with secondary development of WM. A 74 year-old female patient was being followed without any treatment with B-chronic lymphocytic leukemia (CLL)-stage 2 since December, 2012. After April 2013, she started to experience autoimmune hemolytic anemia frequently which responded to treatment of steroids. She had progression in leukocytosis and B symptoms in last months of 2013, and was given rituximab-chlorambucil treatment with the diagnosis of CLL progression. Clinical and hematological response were achieved with the treatment. Her lymphadenopathies were regressed in size and number in computerised tomography scanning. After a follow-up time for 2 months, she had a pathological diagnosis of basal cell cancer of which the result of excisional biopsy from right nasal alar wing. She had hypersedimentation (105 mm/h) and rouleaux formation after 3 months. She had servical lymphadenopathies in size of 1-1.5 cm approximately, which were old. Her traube 's space was dull. There was no finding of hyperviscosity. Globulin was 5.1 gr/dl. Albumin was 4 gr/dl. Serum immunofixation test was showing paraproteinemia of hyperimmunoglobulinemia M (4370 mg/dl).

Kappa/lambda ratio in serum and urine was 33 and 17, respectively. She had no proteinuria, no B symptoms, or any other symptoms. There were %9 plasma cells, and %25-30 lymphoid cells with scanty cytoplasm and nuclei with irregular contours in bone marrow biopsy. Bone marrow immunophenotyping revealed: CD38(+) Kappa(+), CD(20) CD5 (+). Her leukocyte level was: 3970/McL and Lymphocyte level was: 1900/McL Hb: 12.3 gr/dl Plt: 149000/McL. Creatin, calcium, LDH, liver enzyme levels were within normal ranges. She had the diagnosis of WM with these findings. According to our assessment, she had no indication of treatment. She is still in our close follow-up without any treatment.

**Keywords:** Waldenström macroglobulinemia, B-CLL

PS-014

Abstract:0145

### MONOSOMY 16: IS A NEW PROGNOSTIC FACTOR FOR CLL?

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**Introduction:** Chronic lymphocytic leukemia (CLL) is the most common adult leukemia accounting for nearly 30 % of all leukemias. In addition to the clinical findings, genetic parameters have been shown to play a role in prognosis. Monosomy 16 is very rare genetic disorder in CLL. A patient with progressive CLL accompanied with monosomy 16 was reported.

**Case:** A 51 year-old male patient was presented to outside institution with fever and swelling in the neck in December 2013. Lymphocytosis and multiple lymphadenopathies were detected and he was diagnosed as stage 3B CLL with bone marrow biopsy and flowcytometric analysis. He was treated with 6 course of R-CHOP (Rituximab, vincristin, cyclophosphamide, doxorubicin, prednisolon) chemotherapy and he was assessed as refractory. Mini-BEAM (carmustine, etoposide, cytarabine, melphalan) chemotherapy was administered and peripheral stem cell was collected after the treatment but no response was observed. He was referred to our clinic. Bone marrow biopsy was confirmed. Cytogenetic tests from bone marrow aspiration was resulted as 46XY 13, 45XY (-16) 3. Del 17 was negative with FISH. Two cycles of RFC (Rituximab, fludarabine, cyclophosphamide) was administered but no response was detected. FLAG-IDA (Fludarabine, cytarabine, idarubicin) was started 1 month ago, he is still leukopenic and neutropenic. DIC and GIST bleeding was evolved in the follow-up. He has thrombosis in right axillary, subclavian and internal jugular veins.

**Discussion:** If sensitive tests are used chromosomal abnormalities can be detected in the majority of CLL patients. Certain genetic abnormalities are found to be associated with prognosis. Patients will have more aggressive disease with complex genetic changes. The most common abnormalities are 13q and 17p deletion, and trisomy 12. The relationship with monosomy 16 and CLL prognosis is unknown. The progressive and refractory disease in our patient had aroused the 'monosomy 16 may be a new prognostic factor in CLL' idea.

**Keywords:** Chronic lymphocytic leukemia, monosomy 16

PS-015

Abstract:0155

**ACUTE TUMOR LYSIS SYNDROME IN A PATIENT WITH CHRONIC LYMPHOCYTIC LEUKEMIA**

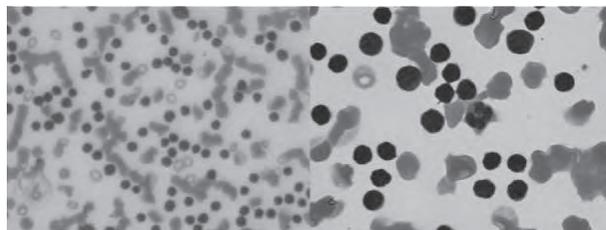
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Objectives: Tumor lysis syndrome (TLS) is a metabolic derangement which can be seen during course of the hematological malignancies. Generally, it occurs in treatment period. However, TLS may develop spontaneously in the patients with bulky lymphadenopathies, high white blood cell (WBC) counts and preexisting renal disease. Spontaneous TLS is frequently described in acute lymphoid leukemia, aggressive lymphomas and solid malignancies. It is extremely rare in patients with the chronic lymphocytic leukemia (CLL). Herein, we present a patient with CLL who develops spontaneous TLS.

Case Report: A 68 years old male with the history of CLL for 3 years was presented to our emergency department with complaints of fever and dispnea. Clinical examination revealed arterial blood pressure of 130/70 mmHg, heart rate of 96 bpm, body temperature of 36.2°C, cervical, axillary multiple lymphadenopathies and minimal splenomegaly.

Laboratory finding revealed hemoglobin 7.1 g/dL, WBC  $403.6 \times 10^9/L$ , platelet count  $30 \times 10^9/L$ , urea 60 mg/dL (normal 8-23), creatinine 2.32 mg/dL (normal 0.5-1.1), calcium 8 mg/dL (normal 8.8-10.6), phosphate 3.8 mg/dL (normal 2.5-4.5), uric acid 9.75 mg/dL (normal 3.5-7.2), sodium 138 mEq/L (normal 136-146), potassium 5.3 mEq/L (normal 3.5-5.1). Liver function tests were with in normal limit. In the peripheral smear it was seen 90% mature lymphocytes (Figure1).

The peripheral blood smear of the patient



The peripheral blood smear, 90% of small mature lymphocytes.

He was admitted to hospital with diagnosis of pneumonia, CLL and spontaneous TLS. He was started hydration, allopurinol and antibiotic treatment for pneumonia. In a period of 10 days, WBC count, uric acid, serum creatinine levels and the other abnormal parameters were improved.

After treatment of pneumonia and the recovery of renal functions it was started fludarabine, cyclophosphamide and rituximab to the patient.

Conclusions: TLS is characterized by elevated serum levels of uric acid, potassium, phosphate, and serum creatinine. Those abnormalities and renal dysfunction are due to release of intracellular contents of tumor cells. Although TLS in CLL is rare, it has been observed after treatment with either chlorambucil, fludarabine, rituximab or steroid. However, there are limited number of cases reported in literature about spontaneous TLS

in CLL. In our patient we diagnosed spontaneous TLS prior to chemotherapy. It was treated with hydration and allopurinol.

Spontaneous tumor lysis syndrome is rarely seen in CLL. Our patient represents the case of leukocytosis and spontaneous tumor lysis syndrome that successfully managed with FCR and supportive care. Especially in the CLL cases with hyperleukocytosis, spontaneous TLS must be keep in mind.

**Keywords:** Spontaneous tumor lysis syndrome, chronic lymphocytic leukemia

PS-016

Abstract:0203

**CLL WITH INFILTRATION OF EAR LOBES**

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A 46-year-old man presented with a 6-month history of palpable masses in his bilateral cervical, axillary regions and swelling on his ear lobes. Physical examination revealed rubbery lymphadenopathy in cervical, axillary and inguinal regions, multiple, firm, violaceous nodules in both ear lobes. Review of his systems was unremarkable. Laboratory results showed marked lymphocytosis and mild anemia. Bone marrow biopsy specimen revealed infiltration of atypical lymphoid cells that were positive of CD20, CD23 and CD5. Flow cytometric analysis of peripheral blood was consistent with B-CLL (monoclonal B-cell population that co-expressed CD20, CD23 and CD5). Histopathologic examination of the nodular lesion of the right ear lobe showed B-CLL infiltration. The patient was treated with cyclophosphamide, vincristine, and prednisone. After 6 cycles of chemotherapy there was significant improvement in the lesions.

**Keywords:** CLL, ear

Photograph of the patient



Infiltration of the left ear lobe

PS-017

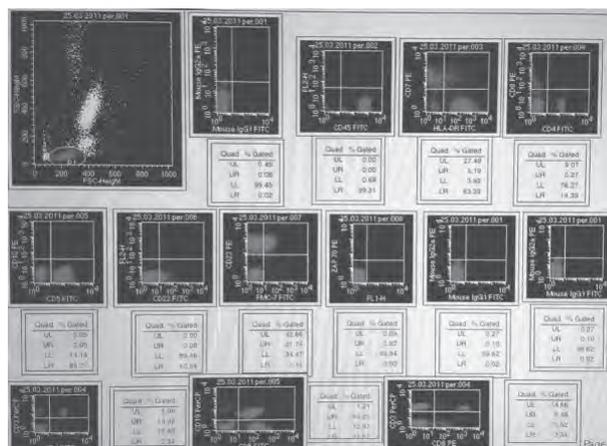
Abstract:0232

**CHRONIC LYMPHOCYTIC LEUKEMIA DEVELOPING IN A CASE OF CHRONIC MYELOGENOUS LEUKEMIA: A RARE COEXISTENCE**

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Chronic lymphocytic leukemia (CLL) and chronic myelogenous leukemia (CML) are common leukemia types in adult. But the coexistence of these diseases is very rare. These conditions can occur either sequentially or simultaneously. We present a patient who developed a CLL four years after the diagnosis of chronic phase CML. An asymptomatic 69 year old man, was referred to Istanbul Medical Faculty Hospital in 2007 because of high leukocyte count: white blood cell (WBC) count  $50 \times 10^3/L$  (%71 neutrophils, %23 lymphocyte, %6 monocyte), platelet count  $453 \times 10^3/L$ , hemoglobin 13,8 gr/dl and mean corpuscular volume 87 fl. There was no splenomegaly and lymphadenopathy on physical examination. Bone marrow biopsy was hypercellular and consistent with chronic phase chronic myeloid leukemia (CML). Cytogenetic study showed Philadelphia chromosome (t(9;22)). A diagnosis of chronic phase CML was considered with intermediate risk (Sokal) and imatinib 400 mg/day was started. At the first year the patient had achieved major molecular response. 4 years later after the imatinib treatment, a WBC count elevation was observed. Peripheral blood smear and immunophenotyping was consistent with chronic lymphocytic leukemia (CLL). Prognostic markers (17p,11q,13q and trisomy12) of CLL were not positive using FISH and any cytogenetic anomaly was not detected. Clinical evaluation was consistent stage 0 (Rai) and stage A (Binet); and CLL did not require therapy. The concordant occurrence of CLL and CML which condition is presented as only case reports in the literature. The genomic explanation of this coexistence is not yet adequately understood.

**Immunophenotyping analysis**



Some authors suggest that leukomogenic affect of therapy in the cases of CLL have been associated with hematological neoplasm. Crooks et al. present that increased IL 3 level in CML patients was associated with increased production of B lymphoid progenitors. These theories have failed to explain when these two diseases

have occurred simultaneously without prior history of any disease. We present a patient who developed CLL 4 years after he diagnosis of chronic phase CML. Imatinib mesylate 400 mg/day didn't affect leukocyte count when CLL was detected. No specific therapy was given for CLL. When leukocytosis in CML patient is detected who is achieve deep molecular response CLL should come to mind.

**Keywords:** chronic lymphocytic leukemia, chronic myelogenous leukemia

PS-018

Abstract:0234

**PROGNOSTIC PARAMETERS OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS IN OUR CENTER**

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**Objective:** Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults. Multiple factors affect the clinical course of CLL. Currently, the most important traditional prognostic factor include clinical staging and the most important novel markers include karyotypic aberrations. The 13q14 deletion is the most common genetic abnormality in CLL. In this study, we evaluated the demografic and prognostic factors of patients such as age, gender, lymphocyte doubling time (LDT), CD38 and ZAP-70 expression, type of bone marrow infiltration pattern 62 CLL patients who were observed from 2005 to 2015 in hematology department of Baskent University Faculty of Medicine.

**Methods:** Demographics and clinical, laboratory parameters of 62 patients who admitted to the hematology department were investigated between 2005 and 2015 retrospectively.

**Results:** 40 patients were male (% 64,51), 22 patients were female (%35,48). The age range at the onset of chronic lymphocytic leukemia is from 43 to 93. The median age was 67. While 28 patients were being observed without treatment, 34 patients were treated with oral or iv multidrug chemotherapy. At the time of diagnosis, 20 (32.25%) patients were at Binet stage A, 32 (51.61%) were at stage B, 10 (16.12%) were at stage C. After the first line Chemotherapy (iv or oral chemotherapy agents); partial remission for 16 (47.05%), complete remission for 13 (38.23%), progression for 5 (14.70%) patients were observed. 13 (20.96%) patients died during the follow-up. Cytogenetic analysis was performed in 55 patients. Of these patients, 30 (54.54%) have no cytogenetic abnormality, 15 have (60%) del(13q14), 7 have (28%) trisomy 12, 2 have (8%) del(11q22) and 1 patient (4%) has del(17p13) respectively. There was no relationship between the expression of zeta-associated protein (ZAP-70) (p=0,4) and the  $\beta 2$  microglobulin ( $\beta 2$  MG)(p=0,09) with survival but the serum LDH level (p=0,006), absolute lymphocyte count more than  $50000/\mu l$  (p=0,041), expression of CD38 (p=0,023), the stage of disease (p<0.0012), diffuse infiltration pattern of bone marrow (p=0,038) was inversely related with the survival. Survival in male patients was significantly less than female patients (p=0.023) **Conclusion:** Multiple factors affect the clinical course and survival of CLL. Some of these; absolute lymphocyte count, bone marrow infiltration pattern, elevated LDH, lymphocyte doubling time;  $\beta 2$ -microglobulin; genetic markers of tumor cells, gene abnormalities, the

expression of CD38 and ZAP70. We founded same results mentioned before in literature in our CLL patients.

**Keywords:** chronic lymphocytic leukemia, prognostic parameters

## Hodgkin's Lymphoma

PS-019

Abstract:0181

### FOETAL MALFORMATIONS IN THE SECOND PREGNANCY AFTER COMBINED MODALITY TREATMENT FOR HODGKIN'S DISEASE: CASE REPORT

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**Introduction:** Although several studies reported in the literature showed no or slightly increased risk of congenital abnormalities among newborns of women previously treated for Hodgkin's disease compared with the general population, abnormalities do occur and sometimes they are very odd and difficult. This is the first report of such specific malformations find in the literature.

**Case:** We report a case of female patient, 25-year-old, presented with Hodgkin's disease, nodular sclerosis subtype, stage IIIA. The patient received chemotherapy according to ABVD protocol – 6 cycles. Thereafter she received 3600 cGy. The first and normal pregnancy occurred after 36 months. Second pregnancy occurred 87 months after completion of treatment. At 13th gestational week ultrasound assessment revealed malformations and induced abortion was performed. A male foetus with malformations on the head such as proboscis, cyclopia and omphalocele on the front abdominal wall containing liver and small bowels was found.

**Conclusion:** I consider that this case is important in bringing the potential late side-effect to the attention of both patients and doctors. Also, this case reveals odd and difficult malformations as potential birth outcome. Doctors and patients should be alert for the risk of congenital abnormalities in newborns of women previously treated for Hodgkin's disease, and should check for them during pregnancy, at birth, in early childhood, or in adulthood.

**Keywords:** Foetal malformations, Hodgkin's disease

PS-020

Abstract:0189

### PHAGOCYTIIZED NEUTROPHIL FRAGMENTS IN THE BONE MARROW

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A 48-year-old woman was admitted to a special hospital suffering from a mass in her left supraclavicular area. On physical examination, there was no organomegaly. Complete blood count revealed the following Results: Hb, 11.7 g/dL; Hct, 32%; leukocytes, 11.1 x 10<sup>9</sup>/L; neutrophils, 8.98 x 10<sup>9</sup>/L; platelets, 542 x 10<sup>9</sup>/L. Peripheral

blood smear revealed no blasts or atypical cells. The erythrocyte sedimentation rate was 37 mm/h. All biochemical tests were within normal ranges, except for elevated lactate dehydrogenase (293 IU/L, range: <246) and C-reactive protein (8.67 mg/L, range: 0-5). Excisional biopsy of the left supraclavicular lymphadenopathy was performed and the histopathological diagnosis was nodular sclerosis hodgkin lymphoma. A staging bone marrow biopsy revealed significant increment in cellularity (80%) and phagocytized neutrophil fragments in macrophages. Standard ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) regimen was initiated.

Bone marrow macrophages containing other cells, or fragments of other cells are termed as "fragmentophages". This condition is associated with malignancies, especially Hodgkin lymphoma, and these cells are less common in marrow samples for nonneoplastic conditions. The recognition of this association may provide a clue for the underlying malignancy 1.

**Keywords:** Phagocytized neutrophil fragments, hodgkin lymphoma

## Multiple Myeloma

PS-021

Abstract:0113

### A MULTIPLE MYELOMA CASE WITH POOR PROGNOSIS

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**Introduction:** Multiple myeloma is characterized with neoplastic monoclonal immunoglobulin secreting plasma cell clones. Disease may present with anemia, bone pain, creatinine elevation, fatigue, hypercalcemia. Bone marrow investigation is basic diagnostic tool. Presence of more than 60% plasma cells in bone marrow investigation indicates more than 80% risk of organ damage in two years and overall survival about 7 months. Herein, we aimed to report a multiple myeloma case who had 90% plasma cell at diagnosis and had very poor prognosis.

**Case Report:** 60 years old men applied to hospital in December 2014 with fever and fatigue. Laboratory test was reported as; hemoglobin 8.9 gr/dl, leucocyte 5.6 x10<sup>3</sup>/µl, platelet 285 x10<sup>3</sup>/µl, ALT 20 u/l, AST 25 u/l, ALP 43 u/l, GGT 15 u/l, total protein 11.5 g/dl, albumin 2,6 g/dl, LDH 212 u/l, creatinine 0.6 mg/dl, calcium 10.2 mg/dl. His anemia parameters were normal. IgG level was 10800 mg/dl and beta-2 macroglobulin was 3428 ng/ml. Gamma pike and monoclonal Ig-G kappa was detected in electrophoresis. 90% plasma cell detected in bone marrow investigation and also MUM-1 was detected in 90% of cells. VAD chemotherapy was started in 22 December 2014. He again applied to hospital with abdominal pain just one day after discharge. Pericholecystic abscess and toxic hepatitis were observed in tomography. Echocardiography revealed normal systolic functions and he was taken to intensive care unit because of neutropenic fever. After the exclusion of Adriamycin toxicity, sepsis began. Plazmapheresis performed for hyperviscosity. Acute renal failure has developed with cerebral emboli. He was treated with dialysis,

however vegetation was detected in aortic valve. He did not respond treatments well and died in January 2015.

**Discussion:** Our patient had some of the poor prognosis criteria that was proposed by "Mayo Clinic"; these were decreased serum albumin and hemoglobin, elevated beta-2 microglobulin and high bone marrow plasma cell ratio. Also, our patient had second stage disease when evaluated with international staging system which proposes median overall survival as 44 months. However, our case died just in one month after diagnosis without toxicity of the chemotherapy. In literature, the relationship between poor prognosis and high plasma cell ratio in bone marrow investigation was reported. Likely our patient had 90% plasma cell at diagnosis. To conclude, for all of this reasons especially in myeloma patients with high risk factors, there is distance to be covered in the management of the treatment.

**Keywords:** Multiple myeloma, poor prognosis

PS-022

Abstract:0122

### **LENALIDOMIDE IN PATIENTS WITH REFRACTORY AND RELAPSED MULTIPLE MYELOMA**

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Multiple myeloma (MM) - malignant disease, which morphological substrate of plasma cells, has a chronic course with periods of improvement (remission, plateau) and subsequent binding relapses - the progression of the disease. Remissions with each relapse are becoming shorter, until it will come resistant relapse - end-stage disease. Disease characterized by a high mortality rate - median survival time of 3.5 - 4 years. However, with the advent of new drugs (bortezomib, thalidomide, lenalidomide) was able to substantially increase the period of disease control, survival and life expectancy of patients.

**Materials and methods:** In Hematological Research Center (Moscow, Russia) 22 patients had a resistance of disease or had a relapse of the disease. All patients used different programs of chemotherapy with Bortezomib and Thalidomide regimens. Prior therapies included in the earlier phases of the program VAD, PAD, VMP, VCD, TCD, and TCM. Male - 15 (mean age - 57 years (43-83)), women - seven (mean age - 63 years (52-82)). Distribution of patients by immunochemical variant was Gk + BJK (5), GA + BJA (6), GA + Gk + BJK (1), Gk (3), GA (2), BJK (2), BJA (2), AA + BJA (1), DA + BJA (1). In addition, the stages of the disease by Durie-Salmon: IIA (2), IIIA (4), IIIB (16). The diagnosis of MM established in 20 patients, one patient diagnosed with plasma cell leukemia and one patient POEMS-syndrome. In three patients had primary resistance, first relapse in 6 patients, in 12 - second and subsequent relapses. The majority of patients used the conventional scheme of appointment of lenalidomide and dexamethasone, one RVD, and another one RAD. 4 patients underwent bone marrow transplantation (3 - AutoBMT, 1 - AlloBMT) - lenalidomide monotherapy used as maintenance therapy. In six patients had severe renal failure - creatinine level greater than 400 mmol/l. The duration of therapy with lenalidomide was from three to 23 months. The average duration of therapy 14 months.

**Results and conclusion:** The most effective treatment with lenalidomide in patients with resistant to first-line

therapy in first relapse after prolonged use. In this group, all patients were alive at the time of the study and continue treatment. Lenalidomide was effective in a patient with POEMS-syndrome and plasma cell leukemia. Using of Lenalidomide in patients with severe renal insufficiency requires caution because of possible development of pancytopenia. In our cases, the development of agranulocytosis observed in three patients; reduction of granulocytes was short and did not require the introduction of G-CSF. Thrombotic complications observed in two patients. Lenalidomide, which is used in the terminal relapse ineffective (three patients died after 6 - 7 - 12 months), median - 8.5 months.

**Keywords:** Multiple myeloma, lenalidomide

PS-023

Abstract:0128

### **BORTEZOMIB INDUCED CONGESTIVE CARDIAC FAILURE IN A PATIENT WITH MULTIPLE MYELOMA**

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**Introduction:** Bortezomib is a proteasome inhibitor. Its cardiac side effects are however not well known. The incidence of cardiac failure associated with bortezomib therapy in clinical trials remains incidental. We describe a 82 years old patient who presented with congestive heart failure associated with bortezomib treatment because of rarity.

**Case:** A 82 years old male patient was admitted to our clinic with low back pain, weakness. He had no prior cardiac history and no additional risk factor except from his age for cardiac disease. On evaluation of his routine laboratory tests, he was found to have haemoglobin of 8.7 g/dl, haematocrit of 26.6%, white blood cell (WBC) count of 10.200/mm<sup>3</sup> and platelet count of 148000  $\mu$ /l. Level of serum urea was 67 mg/dl, creatinine 3 mg/dl, uric acid 11.7 mg/dl, total protein 9.5 mg/dl, albumin 2.8 mg/dl, aspartate aminotransferase 27 UI/L, alanine aminotransferase 13 UI/L, gamma glutamyl transferase 14 U/L. Bone survey graphics confirmed diffuse osteopenia. Electrocardiography was normal. Due to the presence of severe anaemia and osteopenia, multiple myeloma was suspected. Serum protein electrophoresis showed monoclonal protein band. Serum and urine immunofixation electrophoresis showed monoclonal IgG-Kappa immunoglobulin bands. Beta-2 microglobulin level was 7.49. Bone marrow aspiration and biopsy confirmed the diagnosis of International Staging System stage III multiple myeloma. Transthoracic echocardiography revealed a left ventricular EF value of this patient >55%. The patient received three cycles of bortezomib 1.3 mg/m<sup>2</sup> on days 1, 8, 15, 22 of each cycle and 40mg/gün dexamethasone (days 1 to 4). However, after the three cycle of therapy the patient reported increased fatigue, severe dyspnea, orthopnea and mild lower extremity edema. He did not experience any chest pain or myalgia. The physical examination pulmonary auscultation revealed bi-lateral basilar crepitation. There was mild peripheral pedal edema up to ankles bilateral. An ECG value of this patient did not show signs of ischemia. The chest X-Ray showed an increased heart-chest ratio. An echocardiography performed that global hypokinesia. Ejection fraction was estimated to be 30-35%. Coronary angiography was not performed. The patient consulted to cardiology clinic.

The patient was treated for congestive heart failure with diuretics, angiotensin-converting enzyme inhibitors, beta blocker. The patient responded well to this treatment. Bortezomib treatment was stopped. His anti-myeloma treatment was switched to melphalan.

Conclusions: Congestive heart failure is a rare but important toxicity associated bortezomib. Patients with history of cardiac conditions or use of anthracycline drugs may be at increased risk for bortezomib related congestive heart failure. Therefore, as Bortezomib is a new and promising therapy for MM patients, we recommend close monitoring of the cardiac parameters in patients undergoing this therapy.

**Keywords:** Bortezomib, multiple myeloma

PS-024

Abstract:0139

**HEPATITIS C VIRUS INFECTION IN PATIENTS WITH MULTIPLE MYELOMA**

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An association between chronic hepatitis C (HCV) infection and essential mixed cryoglobulinemia and non-Hodgkin lymphoma (NHL) has been reported briefly. However, a causative role of HCV in malignant lymphoproliferative disorders has not been established. NHL and Multiple myeloma (MM) may develop in cases of chronic hepatitis C. Two cases of MM will be presented in whom diagnosed during the follow up of hepatitis C virus infection.

Case1: 55 year old woman, eight years ago left nephrectomy has been done because of chronic pyelonephritis. Four months ago she has been diagnosed hepatitis C infection. She was put on ribavirin and pegylated interferon alfa 2a therapy. During the follow-up she developed back pain and acute renal failure. After the differential diagnosis made by gastroenterology department MM diagnosed. Bone marrow aspirate noted 70% plasma cells. Laboratory results were as: Hb: 10.3gr/dL, WBC: 2900/mm<sup>3</sup>, platelets: 141000/mm<sup>3</sup>, BUN: 55, creatinine: 2.9mg/dl, calcium: 11.1mg/dL, albumin: 3gr/dl. liver functions were normal, HCV RNA1.21x10<sup>3</sup>. Kappa monoclonality was established by urine immunofixation. She did not have 13q and 17q deletions. PET analysis revealed diffuse F-18 FDG uptake in sternum, clavícula, scapula, iliac crests, sacrum, costas and vertebral column. As she had renal failure; to use bortezomib as the first line therapy permission form for the ministry of health has been fulfilled. Bortezomib, adriablastina, dexamethasone (PAD) regimen initiated. After 4 cycles of PAD therapy she has been transplanted. She is well in condition. The renal and liver functions are in normal limits.

Case2: 65 year old woman, she was referred to hematology clinic because of progressive fatigue, back pain. Physical examination revealed no pathologic finding. Hb: 9.3gr/dl, WBC: 2000/mm<sup>3</sup>, platelets: 242000/mm<sup>3</sup>, ESR: 104mm/hr, creatinine 0.75mg/dl, ALT: 66, AST: 79, calcium: 7.5mg/dl, Total protein: 12.4, albumin: 2.8, beta 2 microglobulin: 9143, HCV RNA: 5.5x10<sup>5</sup>. Osteolytic lesions found on cranium X ray films. Bone marrow examination revealed 80% plasma cell infiltration. She did not have 13q and 17q deletions. She has been diagnosed as IgG kappa stage 3A multiple myeloma. She has been consulted by gastroenterology department no

antiviral therapy initiated. Because of the patients status MPT therapy for primary pathology has been begun. She is still under control. As she couldn't tolerate melphalan; she is on thalidomide and prednisolon therapy for the 5th cycle. She is well in condition. Discussion: An association between chronic HCV infection and MM was found in epidemiological studies. Hepatitis C is lymphotropic and may replicate in lymphocytes and hepatocytes. The second portion of the HCV envelope binds to CD81 which triggers B cell hypersensitivity and antibody production. As the close relation between lymphoproliferative disorders and HCV; these patients must be followed up in a regular basis.

**Keywords:** Multiple myeloma, hepatitis C infection

PS-025

Abstract:0140

**A RARE PRESENTATION OF RELAPSE MULTIPLE MYELOMA: ACUTE RENAL FAILURE DUE TO RENAL EXTRAMEDULLARY PLASMACYTOMA WITHOUT BONE MARROW INVOLVEMENT**

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Introduction: Extramedullary plasmacytoma (EMP) is a rare malignant neoplasm which may present as the main symptom of MM, develop during the course of MM or occasionally occur as solitary tumor. Myeloma relapse as EMP without another sign of relapse is known as a rare condition, Herein we describe a case of acute renal failure due to renal extramedullary plasmacytoma without bone marrow involvement, as a presentation of relapsing multiple myeloma.

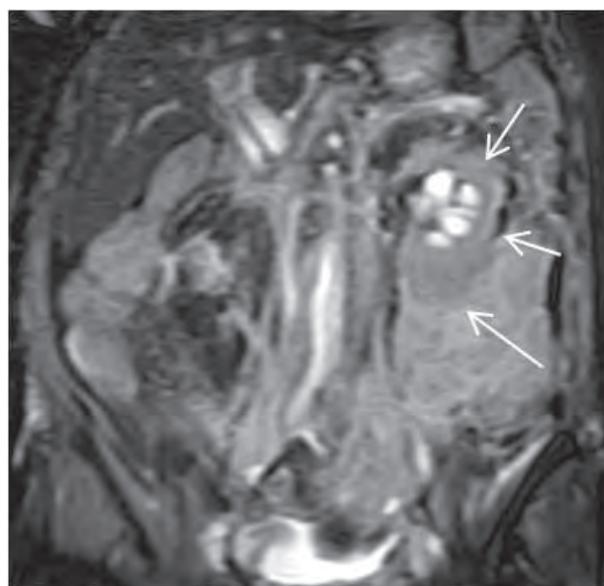
Case: 80 years-old female patient was first admitted to our clinic with acute renal failure and diagnosed as lambda light chain multiple myeloma (MM) with bone marrow biopsy at 2012. Her kidneys were detected normal without any abdominal mass in ultrasonography (USG). After 2 courses of bortezomib and dexamethasone (VD) treatment and two weeks of hemodialysis, renal functions showed partial regression, but bortezomib had to be interrupted due to neuropathy. After four months of treatment with melphalan and prednisolone (MP), the patient discontinued follow-up. Her last creatinin was below 2 mg/dl and she had no anemia but her last response status was not documented. After 2 years without complaint, the patient referred to our clinic for acute renal failure requiring dialysis. Monoclonal spike in gamma globulin region was detected in protein electrophoresis as well as lambda monoclonal light chain in serum and urine immunofixation electrophoresis but bone marrow biopsy was not consisted with MM with only 5% plasma cell percentage. A mass lesion, measured 12x4 cm starting on the right kidney, continuing along the Gerota's fascia and adjacent muscle tissue, was detected on renal USG and contrasted magnetic resonance imaging (MRI). Similar

invasion was surrounding the urethra and aorta reaching perirectal area (Fig 1). Biopsy taken from right renal mass was consisted of monoclonal plasma cells and proved etiology of renal failure was renal EMP. The patient who did not accept chemotherapy but had regular hemodialysis, died because of ischemic stroke and serious pneumonia, one month after relapsing as renal plasmacytoma.

**Discussion:** Although renal injury is a common feature in plasma cell dyscrasias, the occurrence of an EMP in the kidney is an extremely rare mechanisms of this injury. Binnani et al. reported relapse of MM as a massive plasmacytoma in a transplanted kidney causing acute renal failure, as the only case in the literature. Zhang et al also described kidney as a rare localization for plasmacytomas reporting only 24 cases in literature. In this review, most of the renal EMPs (15 of 24) were solitary and solitary EMPs have relatively better prognosis than EMPs which occur during the course of a MM. This fact may explain the aggressive local invasion and fatal course of our renal EMP compared with indolent course of the very few renal EMPs in the literature.

**Keywords:** Renal plasmacytoma, multiple myeloma

Figure 1



T2 coronal contrasted MRI: Mass lesion starting on the right kidney, continuing along the Gerota's fascia and adjacent muscle tissue

PS-026

Abstract:0157

### A MULTIPLE MYELOMA CASE PRESENTING DURING PREGNANCY

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**Introduction:** Multiple Myeloma (MM) is a hematologic malignancy of elderly, representing %10 of hematologic malignancies. The average age for the diagnosis is 65

and 2% of the cases are under 40. MM is extremely rare in pregnancy.

**Case:** Our patient is 36 years old female, gravida 10 parity 3, no relevant medical or surgical history. During the first trimester of her pregnancy, she had back pain, bilateral leg pain especially at left side and numbness in lower extremities. The pain was no relieved with analgesics and related to the pregnancy. Upon the increase of the pains during the third trimester with more movements, compression fractures of L2-L5 vertebrae were diagnosed on the lumbar-spinal MRI. At 34 weeks of her pregnancy, the patient with irregular follow-ups had 2 units of erythrocyte transfusions due to the deep anemia. Further investigations were not made for the anemia etiology. The patient examined for a healthy post-delivery anemia with a term caesarean/section, was referred to our center with the pre-diagnosis of MM due to the high sedimentation rate and switch of albumin/globulin ratio. At the admission, her laboratory findings can be summarized as; hemoglobin level 7,8 g/dl, total protein 12 g/dl, albumin 3,2 g/dl, creatinine 0,68 mg/dl, total calcium 10,94 mg/dl, CRP 13 mg/l, beta-2 microglobulin 4.42 mg/l and multiple lytic lesions and extensive osteoporosis at x-ray imaging. Gamma peak in the protein electrophoresis. Diffuse infiltration with atypical plasma cells at bone marrow sampling was observed. A chemotherapy was planned with the diagnosis of ISS stage II Multiple Myeloma.

**Discussion:** MM rarely occurs in pregnancy and complications; such as, MM like renal failure, bone lesions, bleeding and sepsis can be seen. Most of the patients reported in literature show that the patients did not have therapy before delivery. In the management of MM during pregnancy, organ damage related to the MM, severity of the disease, prognosis and the mother's choice should be outlined. Without considering the therapy related to fetal adverse events, the mother's life should be protected primarily. Dexamethasone is a reasonable non-toxic choice in case of a delivery. Due to the frequent involvement of pelvic and spinal bones, caesarean section is the preferred method of delivery. Specific chemotherapy should be started as soon as possible after the delivery. Despite the fact that our patient has MM findings during the pregnancy, the delivery occurred without any treatments. After the delivery, the diagnosis was made and the patient started chemotherapy. In vitro studies have shown that anti-estrogen agents deteriorate the growth of myeloma derived cell lines as well as they stimulate the apoptosis in myeloma cells. It is known that pregnancy can also facilitate the utilization of growth factors for the MM cells, and increased levels of Interleukin-6, Insulin-like growth factor-1 may accelerate the growth of malign plasma cell clone.

**Keywords:** multiple myeloma, pregnancy

PS-027

Abstract:0158

**MIR-17-92 GENE CLUSTER MAY BE PROGNOSTIC BIOMARKER IN MULTIPLE MYELOMA?**Handan Haydaroglu Şahin<sup>1</sup>, Sacide Pehlivan<sup>2</sup>, Mustafa Pehlivan<sup>1</sup>, Seval Kul<sup>3</sup>, Vahap Okan<sup>1</sup>, Sibel Oğuzkan Balcı<sup>4</sup><sup>1</sup>Gaziantep University, Faculty of Medicine, Department of Hematology, Gaziantep, Turkey<sup>2</sup>Istanbul University, Faculty of Istanbul Medicine, Department of Medical Biology, İstanbul, Turkey<sup>3</sup>Gaziantep University, Faculty of Medicine, Department of Biostatistics, Gaziantep, Turkey<sup>4</sup>Gaziantep University, Faculty of Medicine, Department of Medical Biology, Gaziantep, Turkey

Multiple myeloma (MM) is the B cell lymphoproliferative disease that is characterized by a single plasma cell produce monoclonal immunoglobulin (Ig) or Ig fragments (M protein) and neoplastic proliferation of plasmacytoid cell clones. Complex genetic and epigenetic abnormalities are also involved in the etiology of the disease. Micro RNAs (miRNAs) are small non-coding RNA molecules which are taking part in the cell proliferation, cell differentiation, organogenesis and apoptosis. Also miRNAs plays a role in the development of various diseases such as cancer, cardiac and infectious diseases. MiRNAs have a crucial role in the regulation of hematopoiesis and hematologic cancers and acts as oncogenes or tumor suppressor genes. MiR-17-92 gene cluster members are oncogenic miRNAs which play a critical role in the life cycle of B cells and is associated with MM. This study aimed to investigate the relationship between the expression levels of the miR-17-92 gene cluster members which are miR-17, miR-18a, miR-19a, miR-19b, miR-20a and miR-92 with response to treatment of MM patients. miRNAs expression levels obtained from the peripheral blood samples were taken at pre-treatment and post-treatment from 30 patients who were diagnosed with MM in University of Gaziantep Faculty of Medicine, Department of Hematology and 33 healthy controls determining by a method of the hybridization-based microarray. The expression levels compared statistically by non-parametric methods (Wilcoxon and Mann Whitney U Test). When compared to the patients and control groups; in the pre-treatment samples; miR-17, miR-18a, miR-19 and the miR-20a gene expression levels were increased compared to controls ( $p = 0.001$ ). MiR-19b was not different ( $p = 0.473$ ), the miR-92a was lower in the patients than the controls ( $p = 0.006$ ). When the control samples compared with post-treatment samples; miR-17 was increased compared to controls ( $p = 0.001$ ), while miR-19a and miR-92a were decreased significantly ( $p=0.001$ ), miR-18a, miR-19a, and miR-20a were not different ( $p=0.164$ ,  $p = 0.369$ ,  $p = 0.655$ , respectively). When the compared pre and post-treatment groups; the gene expression levels of miR-18a, miR-19a, miR-19b, miR-92a and miR-20a were significantly decreased in post-treatment samples ( $p=0.001$ ) while the miR-17 was increased ( $p=0.002$ ). The expression levels of the miR-17-92 gene cluster members were found to be significantly decreased in the patients with respond to the treatment ( $p=0.001$ ,  $p=0.007$ ) except miR-17. We concluded that miR-17-92 gene cluster members may be associated with response to the MM treatment and can be used in MM prognosis as a marker. According to our preliminary findings, further researches are needed

to confirm the exact role of miR-17-92 gene cluster in response to MM treatment.

**Keywords:** Multiple Myeloma, miR-17-92 gene cluster

PS-028

Abstract:0159

**NON SECRETORY MULTIPLE MYELOMA AND AL-AMYLOIDOSIS**Özlem Beyler Kılıç<sup>1</sup>, İhsan Ergün<sup>2</sup>, İrmak Sayın<sup>1</sup>, Ali Kemal Oğuz<sup>1</sup>, Şebnem Kalay<sup>1</sup>, Meltem Aylı<sup>3</sup><sup>1</sup>Ufuk University Faculty of Medicine, Internal Medicine Department, Ankara, Turkey<sup>2</sup>Ufuk University Faculty of Medicine, Nephrology Department, Ankara, Turkey<sup>3</sup>Ufuk University Faculty of Medicine, Hematology Department, Ankara, Turkey

Introduction: Multiple myeloma (MM) is a hematological neoplasm which is originated from B cells in bone marrow and characterized from malignant proliferation of a group of clonal plasma cells that secrete monoclonal immunoglobulin or light chain immunoglobulins (M-protein). In Non-Secretory Multiple Myeloma (NSMM) M protein does not exist in serum and urine but monoclonal cells increase in bone marrow. Amyloidosis is a disease which is made up of accumulation of the low molecular weight subunits of normal serum proteins. In AL-amyloidosis, fibrils that aggregate and cause tissue damage are made up of monoclonal light chain fragments. In NSMM, AL-amyloidosis development is very rare. We present a case of NSMM with AL-amyloidosis.

Case: 74 year old male presented with swollen legs, difficulty in walking and chronic tense edema. Medical history included well controlled hypertension. Home medication included Valsartan/hydrochlorothiazide and Doxazosin. On physical examination, he had truncal obesity, severe edema in pretibial region. Laboratory investigations at the time of admission (complete blood count, basic metabolic panel, thyroid function tests, hepatic panel) were normal. 300 mg/dl proteinuria in the urine dipstick test was detected. 4.6 g proteinuria was found in 24-hour Urine Protein Test. He had normal serum and urine immunofixation as well as a normal serum FLC (Free Light Chain) ratio. Immunologic assessments are in Table-1. Renal biopsy was performed; PAS positive depositions and amyloid depositions with Congo Red were seen in glomerules and vessels. Kappa chain showed mild staining despite lambda light chain showed strong staining. Patient was diagnosed with lambda light chain type AL-amyloidosis. Bone marrow aspiration and biopsy was performed. One type mature plasma cell infiltration was found to be 25% ratio in bone marrow biopsy which is coherent with MM. Both FISH (Fluorescence In Situ Hybridisation) and conventional cytogenetic analysis showed normal karyotype (46XY). Chemotherapy with bortezomib (1.3 mg/m<sup>2</sup>, 4 days) and dexamethasone (40 mg/day, 4 days) were administered twice, once in every 28 days. At the end of the second cycle of chemotherapy, control bone marrow aspiration and biopsy showed that the plasma cell ratio was 4%. He was directed to autologous stem cell transplantation.

Discussion: 3% of patients with MM are NSMM. AL amyloidosis has been described very rare in cases of NSMM. The most common form of AL amyloidosis is renal involvement which present as nephrotic syndrome as in our case. This patient had NSMM together with

25% plasma cells in the bone marrow and the presence of osteolytic bone lesions.

**Conclusion:** In patients with nephrotic-range proteinuria, the first diagnose that comes to mind is AL-amyloidosis. In the absence of the patient's serum and urine monoclonal paraprotein and monoclonal immunoglobulin, AL-amyloidosis and the underlying NSMM or oligosekrotory MM should be kept in mind.

**Keywords:** AL amyloidosis, Multiple myeloma

#### Immunologic assessments

Parameters	Value	Parameters	Value
Ig G (gr/L)	5,7(5,4 - 18,2)	ANA	Negative
Ig A (gr/L)	1,19(1 - 6)	c-ANCA	Negative
Ig M (gr/L)	0,63(0,2 - 2,4)	p-ANCA	Negative
C3c (mg/dL)	82(82 - 185)	Lambda light chain (mg/dL)	93(90 - 210)
C4 (mg/dL)	23(15 - 53)	Kappa light chain (mg/dL)	170(170 - 370)

PS-029

Abstract:0160

### INCREASED ISCHEMIA MODIFIED ALBUMIN LEVELS IN PATIENTS WITH MULTIPLE MYELOMA

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**Objectives:** Albumin has a binding site of transitional metal ions, including cobalt and copper, on its N-terminal region. Ischemic and oxidative stress modifies this terminal peptide irreversibly to a dysfunctional form, known as ischemia modified albumin (IMA). Reactive oxygen species (ROS) levels increase with inflammation and oxidative stress. Increased levels of ROS can be cytotoxic and may cause cellular damage, cell death, mutations, and chromosomal aberrations. ROS are found in the development stages of carcinogenesis and there is a growing interest about this subject. In recent years, the role of IMA as a new marker for diseases linked to inflammation have described in several researches. The aim of the present study was to investigate the levels of a novel marker IMA in patients with multiple myeloma (MM). **Methods:** A total of 21 patients with MM (6 females and 15 males; mean age 55.4±8 years) and 32 control healthy subjects (20 males and 12 females; mean age 68±7 years) were selected for the detection of IMA levels.

**Results:** IMA levels were evaluated in patients and controls. In patients with MM, mean serum concentration of IMA was found to be significantly higher than those of control healthy subjects (0.894±0.273 AU vs 0.379±0.08 AU, p <0.001).

**Conclusion:** This study suggested for the first time that patients presenting with MM had elevated levels of IMA when compared to healthy controls. MM is a hematological malignancy which causes the most prominent inflammation among other malignancies. This is due to

the intense cytokine release from stromal cells. Because IMA levels used as a marker of inflammation, high levels of IMA in MM patients may be used as a sign for the disease activation. Further studies are needed to demonstrate the relationship between IMA and MM.

**Keywords:** Ischemia modified albumin, multiple myeloma

PS-030

Abstract:0162

### CASE REPORT SEVERE CARDIAC AND PULMONER TOXICITY DUE TO CARFILZOMIB THERAPY

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**Background:** Carfilzomib is a second generation selective proteasome inhibitor for the treatment of relapsed and refractory multiple myeloma. Carfilzomib is approved as a treatment option for relapsed or refractory cases who received at least two prior chemo regimens and still have a disease progression. Cardiovascular and pulmonary adverse events due to carfilzomib therapy have been rarely reported but these complications may have clinical significance.

**Case:** A 65-years old male was initially diagnosed with MM in 2005 for which he received multiple chemotherapies and autologous bone marrow transplantation in 2009 and second transplantation was in 2012. In December 2014 he was diagnosed with relapsed / refractory Multiple Myeloma who had already bortezomib, bendamustin, melphalan 100mg, lenalidomide treatments and still have hyperviscosity symptoms. So we started to carfilzomib therapy (20mg/m<sup>2</sup>) on days 1-2, 8-9 and 15-16.

Before the carfilzomib therapy contrast echocardiography was performed. Mean pulmonary arterial pressure on Echo was 34+5 mmHg and mild tricuspid regurgitation was found. During the first course of carfilzomib, on day 16, abdominal pain and dyspnea on exertion have been developed. On abdominal ultrasonography the hepatic vein are dilated and vena cava inferior engorged. These findings suggested the hepatic congestion due to cardiac failure. On the second echocardiography, the mean pulmonary arterial pressure was 60+15 mmHg and severe tricuspid regurgitation was found. Due to these clinical findings the therapy did not continued.

**Conclusions:** While cardiac and vascular-related adverse events were reported in patients with relapsed and/or refractory multiple myeloma who were treated with carfilzomib, most patients had a history of the specific cardiac or vascular adverse event they exhibited and demonstrated an improvement or resolution in symptoms after the discontinuation of therapy. Appropriate screening and monitoring could potentially allow at-risk patients to benefit fully from treatment with carfilzomib.

**Keywords:** Multiple myeloma, carfilzomib

PS-031

Abstract:0169

**CASE OF A MULTIPLE MYELOMA PATIENT WITH LIVER INVOLVEMENT AS A SPACE OCCUPYING LESION**

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Multiple myeloma is a malignant plasma cell dyscrasia and the second most common hematological malignancy after Non-hodgkin lymphoma. Extramedullary involvement in multiple myeloma can be seen. The reported incidence of extramedullary disease is %7 and it is increasing at newly published reports. Liver involvement as space occupying lesions in multiple myeloma is a rare condition. The literature published about this issue thus far is limited to few reports and case series.

We report a case of 58-year-old multiple myeloma patient with pathologically proven liver plasmocytoma which is determined on his 7th year of following period.

A 58-year old man presenting with back pain was diagnosed with Ig-G kappa type myeloma in 2006. He was initially treated with VAD and VCD chemotherapy. During follow-up he was accepted as at remission until March 2013, when he experienced relapse and developed extramedullary plasmocytoma on his right thoracic region. After DT-PACE chemotherapy, this lesion disappeared. During follow-up his serum creatinine level increased and an abdominopelvic USG was performed for etiology. USG revealed multiple hypoechoic masses on his liver which are incidentally seen in July 2013. Percutaneous US-guided liver biopsy was performed and plasma cell infiltration was seen. After 3 cures of DT-PACE therapy, abdominal CT showed enlargement of lesions at liver. Carfilzomib chemotherapy was planned for the patient because of refractory multiple myeloma. After first carfilzomib therapy, he died because of pneumonia induced septic shock.

Extramedullary involvement can be observed in multiple myeloma patients. The reported incidence of EMD is %7 and it is increasing at newly published reports. Autopsy reports show a higher incidence with %63 extraosseous involvement in one study. Liver involvement of multiple myeloma can be seen as diffuse cell infiltration or single/multiple space occupying nodular lesions as plasmocytomas. These lesions may cause extrahepatic biliary obstruction, ascites, jaundice and hepatomegaly.

Plasma cell infiltration of the liver can be detected in up to 45% of patients with multiple myeloma at autopsy series. However, nodular liver plasmocytoma is a clinical rarity. There are only a few reports and case series in literature about liver plasmocytoma in myeloma patients. These space-occupying lesions mimic metastatic lesions on imaging techniques.

Clinical and radiological manifestations of liver involvement in MM can vary. So, biopsy is often required for diagnosis.

In some reports, liver plasmocytoma is detected by elevation of transaminase levels or cholestatic signs. In some cases, like we report in this case, there is no elevated enzymes or cholestasis and liver lesions are detected incidentally in living patients.

**Keywords:** Multiple myeloma, liver involvement

PS-032

Abstract:0174

**CARFILZOMIB TREATMENT RESULTS AT THE OUR PATIENTS WITH RELAPSE- REFRACTORY MUTIPL MYELOMA**

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Introduction: Multiple myeloma (MM) is going to be better understood biology and disease come to a position that allows the treatment of an increasing number of targets. Second line proteasom inhibitors of carfilzomib; previously bortezomib and have received treatment at least twice, including an immunomodulatory agent, and during treatment or have relapsed multiple myeloma after treatment within 60 days from the end of this year for the use of patients received FDA approval quickly. So we evaluate our response to treatment relapsed refractory multiple myeloma patients treated with carfilzomib and we present purpose to contribute to the literature.

Material-Method: In our study, Dokuz Eylül University Faculty of Medicine in hematology clinical applied relapse-refractory multipl myeloma diagnosis, 12 patient recived treatment with carfilzomib. MM diagnosis of demographic, clinical, laboratory and cytogenetic features and overall survival of MM treatment (OS) and progression-free survival (PFS) was examined on the effect retrospectively.

Results: Median follow-up time was 64months, 5 of which were female(41.7%)and 7 were male(58.3%) and the median age was 60. When the stage to give 58.3%ISS2,41.7% ISSIII.According to the Durie-Salmon stageIIIA66.7%,16.7% with stageIIIB,stageI to 8.3%,8.3% in stageIII.66.7%of patients had been made since the transplantation of autologous stem cells.66.7%VAD as first-line treatment,16.7% cyclo-dexa,bortezomib-dexa8.3%,8.3% given MPT.When the first-line treatment response of66.7%PR,33.3%VGPRwas followed.As second-line therapy received bortezomib-dexa therapy in since66.7%,16.7%since the thalidomide, lenalidomide-dexa8.3%,8.3%MPV had received.Response to second line treatment of75%PR,8.3%CR,8.3%VGPR is,no response was observed of8.3%.About 50% of patients had recived radiotherapy.3 patients had recived treatment carfilzomib in 4th line and 5 patients had recived treatmentcarfilzomib in 5th line, the other 4 patients received carfilzomib as 6th line treatment.One patient had complete carfilzomib 12 cure and has begun to establish stable disease pomalidomid treatment takes.Carfilzomib treatment response of 50%had stable disease,25% PR,25% were found to be unresponsive.When Carfilzomib treatment of the observed side effects may be evaluated is not observed in 66.7%,16.7%cytopenia,%8.3skin rash,%8.3pulmonary HT.75%of the patients live,that25% of all dead.Median OS was observed 79.25 ±13.67 months and 5 year OS 58%.Median PFS was observed 12.97±4.2 months and 1 year PFS 21%.

Conclusion: In conclusion; relapsed refractory multiple myeloma is prolonged survival in patients treated with the new-generation.Positive effects on survival of the teratment carfilzomib was observed in our patients.Our

small case series is presented to make a contribution to the literature.

**Keywords:** Multipl myeloma, carfilzomib

PS-033

Abstract:0207

**CLINICAL CHARACTERISTICS AND OUTCOME OF CENTRAL NERVOUS SYSTEM INVOLVEMENT AMONG PATIENTS WITH MULTIPLE MYELOMA**

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Introduction: Involvement of central nervous system (CNS) is a rare finding observed 1%. It is usually associated among patients with advanced multiple myeloma (MM) and is known to have a poor prognosis. Patients may present with different neurological findings. Diagnosis is based on pathological evaluation of biopsy material, cerebrospinal fluid (CSF) analysis. Magnetic resonance imaging (MRI) may show typical leptomeningeal enhancement and contrast uptake. Herein, we present a detailed descriptive analysis on the CNS MM cases diagnosed and treated in our institution.

Methods: We present five MM patients diagnosed with CNS involvement during 2013-2014. Hospital records are searched retrospectively.

Table. Patient Characteristics

Pt no	Age/ Sex	Previous lines of therapy (n)	Heavy/Light Chain	FISH	Disease status at CNS MM	Treatments	Response	OS (mon)
1	65/M	1	No/urine kappa	Unknown	PR	RT	N/A	1
2	37/F	0	IgA/kappa	del13q+ del17p+	Untreated	Bortezomib DCEP/ ASCT/RT	PR/Refractory/ N/A	8
3	50/M	3	IgG/kappa	del13q+ t(4:14)	Relapsed	RT/BendaThalDex	PR/ Refractory	2
4	34/M	7	No/kappa	del13q+	Relapsed	RT/DCEP/Thal/ Carfilzomib	Refractory/ Refractory	12
5	62/M	7	IgG/kappa	del13q+	Relapsed	DCEP	Refractory	1

Radiotherapy: RT, DCEP: Dexamethasone, Cyclophosphamide, Etoposide, Cisplatin

Results: The median age of group is 50 (range, 34-65), Female/Male:1/4. The median time from symptomatic MM diagnosis to CNS involvement is 26 months (range, 0-72). Patients received median 3 lines of therapy prior to CNS involvement (range,0-7). The average albumin, LDH, beta-2 microglobulin at diagnosis are as follows, respectively: 2,86 g/dl (2.6-3.8), 159 U/L (116-226), 3,88 mg/L(1.97-6.21). Median LDH increased to 349 U/L(142-976) at the time of CNS involvement. The patient characteristics are shown in Table. Symptoms were: headache, aphasia, ptosis at admission. Frontal lesion was detected in three whereas one patient had orbital, one patient had occipital lesions. Flow-cytometric analysis in cerebrospinal fluid could be performed in one patient, showed diffuse plasma cell infiltration. All patients, except for one who died due to septicemia, received Radiotherapy plus a chemotherapy including anthracycline, Etoposide, Cisplatin, Bendamustine, High dose steroids and Bortezomib or IMiDs depending on the last line of treatment. Patients also received intrathecal Dexamethasone, Cytarabine and Methotrexate. One patient's clinic was complicated with documented Cryptococcal meningitis. All patients died within a median survival of 4.8 months.

The longest survivor was the only patient who was able to receive Carfilzomib.

Conclusion: CNS involvement of MM has a short median OS. Plasma cell dissemination to CNS is still a sign of aggressive MM even in the era of Bortezomib, Carfilzomib and new IMiDs.

**Keywords:** Autologous Stem Cell Transplantation, amyloidosis

PS-034

Abstract:0213

**DIFFUSE LARGE B CELL LYMPHOMA AND PLASMA CELL MYELOMA DEVELOPING YEARS AFTER THERAPY FOR HODGKIN LYMPHOMA**

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Objectives: A spectrum of lymphoid malignancies may rarely develop in the same patient. Multiple myeloma and lymphomas as secondary malignancy after treatment of hematologic malignancies are very infrequent. We report a case who had been treated for Hodgkin Lymphoma (HL) years ago and developed Non-Hodgkin lymphoma (NHL) and multiple myeloma (MM) consecutively four-year apart. Case: A 68 year-old- man was treated successfully with 6 cycles MOPP-ABVD for HL in 1993. Eighteen years after the treatment, he was admitted with the complaints of dispnea and cough. During diagnostic workup at CT imaging cervical, mediastinal and intraabdominal multiple pathologic LAP, splenomegaly were identified. A nodule of 2.5 mm sized and narrowing the passage was seen inside the bronchus at bronchoscopic examination. Biopsy of the nodule was consistent with diffuse large B cell lymphoma. He achieved a partial remission after 8 cycles of R-CHOP. Splenomegaly remained at 200 mm size, which was concluded to be as a result of portal hypertension. Two years after completion of therapy, the patient was admitted with back and bone pain at right leg. In MRI a solid mass involving L3 vertebral corpus was reported. He was investigated for disease progression. Initial labs revealed a WBC count of 10.7 x10<sup>9</sup>/L, elevated absolute neutrophil count of 8.5x10<sup>9</sup>/L, hemoglobin 9.7g/dL, platelet count of 233 x10<sup>9</sup>/L, creatinine 1.0 mg/dL, calcium 9.1 mg/dL, total protein 6.3mg/dL, albumin 3.3 mg/dL, lactate dehydrogenase 307 U/L and beta-2-microglobulin 10.9 mg/L, CRP 132 mg/dl and ESR 86 mm/hr. Peripheral blood smear was unremarkable except rouleaux formation. Renal and hepatic function tests were in normal ranges. On ultrasound he had reactive appearing supraclavicular, axillar and inguinal lymphadenopathies maximal 1.8cm sized and pathology of supraclavicular excisional biopsy could not demonstrate any involvement of lymphoma. Evaluation for anemia revealed a peak at beta2 band at serum protein electrophoresis. Serum and 24 hours urine immunofixation demonstrated free light chain lambda. Bone marrow biopsy revealed dense atypical plasma cell infiltration and immunohistochemical staining with CD138 and lambda confirmed monoclonality. Multiple myeloma was diagnosed and classified as stage III according to International scoring system.

Discussion: In lymphoma patients, an increased incidence for secondary malignancies is described as a result

of cytotoxic chemotherapy. Three types of secondary malignancy are recognized: Solid tumors, leukemias and NHL. MM was an unexpected presentation in this patient. It is difficult to differentiate between secondary malignancy and a second primary, but a common pathogenetic mechanism may be responsible from a spectrum of lymphoid malignancies occurring in the same patient. The increased risk for a second neoplasm in HL underscores the importance of continued monitoring of these patients.

**Keywords:** Multiple myeloma, diffuse large B cell lymphoma

PS-035

Abstract:0216

**SUCCESSFUL TREATMENT OF EARLY DIAGNOSED STRONGYLOIDES STERCORALIS PULMONARY INFECTION IN A PATIENT WITH MULTIPLE MYELOMA**

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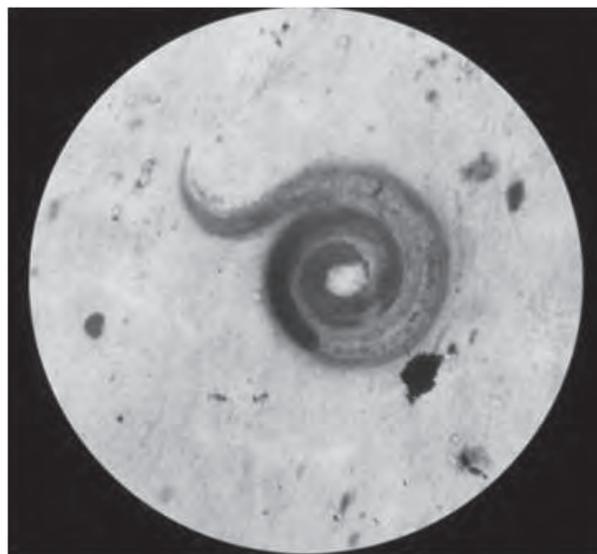
**Introduction:** Strongyloides stercoralis (*S. stercoralis*) is an intestinal nematode, which is seen endemically in tropical regions, and mostly as sporadic cases only in immunosuppressive patients in temperate regions such as Turkey. It is most important helminthic infection that can be fatal in immunosuppressive host. Here we report a case of pneumonia which is diagnosed early and treated? due to *S. Stercoralis* infection in a patient under treatment for multiple myeloma.

**Case:** A 78-years-old male patient, who is followed with refractory multiple myeloma (MM) for three years and treated with lenalidomide and dexamethasone as third-line treatment, presented with loss of appetite, intense, thick brown sputum and hemoptysis. Physical examination revealed diminished breath sounds especially in the left lower zone and excoriation due to scratching in different parts of body. Although patient did not have remarkable laboratory findings associated with active infection, patient was hospitalized due to symptoms and physical findings suggesting pneumonia. He was considered as immunosuppressive and empiric levofloxacin therapy was started. Microscopic examination of sputum with gram and giemsa staining revealed mostly polymorphonuclear leukocytosis and larvae of *S. Stercoralis* (Figure 1). Sputum and stool cultures were negative for any pathogen. Although there was not any infiltrative image in chest radiograph, computed tomography (CT) demonstrated relatively protected subpleural regions and ground-glass opacities decreased towards the lower zone. Albendazole 1x400 mg 3 days, then 1x200 mg for 21 days and human immunoglobulin (IVIG) (400 mg/kg for once) treatment was planned. At the end of the first week patient's clinical condition improved and larvae were completely disappeared in sputum examinations.

**Discussion:** Strongyloidiasis, has caused by *S. Stercoralis*, is a nematode infection caused by parasitic infection of larvae in various organs and adult form in the lumen of small intestine. Infection can be seen as asymptomatic eosinophilia in the immunocompetent host or disseminated disease in the immunocompromised host. Infection, usually starts by penetration of filariform larva to the skin from soil but it leads to minor cutaneous symptoms. Its thought that humoral immunodeficiency has a role in development of disseminated disease. Pulmonary involvement is the most important manifestation of disseminated disease and symptoms may include cough, thick sputum, dyspnea and hemoptysis. Pulmonary hemorrhage and respiratory failure can be seen in the absence of early treatment. To our knowledge there are only 16 reported *S. stercoralis* cases in multiple myeloma patients in literature which all had a fatal course or required admission to intensive care unit. In our case, early diagnosis by demonstrating larvae in sputum and initiation of antihelminthic treatment together with IVIG, may have contributed to disease improvement.

**Keywords:** Strongyloides stercoralis, multiple myeloma

Figure 1



Rhabditoid larva of *S. stercoralis* in giemsa stained sputum

PS-036

Abstract:0218

**CELLULITIS-LIKE SWEET SYNDROME PRECEDING MULTIPLE MYELOMA: A CASE REPORT**

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Sweet syndrome is a reactive neutrophilic dermatosis characterized by fever, multiple erythematous painful plaques and nodules, neutrophilia, high levels of serum

inflammatory markers, and a dense diffuse-mature neutrophilic infiltration typically localized to the upper dermis. The skin eruption usually occurs on the face, neck, chest, and upper extremities, mostly in the middle-aged females. The disease is generally idiopathic but it may also be seen as a paraneoplastic syndrome in 20-25% of the cases. It can precede, follow, or appear concurrent with the diagnosis of the malignancy. Cutaneous manifestations in this condition tend to have a more widespread distribution pattern, including involvement of the lower limbs. While malignancy-associated Sweet Syndrome is most commonly observed in acute myelogenous leukemia, association with Multiple myeloma is relatively rare in the medical literature.

In this case, a 64-year-old man who was admitted to Adiyaman University Medical School Infection Clinic due to his painful red rashes affecting his right leg and buttock for nearly one month, was presented. He had treated with oral and intravenous broad-spectrum antibiotics previously however he was unable to recover. A skin biopsy specimen analysis revealed that marked edema of the upper dermis with a diffuse inflammatory infiltration of the upper dermis consisting mainly of neutrophils. Based on skin biopsy, clinical appearance, laboratory investigations and histological findings, we concluded that patient was diagnosed as Sweet syndrome. His situation was also consulted with hematology clinic and a further diagnosis of Multiple myeloma was also named. As a conclusion, we consider that physicians must be aware of this cases with skin lesions and fever may be a first sign of an underlying malign disease.

**Keywords:** Sweet syndrome, Multiple myeloma

PS-037

Abstract:0223

**A CASE OF POEMS SYNDROME WITH DEBILITATING NEUROLOGICAL INVOLVEMENT AND FDG-AVID BONE LESIONS WHO HAD AN UNUSUALLY RAPID SYMPTOMATIC RECOVERY WITH UP-FRONT CYCLOPHOSPHAMIDE AND DEXAMETHASONE THERAPY BEFORE ASCT WITH HIGH DOSE MELPHELAN**

Ömür Gökmen Sevindik, Ümit Kan, Celal Acar, Doğuş Türkyılmaz, Süreyya Yiğit Kaya, Şerife Medeni Solmaz, İnci Alacacıoğlu, Özden Pişkin, Mehmet Ali Özcan, Fatih Demirkan, Bülent Ündar, Güner Hayri Özsan  
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A 43 years-old male patient admitted to his family physician with the complaints of progressive difficulty in walking due to muscle weakness mainly effecting lower limbs, and erectile dysfunction. Patient indicated that he had first realized these symptoms 6 months ago and the intensity had gradually increased. His family physician referred the patient to our neurology clinic. Detailed history revealed a newly diagnosed type 2 Diabetes Mellitus which was treated with metformin, no alcohol or tobacco abuse and no family history of any malignancies or autoimmune disorders. Physical examination revealed hyperpigmented skin lesions, hepatosplenomegaly, stocking-glove sensory loss mainly localized to distal limbs and grade 2-3 muscle weakness mainly effecting lower extremities, bilateral drop-foot and loss of bilateral DTRs at lower extremities. EMG revealed sensorimotor

polyneuropathy with axonal degeneration. Neuroimaging was normal and lumbar puncture revealed an increase at the CSF protein and IgG levels. All autoimmune markers were negative and age specific screening of possible malignancies revealed no pathological features. Laboratory exams revealed a mild erythro-thrombocytosis, a decreased level of Vit B12, an inverted albumin globulin ratio, decreased total and free testosterone and increased ACTH. JAK2V617F mutation was negative. Monoclonal gammopathy with an M-spike of 2.5 g/dL was detected with serum protein electrophoresis and immunofixation revealed IgG lambda monoclonal gammopathy. His quantitative immunoglobulin levels were as follows, IgG 2652 mg/dL, IgA 168 mg/dL, IgM 250 mg/dL with an FLC ratio of 1.06. Patient was then evaluated by our consultant hematologist with a pre-sumptive plasma cell disorder. Bone marrow examination was ordered and revealed 5% plasma cells staining equally with kappa and lambda with a normal cellularity and grade 1 reticulin fibrosis. Conventional bone survey and CT revealed multiple mixed sclerotic and lytic bone lesions, with soap-bubble appearance, distributed to the pelvic and iliac bones (Figure 1). These lesions were FDG avid with SUVmax values ranging between 4.5 and 6.5. Biopsy from the bony lesion localized to the iliac bone showed an increased osteoid formation, production of new bone which resembles woven bone with lymphocytic aggregates and plasma cell rim. Consultation with endocrinology has confirmed the diagnosis of hypogonadotropic hypogonadism and type 2 diabetes mellitus. Patient was diagnosed as POEMS syndrome with fulfilling the criteria needed to establish the diagnosis. Patient was put on to cyclophosphamide dexamethasone upfront therapy. After two cycles of cyclophosphamide and dexamethasone patient is able to walk without any kind of external support. All symptoms are gradually recovering. We are planning to proceed with autologous hematopoietic stem cell transplantation with high dose melphelan conditioning.

**Keywords:** POEMS syndrome, polyneuropathy

Figure 1



FDG-avid mixed lytic-sclerotic bone lesions.

PS-038

Abstract:0227

**LENALIDOMIDE DESENSITIZATION IN TWO PATIENTS WITH TWO DIFFERENT REACTION TYPES**Semra Demir<sup>1</sup>, Müge Olgaç<sup>1</sup>, Derya Ünal<sup>1</sup>,Aslı Gelincik<sup>1</sup>, Bahauddin Çolakoglu<sup>1</sup>,Suna Büyükoztürk<sup>1</sup>, Meliha Nalcacı<sup>2</sup><sup>1</sup>Istanbul University Istanbul Faculty of Medicine Internal Medicine Department, Division of Immunology and Allergy<sup>2</sup>Istanbul University Istanbul Faculty of Medicine Internal Medicine Department, Division of Hematology

**Introduction:** Lenalidomide is an immunomodulatory agent which is approved to treat multiple myeloma (MM), myelodysplastic syndrome and mantle cell lymphoma. Although generally well tolerated, lenalidomide can cause hypersensitivity reactions such as urticaria and maculopapular or morbilliform lesions. If there is no effective alternative agents desensitization is the choice of treatment. Here we present two cases who experienced different hypersensitivity reactions to lenalidomide and were desensitized successfully with different protocols.

**Case 1:** A 70 years old female patient diagnosed with IgG kappa type MM was given lenalidomide and dexamethasone after first line therapies, including bortezomib dexamethasone and oral cyclophosphamide failed. On the third day of lenalidomide urticaria developed. Drug was discontinued and the patient was treated with antihistamines and methylprednisolone. Considering that urticaria was an immediate type of reaction, the patient was successfully desensitized to 25 mg lenalidomide in a tertiary outpatient allergy clinic using a previously published rapid protocol. As lenalidomide is used for 21 days with 10 days interval, desensitization procedure had to be repeated after every interval.

**Case 2:** A 75 years old male patient with IgG kappa type MM experienced eczematous rash on the second week of lenalidomide treatment. His symptoms resolved after discontinuation of the drug and methylprednisolone treatment. Because a delayed type hypersensitivity reaction was diagnosed, a previously published slow desensitization protocol of 6 weeks was started for tolerance induction. However on the third day of the fourth week similar lesions developed. Therefore protocol was tailored to the patient, incrementing doses more slowly and desensitization was achieved in eight weeks. Consequently, the patient had to continue taking lenalidomide 10 mg/day without interval (almost half of daily recommended dose in the treatment protocol) to keep his tolerance status maintained.

**Conclusion:** Desensitization is an important treatment modality and can be used in both immediate and nonimmediate types of hypersensitivity reactions caused by agents of which alternatives are less effective or absent. Desensitization protocol must be determined according to the drug and the patient's previous reaction. Immediate type hypersensitivity reactions can be desensitized in one or two days while repetitive slowly increasing doses lasting for weeks can be required for delayed type reactions.

**Keywords:** Lenalidomide hypersensitivity, drug desensitization

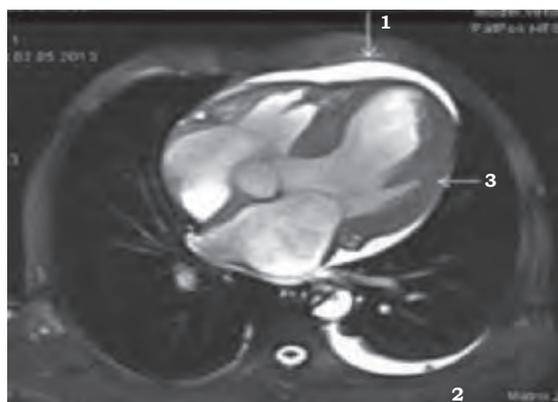
PS-039

Abstract:0251

**PRIMARY SYSTEMIC AMYLOIDOSIS TREATED BY THE COMBINATION OF BORTEZOMIB WITH DEXAMETHASONE AND CYCLOPHOSPHAMIDE: A CASE REPORT**Mahmut Tobu<sup>1</sup>, Çiğdem Altınel<sup>1</sup>, Melda Cömert<sup>1</sup>,Mine Hekimgil<sup>2</sup>, Selen Bayraktaroglu<sup>3</sup>,Hatice Demet Kiper Ünal<sup>1</sup><sup>1</sup>Ege University Medical Faculty, Hematology Department, Bornova-İzmir, TURKIYE<sup>2</sup>Ege University Medical Faculty, Pathology Department, Bornova-İzmir, TURKIYE<sup>3</sup>Ege University Medical Faculty, Radiology Department, Bornova-İzmir, TURKIYE

**Introduction:** Cardiac involvement is seen frequently in primary amyloidosis (AL) and associated with progressive heart failure and poor prognosis. Combination of bortezomib with dexamethasone and cyclophosphamide is an effective treatment choice for the patients with cardiac amyloidosis.

**Case:** 60 years old male was admitted with progressive muscle weakness in upper and lower extremities and diagnosed as demyelinating sensory and motor neuropathy in neurology department. Laboratory analysis demonstrated mild anemia and kappa light chain monoclonal gammopathy. He was referred to hematology department. Bone marrow aspiration and biopsy were performed and 10% monotypic kappa (+) plasma cells and Congo red (+) amyloid deposits were observed. ECG showed low voltage in all leads. In ecocardiography; LVEF: 25%, RVEF: 40%, SPAP: 40 mmHg were found and LV concentric hypertrophy, global LV and RV hypokinesia and dilated heart chambers were notified as expected for cardiac amyloidosis. Cardiac MRI demonstrated the significant increase of myocardial thickness both for right and left ventricles (Figure 1). After one cycle of Bortezomib (1.3 mg/m<sup>2</sup>/day), D(1, 4, 8, 11) + Cyclophosphamide (300 mg/m<sup>2</sup>/day, D(1, 15) + Dexamethasone 25 mg/m<sup>2</sup>/day, D(1, 4, 8, 11) combination therapy control ecocardiography showed a remarkable recovery with LVEF: 38%, RVEF: normal, SPAP: 30 mmHg. Totally three cycles of mentioned regimen were performed and the patient's ECG performance status was apparently improved. Unfortunately during follow up, he died due to pneumoniae and respiratory failure.

**Cardiac MRI**

SSFP (Steady State Free Precession) pericardial effusion (white arrow 1), pleural effusion in left hemithorax (red arrow 2), significant increase in myocardial thickness of ventricles (yellow arrow 3).

Discussion: The role of conventional therapy with alkylating agents for primary amyloidosis is limited. Bortezomib based therapies are preferable and effective treatment choice in this group. Furthermore patients who are ineligible for autologous stem cell transplantation may regain transplant choice by improving cardiac outcomes by initial treatment. More data from larger case series are needed to show the efficacy of bortezomib in cardiac amyloidosis.

**Keywords:** Amyloidosis, bortezomib

PS-040

Abstract:0252

**CHROMOSOME 1 ABERRATIONS IN MULTIPLE MYELOMA: A SINGLE CENTER EXPERIENCE**

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Objectives: One of the most frequent chromosomal aberrations in multiple myeloma (MM) is the structural aberrations of chromosome 1. 1q gain and 1p loss are closely related with shorter survival. In this study, we aimed to investigate the frequency of combined 1p deletion and 1q gain in MM patients.

Methods: G-banding and FISH analyses were carried out on 80 bone marrow samples of 78 MM patients referred for genetic analysis between January 2013 and December 2014. We also performed FISH analyses to all patients by using LSI 1q21/1p32.3 (Cytocell), LSI 13q34 (Vysis), LSI TP53(Vysis) and LSI IGH Dual Color Break Apart (Vysis) probes.

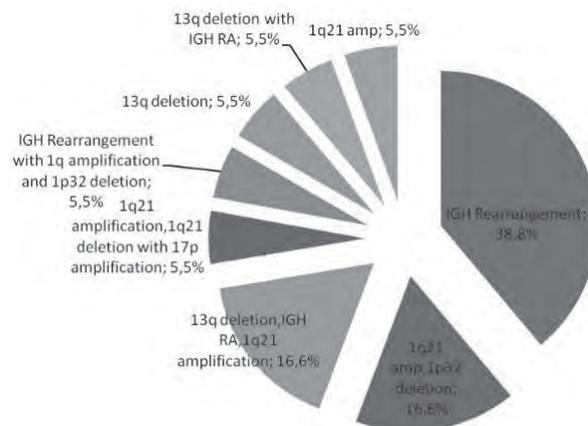
Results: Of the 78 MM patients, 33 (42.3%) were male and 45 were (57.7%) female, with an age range of 35–84 years. One male and one female patient had two consequent bone marrow samples, hence, the number of samples were 80. Lymphocyte cultures were unsuccessful in 5 samples. Normal karyotypes were found in 69/75 (92 %) samples. The frequency of cytogenetic abnormalities was 8% (6/75). Five samples had complex karyotypes and one sample had 6q deletion as a sole abnormality (Table 1). We detected chromosomal abnormality in 22.5% (18/80) of the examined samples by FISH. The frequency of 1q21 copy gain was 10% (8/80). Copy gain at 1q21 region was detected in 8 (44.4%) samples. We did not detect deletion at 1p32.3 region in one of these samples. We observed copy gain at 1q21 and deletion at 1p32.3 region in 3 samples, as the sole abnormality. We detected additional abnormalities to chromosome 1 aberrations in 4 samples. Three of them were together with del(13q14) and IGH rearrangement and one was together with IGH.

Conclusion: The frequency of 1q21 aberrations (10%) in our cohort was lower than the previous studies (40%). This could be due to the heterogeneity of the disease. Although 1q copy gain is usually frequent at progressive disease, we detected this in only one patient during relapse. This patient had a normal karyotype. This finding emphasizes the advantage of molecular cytogenetics in diagnosis. We found 1q21 copy gain in 10% (8/80) of the samples. Among these, 37.5% (3/8) had a complex

karyotype. The remaining five patients had normal karyotypes. It is known that both 1q21 copy gain and complex karyotype are separately related with poor prognosis. We think, these genetic changes will play role in disease follow-up and management. To our knowledge, the frequency of 1q21 copy gain combined with 1p32 deletion has not been reported in the literature. Frequency of combined 1q21 copy gain and 1p32 deletion was 8.7% in our study. Although it seems to be high, because of the small study group, it is hard to conclude about its effect in disease pathogenesis. We concluded clinical evaluation and data are essential before deciding the effects of chromosome 1 aberrations in MM. Thus, studies with large cohorts are needed.

**Keywords:** Multiple myeloma, chromosome 1 aberration

The frequency of the abnormal FISH results.



The karyotype and FISH results of patients with cytogenetic abnormalities.

Patient no.	Karyotype	FISH
1	46,XX,der(7)3,der(9)2,der(18)3,incp7/46,XX13	Normal
2	46-48,XX,der(1)t(1q:?),der(7),der(9),-14,+mar,incp18/46,XX2	IGH rearrangement, 1q21 copy gain, 1p32 deletion
3	48-54,XY,de(1p)x2,+del(3q),der(6)t(6p:?),+11,+15,+18x2cp3/46,XY17	1p32 deletion
4	51,X,-X,+3,+5,+6,+7,+9,-13,der(14)t(14q32:?),+15,+der(18)t(1q:18qter),+19,-20,-22,+mar5/46,xx15	IGH rearrangement, 1q21 copy gain, 1p32 deletion, 13q deletion
5	46,XY,del(6q)	Normal
6	83-87,XX,+der(1)del(1p)x2,+der(9)t(9p:?)x2,-16,-16,+der(16)x2,+del(20q),+der(22)t(22p:?),+2marcp3/46,XX17	1q21 copy gain

PS-041

Abstract:0253

**CYTOGENETIC FOLLOW-UP OF A MULTIPLE MYELOMA PATIENT**

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Objectives: Chromosomal aberrations are frequently seen in Multiple Myeloma (MM) and play an important role in patient outcome and management of disease. The

genetic characterization of MM is very complex. Although cytogenetic evaluation has been usually performed at initial diagnosis, chromosomal instability may occur during disease progress. We report cytogenetic results of a MM patient during follow-up.

Methods: A 58 years old female patient presented with a history of bone pain at lumbar site. Bone marrow aspirate analysis revealed multiple myeloma histopathologically. Conventional cytogenetic analysis and fluorescence in situ hybridization (FISH) analysis was performed. We used LSI 1q21/ 1p32.3(Cytocell), LSI 13q34 (Vysis), LSI TP53/CEP 17 (Vysis) and LSI IGH (Dual Color Break Apart,Vysis) probes. The patient received Vincristine-Doxorubicin-Dexamethasone (VAD) regimen for 4 cycles and showed a complete response after VAD regimen followed by intense dose chemotherapy with peripheral blood stem cell support (PBSC). Complete remission was achieved and patient was followed up without treatment. She referred with bone pain after 2 years. According to bone marrow aspiration, she was accepted as relapse. The patient received Bortezomib-Cyclophosphamide-Dexamethasone (VCD) regimen for two cycles and attained a partial remission. Thus, protocol changed and the patient received Revlimid-Velcade-Dexamethasone (RVD) regimen for two cycles and showed a very good partial response. Second adjuvant dose-intensive chemotherapy with PBSC was administered after RVD regimen. The patient referred with diffuse bone pain after 1 year achieving the complete remission. Radiologic examinations showed osteolytic lesions. Laboratory findings revealed an increased serum calcium level. The patient received Bortezomib, Lenalidomide and Dexamethasone (VRD) protocol.

Results: Cytogenetic abnormality was not observed by cytogenetic and fluorescence in situ hybridization (FISH) analysis at diagnosis. Two years later at the second referral both cytogenetic and FISH analysis were also normal (first relapse). The patient with suspicion of clinical relapse referred third time at 48th months after diagnosis. Karyotype revealed normal, but 1q21 copy gain with IGH(14q32) rearrangement was observed by FISH.

Conclusion: Chromosome 1q gain is associated with complex karyotype and poor-risk genetic features. This finding during relapse and progression of disease was more frequent than in newly diagnosed MM. Our patient's conventional cytogenetic and MM FISH panel analysis at initial disease was normal. However, chromosome 1(q21) copy gain with IGH(14q32) rearrangement was demonstrated by FISH analysis during relapse. Our results suggest that chromosome 1q alterations result in aggressive behavior of the disease. The importance of conventional cytogenetics and FISH analyses in MM follow-up are important.

**Keywords:** Multiple Myeloma, cytogenetic

PS-042

Abstract:0256

**AUTOLOGOUS STEM CELL TRANSPLANTATION AND PRIMARY SYSTEMIC LIGHT CHAIN (AL) AMYLOIDOSIS: SINGLE CENTER EXPERIENCE**

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Introduction: Primary systemic light chain (AL) amyloidosis is a plasma cell disorder effecting multiple organs and tissues. The survival of patients with AL is highly dependent on cardiac dysfunction. Mayo Clinic risk assessment is helpful tool in selection of patients who will benefit from high dose Melphalan with support of autologous stem cell transplantation. Herein, we present the outcome of our AL amyloidosis patients.

Materials-Methods: We retrospectively included 10 patients diagnosed with AL amyloidosis at Ankara University School of Medicine Department of Hematology between 2007-2014.

Table 1. Patient Characteristics

No	Age	Cardiac/ Renal Involvement	Other Organ Involvement	Risk Score	Treatments prior to ASCT	Conditioning regimen/ Mobilization agent	Response (hematological)	OS/FU
1	69	+/+	GI	1	None	Mel140/GCSF	NA	4/4
2	54	+/+	Skin,soft tissue, neurological, GI	2	VAD/MelDex/ VMelDex	Mel100/Cy-GCSF	PR	21/21+
3	58	+/-	GI	3	-	Mel100/ GCSF+ Plerixafor	PR	16/16+
4	46	+/+	GI	2	-	Mel140/GCSF	PR	60/50+
5	50	+/+	GI	-	VAD	Mel100/Cy-GCSF	NA	42/42+
6	57	-/+	-	-	VAD	Mel200/GCSF	VGPR	96/60+
7	49	-/+	-	1	MelDex	Mel200/Cy-GCSF	NA	18/18+
8	56	-/+	-	1	MelDex	Mel200/ GCSF+ Plerixafor	NA	12/22+
9	50	+/+	GI	3	VCD	None	NA	3/3
10	50	-/+	-	1	MelDex	None	PR	5/5+

Results: Patient characteristics are listed in table 1. The median age was 54 years (range 46-69), five of them were male. Six patients had cardiac whereas nine patients had renal involvement at diagnosis. Five of the patients diagnosed with kappa free light chain others had lambda free chain disease. Median eGFR (estimated with the modified MDRD equation) was 86 ml/min/1.73 m<sup>2</sup> (range, 28-129). At diagnosis median NTproBNP level was 1039 ng/L (range 76-4905), median difference between involved and uninvolved free light chain (dFLC) was 403 mg/L. According to cardiac biomarkers 40% of the patients were in Mayo stage-1, 20% were in stage 2 and 10% were stage 3. Eight patients received high dose therapy and underwent autologous stem cell transplantation (ASCT). The median time from diagnosis to ASCT was 8 months (range 3-14 months). Except 1 patient, all patients received chemotherapy prior to ASCT as follows: Melphalan-Dexamethasone (MelDex) (n=2); Vincristine, Adriamycin, Dexamethasone (VAD)(n=2) VAD and Mel Dex (n=1); unknown (n=2). Two patients are currently receiving induction with Mel Dex+VCD prior to stem cell mobilization. Only three patients could be mobilized with G-CSF alone. The median CD34 count was 6.33 x10<sup>6</sup>/

kg (2.8-9.88x10<sup>6</sup>/kg). Melphalan dose was reduced according to the renal or cardiac functions. Median time for neutrophil engraftment was 15 days (range, 11-19) while platelet engraftment was achieved in median of 16 days (range, 11-23). One of two patients died on 11th day due to sepsis other died prior to stem cell transplantation because of intestinal perforation. 50% of patients achieved a hematological response (40% PR, 10% VGPR). The median 24 hour urine protein decreased from 7018 mg/day to 215 mg/day. Following a median follow-up of 23 months (3-60 months) overall survival of the group was 28 months (3-96 months).

**Conclusion:** ASCT is an effective therapy for primary amyloidosis. Our results based on experience among patients having low risk scores and preserved cardiac function favor the role of ASCT. Overall treatment of primary Amyloidosis is likely to evolve as earlier diagnosis and new pre-transplant therapies become available.

**Keywords:** Amyloidosis, autologous stem cell transplantation

PS-043

Abstract:0266

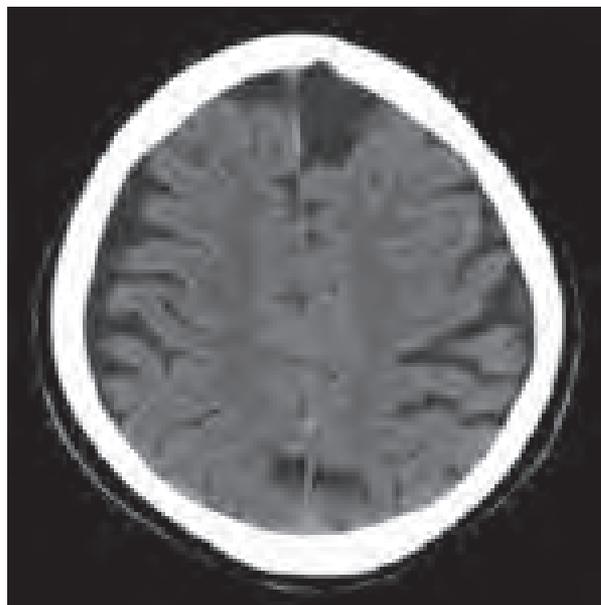
### CENTRAL NERVOUS SYSTEM RELAPSE AFTER CHEMOTHERAPY IN A PATIENT WITH MULTIPLE MYELOMA

Şerife Solmaz Medeni, Can Özlü, Gülsüm Akgün  
Çağlayan, Sinem Namdaroğlu, Oktay Bilgir  
İzmir Bozyaka Eğitim ve Araştırma Hastanesi

We report a case of central nervous system relapse with intraparenchymal, dural and leptomeningeal involvement after high dose chemotherapy. A 72 year old man received two cycles of bortezomib and dexamethasone as an induction treatment for stage IIIA IgA kappa multiple myeloma. Partial response achieved after two cycles of bortezomib and dexamethasone and continued to treat. Biphosphonate treatment was given because of lytic bone lesions. 4 months after bortezomib and dexamethasone therapy increased plasma cells in the bone marrow was observed. Patients were considered refractory multiple myeloma and began therapy with lenalidomide and dexamethasone. Two months after lenalidomide -dexamethasone therapy the patient admitted with sudden transient loss of vision, headache. Cranial computer tomography imaging revealed mass lesion occupying bilateral frontal lobes, was determined mega cisterna magna, both cerebral hemispheric cortical sulci were detected especially in the parietooccipital expanded to be more specific. Heterogenous dural and leptomeningeal infiltration was detected. After lenalidomide therapy symptoms related with central nervous system involvement and cytopenias related bone marrow infiltration got worse. The patient was lost from sepsis and renal failure. **Conclusion:** Central nervous system involvement of multiple myeloma is rare. It may manifest as dural myeloma or intraparenchymal infiltration or with diffuse leptomeningeal involvement. In the literature there is no standard therapeutic approach regarding the central nervous system relapse after high dose therapy and median survival of patients with CNS relapse is 2 to 3 months. There are no treatment guideline for central nervous system myelomatosis in the literature. Systemic chemotherapy regimens, radiotherapy and intrathecal chemotherapy were tried, but no long survival. The central involvement of myeloma patient is presented for contributions to the literature.

**Keywords:** central nervous system, multiple myeloma

Cranial computer tomography



PS-044

Abstract:0267

### COEXISTENCE OF MYELOYDYSPLASTIC SYNDROME AND MULTIPLE MYELOMA

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**Introduction:** The coexistence of both diseases are rare, It has been known that coexistence of multiple myeloma (MM) and myelodysplastic syndrome (MDS) may occur as a complication of treatment. Due to treatment with alkylating agents myelodysplastic syndrome (MDS) may occur in multiple myeloma patients. Chemotherapy induced MDS (secondary MDS) is more resistant to therapy and have a poor prognosis. Here we report a case of coexistent MM and MDS in a patient without history of treatment with any cytotoxic drugs or radiation therapy.

**Case:** A 63-years-old female was presented to our clinic with fever and weakness. Her blood counts were: WBC: 1.51x 10<sup>9</sup>/l - HGB: 8g/dL - MCV 85.4 fl. - PLT: 71,000/mm<sup>3</sup>. Her serum total protein elevated at 10.2 g/dl albumin 4 g/dl. Serum iron levels were normal and serum ferritin was elevated at 539 ng/ml. The peripheral blood smear showed hypochromic and normochromic cells, anisopoikilocytosis, tear-drops and target cells. Bone marrow biopsy showed 70 % lambda positive plasma cell infiltration and were tri-lineage cellular dysplastic features, which included multinucleated erythroblasts, pseudo-Pelger anomaly, hypersegmentation of neutrophils and dysplastic megakaryocytes with hypolobulated nucleus. Ringed sideroblasts and 10-19 % myeloblasts were seen. These findings were characteristic for MDS RAEB II and MM. Karyotype was normal: 46, X, X. FISH analysis did not find any chromosomal abnormality. Serum immunoglobulins showed an elevated IgG (37.6

g/l) with increase in lambda free light chains (4.68 g/l), serum protein electrophoresis and Serum immunofixation studies showed a monoclonal IgG lambda. A radiologic full body-bone survey showed no lytic lesions. Urine protein electrophoresis showed a monoclonal lambda light chain with.....g/day of lambda light chains in the urine. Her radiologic full body-bone survey showed no lytic lesions. Our patient was diagnosed with coexistence MM IgG Lambda and MDS-RAEB II. We started treatment for her multiple myeloma with bortezomib and dexamethasone. After receive 4 cycles of with bortezomib and dexamethasone-containing regimen then patient achieve Complete response (CR).

Conclusion: In this case we reported a patient diagnosed with coexistence both MM and MDS. presence the coexistence of both diseases originating from different cell lines, may not be rare as they known. We suggest that mm patients administrating with cytopenie should be evaluated for coexistent myeloid neoplasms In this case we aimed to engage that mm patients administrating with cytopenie should be evaluated for coexistent myeloid neoplasms. In literature there are some similar reports, support this

**Keywords:** Myelodysplastic syndrome, multiple myeloma

PS-045

Abstract:0269

**COLLET-SICARD SYNDROME AND MULTIPLE MYELOMA ASSOCIATION**

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Introduction: Multiple myeloma is a plasma cell malignancy that characteristically involves extensive infiltration of bone marrow (BM), with monoclonal protein and presence of end-organ damage. Collet-Sicard syndrome is a rare disease which is characterized by Unilateral paralysis of the last four cranial nerves (IX-XII). Few cases have been reported in the literature of Collet-Sicard syndrome due to Multiple myeloma. We reported in this case a patient with multiple myeloma who presented presented an atypical neurologic feature, the Collet-Sicard syndrome.

Case: A 48-year-old woman complained of headache, dysphagia and speech impairment. He had no medical history (i.e. trauma, hypertension, diabetes) and head trauma She didn't have any pathological signs in her neurological examination. Her blood counts- WBC:10850/mm<sup>3</sup>, Hb:10.9g/dl, Plt:297000 mm<sup>3</sup>, crea:0.92mg/dl, AST:19 U/L, ALT:13 U/L, LDH:183U/L, Sedim:51 mm/h, IgG: 842mg/dl, IgA:130mg/dl, IgM:460mg/dl, ca:11.2mg/dl, Albumin:4.4mg/dl, b2microg:4.4mg/l. Bone marrow aspiration revealed 85% plasma cells. Cranial and cervical magnetic resonance filmed because of neurological symptoms in patient. Magnetic resonance revealed mass lesion occupying right cerebellopontin angle and another mass lesion occupying occipital bone and infiltration cerebellum. Thorax and abdomen computer tomography revealed lytic lesions in the spine and ribs. 9,10,11,12 cranial nerve involvement was detected in the patient. She was accepted ISS stage II, Durie salmon stage IIIA light chain multiple myeloma. Patient didn't receive in operation. Neurology accepted as a patient in the present Collet-Sicard syndrome. Central nervous system involvement in patients with multiple myeloma began localized

radiotherapy and combined systemic chemotherapy. Systemic chemotherapy continues at the patient.

Conclusion: Central nervous system(CNS) involvement of myeloma is uncommon and is observed in approximately 1% of cases. There are no established treatment guidelines for CNS myelomatosis. Collet-Sicard syndrome with head trauma, prostate adenocarcinoma, the jugular carotis tumors and multiple myeloma was observed in the literature. Collet-Sicard syndrome and multiple myeloma diagnosed patient was presented for our contribution to the literature.

**Keywords:** Collet-Sicard syndrome, multiple myeloma

**Myeloproliferative Disorders**

PS-046

Abstract:0084

**THE RELATION BETWEEN MULTIDRUG RESISTANCE GENE (MDR1- C343T) POLYMORPHISMS AND THE RESPONSE TO TYROSINE KINASE INHIBITORS (TKIS) IN EGYPTIAN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML)**

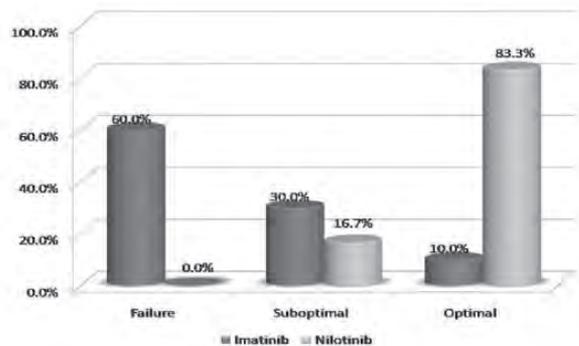
Hend Nabil Ellithy<sup>1</sup>, Mervat Mohamed Mattar<sup>1</sup>, Yasser El Nahas<sup>2</sup>, Asmaa Abd El Hamid<sup>1</sup>

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Introduction: P-glycoprotein (Pgp), the gene product of Multi drug resistance gene 1 (MDR1), is an ATP-driven efflux pump contributing to the efficacy of drugs that are P-gp substrate such as imatinib and nilotinib. The inappropriate expression of MDR1 has been implicated in resistance to imatinib in chronic myeloid leukemia (CML) patients. MDR1 single nucleotide polymorphisms (SNPs) are associated with imatinib efflux and clearance. Response to every tyrosine kinase inhibitors (TKIs) may be affected by MDR1 gene polymorphisms. Detection of these polymorphisms may help to individualize best TKIs to achieve the optimal response. Aim of the work: To investigate the impact of MDR1 gene C3435T polymorphisms, in the response to Imatinib and Nilotinib in upfront CML patients.

The Molecular Response Achieved at Month 12 among Patients carrying TT Genotypes in Nilotinib and Imatinib Arms



Materials-Methods: we detected the MDR1-C3435T genotypes using PCR Restriction Fragment Length Polymorphisms (PCR-RFLP) in 74 newly diagnosed CML patients. Thirty-one patients received nilotinib 300 mg

bid and forty-three patients received imatinib 400 mg od. Molecular response was prospectively assessed every 3 month for 1 year using Real-time quantitative polymerase chain reaction (RQ PCR). Molecular response was assessed in relation to detected polymorphisms.

Results: Unlike CC and CT genotypes, patients carrying TT genotype of MDR-C3435T polymorphism who treated with nilotinib, showed significantly higher major molecular response (MMR) at month 12 (BCR-AB1 % < 0.1%), compared to those treated with imatinib (83.3% vs 10%, P value= 0.001).

Conclusion: Detection of MDR1-3435 TT genotype in CML patients, May predicts better response to nilotinib over imatinib

**Keywords:** CML, MDR1-C3435T-polymorphisms

PS-047

Abstract:0092

**A CURIOUS CASE OF OVARIAN NEOPLASM ACCOMPANYING THERAPY-RELATED ACUTE MYELOID LEUKEMIA**

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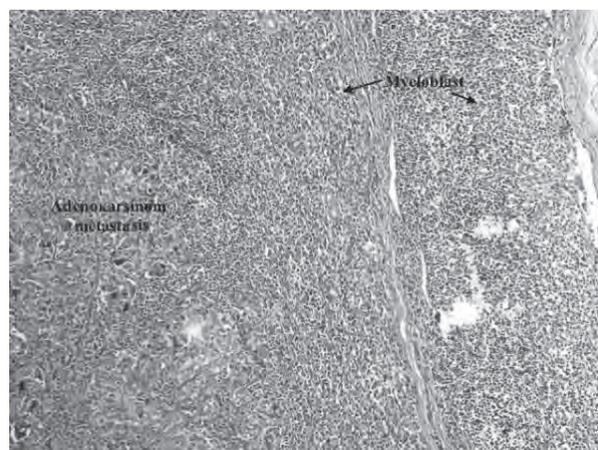
<sup>3</sup>Cukurova University,Department of Pathology, Adana Turkey

Alkylating agents leukemogenic potential in hematology is noteworthy. Both ovarian cancer and acute myeloid leukemia are challenging diseases for physician. Moreover, the treatment of each disease alone is displeasing the coexistence is more weary. 63 years old female patient was administered chemotherapy regimen including paclitaxel combined carboplatin following operation in 2012. She was admitted to hematology department with weight loss and bicytopenia accompanying leukocytosis. Physical examination revealed that inguinal lymphadenopathy was nearly 2 cm in diameter. The admission values were summarized at table 1.Flow cytometry from peripheral blood sample resulted expression of CD13 and CD33 were 93% and 83% respectively. MPO,CD14,CD20,CD19,CD3,CD23 and TdT were negative. Bone marrow biopsy was consisted with acute myeloid leukemia with 25% blasts. Meanwhile, lymphadenopathy was operated and the pathology revealed myeloblast infiltration and metastatic adenocarcinoma of ovarian cancer (figure 1). Hypermetabolic lymph nodes were noted on both sides of cervical, mediastinum and inguinal regions.The therapy was modified for both aml and ovarian cancer. Fortunately, ovarian cancer is thought to be sensitive for chemotherapy and the alkylating regimen with taxan family which are used for therapy. Moreover, they have potential complications like leukemic transformation. Although prognosis are mainly determined by cytogenetic findings, treatment related with acute myeloid leukemia(t-AML) are rarely seen and has poorer prognosis. t-Aml is thought to be a late complication of cytotoxic therapy depending on exposure to various chemotherapeutics and their cumulative doses. Rowe stated on his studies that secondary leukemias should be treated with allogenic stem cell transplantation as soon as possible if appropriate donor is found.Yet, our

patient did not have appropriate donor so chemotherapy was chosen. Even if the future of mmedicine is promising, relationship of profit and loss should be assessed before starting any chemotherapy in oncology patients. After the patient is fully informed and risk assessment achieved, the long term survival and treatment plan can be managed more wisely.

**Keywords:** Ovarian cancer, acute myeloid leukemia

Figure 1



lymph node-adenocarcinoma metastasis and myeloblast

Table 1

WBC:24,7 µl	Ne#:22,5. 10 <sup>^</sup> 3µl	BUN: 22 mg/dl
Hgb:4,6 gr/dl	Ly#: 0,2 10 <sup>^</sup> 3 µl	Cr: 1,2 mg/dl
Hct:14,5 %	AST/ALT: 12/7 U/L	Vit B12: 613 pg/ml
Plt: 26 µl	Ferritin: 1174 ng/ml	Folate: 5,5 ng/ml
Ca 15.3: 16,1 U/ml	Ca125:38,5 U/ml	Sed:56

The laboratory values on admission

PS-048

Abstract:0114

**SUCCESSFUL TREATMENT OF LYMPHOBLASTIC CRISIS IN A PH POSITIVE CML PATIENT WITH NILOTINIB AND AZACYTIDINE COMBINATION**

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Introduction: Tyrosine kinase inhibitors (TKI) open a new are in treatment of chronic myeloid leukemia (CML) but treatment results of blast crisis of CML remains unsatisfactory. Azacytidine is currently available hypometilating agent used in myelodiplasia with excess blast and acute myeloid leukemia patients. Here we report an elderly patient who presented with lymphoblastic crisis of CML and successfully treated with the combination of a second generation TKI and azacytidine.

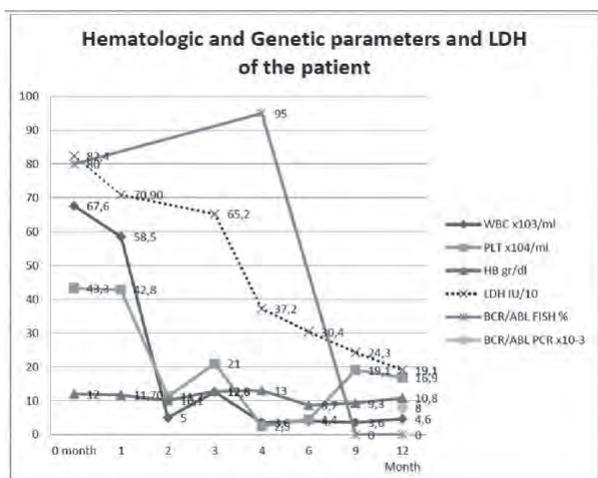
Case: Seventy three year old female patient presented to hematology outpatient clinic with fatigue lasting for two months. The patient was diagnosed as Ph positive CML. Initial therapy was decided as first generation TKI; imatinib mesylate 400 mg once a day. At the end of second month of therapy she reached complete hematologic

response. At the end of third month of therapy her blood count revealed an increase at WBC up to  $12.000 \times 10^3/\text{ml}$  and peripheral smear examination detected 48% blastic cells. Bone marrow examination was done for cytogenetic, flow-cytometric and morphological evaluation. Morphological investigation of bone marrow aspiration revealed 93 % lymphoblastic cells with coarse chromatin, single nucleoli, basophilic narrow cytoplasm without granules. Flow-cytometric analysis of blastic cells was CD3,CD5 and CD7 positive. FISH was 95% positive for Ph chromosome. The patient diagnosed with T lymphoblastic crisis of CML. Dasatinib 70 mg 1x2 po daily plus azacytidine 75 mg/m<sup>2</sup>/day sc for one week and repeated in 4 weeks apart. At the end of first cycle the patient reached hematologic remission. At the end of second cycle lymphoblasts were cleared from bone marrow. At the end of 6th cycle the patient reached complete cytogenetic response. BCR/ABL gene was still detectable at the end of 8 cycle of the therapy. The patient was well tolerated the therapy although she had grade 2 anemia and grade 3 neutropenia and thrombocytopenia. Dasatinib therapy dosage reduced to 100mg/day at the end of 6th cycle due to neutropenia and thrombocytopenia. Laboratory results summarized at figure 1.

**Discussion:** Conventional treatment of BC with interferon, hydroxyurea, alkylating agents and other chemotherapeutics has been unsatisfactory resulting in only transient response and prolonging survival only marginally once BC has been diagnosed. Combination of imatinib with chemotherapy does not yield an additional survival benefit to overall survival in small patient groups of blastic phase CML. There is limited data for patients with BC CML with combination of second line TKI and azacytidine which come from case summaries in literature. It may be feasible in unfit BC CML patients in spite of conventional chemotherapy and may increase the rate of response to therapy prolong survival. Here we want to report a BC CML case with T lymphoblastic crisis which respond to second generation TKI and azacytidine and achieved complete cytogenetic response.

**Keywords:** CML, blastic crisis

**Figure 1:** The laboratory parameters of the patients that show hematologic and cytogenetic response to dasatinib and azacytidine combination. WBC: White Blood Cell count, PLT: Thrombocyte count, HB: Hemoglobin, LDH: Lactate dehydrogenase, FISH: Fluorescence in situ hybridization



PS-049

Abstract:0115

**MELANONYCHIA SECONDARY TO HYDROXYCARBAMIDE: AN ESSENTIAL THROMBOCYTOSIS CASE**

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**Introduction:** Hydroxycarbamide is used in the treatment of essential thrombocytosis and other myeloproliferative disorders. Melanonychia is one of the cutaneous abnormalities including nail changes that could develop with long-term treatment with hydroxycarbamide. Herein, we aimed to present an essential thrombocytosis case with melanonychia that developed secondary to long-term hydroxycarbamide treatment.

**Case Report:** 63 year old woman applied to hospital in July 2012 with bruises on chest without trauma. It was learned from her anamnesis that she also had large bruises after trauma. Platelet levels were around 600000/ $\mu\text{l}$  and JAK2 mutation was positive. She was diagnosed as essential thrombocytosis and she was given hydroxycarbamide treatment. There were no complications and she was on periodic follow-up by our clinic. However in July 2014 she started to feel pain on her toes. Clinical examination revealed melanonychia on her nails (Figure 1). Hydroxycarbamide treatment was discontinued because of pain and she was given Anagrelide treatment. Anagrelide was well tolerated and our patient is still on clinical follow-up with this treatment.

**Figure 1**



*Melanonychia of nail*

**Discussion:** The pathogenesis of melanonychia secondary to long-term hydroxycarbamide treatment is not yet well understood. The mechanism of the involvement of nail is not clear. In literature, some investigators suggested that genetic factors, induction of melanocytes and some changes in nail matrix could be the reason of hydroxycarbamide related melanonychia. In a study that was conducted with 993 patients of myeloproliferative neoplasms (half of them were essential thrombocytosis) treated with hydroxycarbamide, mucocutaneous side effects were reported in 8.3% of the patients. These mucocutaneous side effects include, skin ulcers, oral ulcers and skin infiltration. In this study, median time for occurrence of early toxicities was found 2.1 months after initiation and median time for occurrence of late

toxicities were found as 38 months after initiation of hydroxycarbamide. It was also reported in this study that nearly 50% of the patients discontinued hydroxycarbamide permanently because of side effects. Our patient has suffered color changes in her nails as well as pain that made us to be doubtful for a beginning of ulceration besides melanonychia. Maybe early clinical reaction of discontinuation of the drug has prevented more severe side effect like ulceration in our patient. Also side effect of hydroxycarbamide has developed more slowly in our patient compared to other patients in the mentioned study. To conclude, long-term hydroxycarbamide treatment can cause mucocutaneous side effects and more studies should be done in future in order to reveal the underlying mechanism.

**Keywords:** Melanonychia, Hydroxycarbamide

PS-050

Abstract:0131

### A RARE CASE: IDIOPATHIC HYPEREOSINOPHILIA WITH HIGH LEUKOCYTE COUNT

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*ondokuz Mayıs University, Hematology Department*

Hypereosinophilia (HE) has generally been defined as a peripheral blood eosinophil count greater than 1,500/mm<sup>3</sup> and may be associated with tissue damage. In contrast to HE syndrome, idiopathic HE (IHE) is the preferred term when end-organ damage is absent. The definitive hematologic manifestation of IHE is sustained eosinophilia of 30% to 70%, with total leukocyte counts ranging from 10,000/mm<sup>3</sup> to 30,000/mm<sup>3</sup>. However, extremely high leukocyte counts are not uncommon, which was seen in the presented case, they are often associated with a poorer prognosis.

Case: At first time, a 67-year-old male patient presented to a dermatology department because of widespread pruritus on all his body 6 months ago. Although oral and topical anti-histaminic drugs were given, the complaints were persisting and he was referred to internal medicine department in the same hospital. In his laboratory examination, WBC 55 700 / $\mu$ L (eosinophilia 49 000/ $\mu$ L) hemoglobin 16.2 g/dl, thrombocyte 282 000 / $\mu$ L were found and he was referred to our hematology department. There was no sign and symptoms of end-organ damage. PET-CT, echocardiography and myocardial perfusion syntygraphy examinations were normal. Bone marrow aspiration and biopsy revealed infiltration of eosinophilia and pre-eosinophilic cells. Except increased eosinophil number, there was no other abnormality in flowcytometric study. Cytogenetic examinations including JAK-2, PDGFRA and PDGFRB were normal. In sight of those finding IHE was diagnosed and prednisolone treatment was started. After eosinophil number and percentage were reversed to normal range prednisolone were tapered and later stopped. But after a few weeks later eosinophil number started to increase and hydroxyurea was initiated. By this treatment eosinophil number are under control and the patients has no sign and symptom of organ involvement after 6 months.

In IHE, the goal of therapy is to mitigate eosinophil-mediated organ damage, which may appear after many years.

**Keywords:** Hypereosinophilia

PS-051

Abstract:0156

### A CASE OF RAMSAY HUNT SYNDROME WHICH HAS DEVELOPED DURING RUXOLITINIB THERAPY

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Introduction: Myelofibrosis is a member of chronic myeloproliferative neoplasms and is characterized by accumulation of reticulin and collagen in the bone marrow. It is a clonal disorder of hematopoietic stem cells. Disease may develop primary or secondary to essential thrombocytosis and polycythemia vera. Bone marrow fibrosis, leukoerythroblastosis, extramedullary hematopoiesis, hepatomegaly, splenomegaly and anemia are the major clinical findings for disease. Ruxolitinib is a JAK1/JAK2 inhibitor that is approved for the treatment of myelofibrosis. It is a successful treatment option in the regression of splenomegaly and constitutional symptoms due to the myelofibrosis. In patients using ruxolitinib opportunistic infections and viral infections occur. Here we report a patient with Ramsay Hunt Syndrome while on ruxolitinib treatment. This syndrome characterized by vesicular lesions on the ear, ear pain and peripheric facial paralysis. Reactivation of latent Varicella zoster virus on geniculate ganglion causes this syndrome.

**Ear lesions**



*Herpetic lesions on the external ear*

Case: 68 years old female patient was admitted to emergency department with dizziness, left facial paralysis and herpetic lesions on the left side of face and left external ear. One week ago, patient was admitted to outside institution because of herpetic lesions and valacyclovir was started. The patient was diagnosed

with polycythemia vera in 1995. JAK2 V617F mutation was positive. Myelofibrosis was detected with bone marrow biopsy in 2011. In March 2013, Ruxolitinib was started with 40mg/day. Drug dose was reduced two times due to anemia. The patient was using 20 mg/day Ruxolitinib when viral infection occurred. On physical examination there was limitation of the left facial movements and herpetic lesions on the left side of face and left ear. Her hemogram had been as follows; Hb:11.6 g/dl, WBC:18420/ $\mu$ L, Plt:117000/ $\mu$ L, biochemical laboratory results were normal. Electroneuromyography was performed and early period axonal damage findings were detected on left facial nerve. Contrast-enhanced MRI of the ear was normal. Left peripheral facial paralysis due to viral infection was diagnosed. We continued valacyclovir 1000 mg three times in a day and methylprednisolone 80 mg/day and didn't change ruxolitinib dose. In a few week herpetic lesions regressed, facial movements got partially better. We decided to continue antiviral drug as prophylactic treatment. Here we wanted to point out that we should take care about viral infections on people who are using JAK1/JAK2 inhibitor drugs.

**Keywords:** Ruxolitinib, Ramsay hunt syndrome

PS-052

Abstract:0165

### CONCOMITANT OCCURRENCE OF DEL5Q AND JAK2V617F MUTATION IN MYELODYSPLASTIC SYNDROME: A NEW CLINICAL ENTITY AND RESPONSE TO LENALIDOMIDE

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**Objectives:** The interstitial deletion of the long arm of chromosome 5–del(5q)– is the most frequent cytogenetic abnormality observed in myelodysplastic syndromes (MDS). The concomitant presence of del(5q) and JAK2V617F mutation is very rare, patients have distinct, but still not well-defined, morphological and clinical characteristics of an overlap MDS/MPN disorder. The efficacy of lenalidomide has been established in RBC transfusion dependent patients with IPSS low/intermediate-1 risk del5q MDS.

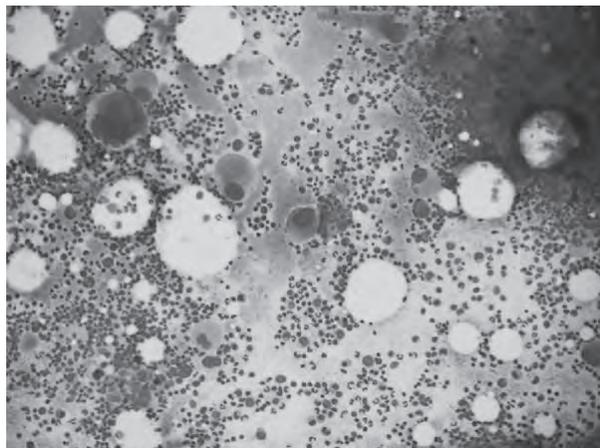
**Case:** We report a 67-year-old female patient who was admitted to hematology clinics with fatigue. She had macrocytic anemia (Hb 7.7 gr/dl, MCV 100 fL), thrombocytosis ( $744 \times 10^9/L$ ). Her physical examination was unremarkable except paleness. Work up for anemia did not reveal any nutritional deficiency or hemolysis. Physical examination did not reveal any enlargement of liver or spleen. Bone marrow biopsy revealed increased number of dysplastic megakaryocytes and grade II reticulin fibrosis. BCR/ABL1 rearrangement was negative, while the JAK2V617F mutation was identified. The patient was started danasin, methyl prednisolon and aspirin with diagnosis of primary myelofibrosis. However, treatment was discontinued at the end of 16 weeks due to unresponsiveness. Moreover, the patient started RBC transfusions for symptomatic anemia and hydroxycarbamide for

increased thrombocytosis of  $1.139 \times 10^9/L$ . Her transfusion requirement increased to 2 units RBC every 3 weeks and a transfusional iron overload developed which necessitated addition of deferasirox. The second bone marrow biopsy was moderately hypercellular with E/M= 1:1. Dysplasia was observed in >10% of erythroid and <10% of the myeloid cells. There was marked hyperplasia of morphologically atypical megakaryocytes with monolobated nuclei. Blast cells were <5%, no remarkable increase in reticulin fibers. These findings were consistent with refractory cytopenia with multilineage dysplasia. FISH for del5q(q13q31) revealed positive in 37% of the cells analyzed. Hydroxycarbamide was stopped and the patient was started lenalidomide 10 mg/day for 21 days every 28 days and enoxaparin 0.4 cc/day for thromboprophylaxis. At the end of two cycles, patient's platelet count dropped to normal levels and a mild improvement in transfusion requirement could be noticed. No adverse events were observed except for a mild and transient neutropenia after the first cycle. Patient is currently receiving the third cycle of lenalidomide.

**Conclusion:** MDS/MPN with concomitant del5q and JAK2V617F is a very rare entity. Awareness to search for both chromosomal abnormality is warranted when common morphological and clinical features are encountered: macrocytic anemia, remarkable thrombocytosis, no splenomegaly, hypercellular marrow with increased number of hypolobated megakaryocytes, no evidence of relevant fibrosis or ring sideroblasts and good response to lenalidomide.

**Keywords:** del5q, JAK2

Figure 1.



Atypical megakaryocytes with monolobated nuclei

PS-053

Abstract:0170

### SUCCESSFUL TRANSFECTION OF JAK2V617F LENTIVIRUS

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**Objectives:** The Janus Kinase 2 gene (*JAK2*) codes for a tyrosine kinase (*JAK2*) that is associated with the cytoplasmic portion of a variety of transmembrane cytokine

and growth factor receptors important for signal transduction in hematopoietic cells. Signaling via JAK2 activation leads to cell growth and differentiation. *BCR-ABL1*-negative myeloproliferative neoplasms (MPNs) frequently harbor an acquired single nucleotide mutation in *JAK2* characterized as Val617Phe (V617F) in the protein. This mutation is identified overall in approximately two-thirds of all MPNs. Lentiviral vectors are commonly used as gene delivery vehicles. In this study, it is aimed to produce JAK2V617F virus.

**Methods:** *E. coli* DH5 $\alpha$  strain was transformed with lentiviral transfer vectors (LTVs) which have the wild type (wt) *JAK2* or *JAK2* with V617F mutation insert, which also have *GFP* (Green Florescent Protein) gene. LTV that carry only *GFP* insert, and two additional plasmids (pCI-VSVG, pCPRDEnv) required for viral packaging were also transferred into the bacteria separately by electroporation. Some colonies of transformed bacteria were selected and produced in LB medium. Plasmid DNAs were isolated from bacterial cells and subjected to restriction digestion to confirm correctness of the plasmids. Next, 293T (Human Embryonic Kidney) endothelial cells were transfected with the isolated plasmid vectors using lipofectamine. Transfection efficiency was evaluated by flow cytometry analysis.

**Results:** The plasmid DNAs (*JAK2*wt-GFP, *JAK2*V617F-GFP, GFP, pCI-VSVG, pCPRDEnv) were confirmed by digestion with proper restriction enzymes on the agarose gel electrophoresis. 60% and/or higher transfection efficiency was detected in the each cell line with *JAK2*wt-GFP, *JAK2*V617F-GFP and only GFP by flow cytometry analysis. Viral particles were harvested from the supernatant of these cell lines by ultracentrifugation. The green color of GFP proteins were also detected under the light microscope.

**Conclusion:** Lentiviral vector plasmids were cloned in *E. coli* cells and lentiviral particles were produced in 293T cells successfully. This method provides variety of opportunities for understanding the effects of *JAK2*V617F mutation on individual cell types including hematopoietic cell and stem cell. In conclusion, our study could provide an understanding of the *JAK2*V617F mutation effects on the cells in the context of MPNs.

**Keywords:** *JAK2*V617F mutation, Lentivirus

PS-054

Abstract:0250

#### GENE EXPRESSION ANALYSIS OF *JAK2*V617F MUTATION IN POLYCYTHEMIA VERA

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**Objectives:** Detection of mutations activating JAK-STAT pathway identified tyrosine kinase activation causing Myeloproliferative Neoplasm (MPN) pathogenesis. *JAK2*V617F mutation is seen in 95% of Polycythemia Vera (PV) patients. JAK activation stimulates cellular events like cell differentiation, proliferation and apoptosis. JAK-STAT pathway gene expression profiling is critical for the gene analysis. In this study it is aimed to

understand the effects of genes in JAK-STAT signaling, apoptosis, immune response, cell growth and maintenance by JAK-STAT PCR array.

**Methods:** Gene profiling was started with 3 different PV patients that were wild-type, heterozygote and homozygote mutant for *JAK2*V617F mutation. Mononuclear cells (MNC) for patients were taken from liquid nitrogen and thawed. After RNA isolation, cDNA synthesis was performed prior to real-time PCR. SYBR Green based detection was accomplished. 96-well format PCR array made it possible to profile 84 genes utilizing the threshold cycle values from the light cycler, fold changes were calculated for each genes comparing with control.

**Results:** Expression level of housekeeping genes that were ACTB, B2M, GAPDH, HPRT1 and RPLP0 examined and quality was checked for PCR Array reproducibility, RT efficiency and gDNA contamination. To obtain fold change of gene expression for homozygote and heterozygote mutant, wild type patient was used as a control. *CDKN1A* was 4 fold downregulated in heterozygote than homozygote mutant and *FAS* was about 2 fold downregulated in heterozygote than homozygote mutant patient. *OSM* was 1.5 fold upregulated in homozygote mutant but, downregulated in heterozygote patient. *OSM* encodes a growth regulator that inhibits the proliferation of a number of tumor cell lines. Protein encoded by *CDKN1A* functions as a regulator of cell cycle progression at G1 and protein encoded by *FAS* has central role in the physiological regulation of apoptosis. It was shown that activating JAK mutations are major molecular events in human hematological malignancies. *JAK2*V617F mutation caused a reduction in the gene expression level in the JAK-STAT pathway.

**Conclusion:** The study was carried out with a limited number of patients but it is important for the understanding of the effects of the mutation on the genes and gene expression profiling technology for analyzing the panel of genes in signal transduction pathways, biological processes and disease related gene networks. Further studies are going to be advanced by working with more patients.

**Keywords:** JAK-STAT pathway, Polycythemia Vera

PS-055

Abstract:0258

#### IMPORTANCE OF MPL MUTATION ANALYSIS: AN ADVANCED AGE FEMALE MPN PATIENT

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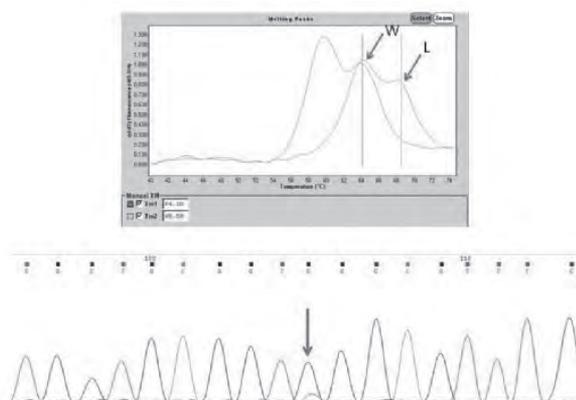
**Objective:** BCR-ABL1 negative Myeloproliferative neoplasms (MPN's) includes polycythemia vera (PV), primary myelofibrosis (PMF) and essential thrombocythemia (ET). Janus kinase 2 (*JAK2*), calreticulin (*CALR*) and myeloproliferative leukemia virus oncogene (*MPL*) are the molecular markers used for the molecular diagnosis of MPN. However, the *CALR* and *MPL* mutations have not been classified yet in the WHO diagnostic criteria for BCR-ABL1 negative MPN's. Here we discuss the importance of *MPL* in this group of diseases over an example.

**Method:** Karyotype analysis and FISH (-5/5q-, 20q-, +8, -7/7q-) were performed on cultured bone marrow cells. *JAK2* V617F mutation analysis and BCR-ABL1

p210 fusion transcript detection were performed by Real-Time PCR (RT-PCR), and Quantitative Real-Time PCR (q-RT-PCR), respectively. JAK2 exon 12 was analyzed with Sanger sequencing. Melting curve analysis was performed for detecting MPL W515K/L and S505N mutations. MPL mutation was also confirmed by Sanger sequencing.

Results: An 83-year-old woman who had anemia and thrombocytosis was referred to our department with myeloid neoplasm pre-diagnosis. FISH, JAK2 V617F mutation analysis and BCR-ABL1 p210 fusion transcript detection were performed. Karyotype and FISH analysis revealed normal results. BCR-ABL1 p210 fusion transcript was under the limit of detection and the CML pre-diagnosis was excluded according to this result. JAK2 V617F and JAK2 exon 12 mutation analyses were performed sequentially, and the results were normal. MPL mutations were analyzed and W515L (rs121913615) mutation was detected (Figure 1A). Presence of this mutation was also confirmed by Sanger sequencing (Figure 1B).

**Molecular genetic analysis of the MPL gene**



(A) Melting curve and (B) Sanger sequencing analyses

Discussion: Recent studies have been focused on new molecular indicators related to signaling pathways in hematopoietic system to elucidate MPN pathogenesis. Although there is no evidence between MPL mutations and its triggering effect on MPN pathogenesis, MPL plays role in the activation of cytokine-regulated intracellular signaling pathways like JAK2. Because of this reason, MPL mutation analysis was added to molecular diagnosis algorithm in our clinic and resulted in a positive result in this current patient.

**Keywords:** MPL, Myeloproliferative neoplasms

PS-056 Abstract:0259

**IMPORTANCE OF MPL W515L MUTATION IN DIAGNOSIS OF MYELOFIBROSIS: A CASE REPORT**

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Introduction: Myeloproliferative neoplasms (MPN) are heterogeneous group of disorders. While JAK2 V617F is the predominant disease-associated allele in Philadelphia chromosome negative MPN, approximately 10% of patients meeting the clinical criteria for polycythemia vera (PV) and 50% of essential thrombocytosis (ET) and primary myelofibrosis (PMF) do not present this mutation. Genetic studies continue to determine the responsible oncogenic events. Recently, two other related mutations have been described in patients with JAK2 V617F-negative PMF/ET (MPL W515L/K mutation) or PV (JAK2 exon 12 mutations). We herein describe a patient diagnosed as PMF with the help of MPL W515L mutation analysis.

Case: An 83 year old woman was referred for having anemia and thrombocytosis for last 10 months. Past medical history revealed hypertension for 15 years. A complete blood count showed the following Results: hemoglobin, 10.6 g/dl (normal range, 12.0-15.0 g/ dl); hematocrit, 33,65% (normal range, 36.1-44.3%); mean corpuscular volume 87,2 fl (normal range, 80-100 fl); platelet count, 550x10e9/l (normal range, 150-400x10e9/l); and a normal white blood cell and differential count 9.2 x10e9/l (normal range, 4-10 x10e9/l). An iron panel demonstrated slightly elevated ferritin level (171 mg/l; normal range, 5-148 ng/ml) and normal transferrin saturation (24%; normal range, 15-55%). Folic acid was 4,7 ng/ml, vitamin B12 630 pg/ml and creatinine 1,21 mg/dl. Abdominal computed tomography showed a spleen size of 140 mm. No underlying disease was detected for thrombocytosis. A bone marrow aspiration and biopsy demonstrated mild hypercellularity with dysplasia and increased megakaryocytes. There was grade 2/3 fibrosis with retuculin. Cell morphology and ringed sideroblast ratio could not be examined because of suboptimal aspiration. CD34+ was positive in 4% of the cells. Differential diagnoses were unclassified MDS/MPN overlap syndrome or PMF. Karyotype analysis and fluorescence in situ hybridization analysis (-5/5q-, 20q-, +8, -7/7q-) were performed on cultured bone marrow cells. JAK2 V617F mutation analysis and BCR-ABL1 p210 fusion transcript detection were performed by Real-Time PCR (RT-PCR), and Quantitative Real-Time PCR (q-RT-PCR), respectively. JAK2 exon 12 was analyzed with Sanger sequencing. Melting curve analysis was performed for detecting MPL W515K/L and S505N mutations. MPL mutation was also confirmed by Sanger sequencing. Karyotype and FISH analysis revealed normal results. There was no evidence of a chromosome JAK2 mutation, JAK2 exon12 mutation, MPL W515K mutation or BCR-ABL fusion gene. The patient was found positive for MPL W515L mutation. Therefore she was diagnosed as PMF.

Discussion: Recent studies have been focused on new molecular indicators related to signaling pathways in hematopoietic system to elucidate MPN pathogenesis. The MPL gene encodes the thrombopoietin receptor. Although there is no evidence between MPL mutations and their triggering effect on MPN pathogenesis, MPL plays a role in the activation of cytokine-regulated intracellular signaling pathways like JAK2. MPL mutations W515L and W515K were found in approximately 5% of patients with PMF and 1% of those with ET and none of the patients with PV. Previous studies showed that the MPL W515L mutation was associated with an older age, a lower hemoglobin level and higher platelet count as in our case. However, its impact on prognosis is not clear.

These mutations may be helpful for diagnosing clonal disease in MPN. More studies are needed to understand their clinical relevance.

**Keywords:** MPL mutations, primary myelofibrosis

PS-057

Abstract:0275

### CHARACTERIZATION OF CANCER STEM CELLS IN POLYCYTHEMIA VERA

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**Objectives:** Myeloproliferative neoplasms (MPN) are clonal bone marrow stem cell disorders characterized by proliferation of abnormal myeloid, eritroid and megakaryocyte cell lines. MPN includes polycythemia vera (PV), essential thrombocythosis and primary myelofibrosis. JAK2V617F mutation, converted from Valin to Phenylalanine, occurs in MPN patients with a high range 50% to 98%. This mutation, occurred without JAK2 cell stimulant, leads to permanent activation and cell proliferation. Hematopoietic stem cell antigen CD133 is a biological marker for cancer stem cells. CD34 is hematopoietic progenitor cell antigen.

We aimed to investigate the presence of the JAK2V617F mutation in both stem cells compartments and mononuclear cells of PV patients in this study. Method

The mononuclear cells were isolated by ficoll-gradient method in three PV patients' peripheral blood samples taken by phlebotomy. Those cells were stained by a group of cell surface marker and selected in cell sorter. Initially CD45- cells were gated and then CD133+CD34-, CD133-CD34+, CD133+CD34+, CD133-CD34- stem cell compartments sorted by cell sorter. In order to investigate JAK2V617F mutation in mononuclear cells and CD133+CD34-, CD133-CD34+, CD133+CD34+, CD133-CD34- stem cell compartments, allele specific Nested PCR was performed.

**Results:** JAK2V617F mutation analysis were compared between mononuclear cells and different stem cell compartments. According to this comparison there has been no difference for JAK2V617F mutation screening between mononuclear cells and different stem cell compartments.

**Conclusion:** As a result of detailed research in terms of JAK2V617F mutation is revealed that there has been no differences between stem cells compartments and mononuclear cells of PV patients.

In the future treatment of PV, detecting JAK2V617F mutation whether it occurs in early stages of hematopoiesis might will be important. The mutation with early stages in cell development of PV, might be present years before PV become clinically apparent. This research performed limited number of patient the increased number of patient would enhance our knowledge.

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**Keywords:** Polycythemia Vera, Stem Cell

## Non-Hodgkin's Lymphoma

PS-058

Abstract:0094

### PROGNOSTIC SIGNIFICANCE OF THE BASELINE ABSOLUTE NEUTROPHIL/LYMPHOCYTE RATIO IN NON-HODGKIN LYMPHOMA

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**Objective:** The absolute neutrophil/lymphocyte ratio (NLR) at diagnosis has been shown to be a simple, inexpensive prognostic factor for survival in solid tumors. We studied NLR at diagnosis as a prognostic factor for patients with non-Hodgkin lymphomas.

**Patient and Methods:** We performed a retrospective analysis including 33 patients with non-Hodgkin lymphoma without bone marrow involvement at diagnosis, and 33 controls without lymphoma. The absolute neutrophil count (ANC), and absolute lymphocyte count (ALC) were obtained from the complete blood cell count using the Beckman Coulter automated hematology analyzer (Tenay, USA) at the time of the diagnosis of NHL. The parameters tested in the present study included B symptoms (Fever >38°C; drenching sweats; and weight loss > 10% of normal body weight), baseline NLR, International Prognostic Index (IPI) score, extranodal sites >= 2, lactate dehydrogenase (abnormal versus normal levels), Eastern Cooperative Oncology Group (ECOG) performance status >= 2, and disease stage (I/II versus III/IV). A receiver operating characteristic (ROC) curve of NLR according to inflammation markers at diagnosis was generated to determine the cutoff value. The area under the curve was recorded as 0.786 (95% CI, 0.667-0.877) (Figure 1). A NLR value of 2.3 corresponded to the maximum combined sensitivity and specificity on the ROC curve (73% sensitivity and 79% specificity). The patients were divided according to the NLR at diagnosis: the group with a NLR of < 2.3 contained 9 patients (27.3%), and the group with a NLR of >= 2.3 consisted of 24 patients (72.7%).

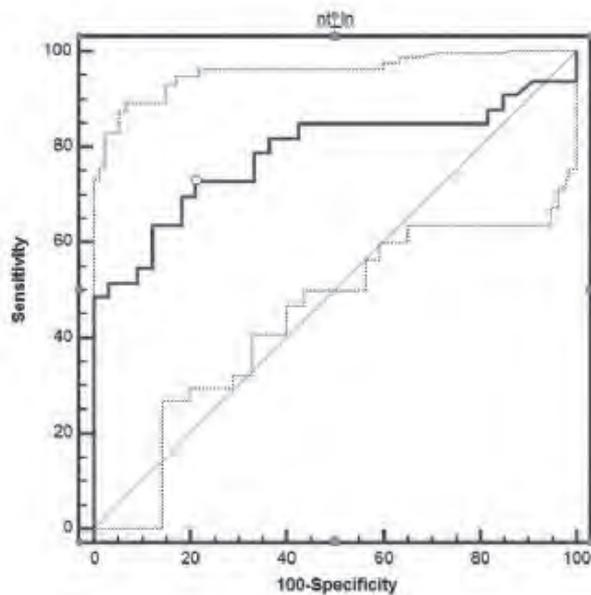
**Results:** Non-Hodgkin lymphoma patients presented with significantly higher levels of ANC, platelet count, NLR, platelet/absolute lymphocyte ratio (PLR), sedimentation rate, and lactate dehydrogenase (LDH) levels, but lower ALC compared to controls, at the time of the diagnosis (Table 1). NLR was positively correlated with white blood cell count, LDH, PLR, and sedimentation rate, whereas NLR showed a negative correlation with hemoglobin levels at diagnosis (Table 2). Patients with a NLR of >= 2.3 at diagnosis were more likely to present with higher levels of LDH and PLR, whereas more likely to present with lower ALC. Patients with a NLR of >= 2.3 and patients with a NLR < 2.3 showed similar overall survival (OS) and progression-free survival (PFS) rates.

**Conclusion:** Obtaining NLR from a complete blood count at diagnosis is quite simple, widely available, and can be easily used in clinical practice, especially in resource-poor areas. NLR was significantly higher in non-Hodgkin lymphoma patients compared to controls,

and was positively correlated with inflammatory markers, LDH and PLR. However, in this study, NLR was not found to be a prognostic marker to assess OS or PFS. Extended future studies are needed to better clarify this association.

**Keywords:** Non-Hodgkin lymphoma, neutrophil/lymphocyte ratio

**Figure 1.** Receiver operating characteristics and area under the curve (AUC) analysis of the ratio of absolute neutrophil count to absolute lymphocyte count at the diagnosis of non-Hodgkin lymphoma (AUC= 0.786, 95% confidence interval= 0.667-0.877).



**Table 1.** The comparison of control and non-Hodgkin lymphoma patients in terms of the laboratory variables

Variables	Control	NHL	P
Age (in years)	51.87 ± 7.7	56.67 ± 22.6	0.246
Gender (Male/Female)	21/12	13/20	0.049
WBC (x109/L)	7.09 ± 2.6	7.35 ± 5.0	0.137
ANC (x109/L)	4.00 ± 1.7	4.92 ± 4.9	0.024
ALN (x109/L)	2.11 ± 0.7	1.28 ± 1.1	0.001
PLT (x109/L)	243.00 ± 59.5	282.00 ± 135.5	0.024
NLR	1.72 ± 0.7	3.34 ± 4.2	0.001
PLR	113.96 ± 42.3	190.54 ± 158.9	0.001
Sedim (mm/h)	17.0 ± 18.5	77.50 ± 78.5	0.001
LDH (U/L)	178.00 ± 41.5	590.24 ± 473.8	0.001

Abbreviations: NHL: Non-Hodgkin lymphoma; P= p value; WBC: White blood cell; ANC: Absolute neutrophil count; ALN: Absolute lymphocyte count; PLT: Platelet; NLR: Ratio of absolute neutrophil count to absolute lymphocyte count; PLR: Ratio of platelet count to absolute lymphocyte count; LDH: Lactate dehydrogenase

**Table 2.** Correlation analysis of the ratio of absolute neutrophil count to absolute lymphocyte count (NLR)

Variables	P	r
WBC	0.015	0.299
Hb	0.003	-0,357
PLT	0.473	0.090
LDH	0.001	0.563
Beta 2 microglobulin	0.287	0.201
PLR	0.001	0.574
Sedimentation rate	0.047	0.250
Age at diagnosis	0.716	-0,066
IPI score	0.698	0.071
Disease stage	0.346	0.169
ECOG performance status	0.467	0.131
Chemotherapy cycles	0.453	0.157

Abbreviations: P: p value; r: Correlation coefficient; WBC: White blood cell; Hb: Hemoglobin; PLT: Platelet; LDH: Lactate dehydrogenase; PLR: Ratio of platelet count to absolute lymphocyte count; IPI: International prognostic index; ECOG: Eastern Cooperative Oncology Group

PS-059

Abstract:0096

**PRETRANSPLANT ELEVATED SERUM FERRITIN LEVELS MAY PREDICT POOR PROGNOSIS IN PATIENTS WITH LYMPHOMA THAT UNDERWENT AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (AUTOHSCT). SINGLE CENTER EXPERIENCE**

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**Introduction and Aim:** Blood transfusions to correct anemia may cause undesired accumulation of iron, which is an important element for the function of human body and this iron overload also causes many complications ranging from mortality. Our goal is assessment of the association between pretransplant iron overload and survival in patient that underwent autologous hematopoietic stem cell transplantation (autoHSCT).

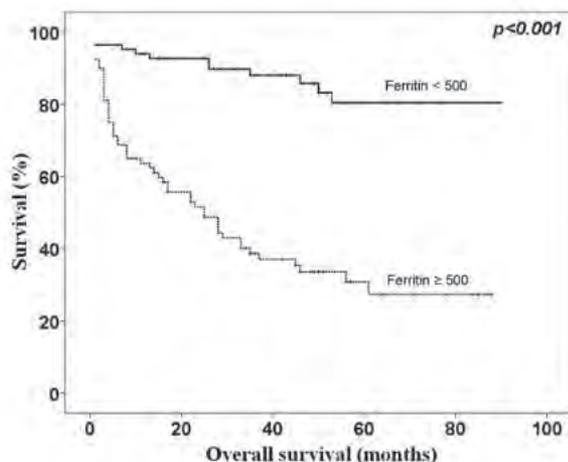
**Patients (Materials) and Methods:** 165 patients with lymphoma, who underwent autoHSCT between the years of 2007-2014, were included in study. Ferritin levels were used to determine iron status and the cut-off value was 500 ng/ml. The relationship between iron overload and survival was assessed by statistical analysis.

**Results:** In high-ferritin group, compared with low-ferritin group, median overall survival (OS) and disease-free survival (DFS) are both resulted inferior. (OS, 20 range 1-88 vs. 42 range 1-90 months; DFS, 10 range 1-88 vs. 39 range 1-90 months, respectively, p= <0,001). There was no significant differences between both groups in infections and engraftment days. The number of patients who survive was 101 (%61.2) and 73 of these were in low-ferritin group, 28 patients were in high-ferritin group. In 64 (%38.8) patients who died, 12 (%14.1) patients were in low-ferritin group, while 52 (%65.0) of them were in high-ferritin group (p= <0,001).

Conclusion: OS, DFS and 100-day mortality results were significantly lower in patients with high ferritin levels ( $>=500$  ng/ml,  $p>0.005$ ).

**Keywords:** Auto HSCT, ferritin

Figure 1



Kaplan-Meier analysis of patients with ferritin levels below and above 500ng/ml. ( $p < 0.001$ )

PS-060

Abstract:0100

## TWO CASES WITH HEPATOSPLENIC T CELL LYMPHOMA

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**Introduction:** Hepatosplenic T cell lymphoma (HTCL) accounts for 1% of non-Hodgkin lymphomas. Only around 100 patients have been reported in the literature. The disease has characteristic features like hepatosplenomegaly and expression of T-cell receptors (TCR). Herein, we aimed to report two cases with hepatosplenic T cell lymphoma in which both cases treatments were planned as allogeneic bone marrow transplantation.

**Case 1:** In May 2014, 25 year old men first time applied to hospital with fatigue and anorexia. In his anamnesis, B symptoms were present. Abdominal CT revealed massive splenomegaly. Bone marrow aspiration was reported as myeloid shift to the left and increase in CD3+ cells. Liver wedge biopsy and splenectomy was performed and he was diagnosed as hepatosplenic T cell lymphoma. He was given 2xCHOP regimen. In September 2014, bone marrow investigation was reported as presence of 30% lymphoid cells. Then he was given HyperCVAD chemotherapy regimen and allogeneic bone marrow transplantation was planned after the completion of chemotherapy protocols.

**Case 2:** In April 2014, 35 year old women first time applied to hospital with fatigue and feeling of a mass in left upper quadrant. In her anamnesis, B symptoms were present. Pancytopenia was observed in laboratory tests. In physical examination splenomegaly was detected and ultrasonography revealed the spleen as 210 mm, liver as 165 mm. Splenectomy was performed and she was diagnosed as hepatosplenic T cell lymphoma. PET-CT

revealed the disease involves only the spleen. CHOEP regimen was started. Allogeneic bone marrow transplantation was planned after the completion of chemotherapy protocols.

**Discussion:** HTCL has very poor prognosis and because of low prevalence there is no evidence based treatment recommendations. There are some clinical trials for peripheral T cell lymphoma (PTCL) but only a few study includes patients with HTCL. However HTCL is a subtype of PTCL so HTCL patients may respond differently for PTCL treatment approaches. Therapy recommendations for PTCL were based on experience in B and other types of T-cell lymphomas. CHOP and HyperCVAD regimens before bone marrow transplantation were the most common treatment options. In PTCL, overall response rates to CHOP regimen is around 60%. For patients younger than 60 years, etoposide is suggested to be added to CHOP regimen, so although overall survival does not change, event-free survival is improved. When we look at the limited data on HTCL, only 50% could reach complete remission with an overall survival around 1 year. Although the data on bone marrow transplantation (BMT) in HTCL patient is limited, the results of sole chemotherapy treatment is very poor. So, autologous or even allogeneic bone marrow transplantation should be considered in HTCL patient who could achieve complete remission. We also planned in our cases allogeneic BMT after the completion of CHOP and HyperCVAD chemotherapy regimens.

**Keywords:** Hepatosplenic T cell lymphoma

PS-061

Abstract:0103

## EFFECTS OF CONCURRENT EXPRESSION OF MYC AND BCL-2 ON THE TREATMENT AND PROGNOSIS IN EXTRANODAL DIFFUSE LARGE B CELL LYMPHOMA

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**Objective:** We aimed to investigate the effects of immunohistochemical and molecular presence of double-hit lymphomas (DHL) (combined expression of myc and bcl-2) on overall and progression-free survival rates of patients with extranodal diffuse large B-cell lymphoma (DLBCL).

**Material-Methods:** A total of 31 patients (17 female, 14 male; mean age 57 years) with diagnosis of extranodal DLBCL were included into the study (patients in the 2008-2013 period, Karadeniz Technical University Medical School). Patients transforming from low grade B cell lymphoma, and patients with HIV positivity were not included. In a retrospective manner, patient characteristics were noted (age at diagnosis, sex, sites of extranodal involvement, stage, high-risk group, histopathological diagnosis, IPI score, LDH level at diagnosis, bone marrow involvement, and treatment modalities). Histopathological specimens underwent immunohistochemical (bcl-6, bcl-2, myc, CD10, Mum-1) and molecular (bcl-2 and myc, by means of PCR) analysis.

**Findings:** DHL was observed immunohistochemically in only one patient, while molecular studies found

6 cases. Three-month overall survival rates were 50% and 88% in DHL positive and negative groups, respectively. Six-month overall survival rates were 16% and 76% in DHL positive and negative groups, respectively. Progression-free 3-month survival rates were 51% and 88% in DHL positive and negative groups, respectively. Progression-free 6-month survival rates were 33% and 76% in DHL positive and negative groups, respectively. No relation with histopathological type of the disease was noted.

We conclude that DHL presence in patients with extranodal DLBCL was an independent factor leading to shortened overall or progression-free survival.

**Keywords:** Double-hit lymphomas, Extranodal Diffuse Large B Cell Lymphoma

PS-062

Abstract:0110

### DIFFUSE LARGE B-CELL LYMPHOMA WITH EXTENSIVE CUTANEOUS RELAPSE: A CASE REPORT

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**Introduction:** The majority of relapses in diffuse large B cell lymphoma (DLBCL) occurs during the first two years after completion of treatment. Herein, we aimed to report a DLBCL case that had extensive cutaneous relapse who had no skin involvement previously.

**Case Report:** A 59-year-old man applied to our clinic in April 2014 with fatigue. Clinical examination revealed splenomegaly and cervical lymphadenopathies. Laboratory tests revealed anemia. HIV test was negative. Cervical lymph node biopsy revealed CD20+, BCL2+, MUM1+, BCL6+ high grade B lymphoproliferative neoplasm. After FISH investigation specimen was considered as diffuse large B cell lymphoma. No involvement were detected in marrow biopsy. PET-CT was performed for staging. Involvement in spleen, bone marrow, serical lymph nodes, bilateral axillary lymph nodes, toracal lymph nodes, portal, paraaortic, paracaval, bilateral inguinal lymph nodes, sternum, C2 vertebra, clavícula, acromion and left humerus were detected with SUV max value above 4. He was considered as stage 4 disease and R-CHOP regimen were given 4 cycles. In August 2014 serical, toracal and abdominal CT revealed regression. R-CHOP were given 3 more cycles. Patient responded to the treatment well with complete resolution of all lymphadenopathies. However in November 2014, the patient had emerging skin lesions that covers nearly all of his body. The lesions were painless and different in diameter with the biggest lesion reaching 5-6 cm (Figure 1). A control PET-CT revealed diffuse cutaneous involvement (SUV max 18.3) with axillary lymphadenopathy involvement (SUV max 14.8). Interestingly this time spleen, inguinal nodes and bone marrow involvement could not be detected. Biopsy was performed from skin lesion that were reported as, CD20+, BCL2+, MUM1+, BCL6+ high grade B cell lymphoma infiltration.

**Discussion:** Non-Hodgkin lymphomas generally relapses in the same involvement sites. In the literature, there are reports of cases who relapsed with CD20- skin involvement after rituximab therapy. Our case differs from these reports with CD20+ relapse after 7 cycles treatment with R-CHOP regimen. At the beginning of the

treatment our patient did not have skin involvement that excludes the diagnosis of primary cutaneous lymphoma. The involvement of skin with CD20+ after 7 cycles of rituximab therapy suggests there is a resistant disease to rituximab which tends to involve the skin. With this viewpoint this is the first case to describe a rituximab resistant diffuse large B cell lymphoma with cutaneous relapse. Also, interestingly disease relapse was not present in our patients' primary involvement sites except axillary region. Moreover, disease relapse occurred in cutaneous region which did not have disease involvement primarily. To conclude, diffuse large cell B lymphomas may relapse extensively with cutaneous involvement even if there is no cutaneous involvement before and this could be a sign of poor prognosis in such cases.

**Keywords:** Diffuse large B-cell lymphoma, cutaneous relapse

Figure 1



Cutaneous involvement

PS-063

Abstract:0111

### THE INFLUENCE OF INTENSIFIED MOBILIZATION CHEMOTHERAPY ON STEM CELL COLLECTION IN FRONT-LINE AUTOLOGOUS STEM CELL TRANSPLANTATION IN NON-HODGKIN LYMPHOMA: DHAP VERSUS HIGH-DOSE CYCLOPHOSPHAMIDE

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High-dose cyclophosphamide (HDC) mobilization is a standard regimen and commonly used to hematopoietic stem cell harvest (SCH) for malignant lymphoma. There are other mobilization regimens such as ESHAP, DHAP, ICE and so on. Intensive mobilization acts more effectively in non-Hodgkin lymphoma (NHL) patients,

but toxicity is greater than non-intensive regimen. DHAP is the cytarabine-based intensified regimen and usually used as salvage therapy for relapsed/refractory NHL patients. We wondered if the intensified regimen could collect more CD34+ stem cells, and improve the outcome of autologous stem cell transplantation (ASCT) compared with standard mobilization in front-line ASCT setting in NHL patients. This is a multicenter, retrospective study to evaluate the efficacy of the DHAP compared with HDC mobilization, in NHL patients undergoing front-line ASCT. We collected the data of patients with NHL undergone front-line ASCT from two different institutions. The primary endpoint was collected CD34+ cell count ( $\times 10^6/\text{kg}$ ). Secondary endpoints included: (1) overall survival (OS); (2) progression free survival (PFS); and (3) engraftment speed. Myeloid engraftment day was the first day of three consecutive days when the absolute neutrophil count (ANC) was  $\geq 0.5 \times 10^9/\text{L}$ . Platelet engraftment day was the first day of three consecutive days when the platelet count was  $\geq 20 \times 10^9/\text{L}$  without requiring platelet transfusion. All patients in two centers received one of the two regimens to mobilize stem cells, DHAP or HDC. In DHAP group, cisplatin  $100\text{mg}/\text{m}^2$ , cytarabine in two pulses at a dose of  $2\text{g}/\text{m}^2$ , dexamethasone  $40\text{mg}$  plus G-CSF ( $5\text{ug}/\text{kg}/\text{days}$ ) were given. In HDC group, cyclophosphamide ( $5\text{g}/\text{m}^2$ ) plus G-CSF ( $5\text{ug}/\text{kg}/\text{days}$ ) were given. Fifty-seven subjects from two centers were included in the analysis. 31 patients received DHAP regimen, and 26 patients received HDC chemotherapy. Median follow up was 51.2 months. There was no statistical difference between two groups in baseline characteristics. Median collected CD34+ cell count was 21.740 95% CI, 16.643-25.096 in DHAP group vs. 15.660 95% CI, 13.508 – 32.039 in HDC group. The CD34+ cell count of DHAP group tended to be greater than HDC, and this was more pronounced in T-cell lymphoma, but it was statistically insignificant ( $p=0.23$ ). Myeloid engraftment day was 13.0 days in both group, platelet engraftment day was 13.0 days in DHAP group, and 15.5 days in HDC group. Toxicity was also similar in the two groups. OS and PFS showed no statistical differences. DHAP mobilization seems to enhance the power to mobilize CD34+ stem cells and shows similar toxicity without statistical significance. OS and PFS of DHAP are not superior to HDC. In front-line ASCT setting in NHL patients, intensified mobilization does not have statistically significant benefit compared with standard regimen than we had expected.

**Keywords:** Non-Hodgkin's lymphoma, Autologous stem cell transplantation

PS-064

Abstract:0118

#### CLINICAL AUDIT OF NON HODGKIN LYMPHOMA PATIENTS TREATED WITH CHOP VERSUS R-CHOP IN MENOFIA – EGYPT

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Background: the addition of Rituximab to conventional chemotherapy CHOP has been proven in a lot of international studies, still the cost of Rituximab is an issue in some countries. This study aims to evaluate the value in addition of Rituximab to CHOP and the importance of

adapting a health finance system to insure regular drug administration.

Methods: This retrospective clinical audit included 311 patients diagnosed with Diffuse Large B Cell Non-Hodgkin's Lymphoma (DLBCL) and treated at two centers (Menofia university hospital and Al Helal hospital for health insurance). Data were collected for patients treated between 2010 and 2012.

Inclusion criteria: A histopathological evidence of (DLBCL) CD 20 +VE. All patients were treated with first line CHOP or R-CHOP All data regarding patient & disease were collected (Age, gender, residence area, job, health insurance status, comorbid conditions, Date of diagnosis, date of start of treatment, stage, presence of extra nodal disease or LDH level, presence of B symptoms, and IPI score, Number of cycles received, regularity of treatment, side effects, and response rate. Progression free survival (PFS) was calculated. Data were analyzed using the chi square test, fissure-exact test, t-test, Anova test. 5% level is used for statistical significance

Results: a total of 311 patients were included 48 patients received R-CHOP while 263 patients received CHOP alone. 70% presented at stage III & IV in both groups, 79% of patients receiving R-CHOP were health insurance patients Median PFS of R-CHOP was 38 months while it was 14 months for the other group with p.value 0.001. Males have a lower PFS in both groups. Increased incidence of grade III& IV neutropenia in R-CHOP arm 22% vs 8%.

Conclusion: Ensuring the regular administration of Rituximab to CHOP in treatment of DLBCL is essential to all groups of patients.

**Keywords:** DLBCL, CLINICAL AUDIT

PS-065

Abstract:0129

#### A RARE CASE OF NASAL TYPE EXTRANODAL NK/T-CELL LYMPHOMA

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Introduction: NK/T-cell lymphoma (NKCL), nasal-type is rare in the United States, representing only 1.5% of non-Hodgkin lymphomas. Classically, patients initially present with nasal obstruction (70%), caused by invasion of the localized lesion into the sinuses and nasal cavities. Initial presentation with persistent sore throat and odynophagia due to oropharyngeal tumor extension is rare, and thus, is often overlooked as viral or bacterial pharyngitis. By studying a case of NKTCL nasal type, we emphasize the need to apply high clinical suspicion for NKTCL, nasal type for early diagnosis and improved survival.

Methods: A case report of a rare presentation of NKTCL, nasal-type is discussed. A literature review is provided to define clinical signs crucial for early diagnosis, appropriate work-up, and expedient treatment of this aggressive, rapidly progressive malignancy.

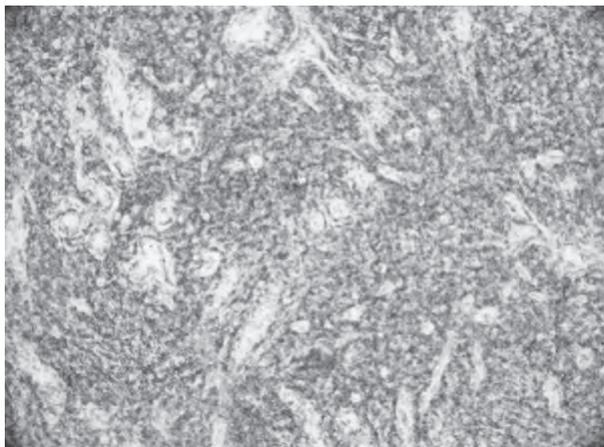
Results: 29 year-old female presented to our clinic with 7 months ago with epistaxis and nasal obstruction complaints, and the patient was diagnosed with Extranodal NK-cell / T-cell lymphoma (NKTCL), nasal

type. Complete remission was achieved with the patient who was stage 1 E after a total 38 Gy radiotherapy.

Conclusions: High clinical suspicion is key to early diagnosis and improved survival of NKTCL, nasal-type. Otolaryngologists who encounter prolonged, complicated cases of pharyngitis or necrotizing sialometaplasia should consider a diagnosis of NKTCL, nasal type, in order to prevent rapid disease progression.

**Keywords:** Nasal Type Extranodal NK/T-cell Lymphoma

Strong staining of diffuse cytoplasmic NK cells with CD 56 (CD56, IHCx200)



PS-066

Abstract:0134

#### A RARE TYPE OF NON-HODGKIN LYMPOMAS: PRIMARY BREAST LYMPHOMA

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Primary breast lymphoma (PBL) is a rare type of non-Hodgkin lymphoma (NHL) representing 0.5% of malignant breast tumors, 1% of all NHL and 2% of extranodal lymphomas. We present a 27-years old woman with PBL. She was operated with a mass in right breast. And the biopsy and immunohistochemical studies are concluded as PBL. She had 6 cycles of immunochemotherapy and still in complete remission. We want to present this case for discussing the clinical, staging and treatment differences between PBL and diffuse large b cell lymphoma (DLBCL). Lymphomas of breast is defined as primary if only breast is involved and secondary (or metastatic)(SBL) if other sites of lymphoma are involved with breast. Most of the PBL are DLBCL. There are clinical, staging and treatment differences between DLBCL and PBL. In staging and definition of PBL, Wiseman and Liao criterias are widely accepted with Ann Arbor Classification.

The chemoimmunotherapy is still gold standart, but there are discussions of addition of radiotherapy and central nervous system prophylaxis.

**Keywords:** Non-Hodgkin lymphoma, primary breast lymphoma

PS-067

Abstract:0138

#### PRIMARY BREAST LYMPHOMA – MAY BE THE LYMPHOMA WITH GOOD PROGNOSIS

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Background: The primary breast lymphoma (PBL) is rare and represents 0.5% of malignant breast tumors, 1% of all NHL and 2% of extranodal lymphomas. The term PBL is used to define malignant lymphomas primarily occurring in the breast in the absence of previously detected lymphoma localizations. Majority of PBL are diffuse large B-cell lymphomas (DLBCL).

Aims: To analyze the clinicopathological features of primary breast lymphoma and correlation between the treatment modality and the outcome. Methods:

The data of 6 patients diagnosed with the primary breast lymphoma and treated between 2008.y and 2012.y. in Institute for oncology and radiology of Serbia, were retrospectively evaluated. Diagnostic criteria for PBL was present breast mass as first and dominant disease manifestation and site of biopsy, disregarding the extent of dissemination. Whole body CT was performed for each patient in initially staging and in response evaluation of initially pathological findings. Bone marrow biopsy was also performed for staging. The treatment modality, the response and its duration (follow up) were registered for all the patients.

Results: All the patients were women. The median age of the patients was 54 years (range 31–75 years). The majority of the patients presented with a sign of tumor mass and palpable lymph nodes. The diagnosis was established by tru cat biopsy (2 patients) or breast-conserving surgery (4 patients). The median tumor size was 7 cm. All the patients were classified as DLBCL. Regarding to R IPI, three patients had very good, one had good and two patients had poor score. The patients with early stage of the disease received III-IV cycles of R-CHOP combined with RT. The patients with the advanced stage of the disease received VI-VIII cycles of Rituximab plus anthracycline based chemotherapy regimen, followed by RT on the residual breast mass. Four patients received CNS prophylaxis. One of them received intravenous methotrexate therapy regarding advanced stage of disease, elevated LDH, age less then 60y, billateral breast involvement and poor IPI score. Other 3 patients received intrathecal methotrexate prophylaxis but none of the patients had positive diagnostic lumbar puncture test or clinical manifestation of CNS involvement. The median follow up was 44 months. The overall response rate was 100% as none of the patients showed disease progression during initial treatment. 2 -year OS was 100%. All the patients achieved CR at the end of treatment and all of them were still alive and without the progression at the end of the follow up period.

Summary: Despite our findings, many authors still advocate the need to improve outcome by improving systemic therapy. The problem of identifying high risk patients and optimal and efficacious CNS prophylaxis still remains. One is sure: there is no indication for mastectomy in the management of PBL.

**Keywords:** extranodal lymphoma, breast

PS-068

Abstract:0142

### MODIFIED R-IDARAM PROTOCOL IN THE PATIENTS WITH PRIMARY CENTRAL NERVOUS SYSTEM DIFFUSE LARGE B CELL LYMPHOMA: SINGLE CENTER EXPERIENCE

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**Introduction:** Central nervous system lymphomas appear as primary or secondary forms. Primary central nervous system lymphomas (PCNSL) are defined as lymphomas that occur without any primary focus in the body typically involving brain parenchyma, intraocular compartment, cranial nerves, leptomeninges and spinal cord and they are aggressive extra nodal non-Hodgkin's lymphomas. PCNSL constitutes 1% of all lymphomas. Diffuse large B cell lymphoma (DLBCL) constitutes more than 90% of primary CNS lymphomas. For the last 30 years the incidence of this group is reported to be increased and most important known risk factors are congenital or acquired immunodeficiency states. Today, in this group the prognosis has improved significantly with the new treatment strategies.

**Methods:** Four patients who were diagnosed as DLBCL and treated in Diskapi Yildirim Beyazıt Research and Training hospital are analyzed retrospectively.

**Results:**

Three of the 4 patients were male. They were all considered as primary CNS lymphoma for they did not have any other involvement. Modified R-IDARAM protocol (Rituximab 375 mg /m<sup>2</sup>/day (day 0.), Cytosine arabinoside 1 g/m<sup>2</sup>/day (day 1. and 2.), Idarubicin 10 mg/m<sup>2</sup>/day (day 1. and 2.), methotrexate (MTX) 3 g/ m<sup>2</sup>/day (day 3.) and Dexamethazone 100 mg / m<sup>2</sup>/day (1st, 2nd and 3rd day) was given in 28 days interval. Folinic acid was added after MTX therapy and G-CSF was given in the seventh day.

2 cases (Case 1 and 4) died during the first cycle. Cranial radiotherapy (36 Gy) was given for the other 2 patients (Case 2 and 3) after 2 cycles of chemotherapy. R-IDARAM chemotherapy was given for 4 cycles. Intrathecal prophylactic therapy was performed at least 2 times for every cycle. CSF examination revealed no pathological cells in none of the patients. Both of the cases (Case 2 and 3) are now being followed in complete remission.

**Discussion:**

Life time of primary CNS lymphoma is 2-3 months without treatment and 4-5 months with surgical treatment alone. Primary treatment strategy is controversial and is not standardized. Surgical treatment is not helpful in most of the patients because of multifocal spread and deep localization. Although response rate of whole brain irradiation is 80-90%, mean life time is about 12-18 months and 5-year survival is 5-18%. Preferred treatment today is high-dose methotrexate (1-8 g/m<sup>2</sup>) in combination with radiation therapy. Methotrexate dose and infusion duration is both important in terms of transition of the brain parenchyma and cerebrospinal fluid (CSF).

Primary CNS diffuse large B-cell lymphoma is a rare disease. In these patients there is no standard treatment

regimen reported in the literature. R-IDARAM protocol is seemed to be effective if toxicity and side effects are managed carefully. but multi-centered prospective studies with higher patient number is needed.

**Keywords:** Primary central nervous system lymphomas, Modified R-Idaram

PS-069

Abstract:0148

### MYD88 EXPRESSION AND L265P MUTATION IN MATURE B-CELL NON-HODGKIN LYMPHOMAS

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**Objective:** MYD88 is a common adaptor protein that is responsible for signaling from several receptors, located either at the plasma membrane or in endosomes. It has been suggested that MYD88 mutations play a role in the pathogenesis of lymphoma. We aimed to determine the MYD88 L265P mutation frequency, the level of MYD88 expression, and their association with clinicopathological parameters in mature B-cell Non-Hodgkin Lymphomas (NHLs).

**Materials-Methods:** A total of 68 patients were included in the study. The presence of MYD88 L265P mutation was analyzed by real-time polymerase chain reaction and direct sequencing. MYD88 expression was evaluated by immunohistochemistry (IHC) according to two different scoring systems.

**Results:** MYD88 L265P mutation was present in 8 (18.6%) of Diffuse Large B-cell Lymphoma (DLBCL) patients. Interestingly, it was observed a significant association between loss of MYD88 expression and advanced stage in both mature B-cell NHL and DLBCL according to first IHC scoring system (p=0.015 and p=0.024, respectively). An association was also seen between MYD88 overexpression and low clinical risk in both mature B-cell NHL and DLBCL according to second IHC scoring system (p=0.027 and p=0.024, respectively). **Conclusion:** MYD88 L265P mutation may be helpful for understanding the pathogenesis of immune-privileged site-associated DLBCLs. The presence of the mutation, together with its protein overexpression, could also use as a prognostic marker in only advanced stage of DLBCLs.

**Keywords:** Mature B-cell Non-Hodgkin Lymphoma, MYD88

APS-070

Abstract:0161

**PERIPHERAL T CELL LMPHOMAS: ANALYSIS OF 11 CASES FROM A SINGLE CENTER**

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Objectives: Peripheral T cell lymphomas (PTCL) are a heterogenous group of clinically aggressive diseases associated with poor outcome. There is no standardized approach to this subtype of T cell lymphomas, because rarity of disease is a big problem in performing clinical studies.

Method: We report the retrospective analysis of 11 patients diagnosed with PTCL according to the WHO classification between 2010-2014 at a single hematology department.

Results: Median age was 60 (31-78) and the male/female was 10/1. The histologic subtypes were as follows: 1 ALK-positive anaplastic large-cell lymphoma (ALCL), 3 angioimmunoblastic T cell lymphoma (AITL), 3 extranodal natural killer (NK)/T-cell lymphoma, 4 peripheral T-cell lymphoma not otherwise specified (PTCL-NOS). Seven patients had advanced stage (Ann Arbor III or IV) disease. According to the Prognostic Index for T-cell lymphoma (PIT): 1 patient was low-risk, 2 patients were low-intermediate-risk, 5 patients were high-intermediate-risk and 3 patients were high-risk. Extranodal involvement was observed in 7 cases (3 nasopharynx, 4 cutaneous) and bone marrow infiltration was observed in 7 cases. At the time of diagnosis median hemoglobin levels were 12.2 (7.3-17.7) gr/dL, leukocyte count 7.8 (2.6-61.3) x10<sup>9</sup> /L, lymphocyte count 1.18 (0.17-3.6) x10<sup>9</sup> /L, platelet count 195 (42-312) x10<sup>9</sup> /L, serum LDH 345 (133-1496) U/L and albumin 2.8 (2.9-5.0) gr/dl. Pathologic evaluation revealed a median Ki -67 index of 60 % (20-90 %).

First line treatment protocols were as follows: CHOP (n=5); CVP (n=1); CHOEP (n=1); SMILE (n=1); involved field radiotherapy (n=2); 1 patient could not be given any chemotherapy because of early death. Two patients died after the first chemotherapy cycle. One patient is still on his first line chemotherapy. Response to first-line treatment could be evaluated in 7 patients and was observed in 4 patients (1 CR; 3 PR). Only one patient with ALK+ ALCL, who after having received involved field RT, has been followed up for 19 months in CR without progression. All other patients progressed at a median of 7 (4-11) months. Four out of 5 patients who received salvage chemotherapy did not respond and could not receive high dose therapy and autologous stem cell transplantation (HDT-ASCT). Only one patient with NK/T cell lymphoma who progressed shortly after first line CHOP chemotherapy, received 3 cycles of SMILE protocol as salvage and has been referred to HDT-ASCT. At present, 3 patients are surviving at a median follow up time of 9 (1-22) months. Patients' data are summarized at table 1.

Conclusion: Patients with PTCL usually present as high risk disease. Even though they respond to the first line chemotherapy, progression in a short time, mainly during the first year after diagnosis, is inevitable. For

subtypes other than ALK+ ALCL which is known to possess favorable outcome, early referral to HDT-ASCT after completion of first line chemotherapy is utmost important.

**Keywords:** Peripheral T Cell Lymphoma

Table.1

PATIENT	AGE	SEX	DIAGNOSIS	ANN-ARBOR STAGE	EXTRANODAL INVOLVEMENT	KI-67 INDEX	PIT GRADE	TREATMENT	RESPONSE	TIME TO PROGRESS (months)	DURATION OF FOLLOW-UP (months)	SURVIVAL
1	61	M	AITL	2A	Nasopharynx	90%	high intermediate	CHOP	PR	9	11	Ex
2	31	M	NK/TCELL LYMPHOMA	2A	Nasopharynx	30%	low intermediate	RT	No Response	4	9	Ex
3	60	M	PTCL,NOS	4A	Cutaneous	20%	high	CHOP	No Response	5	9	Ex
4	48	M	ALK+ALCL	1A	Cutaneous	90%	low	RT	CR	NA	19	Alive
5	74	M	PTCL,NOS		None	NA	high intermediate	CHOP	NA	NA	1	Ex
6	65	M	PTCL,NOS	3A	Cutaneous	80%	high	NONE	NA	NA	1	Ex
7	52	M	NK/TCELL LYMPHOMA	4B	Nasopharynx	30%	high intermediate	SMILE	No Response	NA	1	Ex
8	57	F	PTCL,NOS	4B	None	60%	high intermediate	CHOP	PR	7	14	Ex
9	64	M	NK/TCELL LYMPHOMA	4A	Cutaneous	80%	low intermediate	CHOP	NA	7	11	Alive
10	59	M	AITL	4B	None	30%	high intermediate	CHOEP	PR	NA	2	Alive
11	78	M	AITL	4B	None	70%	high	CVP		11	22	Ex

PS-071

Abstract:0166

**A CASE REPORT WITH MEDIASTINAL GREY ZONE LYMPHOMA**

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Introduction: Mediastinal Grey Zone Lymphoma (MGZL), which is recognized by the 2008 WHO classification as a group of lymphomas, is a clinical and pathological term that demonstrates clinical and biological features between nodular sclerosis hodgkin lymphoma (NSHL) and primary mediastinal B-cell lymphoma (PMBL). It is difficult to diagnose MGZL; clinicians and pathologists tend to work patiently and well organized for this difficult cases. We aimed in this case report to present a patient with MGZL that diagnosed with LN excision and died during treatment with combined chemotherapy. Case: 27 years old male patient was admitted to our hospital with fatigue and pain in back. The patient's physical examination was found servical, inguinal lymph node (LN), hepatomegaly, splenomegaly. Lab studies revealed leukocyte 2.1x10<sup>3</sup>/µL, hemoglobin 7.0 g/dL and platelet 99x10<sup>3</sup>/µL, ESR 21 mm/h, CRP 4.87 mg/dl, LDH 780 U/L. CT scan showed us bilateral 2 cm servical and axiller lymphadenopathy (LAP), splen sized 18 cm, paraaortic, retrocaval, bilateral iliac and obturator LAP 3cm. MRI revealed a mediastinal mass which was compressing spinal cord on T3-T5 vertebral level. Servical LN excision, bone marrow (BM) biopsy performed. The cells whom made the infiltration has large clear chromatin and some of them look like Reed- Stenberg cells. It was stained CD20, CD30, PAX5 positivity, CD15, CD23, CD10 negativity. BM biopsy demonstrated us CD20, CD30 positivity and Bcl-2 negativity. Our patient was diagnosed with MGZL

based on the servical LN spicimens and bone marrow biopsy specimens according to WHO classification 2008. According to ann-arbor clasification our patient was classified with stage IVb. We started patient radiotherapy (RT) for the mass. We treated patient with three cycles of CHOP (cyclophosphamide, doxorubicin, vincristine and methylprednisolone) and one cycle of EPOCH (etoposide+CHOP). After the RT we performed an new MRI to evaluate remission. We found total regression of the mass but all the spinal cord was infiltrated with lymphoma. We gave patient intratecally methotrexat one dose. He was diagnosed with febrile neutropenia after chemotherapy; and a CT scan demonstrated us bilaterally infiltrating of the lungs with oppotunistic fungal infection. We started broad spectrum antibiotics and antifungal agents but our patient did not respond well our treatment and died in the 4th month of treatment. Discussion: In this case report we described a patient with a mediastinal mass and a lymphoma infiltration overlapping between PMLBL and NSHL. Our patient has CD30, CD20, PAX-5 positivity and lack of CD15 staining in the LN biopsy. The optimal treatment is unknown due to the rare of this type of tumor and the prognosis of these patients is poor. Most recent studies have recommended that these tumors are treated with CHOP-like regimens but our patient did not respond this treatment. Further clinical studies need to be done evaluating for treatment options.

**Keywords:** Mediastinal Grey Zon Lymphoma, Primer Mediastinal B cell Lymphoma

PS-072

Abstract:0168

**OVEREXPRESSION OF SOX11 TRANSCRIPTION FACTOR IS A HIGHLY SPECIFIC MARKER FOR MANTLE CELL LYMPHOMA DIAGNOSIS AND CORRELATES WITH CYCLIN-D1 EXPRESSION**

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Mantle cell lymphoma (MCL) is a B-cell neoplasm generally composed of monomorphic small to medium-sized lymphoid cells with the t(11;14)(q13;q32), resulting in abnormal expression of the cyclin-D1 gene in the tumor cells. Mantle cell lymphoma (MCL) accounts for 5–10% of mature B-cell neoplasms. Immunohistochemically, MCL are usually positive for CD5, CD20, and cyclin D1, but are negative for CD10 and CD23. SOX11 is normally expressed throughout the developing nervous system of human embryos and is required for neuron survival and neurite growth. However, the data on the roles of SOX genes in hematopoiesis are very limited. The prognostic role of the transcription factor SOX11 in mantle cell lymphoma is unclear and controversial. In this study, we analyzed absence or existence of SOX11 expression and clinical prognostic role of SOX11, in a total of 171 materials obtained from 160 cases including mantle cell lymphoma and other B-cell neoplasms composed of small lymphocytic lymphoma (SLL), marginal zone lymphoma (MZL), and follicular lymphoma (FL), diagnosed between 2000 and 2012 and evaluated its association with overexpression of cyclin D1. Of the 51 cases diagnosed as MCL, 58 samples were evaluated in the study. The materials were obtained

from 29 lymph nodes ( 11 cervical, 8 inguinal, 7 axillary, 1 supraclavicular and 2 unknown localization) and 29 extranodal sites (9 gastrointestinal tract, 7 tonsil, 2 neck, 2 mammillary, 2 nasopharyngeal, 2 orbital, 1 eye, 1 conjunctiva, 1 lung, 1 maxillary sinus, 1 spleen involvement). Nuclear staining of SOX11 was observed in 48 of 58 (82.75%) mantle cell lymphoma samples. Ten mantle cell lymphoma cases negative for nuclear SOX11 staining were analyzed and were all positive for cyclin D1. In order to evaluate nuclear SOX11 as a possible differential diagnostic marker in MCL, we stained FL (n=29), SLL (n=52) MZL (n=32) and all other B-cell lymphomas (n=113) showed no nuclear positivity. As a result of the study, we have concluded that SOX11 mRNA and nuclear protein expression is a highly specific marker for mantle cell lymphomas.

**Keywords:** mantle, SOX11

PS-073

Abstract:0179

**ADULT T-CELL LEUKEMIA / LYMPHOMA COMPLICATED WITH SAPROCHAETE CAPITATA FUNGEMIA – CASE REPORT**

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Adult T cell leukemia/lymphoma (ATLL) is a rare and often aggressive T - lymphoproliferative disorder, etiologically linked with human T cell lymphotropic virus type-1 (HTLV-1). HTLV-1 is endemic in Japan, Caribbean and Africa, although sporadic cases have been reported elsewhere in the world. ATLL affects almost exclusively adults. There is no gender prevalence. Depending on the disease manifestations, ATLL is classified into several forms: acute, chronic or smoldering. Treatment of ATLL remains a challenge for the clinicians. Patients with ATLL are immunocompromised and develop opportunistic infections that complicate the disease course and make its management even more difficult. Infection with Saprochaete capitata has been reported in patients with hematological malignancies, especially in acute leukaemia. But, so far, infection with Saprochaete capitata in the ATLL patients has not been reported. We report a case of a 54 year old woman presented at the University Clinic of Hematology in Skopje, Macedonia, in July 2014, with intensive itching, coughing, dyspnea and skin tumorous formations. Physical examination revealed neither peripheral adenopathy nor organomegaly. Diagnosis was made upon several investigations. Beside the presence of skin lesions she had elevated white blood cells (55,9 X 10<sup>9</sup> /L) with atypical lymphomonocytoid cells. The bone marrow (BM) smear showed 80-90% lymphoid infiltration. The histopathological finding was - Malignant lymphoma. PCR of BM aspirate showed dominant monoclonal population of T lymphocytes. Immuno-phenotyping of bone marrow cells showed positivity for CD3, CD2, CD4, CD5, CD7, CD10, CD25, CD38 and negative FCM7, CD79b and CD22, confirming ATLL. Treatment started with CHOP protocol due to acute renal failure. In the course of the disease she complained she couldn't walk. MRI of the back bone revealed vertebral sclerosis with spinal stenosis L4-L5. After 3 cycles, treatment continued with Hyper-C-VAD regimen. After the first cycle, she became neutropenic and febrile.

Microbiological finding was *Saprochaete capitata*. Despite treatment, she succumbed to her illness five days from the beginning of the febrile episode and six months from the presentation due to *Saprochaete capitata* fungemia.

**Keywords:** Adult T cell leukemia/lymphoma, *Saprochaete capitata*

PS-074

Abstract:0183

### PRIMARY BREAST LYMPHOMA; A SINGLE CENTER EXPERIENCE

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Primary breast lymphoma (PBL) is a rare disease accounting for 0.4–0.5 % of all breast malignancies. The breast is an uncommon site for non-Hodgkin lymphoma involvement. The term “primary breast lymphoma” (PBL) is used to define malignant lymphomas primarily occurring in the breast without any history of previously detected any other lymphoma localizations. Over 98 % of cases occur in women. We retrospectively examined our case records and found out 7 cases of PBL. These 7 patients were diagnosed as PBL between January 2006 and December 2012. We analyzed their medical records in terms of clinical features, prognostic factors, diagnostic methods and treatment outcomes of these cases. Median age at diagnosis was 55 years (range 17-72 years). All patients were female. The most common histological subtype was diffuse large B cell lymphoma (DLBCL) with total 6 cases. The remaining was anaplastic lymphoma with T cell. B symptoms were present in 2 patients. The IPI scores were determined as 3 in 1 case, 2 in one and 1 in two cases. Three of the patients revealed IPI 0. Pathologic evaluation was done via surgery (lumpectomy) in 2 patients. Six patients underwent core biopsy. Systemic treatment was selected as R-CHOP chemotherapy for 6 patients, CHOP for one. Two of the formerly mentioned 6 patients received adjuvant radiotherapy. Two patients received intrathecal injection. A case who has not received central nervous system (CNS) prophylaxis developed CNS relapse in the 46 months after first remission. She is currently in remission after second line treatment and autologous bone marrow transplantation (ABMT). One of the patients died due to disease progression. To date 5 patients are followed in remission. One patient was lost to follow up when she was in remission with second line treatment owing to her relaps after 13 months from first remission. After entire evaluation median follow-up was found to be 72 (28-102) months. The most common histological subtype in patients with PBL was DLBCL. Combined modalities containing chemotherapy and radiotherapy provides long term survival in PBL treatment. As this is a chemosensitive disease, consecutive morbidity of mastectomy must be avoided and surgery must not be the primary modality of choice for treatment. The patients should be monitored closely for CNS relapse.

**Keywords:** Non-Hodgkin Lymphoma, primary breast Lymphoma

PS-075

Abstract:0186

### A RARE PEDIATRIC CASE OF CUTANEOUS GAMMA/Delta T-CELL LYMPHOMA

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Cutaneous  $\gamma/\delta$  T-cell lymphoma (CGD-TCL) is a recent entity described in the newly revised World Health Organization-European Organization for Research and Treatment of Cancer classification of cutaneous lymphomas. Only a few cases have been reported, of which two pediatric cases. A 15 years old child with a 6 months history of polyadenopathy, cutaneous lesions, general edema and deterioration of general condition was hospitalized. Results from laboratory testing, cutaneous histopathology and immunohistochemistry showed a primary CGD-TCL. Staging was completed by a total body computed tomography. Therapy was planned with SMILE protocol. It is a highly aggressive tumor resistant to chemotherapy, immunotherapy, and radiation therapy. The GDTCL is characterized by a worse prognosis with a median survival of 15 months. Early diagnosis is essential and aggressive therapy is necessary.

**Keywords:** cutaneous / T-cell lymphoma, WHO-EORTC classification

Erythematous infiltrated lesions of the internal face of the thigh



PS-076

Abstract:0194

### PERIPHERAL T CELL LYMPHOMA WITH CNS INVOLVEMENT DURING THE TREATMENT OF MYCOSIS FUNGOIDES- CASE REPORT

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**Introduction:** The peripheral T cell lymphomas (PTCL) are a heterogeneous group of aggressive neoplasms that constitute less than 15 percent of all non-Hodgkin lymphomas (NHL) in adults. PTCL, NOS(not otherwise specified) is the most common subtype of PTCL, accounting for approximately 30 percent of PTCL and approximately 6

percent of non-Hodgkin lymphomas (NHL) overall. Central nervous system (CNS) involvement is a very rare extranodal site for PTCL.

**Case:** 54 year old man who was following with the diagnosis of Mycosis Fungoides, consulted to hematology just he had enlarged servical and inguinal lymph nodes. Excisional removal of this lymph nodes revealed PTCL-NOS. In studies of immunohistochemistry, dyed with CD4 -CD30 diffusely and displayed the loss of CD3-CD8 expression. Diagnosis also supported with flow cytometry showing CD4/CD8:1 CD2-3-5-7 positivity that is compatible with PTCL. Increased FDG uptake detected with PET-CT extensively (servical, axillar, mediastinal, abdominal lymph nodes) and putamen involvement displayed. Due to patient's discordance cranial MR imaging failed. We performed lumbar puncture and cannot establish cytologic positivity in cerebrospinal fluid. Serum LDH level increased minimally. He was stage-4 according to Ann-Arbor and international prognostic score was 1 (IPI-score:1). Because of CNS involvement high dose methotrexate and CHOP protocol (cyclophosphamide, doxorubicin, vincristine, prednisone) has given. After 4 cycles, almost complete metabolic response obtained.

**Discussion:** Most patients with PTCL-NOS present with generalized lymphadenopathy with or without extranodal disease. Approximately 38 percent of patients have nodal disease alone, 49 percent have nodal and extranodal disease, and 13 percent have extranodal disease without evidence of nodal involvement. Important risk factors for CNS involvement include aggressive histologic subtype, advanced stage disease, increased serum LDH level, involvement of more than one extranodal site, and a high IPI score. In most cases, magnetic resonance imaging (MRI) will show abnormal enhancement with in the leptomeninges, brain parenchyma, and/or spine, and CSF analysis can be diagnostic in cases of leptomeningeal involvement. There is no general consensus regarding the preferred induction chemotherapy. The most recommended protocol for PTCL is CHOP. EPOCH is alternative protocol including etoposide that can penetrate at CSF. However if there is CNS involvement, the regimens that includes high-dose systemic therapy suggested (eg, methotrexate, cytarabine) allowing optimal penetration of CSF spaces. Intrathecal (IT) chemotherapy, Radiation therapy (RT) are alternatives for radiographically apparent symptomatic disease. Because of the short duration of remission, consolidation with autologous stem cell transplantation is necessary as soon as possible and this is what we plan for this patient.

**Keywords:** Extranodal, CNS involvement

PS-077

Abstract:0195

### ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA FOLLOWING CUTANEOUS INVOLVEMENT SECONDARY B CELL LYMPHOMA

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**Introduction:** Angioimmunoblastic T-cell lymphoma (AITL) is a rare, aggressive (fast-growing) form of peripheral T-cell lymphoma (PTCL). While AITL only accounts for one percent to two percent of all NHL cases in the United States, it is one of the more common subtypes of mature T-cell lymphomas. Elderly patients are more likely to have AITL. Symptoms of AITL include high fever, night sweats, skin rash, and autoimmune disorders such as autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia (ITP). As a result of these autoimmune disorders, the body's immune system does not recognize, and consequently destroys, its own cells and tissues, such as red blood cells (in the case of AIHA) or platelets (in the case of ITP). Disease behavior is different personally and regression spontaneously. Prognosis is the worst and overall survival is 3 years.

**Case:** A 66-year-old female patient, 18 months ago fever, swollen lymph glands, swelling in the legs, redness of the extremities, referenced with complaints of abdominal pain and weight loss. History of COPD and pulmonary HT, Blood counts were within normal limits. Test of the hepatosplenomegaly investigating found that the largest 4.5 cm lymphadenopathy was detected neck and abdomen. At cervical biopsy was determined to AITL. EBV is determined in large cells. PET CT evaluation after the patient received 6 cycles of CVP was observed almost complete regression. Tracking in the left arm in the proximal biopsy of the rash and skin rash swollen, plaque B cell lymphoma was detected. 2 cycles of treatment is still being followed under the ICE.

**Results:** Rash in AITL patients with secondary can be detected in B-cell lymphoma as a primary lymphoma. AITL should be noted that can make the skin involvement. After treatment of localized developing skin involvement should be kept in mind that the secondary lymphoma involvement could be.

**Keywords:** Angioimmunoblastic T-cell lymphomas, B Cell lymphoma

PS-078

Abstract:0205

### SYSTEMIC T-CELL LYMPHOMA PRESENTING WITH EXTERNAL AUDITORY CANAL AND PAROTID GLAND INVOLVEMENT

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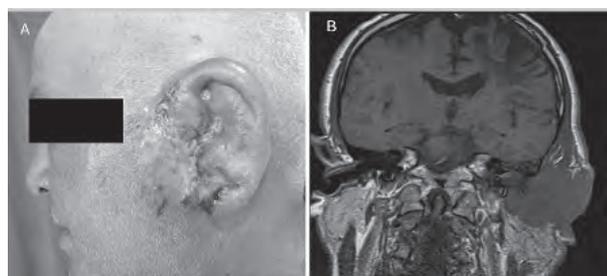
**Introduction:** External auditory canal is an unusual presenting site for lymphomas. There are a few case reports in the literature about the T-cell lymphoma involvement of the external auditory canal. Malignant lymphomas arising from the salivary glands are also uncommon, accounting for approximately 5% of extranodal lymphomas and the majority of them are of B-cell lineage. In this article, we describe the case of a peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) arising from external auditory canal and parotid gland.

Case: A 63 year old man was admitted to the dermatology clinic with suppurative lesion and marked swelling of the left side of his face in front of the ear, left auricle and external auditory canal. The patient's symptoms had begun 6 months ago and had increased in time despite the use of antibiotics. Physical examination revealed edema in left auricle, external auditory canal and preauricular region. A suppurative lesion consisted of ulcerated areas and granulation tissue was observed in external auditory canal and preauricular region (Figure 1A). Otoloscopic examination revealed external otitis and obliteration of the external auditory canal due to compression. Incisional biopsy from the lesion revealed CD3, CD5 and CD30 positive, S100 negative lymphoid cells diffusely filling the dermis. A lobulated mass lesion of 47X39 mm arising from the left parotid and extending to the left auditory canal was detected in computed tomography (CT) (Figure 1B) with accompanying lymph nodes in left subauricular and cervical localization. Case was considered as stage II PTCL-NOS and CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) protocol was started. Despite clinic improvement in lesions after first cycle, PET/CT revealed new cervical lymphadenopathies and pulmonary parenchymal involvement after the fourth cycle, consisted with progressive disease. The second-line treatment was planned as 2 courses of DHAP (Dexamethasone, High dose Ara-C, Cisplatin), followed by allogeneic stem cell transplant. The patient is having the second course at the 8th month of his follow up.

Discussion: Involvement of the external auditory canal and parotid gland is an extremely rare presentation for a lymphoma. A suppurative auricular lesion suggest an infectious disease rather than a lymphoma but T-cell lymphomas which may present with unusual cutaneous manifestations in earlier stages must be kept in mind. Early incisional biopsy may prevent excessive waste of time with antibiotherapies. Patients diagnosed with cutaneous or mucosal lymphoid involvement should be examined carefully for systemic disease because clinical staging predicts prognosis and determines treatment.

**Keywords:** T-cell lymphoma, external auditory canal

Figure 1



suppurative lesion with ulcerated areas and granulation tissue in left auricle, external auditory canal and preauricular region

PS-079

Abstract:0211

### A CASE OF PRIMARY MEDIASTINAL B-CELL LYMPHOMA PRESENTED DURING PREGNANCY

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Introduction: Diagnosis of cancer during pregnancy is a relatively rare phenomenon with an incidence of approximately 1 in 1000-1500 pregnancies. Here, we will describe a case of a 36-year-old woman with primary mediastinal B-cell non-Hodgkin lymphoma (PMBCL) presenting with superior vena cava syndrome.

Case: A 36-year-old pregnant woman (14th week) whose medical history was unremarkable admitted to a different hospital with effort dyspnea and non-productive cough. Radiological investigation was not performed because of pregnancy. Her symptoms toughed to be related to pregnancy and could be psychogenic. At the 21th week gestation, her pulmonary symptoms were progressed with a palpable mass in her right medial thoracic area. Subsequently, the patient underwent thoracic X-ray and magnetic resonance imaging at the same hospital. A 14x11-cm enlarged mass in the left hemithorax was identified in thoracic MRI images. She was recommended for termination of pregnancy. The patient and her family decided to continue the pregnancy. However, emergency cesarean section was performed at 30 weeks gestation because of overt dyspnea, orthopnea and tachypnea of the patient and fetal distress. After delivery, positron emission tomography/computed tomography scans showed a huge mass (235 X 174 X 146 mm) filling the all left hemithorax with a SUVmax: 16.3, and mediastinal hyper metabolic lymph nodes (Fig. 1). Excisional biopsy of the tumoral mass has shown a primary mediastinal Large B cell lymphoma (CD 20+, CD 23+, CD 19-, CD 10-, CD5-). 3 weeks later, she hospitalized to our hospital with superior vena cava syndrome signs and symptoms. Initial blood tests re-vealed normal blood counts and chemistry, except elevated beta-microglobulin level of 2.38 mg/L and elevated LDH level of 498. A R-MACOP-B chemotherapy was started. She was discharged after 2 weeks of the chemotherapy, and thereafter chemotherapy was continued without complication in outpatient setting for 10 more weeks. At the end of the chemotherapy, PET-CT scans showed residual hypermetabolic lesions in the narrowed mass. She underwent salvage chemotherapy with R-DHAP followed by auto - SCT. A complete metabolic response was identified in PET-CT scans performed in 3rd month of Auto-SCT. Then, she was recommended to receive involved site RT to decrease risk of local relapse.

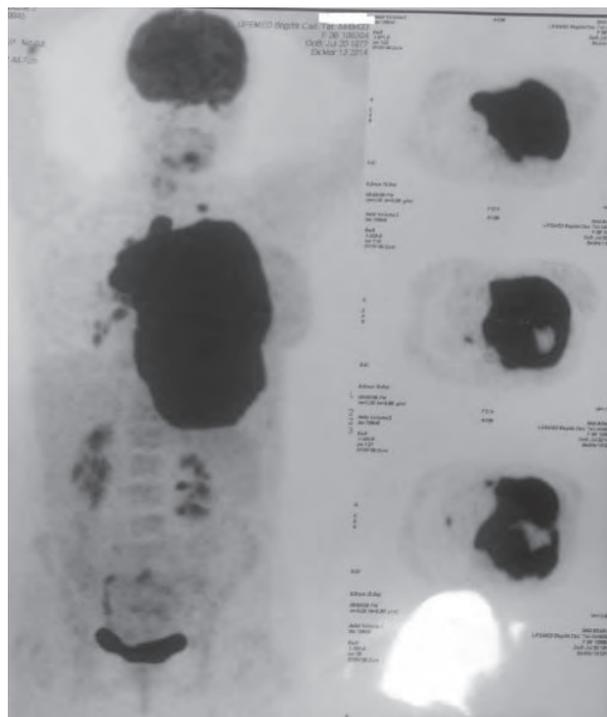
Discussion: Lymphoma, especially NHL is extremely rare in pregnancy for an overall incidence of 5.39 per 100000 births. NHL often present with systemic symptoms such as fatigue, shortness of breath and night sweats, mimicking pregnancy-related features which may result in delayed disease diagnosis. Furthermore, the wish to avoid investigational imaging, aiming to protect the fetus from radiation exposure, may lead to a further delay.

Conclusion: The specific predilection of PMLBCL for young female adults makes it an important entity to be

consider among of the hematological malignancies during pregnancy.

**Keywords:** Lymphoma, Pregnancy

PET-CT findings at the time of diagnosis.

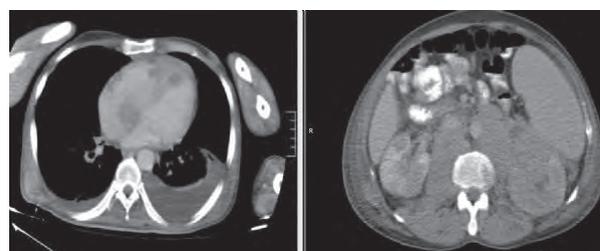


revealed a left surrenal mass (85x50 mm) and multiple retroperitoneal, paraaortic lymph nodes. Additionally, his thoracic CT revealed two hypodense cardiac masses suggestive of myxoma, thrombus or other tumor. One of them was located in right ventricle near the interventricular septum (40x20 mm), the other one was located in right atrium (43x26 mm). Echocardiography confirmed the diagnosis and anticoagulant therapy was started. The masses persisted at six weeks despite anticoagulation and antibiotic therapy. A tru-cut biopsy of the left surrenal mass was performed and diffuse large B cell lymphoma was diagnosed by histopathology of tumor tissue. The patient's clinical picture was considered as Richter's transformation and possible cardiac involvement of lymphoma. He was started on R-CHOP chemotherapy protocol. Numerous lymphoid cells were seen on his peripheral blood smear. He died after second cycle of chemotherapy because of neutropenic fever.

**Conclusion:** The existence of two neoplastic processes in this patient may be coincidental. However, the chance of this situation is small. According to epidemiological studies, the likelihood of developing these independent processes is estimated to be 0.002 per billion per year. For this reason, we believe that the cardiac masses of this patient is likely to be the cardiac involvement of lymphoma. A common pathogenic pathway may lead to the development of both myxoma and CLL in this patient.

**Keywords:** Richter's transformation, Cardiac involvement

Surrenal mass and cardiac masses of the patient



PS-080

Abstract:0214

### A CASE OF RICHTER'S TRANSFORMATION WITH CARDIAC INVOLVEMENT

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**Introduction:** Richter's transformation (RT, Richter's syndrome) was first described in 1928 by Maurice Richter as the development of an aggressive large-cell lymphoma in the setting of underlying chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). Although diffuse large B cell lymphoma is the most common histology seen in patients with RT, Hodgkin lymphoma and T cell lymphomas have also been reported less commonly. We found some cases with lymphoma and cardiac myxoma which was thought to be coincidental in literature. However we couldn't find a case of Richter's transformation with cardiac involvement.

**Case:** A 52 year-old male patient with a history of CLL and CLL induced autoimmune hemolytic anemia for nearly 5 years was admitted to hospital because of abdominal pain localized at left upper quadrant. He was at stage 2 according to Rai staging system. He used steroid intermittently during follow-up for hemolytic anemia. His abdominopelvic CT (computerized tomography)

PS-081

Abstract:0217

### DIFFERENT FACTORS EFFECTING THE DETECTION OF MYD88 MUTATION IN SMALL B CELL LYMPHOMAS WITH PLASMA CELL DIFFERENTIATION

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MYD88 L265P single amino-acid mutation has been reported in ~90% of Lymphoplasmacytic lymphoma Waldenström's Macroglobulinemia (LPL/WM) and / or immunoglobulin M (IgM) monoclonal gammopathies of uncertain significance (MGUS). Although this mutation is not specific for LPL / WM can be used for differential diagnosis on routine practice considering the sensitivity of detection. One problem effects sensitivity is the allelic burden status or the amount of the neoplastic cells within the available sample. Different methods with different sensitivity can be performed for mutation analysis. Both the performed method and the status of materials used for mutation analysis may effect the result of the molecular tests. In this study we aimed to compare the results of

two different methods for the detection of MYD88 L265P mutation on various different tissue samples and previously diagnosed different SBCL.

We included 14 patients with WM, 24 cases of low grade B cell lymphoma of various types showing increased plasma cells (FL, SLL/CLL, MM, NMZL, EMZL), 17 splenic marginal zone lymphoma (SMZL), 10 unspecified MZL and 4 splenic diffuse red pulp lymphoma (SDRPL) cases in the study. Either formalin fixed paraffin embedded tissues or bone marrow smears of involved tissues were used for DNA extraction. Allele-specific polymerase chain reaction (AS-PCR) and Sanger sequencing methods were performed. In order to evaluate specific advantages and disadvantages of both methods, we also considered the number of neoplastic cells in the samples as well as the fixation status. The results were compared.

**Result:** The factors effecting the sensitivity of the results can not be explained by only the sensitivity of the method. Thus the number of neoplastic cells especially for the bone marrow aspirates and the DNA quality for the FFP tissues are other important factors effecting the reliability of the molecular result.

DNA quality, on FFPE preparation depending on the fragmentation status of the extracted DNA is important for the reliability of the detection of the MYD88 L265P mutation. One other issue is the number of neoplastic clone within whole cellular component in the involved tissues. By using AS-PCR it is possible to amplify shorter bp fragments (Max 200bp) than Sanger sequencing. This may lead us to prefer using high sensitive assay like AS-PCR for samples with lower size ladder (100-200 bp) values. In bone marrow biopsies and the tissues with limited disease involvement the amount of neoplastic population is important for getting a reliable molecular result. MYD88 L265P mutation could not be demonstrated in 4 LPL/WM cases in our study. This can be caused by the sampling, since all of the cases were BM smears. The other reason can be the DNA degradation due to the long period storage in the archives. High CT values can be interpreted as reflecting the fragmented DNA or lower amount of neoplastic population.

**Keywords:** Lymphoplasmacytic lymphoma, MYD88

Mutational status obtained by two different methods showed striking difference

	MZL Unspecified	SMZL	LPL/WM	SDRPL	OTHER SBCL	Total
AS-PCR MUTATION	2/10 (20%)	2/17 (11.8%)	10/14 (71.4%)	3/4 (75%)	1/24 (4.1%)	18/67 (26.9%)
Sanger Seq MUTATION	1/10 (10%)	0/17 (0%)	3/14 (21.4%)	0/4 (0%)	1/24 (4.1%)	5/67 (7.5%)

PS-082

Abstract:0219

**METASTATIC ADENOCARCINOMA OF THE STOMACH FOLLOWING HAIRY CELL LEUKEMIA**

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**Introduction:** Hairy cell leukemia (HCL) is a rare, indolent B-cell disorder. Patients with HCL have an increased risk of second malignancy either in a form of synchronous disease or in a form of an increased incidence of a second neoplasm after the treatment of HCL. We report a patient who presented metastatic adenocarcinoma of the stomach following hairy cell leukemia.

**Case:** A 36-year-old male patient was admitted to our clinic suffering from fatigue, fever, weight loss. Splenomegaly was found on physical examination. Laboratory findings were as follows; level of hemoglobin was 8.7 gr/dl, hematocrit 27%, white cell count 1100/mm<sup>3</sup>, platelets 160000 µ/l. Level of serum urea was 17 mg/dl, creatinine 0.9 mg/dl, sodium 133 mEq/L, potassium 4.1 mEq/L, uric acid 3.6 mg/dl, total protein 7.2 mg/dl, albumin 3.2 mg/dl, aspartate aminotransferase 34 UI/L, alanine aminotransferase 48 UI/L, lactate dehydrogenase 200 U/L, cancer antigene 125 (CA-125) 841 U/mL. Examination of peripheral blood smear, only few hairy cells were found. Bone marrow trephine biopsy showed monoclonal B cells with immunophenotype characteristic of HCL (CD25 +, CD103 +, tartrate resistant acid phosphatase +). Due to these findings we thought that HCL was the diagnosis. The patient was treated with cladribine and achieved to complete remission. The patient was admitted to the chest disease department with dispnea, fever after six month later of cladribine treatment. Massive pleural efusion was determined and was performed thorasentesis. Parapneumonic effusion was determined and was initiated antibiotic treatment. But pleural effusion was not improve. Therefore video-assisted thoracoscopic surgery was performed and taked of biopsy. Tuberculosis pleurisy was determined. The patient received anti-tuberculosis treatment for six months and pleural efusion regressed. He complained from dispepsia after three years. Endoscopic examination objectified a tumor with ulceration in the stomach. Histological examination of biopsied specimens resulted in a diagnosis of adenocarcinoma. Dosetaxel, cisplatin, dexametazone and 5-flouro-uracil was given to this patient for six cycles. The patient survived for 6 months, dying form.

**Conclusion:** Elevated second cancer risks in patients with hairy cell leukemia have been primarily attributed to decreased T-cell function caused by chemotherapy and to immune perturbations associated with the underlying disease. A propable second primary malignancy should be kept in mind in cases with a defined malignancy in the presence of unusual symptoms.

**Keywords:** Adenocarcinoma of the stomach, hairy cell leukemia

PS-083

Abstract:0225

**SPONTANEOUS REGRESSION OF A SYSTEMIC ALK (+) ANAPLASTIC LARGE CELL LYMPHOMA CARRYING ALK GENE REARRANGEMENT THAT DEVELOPED AFTER PPD TUBERCULIN SKIN TEST**

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**Introduction:** Here we report an ALK (+) ALCL which spontaneously regressed after an excisional lymph node biopsy and is still in complete remission without treatment.

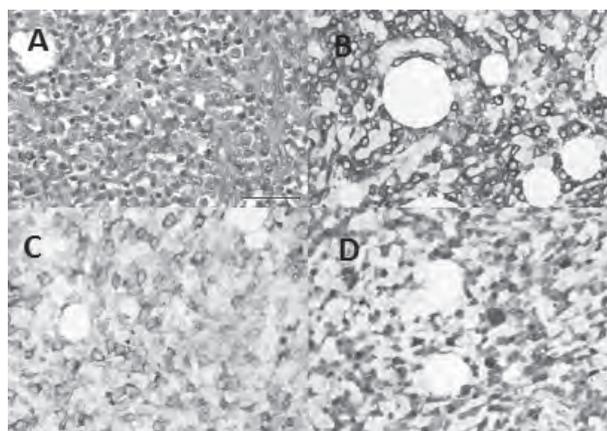
**Case:** A man aged 38 years was admitted to our hematology clinic on receipt of his excisional biopsy pathology report in October 2008. The patient was otherwise healthy and he was asymptomatic. Two months prior to his admittance, in August 2008, he had noticed aching and swelling in his left armpit 2-3 days after a purified protein derivative (PPD) skin test had been conducted to

his left arm. He had also developed high grade fever. The patient's primary care physician first considered it to be an allergic hypersensitivity reaction, and nonsteroidal anti-inflammatory drugs were prescribed. His fever had initially responded to the aspirin and paracetamol; however in the following days the swelling in the left axillary region increased, high grade fever continued and fatigue, loss of appetite, and weight loss added to his symptoms. The patient reported, he had lost approximately 4 kg of body weight during this period. A left axillary ultrasound was performed which showed multiple conglomerated lymph nodes, up to 3x2,5 cm in diameter. With the exception of the multiple conglomerated left axillary lymph nodes, there was no evidence of disease in any other region, in computerized tomography scans of the neck, chest and abdomen. Tuberculosis lymphadenitis was then suspected and an excisional biopsy was performed. The patient reported that, his fever had resolved immediately after the biopsy and that he felt well at the time of admission to the hematology clinic. The result of the biopsy was consistent with ALK(+) anaplastic large cell lymphoma. Fluorescent in situ hybridization using a break apart probe was positive for ALK gene rearrangement. Immunohistochemical staining with ALK, was showing strong nuclear and cytoplasmic staining, which was consistent with t(2;5) (p23;q35). (Figure-1) At the time of admission the patient was in good condition and had no signs or symptoms of peripheral lymphadenopathy, splenomegaly and hepatomegaly. A PET/CT was performed which showed no evidence of disease. Due to the fact that this lesion had developed after a PPD test, and the symptoms had complete resolved immediately after the lymph node excision, with no further evidence of disease present, we decided to follow-up the patient without treatment. In the follow-up we saw no signs of disease progression. The patient is still in complete remission as of January 2015 without having received any kind of treatment.

**Conclusion:** Here we report a case of primary systemic, ALK positive ALCL, carrying ALK gene rearrangement that developed after a tuberculin skin test which spontaneously regressed without any therapy. We emphasize the importance of showing ALK positivity for such cases and highlight that treatment may not be always required in patients who have good prognostic factors.

**Keywords:** ALCL, ALK rearrangement

Figure 1



Neoplastic cells are generally medium-sized. Few of them have eccentric kidney-shaped large nuclei ("hallmark" cells) HE X40 (A); CD30 X40 (B); EMA X40 (C); ALK1 X40 (D).

PS-084

Abstract:0228

**A CASE REPORT: TWO DIFFERENT RARE SEEN MATURE B CELL NEOPLASIA IN THE SAME CASE**

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Multiple myeloma (MM) and diffuse large B-cell lymphoma (DLBCL) are mature B cell neoplasia derived from B cells. In our case, a 78 years old, male patient; was admitted to our hospital with a right inguinal mass and multiple myeloma has been diagnosed with a bone marrow biopsy after an increase in total serum protein, sedimentation and in immunoglobulin G was detected. An excisional biopsy was performed for the inguinal mass. A combination chemotherapy was given for both of the diagnoses after the biopsy result was reported as diffuse large B-cell lymphoma. It has been found to be a rare case when combination of the two B cell neoplasia has been scanned in the literature and has been approved for presentation to intend to contribute understanding B cell oncogenesis.

**Keywords:** Multiple myeloma, diffuse large B-cell lymphoma

PS-085

Abstract:0230

**A CASE OF MARGINAL ZONE LYMPHOMA WITH CONCOMITANT UROTELIAL CANCER AND COAGULATION DISORDERS**

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**Introduction:** Marginal zone lymphomas (MZL) are a group of indolent (slow-growing) non-Hodgkin lymphomas, which account for approximately 12% of B-cells lymphomas. The median age of onset is 65 years. Here we present a case of nodal MZL, one of the 3 subtypes of the disease.

**Methods:** Initial presentation (age = 53 years) was given by light increase in serum aminotransferase activity and presence of hypoechoic lesions in the liver. CT scan confirmed hypodense lesions in the VIII hepatic segment and showed lombo-aortic nodes > 1 cm.

B lymphocytes represented 70% of bone marrow. Immunophenotype was CD20+, CD5-, CD23-.

Histology of abdominal nodes prompted the diagnosis of nodal MZL, hence immunochemotherapy was started.

After 7 years of remission, CT scan showed a 3 cm hypodense node in the right lung and a right ureteral stenosis due to a solid tumour. Bone marrow cytology and biochemical parameters were normal. Ureteral biopsy showed a high-grade transitional carcinoma. The patient underwent surgical removal of right kidney and ureter in October 2013.

**Results:** In December 2013, PET showed hypercapture of the lung mass.

In September 2014, ultrasonography showed hypoechoic hepatic lesions. In October 2014, the lung mass was increased at CT scan and pleural effusion was present, which contained urothelial carcinoma cells. At this stage, coagulation disorders appeared: INR, APTT and D-dimer were increased, while Factor VIII and anti-thrombin III were low. Platelet count was 10.000 /mm<sup>3</sup>.

Bone marrow was again infiltrated by lymphoma cells. Immunochemotherapy was resumed, but gastrointestinal hemorrhage occurred and the patient died for respiratory failure and shock.

Discussions: This case shows that coagulation disorders can arise from both solid tumours via poorly understood mechanisms (paraneoplastic syndromes) and hematologic disease. When both of them are present, chemotherapy may not be sufficient to avoid coagulation failure.

**Keywords:** Coagulation, lymphoma

PS-086

Abstract:0233

**PRIMARY THYROID LYMPHOMA; SINGLE CENTER EXPERIENCE:**

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Primary thyroid lymphoma (PTL) is a rare malignancy with an incidence of 1% to 5% of all thyroid malignancies and less than 3% of all extra nodal lymphomas. It typically presents in 6th decade of life and occurs more commonly in women. The majority of the cases are B cell origin; diffuse large B cell lymphoma (DLBCL) is the most common and it is followed by mucosa-associated lymphoid tissue (MALT) lymphoma. Association with Hashimoto thyroiditis is well documented in the literature especially in MALT lymphoma patients. Combination chemotherapies with or without radiotherapy is mostly preferred strategy although there is not a standard therapy for PTL.

In this case series, we aimed to analyze the characteristics and treatment outcomes of PTL patients in our institution.

7 patients (one male and 6 females) diagnosed as PTL were analyzed. The median age was 52 years (range, 23-80 years). All the patients were diffuse large B cell lymphoma. Four of the patients also had Hashimoto thyroiditis. ECOG scores were 2 and 1 in 3 and 4 patients respectively. 28 % of the patients (2 patients) had high IPI scores. All the patients were treated with combination therapies with rituximab similar to nodal DLBCL patients. Radiation therapy was used only in one patient with a bulky disease in addition to combination chemotherapy. Only one patient with bulky disease relapsed 2 years after first remission and died due to refractory disease. The median follow up of the patients and the progression free survival rates were 17 and 15 months, respectively.

Primary thyroid diffuse large B cell lymphoma is a rare entity. Although there is not a large prospective randomized studies for the treatment of this rare entity, combination chemotherapies with rituximab is an effective treatment with a favorable outcome.

**Keywords:** Thyroid gland, B-cell lymphoma

PS-087

Abstract:0261

**INVESTIGATION OF THE NUMBER OF ABSOLUTE LYMPHOCYTES AS A RISK FACTOR OF HODGKIN AND NON-HODGKIN LENFOMIA**

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Purpose: In general, lymphomas appear as tumors of the lymphoid system. In our study; we investigated the relationship between the number of absolute lymphocytes and the prognosis of 103 lymphoma patients, who have been monitored and received treatment in our hospital's hematology outpatient clinic.

Methodology: All patients diagnosed as Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL), who have been monitored in our hospital's hematology outpatient clinic, were enrolled retrospectively.

Findings: In this study, 103 lymphoma patients were enrolled and 27 of them were diagnosed as HL, 76 of them were diagnosed as NHL. 12 of the HL patients were female and 15 were male. 30 of the NHL patients were female, and 46 were male. For the patients with Non-hodgkin lymphoma; group of patients whose number of absolute lymphocytes were more than 3500 (%53,3) progress or being stable ratio was significantly higher (p=0,003 < 0,05) than the group whose number of absolute lymphocytes were between 0-3500 (%16,4). Non-Hodgkin lymphoma patients who are younger than 60 years-old in the group whose number of absolute lymphocytes were more than 3500 (%66,7) progress or being stable ratio significantly higher than (p=0,012 < 0,05) the group whose number of absolute lymphocytes were between 0-3500 (%12,5). Non-hodgkin lymphoma patients who are older than 60 years-old, there was no significant difference (p=0,205 > 0,05) between the groups of patients whose number of absolute lymphocytes were greater than 3500 and between 0-3500 (Table)

Discussion and Result: For B cell NHL group at diagnoses; higher number of absolute lymphocytes would be an independent risk factor and this topic should be evaluated with a larger spectrum and a larger sample size.

**Keywords:** Lymphoma, absolute lymphocyte Value

Tablo

		Number of Absolute Lymphocytes				p
		> 3500		0-3500		
		n	%	n	%	
Non-Hodgkin Lymphoma	Progressed or Stable	8	53.3%	10	16.4%	<b>0,003</b>
	Remission	7	46.7%	51	83.6%	
Non-Hodgkin Lymphoma	0-60 Years Old	4	66.7%	4	12.5%	<b>0,012</b>
	Remission	2	33.3%	28	87.5%	
Non-Hodgkin Lymphoma	Older than 60 Years Old	4	44.4%	6	20.7%	0,205
	Remission	5	55.6%	23	79.3%	

Table: Non-Hodgkin lymphoma patient group's progress or being stable ratio, Non-Hodgkin lymphoma patient group's progress or being stable ratio

PS-088

Abstract:0270

### AN UNUSUAL PRESENTATION OF DIFFUSE LARGE B CELL LYMPHOMA AS AN URETHRAL MASS AND AN INCIDENTAL PRIMARY SEROUS TUMOR OF THE UTERINE FALLOPIAN TUBE: A UNIQUE COEXISTANCE

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A 68 year-old woman presented with an urethral mass protruding through the external orifice. Punch biopsy specimen was examined and diagnosed as poorly differentiated malignant tumor. Preoperative diagnosis was urethral tumor and bladder tumor, so the patient underwent a radical cystectomy, urethrectomy, total abdominal hysterectomy, bilateral salphingo-oophorectomy, bilateral ilioobturator lymph node dissection. Intraoperative lymph node sampling was examined by a pathologist not familiar with hematopathology, and the frozen section diagnosis was only malignant tumor, without any comment about probable lymphoid malignancy. Histopathologic examination and antigenic evaluation of the specimen revealed the diagnosis of diffuse large B cell lymphoma. Ilioobturator lymph nodes, urethral wall and vaginal wall were involved with neoplastic lymphoid cells. Bladder had only inflammatory changes. Cervix, uterus and bilateral ovaries were not involved with tumor. Endometrium was atrophic, and there were corpus albicanses in bilateral ovaries. There was another tumor 2x1,5x1cm in dimensions, located at the fimbrial portion of the right uterine fallopian tube histopathological examination of which revealed a carcinoma consistent with a serous tumor. The patient was treated with 6 R-CHOP chemotherapy. We present a unique coexistence of a lymphoma with an unusual presentation and an incidental serous tumor of uterine fallopian tube. The patient was unlucky for undergoing such an aggressive surgical treatment like radical cystectomy, urethrectomy, total abdominal hysterectomy, bilateral salphingo-oophorectomy, bilateral ilioobturator lymph node dissection for the diagnosis of lymphoma, but the extensive surgery helped identifying the coexisting tumor of the uterine fallopian tube at an early stage. Careful examination of the intraoperative sampling specimen and recognising lymphoid malignancy can be helpful avoiding unnecessary surgical therapy. This case is being reported due to the unique and unusual simultaneous occurrence of these two tumour entities.

**Keywords:** Lymphoma, serous tumor

PS-089

Abstract:0277

### COMPARISON OF IPI AND NCCN-IPI IN 324 DE-NOVO DLBCL PATIENTS: MULTICENTER RETROSPECTIVE ANALYSIS

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**Introduction:** International Prognostic Index (IPI) was developed in pre Rituximab era, in 1993, to predict the prognosis of aggressive lymphomas. However, IPI has limitations and we need a better prognosis indicating system to anticipate the outcome of patients receiving CHOP+R. National Comprehensive Cancer Network (NCCN)-IPI was recently claimed to be better predictor of prognosis of diffuse large B cell lymphoma (DLBCL). In this retrospective multicenter analysis, we aimed to compare the prognostic significances of IPI and NCCN-IPI in DLBCL patients treated with anthracycline based chemotherapy in Rituximab era.

**Methods:** 324 de-novo DLBCL patients, being older than 16 years-old, with a negative HIV status and no known malignancy, treated with CHOP+R-like chemotherapy between 2002 and 2013 were included in the study. Initial age, LDH level, ECOG performance status, Ann-Arbor stage and extranodal involvement status were analyzed to determine IPI and NCCN-IPI. We classified the patients in four risk groups for both IPI and NCCN-IPI and, we compared progression free survival (PFS) and overall survival (OS) rates between these scoring systems.

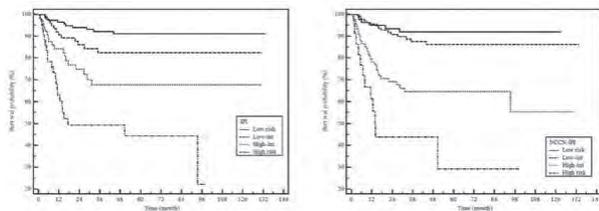
**Results:** The mean age was 53 years old (range:17-90); 36% of 324 patients being older than 60 years old. Fifty-two percent of the cohort had stage III or IV disease and, 50% of patients had high LDH levels. Extranodal involvement of major organs and systems (i.e. bone marrow, CNS, liver, GI tract, lung) was observed in 35.5% of patients. The cohort was categorized as low, low-intermediate, high-intermediate and high risk groups for both IPI (n for each categories was 140-76-66-42 respectively) and NCCN-IPI (n for each categories was 79-131-92-22 respectively). Median follow-up was 44 months and follow-up information was available through September 2015. PFS and OS were compared between risk categories. NCCN-IPI was able to discriminate more accurately high risk category OS compared to IPI; 5-year OS being 29% for NCCN-IPI and 44% for IPI (Figure 1). No difference was observed between OS rates of low risk groups (both 91% at 5-year). (Table 1)

**Conclusion:** Although these scoring systems are essential predictors of prognosis in DLBCL patients, neither IPI nor NCCN-IPI covers molecular subgroups like double-hit and double-expressor lymphomas. The molecular parameters are still not part of scoring systems due to standardization and accessibility issues. We had many scoring systems to predict the prognosis

of aggressive lymphomas and we need to develop better ones. Nonetheless, our “real-life” cohort showed that, although we can discriminate more accurately the high risk patients with NCCN-IPI, it is not superior to IPI in low risk patients in the Rituximab-era.

**Keywords:** NCCN-IPI

**Overall survival for IPI vs NCCN-IPI.**



**Kaplan-Meier survival estimates with hazard ratios (HR) for PFS and OS in 5 years.**

	IPI				NCCN-IPI			
	PFS (%)	HR (95% CI)	OS (%)	HR (95% CI)	PFS (%)	HR (95% CI)	OS (%)	HR (95% CI)
Low Risk	85	1.0	91	1.0	90	1.0	91	1.0
Low-int	72	2.2 (1.17-4.12)	82	2.1 (0.93-4.79)	74	2.1 (0.98-4.47)	86	1.6 (0.62-4.13)
High-int	52	3.6 (2.00-6.72)	67	4.3 (2.04-9.18)	54	5.1 (2.49-10.77)	64	3.2 (2.34-13.57)
High Risk	46	6.6 (3.55-12.25)	44	10.3 (5.00-21.45)	38	10.7 (4.51-25.84)	29	13.2 (4.94-35.53)

## Palliative Care–Supportive Therapy

PS-090

Abstract:0085

### LOW DOSE CYTOSINE ARABINOSIDE AND AZACITIDINE COMBINATION IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA AND REFRACTORY ANEMIA WITH AN EXCESS OF BLASTS (MDS-RAEB2) A SINGLE CENTER EXPERIENCE

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**Introduction:** One-third of elderly (>60 years) acute myeloid leukemia and refractory anemia with excess of blasts (MDS-RAEB2) patients may receive intensive chemotherapy. Treatment alternatives are limited in this patient group due to the potential of severe toxicity. Previous studies have shown that azacytidine and low dose cytarabine treatments may be a good treatment option in these patients. In this study, we retrospectively analysed the AML and MDS-RAEB2 patients who received azacytidine monotherapy and azacytidine and LD-ara-c combination therapy for comparison of response to therapy, survival and toxicity rates and for determining the factors which could affect overall survival.

**Method -patient groups:** A total of 27 patients who were diagnosed with de novo AML and MDS- RAEB2 and who received at least four cycles of chemotherapies were included in the study. The patients were administered 4 cycles of azacytidine or azacytidine + low dose cytarabine combination and data were evaluated retrospectively.

**Results:** When monotherapy and combination therapy groups were compared, pretreatment bone marrow blast count was seen to be greater in combination

therapy group. A statistically significant difference was not detected between groups with regard to response to therapy ratios (p=0.161) (42.9%, 57.1%, respectively). No difference was detected between groups with regard to therapy-related toxicity. Infections were the most common complication. Progression-free survival was 30.3% for azacytidine monotherapy group and 66.7% for combination (azacytidine + LD-ara-c) group. The factors influencing the overall survival were determined as response to first line therapies, more than grade 2 infection, fever and relapse in multi-variance analysis.

**Conclusion:** We consider that this combination therapy may be a well tolerated treatment option in the elderly, vulnerable AML patients whose blast count is high as response to therapy rates, overall survival and toxicities are not different although pre-treatment bone marrow blast count was greater in combination therapy groups compared to monotherapy group.

**Keywords:** Acute myeloid leukemia, azacytidine

PS-091

Abstract:0089

### SAFETY AND EFFICACY OF ANKAFERD HEMOSTAT (ABS) IN CHEMOTHERAPY-INDUCED ORAL MUCOSITIS

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**Purpose:** Oral mucosa is affected from chemotherapeutics easily because of high cell-turnover. Oral mucositis is usually seen in patients with hematologic malignancy who take chemotherapy. Treatment with 5-Fluorouracyl (5-FU), Doxorubicine, Etoposide, Vinblastine and Methotrexate cause treatment-related-mucositis more frequently. Ankaferd Blood Stopper® (ABS) is a folkloric herbal drug extract. It has been used for stopping bleeding in Turkey for many years. In addition to its hemostatic effect, it helps tissue healing and has anti-microbial property. In this study we investigate therapeutic efficacy of ABS in oral mucositis which occurs in adult patients with hematologic malignancy after chemotherapy.

**Methods:** Twenty patients with oral mucositis after chemotherapy was included in this study. Ancaferd was given to patients with grade 3-4 mucositis according to WHO classification patients with grade 3-4 mucositis. 5 ml of ancaferd was given to patients at least four times a day and offered to mouthwash and swallow ancaferd. Age and gender of patients, type of disease, drugs that used in chemotherapy, frequency and amount of ABS, duration of ABS use and healing time of oral mucositis were recorded.

Fig 1.A,B.

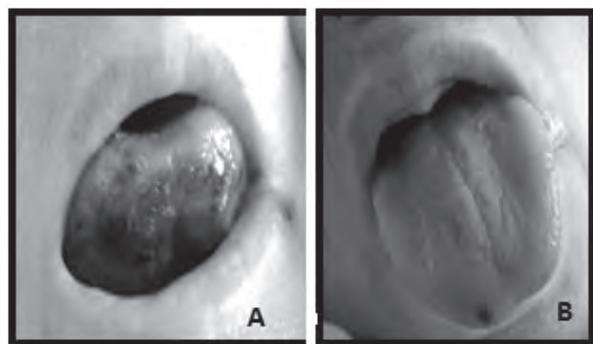


Fig 1.A.Oral mucositis of the patient after induction regimen for acute myeloid leukemia. Fig 1.B. After using ancaferd blood stopper mucositis was healed

Table 1. The characteristics of patients and ABS treatment

No	Age/years	Sex	Type of disease	Chemotherapy regimens	Frequency of ABS	Volume of ABS/ml	Mucositis recovery time/days
1	52	F	AML	Ida+ Ara-c	q8h	100	7
2	49	F	AML	Flu+ Ara-c	bid	100	10
3	60	M	lymphoma	HD-mtx+ Ara-c	bid	50	10
4	19	M	ALL	Mtx+ Ara-c	bid	50	5
5	43	F	AML	Idarubisin	bid	100	10
6	28	F	Lymphoma	HD-mtx+ Ara-c	bid	100	10
7	27	F	Lymphoma	HD-mtx+ gemcitabine	bid	100	10
8	70	M	Hemophagocytosis	Etoposide	bid	50	7
9	23	M	ALL	HD-mtx + Ara-c	q8h	30	3
10	31	M	AML+ Hemophagocytosis	HD-Ara-c+ Etoposide	q8h	100	7
11	47	M	AML	Azacitidine	bid	30	3
12	19	M	ALL	Mtx+ MCP	bid	80	10
13	53	F	AML	Ida+ Ara-c	q6h	80	4
14	57	F	AML	HD Ara-c	q8h	100	5
15	49	F	AML	Ida+ Ara-c	bid	60	4
16	48	F	AML	HD-Ara-c	bid	30	3
17	28	F	AML	Flu+ Ara-c	q8h	100	3
18	58	F	AML	Flu+ Ara-c	q6h	100	7
19	58	F	AML	Ida+ Ara-c	q6h	80	6
20	53	M	ALL	Mtx+ Ara-c	q6h	30	3

F:Female, M:Male, AML: Acute myeloid leukemia, ALL:Acute lymphoblastic leukemia, mtx:Methotrexate HD-mtx: High dose methotrexate, flu: fludarabine, Ida:idarubicine, Ara-c:Cytosine arabinoside, HD- Ara-c: High dose cytosine arabinoside, MCP: mercaptopurine

Results: Eleven patients had used ABS twice a day, five patients had used ABS three times a day and four patients had used ABS four times a day. Amount of used ABS was 30 ml in four patients, 50 ml in three patients, 60 ml in one patient and 100 ml in nine patients. Median extract amount was calculated as 74,50 ml (30-100 ml) and median healing time was 6,6 days(3-10). Except for metallic taste, no side effect was observed in patients.

Conclusions: ABS is an effective agent in chemotherapy related severe oral mucositis in patients with hemathologic malignancy. It shortens healing time with less side effects. It is the first study that shows ABS use and activity in adult patients with chemotherapy related oral mucositis. We hope that ABS will be recommended in routine treatment of chemotherapy related oral mucositis with further randomized controlled studies.

**Keywords:** Oral mucositis, ABS

PS-092

Abstract:0090

**VORICONAZOLE INDUCED HYPOKALEMIC PARALYSIS**

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Visual disturbances, photosensitivity and increased liver enzymes are the most common side effect of VOR, a broad spectrum antifungal agent of triazole family used for treatment of invasive candidiasis and aspergillosis. VOR induced hypokalemia, myopathy, neuropathy and rhabdomyolysis were described. Yet, VOR induced hypokalemic paralysis was not reported.

A 24 -year-old male patient was admitted for one week long myalgia and difficulty in walking for the past four days. He had received reinduction chemotherapy including fludarabine, cytarabine and idarubicin for relapse of acute myeloid leukemia after allogeneic stem cell transplantation. He had received liposomal amphotericin for invasive fungal infection and after 2 weeks, he was switched to VOR 400 mg/day. He was on 10th day of VOR at admission. On neurological examination (NE), there was slightly asymmetrical proximal muscle weakness in lower extremities. Nerve conduction studies (NCSs) revealed decreased in amplitudes of bilateral femoral and peroneal (recorded from extensor digitorum brevis and tibialis anterior muscles) compound muscle action potentials. Other motor (right median and ulnar, bilateral tibial) and sensory NCSs (right median, ulnar, superficial peroneal and sural) were normal. F responses (right median, ulnar, peroneal and bilateral tibial) were normal. Needle EMG showed reduced recruitment and difficulty in sustaining muscle contraction in weak muscles. Potassium was low (2.6 mEq/L) and creatine kinase was high (12,000 U/L). VOR was stopped, alkaline hydration and potassium replacement were made for rhabdomyolysis. Complaints disappeared by 3rd day. Control NE and NCSs were normal. Hypokalemia and myopathy is rare due to VOR. In this patient, there was a temporal relation between introduction of VOR and onset of weakness. Weakness improved after discontinuation of VOR. Consequently, we consider that reversible paralysis was due to hypokalemia induced by VOR. CYP450 enzyme family is the major metabolic pathway for VOR. CYP2C19 activity is highly dependent on genetic polymorphisms. 15–20% of patients of Asian and 3% of patients of European origin have low CYP2C19 activity, resulting in VOR levels as much as 4 times higher compared to subjects who metabolize VOR more extensively.

In our patient, serum VOR level was not measured. Yet, he might have low CYP2C19 activity because of absence of other risk factors causing hypokalemia. Among list of disorders causing acute and reversible attacks of severe muscle weakness, hypokalemic paralysis is uncommon. Most cases are due to familial hypokalemic paralysis. Sporadic cases are associated with different etiologies including renal tubular acidosis, primary hyperaldosteronism, thyrotoxic periodic paralysis, and gastrointestinal potassium loss. Hypokalemia increases hyperpolarization of normal fibers and causes paralysis. Muscle weakness due to drug induced hypokalemia has to be included in the list of side effects of VOR.

**Keywords:** Voriconazole, hypokalemic paralysis

PS-093

Abstract:0091

### ACUTE ONSET DEEP THROMBOCYTOPENIA ASSOCIATED WITH PIPERACILLIN/TAZOBACTAM

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Piperacillin/tazobactam (PT) is a commonly prescribed antimicrobial and is generally considered safe. As well as other betalactam antibiotics, it may cause thrombocytopenia. However, there are only a few reports (1-4) about PT-induced thrombocytopenia in the current literature. Bone marrow suppression (1), antibodies specific for PT (2), immune thrombocytopenia (3) and delayed response to PT (4) are the probable causative mechanisms of this situation. A 67-year-old woman was diagnosed with diffuse large b-cell lymphoma, and was treated with 4 courses of rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) chemotherapy, and maintains a partial remission. She was treated with PT empirically for an open wound 3 x 2 cm in diameter in her right hand. On day 3 of PT therapy her platelet count had fallen to a nadir of 4 x 10<sup>9</sup>/L (72 x 10<sup>9</sup>/L on day 1 of treatment). A clinical diagnosis of PT-induced thrombocytopenia was made and the drug withdrawn. Wound culture was positive for extended spectrum beta-lactamase producing *Escherichia coli*, therefore ertapenem therapy was initiated. After 7 days, a significant improvement in the platelet count was noted rising to 123 x 10<sup>9</sup>/L. Physicians must be aware of drug induced thrombocytopenia especially in long-term hospitalized patients with complex medical problems.

**Keywords:** Piperacillin/tazobactam, thrombocytopenia

PS-094

Abstract:0119

### EFFECTIVENESS OF ANKAFERD BLOOD STOPPER IN PROPHYLAXIS AND TREATMENT OF ORAL MUCOSITIS SEEN IN CHILDHOOD CANCERS AND CORRELATION WITH PLASMA CITRULLINE LEVELS

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**Objective:** In last 3 decades, success rate has been markedly increased in childhood cancers; however, chemotherapy-related adverse events remain to be an important issue. Oral mucositis, one of the toxic effects of chemotherapy, is observed in 52-80% of the children receiving cancer therapy. Ankaferd Blood Stopper (ABS) is an herbal product that is used as a hemostatic agent. In previous studies, it was shown that ABS has antimicrobial, anti-inflammatory effects as well as positive effects on healing of tissue injury. In our study, it was aimed to investigate effectiveness of ABS in prophylaxis and treatment of oral mucositis in patients receiving chemotherapy at childhood. In addition, plasma levels of citrulline, a biochemical marker for mucosal barrier injury, were measured and effectiveness of ABS therapy in mucositis was correlated by quantitative data in addition to clinical assessment.

**Material-Method:** This was a randomized, controlled and open-label study which included 27 patients aged 4-17 years receiving chemotherapy regimens with strong mucotoxic effect. The patients were asked to perform standard oral care (SOC) upon first day of chemotherapy for 10 days and oral mucosa was assessed daily upon completion of chemotherapy based on World Health Organization scale for oral mucositis. In addition, blood samples were drawn immediately before initiation of chemotherapy and at the period where mucositis became most intensive. Same patients receiving same chemotherapeutic agents in the second course of chemotherapy were asked to gargle by using ABS four times daily in addition to SOC. Mucosa ratings were performed before second chemotherapy course and at the period where mucositis became most intensive, and blood samples were drawn to measure citrulline levels in second chemotherapy course.

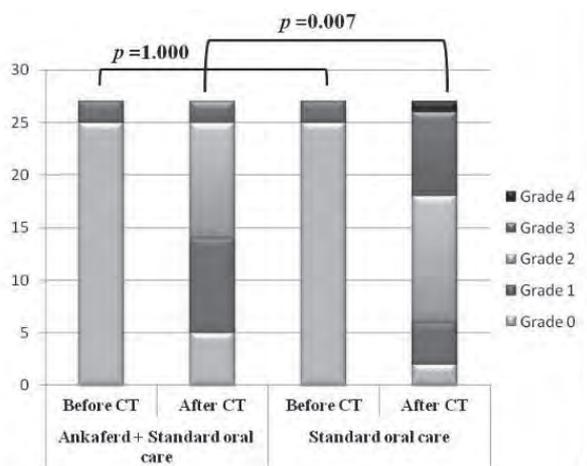
**Results:** The study included 27 patients (mean age: 9.1 ± 4.4 years; 15 boys 56%). Stages of oral mucositis were found to be significantly lower in the second chemotherapy course given SOC plus ABS when compared to first chemotherapy course given SOC alone (p=0.007) (Figure). Mean plasma citrulline level obtained before and after chemotherapy decreased from 44.08 ± 11.20 to 23.99 ± 12.16 nmol/mL in chemotherapy course given SOC alone (p<0.001) while it decreased from 38.67 ± 11.46 to 26.78 ± 11.99 nmol/mL (p<0.001). When extent of decrease in plasma citrulline level was assessed, it was greater in courses given SOC alone compared to those given SOC plus ABS (p=0.009) (Table).

**Conclusion:** ABS can be considered to be an approach with potential benefits, although its effectiveness hasn't been proven in the prophylaxis and treatment of oral

mucositis. Based on our results, ABS exhibited beneficial effects in the prophylaxis and treatment of oral mucositis. However, multi-center experiences and further studies with larger sample size are needed for introduction of ABS into primary oral care and treatment protocols of oral mucositis.

**Keywords:** Ankaferd, oral mucositis

Figure



Table

	1. Chemotherapy cure			2. Chemotherapy cure			p value#
	Before	After	p value	Before	After	P value	
Citrullin (nmol/mL)	44.08 ± 11.20	23.99 ± 12.16	<0.001	38.67 ± 11.36	26.78 ± 11.99	<0.001	0.009

Plasma citrullin levels before and after chemotherapy cure.

## Stem Cell Transplantation

PS-095

Abstract:0095

### THE EVALUATION OF THE EFFICACY AND SAFETY OF ORIGINAL FILGRASTIM (NEUPOGEN®), BIOSIMILAR FILGRASTIM (LEUCOSTIM®) AND LENOGRASTIM (GRANOCYTE®) IN STEM CELL COLLECTION FOR ALLOHSCTDONORS

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**Objectives and Aim:** In this study, we aimed to compare the potency of different G-CSF agents including original filgrastim (Neupogen®), biosimilar filgrastim (Leucostim®) and Lenograstim (Granocyte®) on CD34+ cell mobilisation in patients who underwent allogeneic hematopoietic stem cell transplantation (alloHSCT).

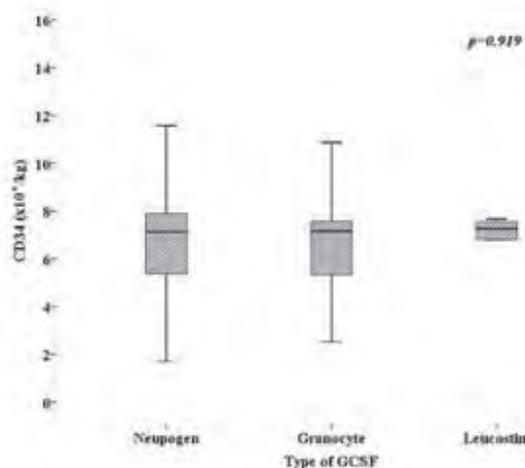
**Materials-Methods:** The data of 243 donors for alloHSCT recipients diagnosed with mostly acute leukemia and myelodysplastic syndromes (MDS) were analysed, retrospectively. Data for stem cell mobilisation has been recorded from patients' files. Patients who received Filgrastim (Neupogen®, Group I), biosimilar Filgrastim (Leucostim®, Group II) and Lenograstim (Granocyte®, Group III) were analysed for total CD34+ cell count at the end of mobilisation procedures.

**Results:** A total of 243 donors and patients for alloHSCT were analyzed retrospectively. The characteristics of the donors are shown in Table 1. 110 (45.3%) of the patients were female, and 133 (54.7%) were male. The diagnosis of the patients were; acute myeloid leukemia (AML) (110 patients, 45.2%), acute lymphoid leukemia (ALL) (61 patients, 25.1%), aplastic anemia (AA) (38 patients, 15.6%), lymphomas (14 patients, 5.7%) and others (20 patients, 8.4%). The median doses of G-CSF agents (µg/kg/day) in PBSC collection in Neupogen® group was; 11.00 (10.00-12.00) in Leucostim® group 10.35 (min-max: 10.00-11.10) and in Granocyte® group 11.00 (min-max: 10.00-11.00). There was no statistical significance among groups (p=0.215). The median number of total collected PB CD34+ cells (x10<sup>6</sup>/kg) was 7.12 (min-max: 5.38-7.90) in the Neupogen® group, 7.27 (min-max: 6.79-7.55) in the Leucostim® group and 7.15 (min-max: 5.34-7.58) in the Granocyte® group. There was no statistically significant difference among groups in term of total collected PB CD34+ cells (p=0.919).

**Conclusion:** Biosimilar filgrastim (Leucostim®) have comparable results to original filgrastim (Neupogen®), and Lenograstim (Granocyte®) in alloHSCT stem cell collection.

**Keywords:** Stem cell collection, biosimilar

Figure 1



Box-plot showing CD34+ cell count (x10<sup>6</sup>/kg)

PS-096

Abstract:0097

### HEPATIC IRON OVERLOAD (HIO) IS STRONGLY CORRELATED WITH SERUM FERRITIN LEVELS AND INFERIOR SURVIVAL IN PATIENTS THAT UNDERWENT ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (ALLOHSCT)

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**Objectives and Aim:** Iron overload results in increased infection, venous-occlusive disease and hepatic dysfunction in allogeneic hematopoietic stem cell transplant (alloHSCT) recipients. Serum ferritin is the most common, easy, non-expensive and non-invasive method for evaluation of iron overload. In this study; our aim was to investigate the relationship between liver iron content and post-transplant prognosis in patients that underwent alloHSCT in our center.

**Materials and Methods:** A total of 50 patients that underwent alloHSCT in Stem Cell Transplantation Hospital, Faculty of Medicine, Department of Hematology, Erciyes University between 2004-2011 were enrolled in the study. The data of the patients' files have been recorded retrospectively. The liver biopsy specimens have been found from the archives of Erciyes University, Department of Pathology and stained for iron content. Graft-versus host disease (GVHD), relapse rates, infections, days of neutrophil and platelet engraftment days, disease-free survival, overall survival and transplant-related mortality rates have been analyzed among groups.

The analysis of the groups have been made using IBM SPSS Statistics 22.0 (IBM Inc., Ill, USA) programme.  $p < 0.05$  has been considered as statistically significant.

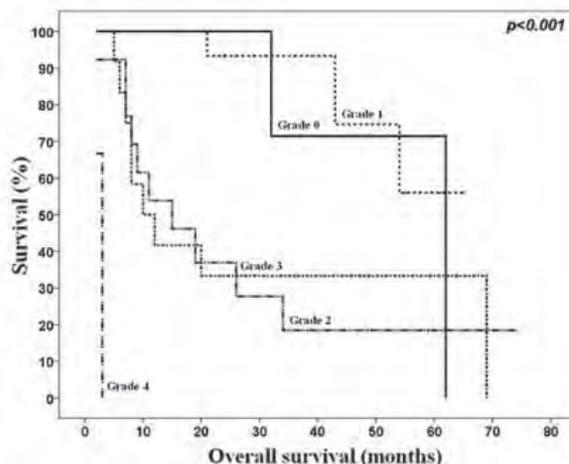
**Results:** A total of 50 patients have been enrolled in the study. Of the patients; 32 were (%64) male, 18 were (%36) female. The diagnosis were; acute myeloid leukemia (AML) was in 24 patients (%48), acute lymphoblastic leukemia (ALL) in 14 patients(%28), multiple myeloma (MM) in 3 patients (%6) and other disorders (Aplastic anemia (AA), myelodysplastic syndrome (MDS), lymphoma, chronic myeloid leukemia (CML) in 9 (% 18) patients. The mean age was found  $34.00 \pm 11.48$  years. For overall survival (OS); it was found (mean) grade 0 patients 53.4 months (41.1-65.7), grade 1 55 months (47.2-64.5), grade 2 patients 25.4 months (11.5-39.4), grade 3 patients 29.3 months (12.3-46.3) and grade 4 patients 2.6 months (2.0-3.3). Overall survival was correlated with the degree of liver iron content and was statistically significant for Kaplan-Meier analysis ( $p < 0.001$ ). Disease-free survival was found (DFS); it was found (mean) grade 0 patients 47.1 months (32.0-62.0), grade 1 patients 36.9 months (21.0-65.0), grade 2 patients 23.5 months (12.0-59.0), grade 3 patients 27.4 months (5.3-59.3) and grade 4 patients 2.6 months (2.0-3.0). For DFS; it was negatively correlated with the degree of liver iron content, nevertheless it was not statistically significant for Kaplan-Meier analysis ( $p = 0.093$ ).

**Conclusion:** Especially in patients with hematological malignancies that underwent alloHSCT, liver iron

accumulation may effect the post-transplant survival rates negatively.

**Keywords:** Liver, alloHSCT

Figure 1



Kaplan Meier analysis of overall survival (OS) in alloHSCT recipients for liver iron content ( $p < 0.001$ )

PS-097

Abstract:0098

### INCREASED BONE MARROW IRON STORE (BMIS) MAY BE A PREDICTIVE MARKER FOR INFERIOR SURVIVAL IN PATIENTS WITH IRON OVERLOAD THAT UNDERWENT ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (ALLOHSCT). SINGLE CENTER EXPERIENCE

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**Background:** Iron overload (IO) is one of the most significant problems as a leading cause of death in patients with leukemia and those who underwent allogeneic hematopoietic stem cell transplantation (alloHSCT).

**Methods:** In the current study, we retrospectively evaluated the bone marrow iron stores (BMIS) in patients who underwent allogeneic hematopoietic stem cell transplantation ( $n = 125$ ). The first available bone marrow biopsy specimens prior to the HSCT diagnosis or date of hospitalization (control group) were assessed in a blinded fashion using a standardized scoring system (1-4).

**Results:** A total of 125 patients were enrolled in the study. 76 (60.8%) of the patients were male and 49 (39.2%) were female. The median level of pre-transplant serum ferritin was 1023.00 ng/mL (min-max: 393.80-1627.50). The OS and DFS were strongly correlated with the degree of BMIS and both data were statistically significant ( $p < 0.001$  and  $p = 0.012$ , respectively). The majority of the patients were diagnosed with acute leukemia (83, 66.4%) and lymphomas (20, 16.0%). The median day for neutrophil engraftment was 14.00 (min-max: 13.00-16.00) days and 11.00 (min-max: 10.00-14.00) days for platelet

engraftment. 50 patients (40.0%) was died to primary disease or secondary complications (infection, bleeding) during post-transplant follow-up.

Conclusion: The validation of BMIS for risk stratification in patients who undergo allogeneic hematopoietic stem cell transplantation may predict posttransplant outcomes.

**Keywords:** Bone marrow iron, HSCT

Figure 1

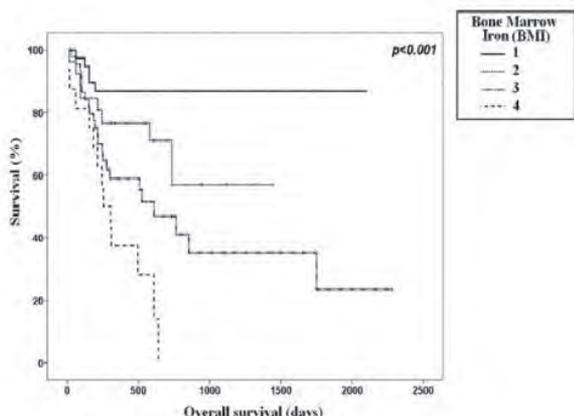


Figure 1 shows the correlation between high BMIS and poor survival in alloHSC recipients ( $p < 0.001$ )

PS-098

Abstract:0104

**EXPERIENCE WITH CIDOFOVIR IN TREATMENT OF CMV INFECTION REFRACTORY TO GANCYLOVIR AFTER ALLOGENEIC STEM CELL TRANSPLANTATION**

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Cytomegalovirus (CMV) infection and disease remain as major complications of allogeneic hematopoietic stem cell transplantation (AHSCT). CMV resistance to gancyclovir (GCV) after AHSCT requires prolonged use of alternative antivirals with outcomes ranging from asymptomatic to severe or fatal disease. Foscarnet (FC) is usually the 2nd choice with rare hematological toxicity in contrast to GCV. There is limited data about tolerability and clinical activity of cidofovir (CDV) in this setting. 43-years old male diagnosed with Ph+ ALL underwent myeloablative peripheral AHSCT from full matched unrelated male donor. At time of HSCT, there was hematological and molecular remission. Donor was CMV Ig G- and recipient was CMV Ig G+. On day (D) 31, grade II acute GVHD involving skin and GIS developed. Methylprednisolone (MP) was initiated. For relapse of GIS GVHD on D79, MP was restarted with mycophenolate mophetil under valgancyclovir prophylaxis. On D135, he was hospitalised for diarrhea. Serum CMV DNA was 2483 copies/ml, so GCV 10 mg/kg was begun. Immunohistochemistry studies on gastric and colonoscopic biopsies were positive for CMV. On 2nd day of admission, fever and hypoxemia developed. Thorax

CT revealed bilateral ground glass opacities. With presumptive diagnosis of pneumocystis jiroveci pneumonia, trimethoprim-sulfometaxazole (TMP-SMX) was started. CMV DNA was 1,727,257 and 950,149 copies/ml at 1st and 2nd week of GCV, respectively. After 3 weeks of TMP-SMX, infiltrates on thorax CT showed significant regression. At 6th week of admission, fever recurred and thorax CT showed new consolidation on right lower lobe. Bronchoalveolar lavage (BAL) cultures remained sterile and BAL CMV DNA was 1909 copies/ml establishing diagnosis of CMV pneumonia. On 7th week of GCV, CMV retinitis was diagnosed and CMV DNA rose significantly (Figure 1). Also, mutation at codon 594 of UL97 gene reported to confer gancyclovir resistance was identified. FC 2x90 mg/kg/day was begun. After 3 weeks, CMV DNA decreased to 10,402 copies/ml and FC was continued as 90 mg/kg/day. GCSF support was required for FC induced neutropenia. By 10th week of FC, CMV DNA steadily decreased to 1122 copies/ml. Repeat ophthalmological examination showed signs of ongoing retinitis. BM biopsy performed due to development of grade 3 neutropenia demonstrated ongoing hematological remission and full donor chimerism. FC was switched to CDV. He has received 2 weekly doses of CDV followed by 3 doses every 2 weeks. By 23rd week of initiation of therapy, CMV DNA was 286 copies/ml. He is still under CDV maintenance.

In suspicion of CMV drug resistance, sending samples for mutation analysis and switch to alternative drug, in most cases, to FC is recommended. In our patient, in whom FC had to be ceased due to the rare side effect of neutropenia, CDV was well tolerated and achieved sufficient response. CDV seems to be an alternative for GCV refractory CMV infection especially in presence of cytopenias.

**Keywords:** Resistant Cytomegalovirus infection, allogeneic stem cell transplantation

Figure 1

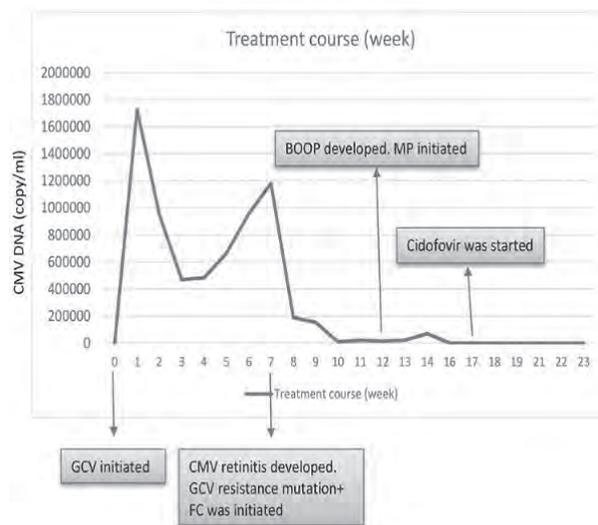


Figure: Quantification of CMV DNA by PCR during follow up

PS-099

Abstract:0105

**REFRACTORY MEMBRANOUS GLOMERULONEPHRITIS AS A MANIFESTATION OF CHRONIC GRAFT VERSUS HOST DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IN A PATIENT WITH HYPOCELLULAR MYELODYSPLASTIC SYNDROME**

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Renal involvement secondary to cGVHD is rare and its usual clinical symptom is nephrotic syndrome. The most common pathological diagnosis of nephrotic syndrome secondary to GVHD is membranous glomerulonephritis. A 52-yr-old female, diagnosed with hypocellular myelodysplastic syndrome unresponsive to immunosuppressive treatment with ATG and cyclosporine (CSa), underwent allogeneic peripheral HSCT from HLA-identical sibling donor with reduced intensity conditioning regimen including busulphan and fludarabine and cyclosporine and metotrexate as prophylaxis of GVHD. Engraftment was sustained and no acute GVHD developed. Assessments on 3rd and 6th months for signs of chronic GVHD (cGVHD) were unremarkable. CSa was tapered and finally withdrawn 7 months after HSCT. Eleven months after HSCT, she developed moderate cGVHD (oral score II, eyescore I, GİS score:1 and skin score:1). Methylprednisolone (MP) 0.5 mg/kg/day PO was initiated. She remained on maintenance MP until 16th month after HCST. On the 22nd month of HCST, severe cGVHD (score:oral 3, eye 2, skin 2, liver 1, lung 2, musculoskeletal 2) was diagnosed and MP 1 mg/kg/day and CSa were started. Three months later, the patient developed a clinical nephrotic syndrome with hypoalbuminemia of 2 g/dl, 6.5 gr/day proteinuria, severe hypercholesterolemia (LDL:504 mg/dl). On physical examination, there was anasarca edema. Cardiac functions on echocardiography were normal. On laboratory tests, complete blood count, creatinine as well as serum Ig G, IgA, Ig M and C3, C4 were normal. Tests for HbsAg, anti HCV, anti HIV and ANA and anti-DNA were negative. Tumor markers and radiological surveys for an underlying malignancy were negative. Renal biopsy contained a total of 14 glomeruli with no global sclerosis. The glomerular tufts showed mild thickening of capillary walls. There were patchy foci of tubular basalmembrane thickening and a mild increase in mononuclear cell infiltrate. Immunofluorescence study showed granular deposits of Ig G and C3 along the capillary basal membrane. These clinical and renal biopsy findings were compatible with cGVHD. Mycophenolatemofetil (MMF) was initiated as the 3rd immunosuppressive agent to the patient, who was already receiving CSa and MP for ongoing severe cGVHD. Yet, massive proteinuria continued despite triple immunosuppression. The patient succumbed to pneumonia 3 months after diagnosis of cGVHD related membranous nephropathy.

Late onset nephropathy may simultaneously develop with other GVHD symptoms. Our case reemphasizes the importance of periodic monitoring of renal function and proteinuria after allogeneic-HSCT. Other possible causes of nephrotic syndrome including drugs, cancer and infections must be ruled out as well.

**Keywords:** Allogeneic stem cell transplantation, membranous glomerulonephritis

PS-100

Abstract:0107

**AUTOLOGOUS STEM CELL TRANSPLANTATION IN ELDERLY PATIENTS (>=60 YEARS) WITH MYELOMA OR LYMPHOMA**

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**Introduction:** Significant therapeutic advances have recently been made in the care of older patients in myeloma and lymphoma. Although there are many elderly patients who are eligible for high dose therapy (HDT) with autologous stem cell transplantation (ASC) rescue, the evidence is inadequate. Therefore we aimed to analyze the efficacy and safety of HDT with ASC rescue in our elderly myeloma and lymphoma patients.

**Patients&Methods:** Between Jan 2003 and Oct 2014, 133 patients (84 M; 49F) admitted to Ankara University Department of Hematology were included in the study. Their diagnosis were as following; 116 plasma cell dyscrasia (114 multiple myeloma, 1 plasmocytoma and 1 primary amyloidosis) and 17 non-Hodgkin Lymphoma (10 Diffuse large B cell, 6 Mantle cell and 1 follicular). We retrospectively evaluated the early toxicity and outcome of the HDT.

**Results:** Median age of the group was 63 years (60-74 ys) while median time from diagnosis to the transplantation was 11.4 months (3.1-188.7 months). Most of the patients had low Sorror Comorbidity Index (CI) (68.4 %). In comparison of the toxicities including oral mucositis, diarrhea, renal failure, liver and cardiac toxicity and the incidences of overall infections, we found only oral mucositis and diarrhea that were more frequent in patients with myeloma than lymphoma (p=0.01 and 0.03, respectively). When similar comparisons were repeated according to age (<65 ys vs >=65 ys), Sorror CI (low vs intermediate and high), the intensity of conditioning regimen for melphalan (standard vs low dose), the rates of toxicities and infection did not change. In addition, engraftment kinetics were not affected by the diagnosis, and age. However, faster neutrophil recovery was observed in low and intermediate Sorror CI compared to high index (median 11 days vs 12 days, p=0.03). Early-transplant related mortality (TRM) was seen only in 5 patients. The diagnosis, age and Sorror CI were not found to have any impacts on both TRM and overall survival.

**Conclusion:** Although the oral and gastrointestinal mucositis were more frequent in myeloma patients, TRM and OS did not differ. Therefore, HDT with ASC rescue was demonstrated to be a safe option in the treatment of myeloma and lymphoma patient in advanced age in our cohort.

**Keywords:** Autologous stem cell transplantation, elderly

PS-101

Abstract:0109

**ALLOGENEIC STEM-CELL TRANSPLANTATION FOR HODGKIN AND NON-HODGKIN LYMPHOMA**

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**Introduction:** Although long term survival rates can be achieved with chemoradiotherapy approaches in lymphoma, one third of the patients can relapse during the follow up. Allogeneic hematopoietic stem cell transplantation (HCT) remains to be the only curative treatment approach for relapsed/refractory lymphoma patients. Here, we present our relapsed/refractory Hodgkin and non-Hodgkin lymphoma patients who required allogeneic HCT.

**Materials and methods:** We retrospectively analyzed 46 relapsed/refractory lymphoma patients (25 NHL;21 HL) who underwent allo HCT rescue treatment Ankara University Department of Hematology BMT Unit. Chi-square test was used in comparison between two groups due to engraftment kinetics, post-transplant complications, post-transplant responses.  $P < .05$  was considered statistically significant.

**Results:** The median age of the patients in NHL group was  $40,2 \pm 14,2$  and  $28,8 \pm 7,9$  in HL group. Peripheral blood was the preferred stem cell source in both NHL and HL patients (24/25, 17/21). Sixteen of twenty five non Hodgkin lymphoma patients received myeloablative conditioning regimen whereas reduced intensity conditioning was the preferred conditioning regimen in HL group. Also, transplantation from unrelated donor was also significantly higher than the NHL group. Fifteen patients had primary refractory disease in both groups. Median follow-up period from transplantation was 26 months (range 4-98) in 46 patients. Complete remission was observed in 5/21(23%) patients in NHL group while 9/21(42%) patients in HL group. Engraftment could not be achieved in 5 NHL patients and 3 Hodgkin lymphoma patients due to death in aplasia period. Median time for neutrophil engraftment was 14 days and platelet engraftment was 15 days for the Hodgkin lymphoma whereas non-Hodgkin lymphoma neutrophil and platelet engraftment median were both 16 days. Acute graft versus host disease (GVHD) was detected in 13/25(52%) patients in NHL group and 10/21(47%) patients in HL group. One year overall survival in NHL group was detected as 40% in NHL group while 55% in HL group.

**Conclusion:** Patients with HL received reduced intensity conditioning regimen more frequently and transplantation was mostly from unrelated donors. In our retrospective study, the allogeneic stem cell transplantation outcomes were found to be similar with the previous reports.

**Keywords:** Allogeneic stem cell transplantation, lymphoma

PS-102

Abstract:0132

**AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN RELAPSED/REFRACTORY LYMPHOMA PATIENTS: SINGLE INSTITUTE EXPERIENCE**

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**Background:** Autologous hematopoietic stem cell transplantation (AHST) is a treatment choice in relapsed/refractory lymphoma patients. We aimed to evaluate our relapsed/refractory lymphoma patients who had AHST retrospectively.

**Patients-Method:** Forty patients with lymphoma between November 2004 & February 2014 were evaluated retrospectively. **Results:** Median age was 33 (range:17-60) with male (n:23) predominance. 22 patients had HL while 18 patients (B-cell lymphoma (n:15), T cell NHL (n:3)) had NHL. The most common subtypes were as nodular sclerosing HL (n:14) and diffuse large cell lymphoma (NHL, n:9). Most of the patients had stage 2 disease (n:16), nodal involvement (n:36). 15 patients had bulky disease. Objective response rate (ORR) was 82,5% for first line chemotherapy. Four patients (T-cell NHL, n:3; Burkitt lymphoma, n:1) had refractory disease. Median relapse-free survival was 11.4 months. ICE was most preferred as salvage chemotherapy regimen (n:26). ORR was 76,3% for salvage chemotherapy. Median CD34 count was  $3.46 \times 10^6/\text{kg}$  (range: 1.5-9.0). Median hospitalization duration was 29 (range:15-72) days. Median values for neutrophil and platelet engraftments were as 11 & 12 days, respectively. 14 patients had infection during hospitalization. 24 patients relapsed, 20 patients died after AHST. 15 patients had chemotherapy after relapse. Median OS was 30,1 months (95% CI: 23.6-36.6). However, there was no difference between HL & NHL patients after AHST (30,2 months vs 31,1 months,  $p=0,44$ ).

**Conclusion:** Relapsed/refractory lymphoma patients have poor prognosis with conventional chemotherapy, but AHSCT is a promising option in these patients with favourable outcomes. Our results are in parallel to the literature with acceptable engraftment durations and infection rates.

**Keywords:** Autologous hematopoietic stem cell transplantation, relapsed/refractory lymphoma

PS-103

Abstract:0221

### SEVERE CHRONIC COLITIS FOLLOWING AUTOLOGOUS - STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA

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**Introduction:** Here we describe a case in a recipient of autologous -stem cell transplantation (Auto-SCT) who developed life-threatening chronic colitis.

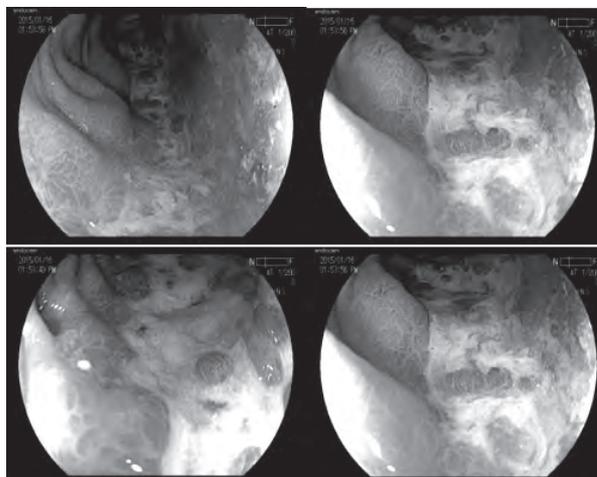
**Case:** A 75-year old male patient with multiple myeloma underwent an Auto-SCT with reduced dose melphalan conditioning. He was experienced any complication during the post-transplant period and neutrophil engraftment occurred on day +11, and discharged from the hospital on day +14. However, he was admitted to the hospital with diarrhea on day +34. Infectious stool studies were negative. CMV PCR testing was negative. Symptomatic treatment with loperamid was given. He was started parenteral antibiotherapy due to septicemia on day +40 and continued 14 days. Thereafter, he developed profuse bloody diarrhea and abdominal pain. Infectious stool studies were negative. Colonoscopy was done and showed severe deep ulcerations in the distal rectum. Several biopsies were obtained for histology and HSV, CMV, and TBC PCR. The PCR studies were negative except for CMV. Histological examination of biopsies revealed severe active chronic inflammation with extensive ulceration and two CMV inclusions were identified, confirming a diagnosis of severe ulcerative CMV colitis. Meantime, peripheral blood sample test for CMV PCR revealed 3824 IU/ml copy. Intravenous ganciclovir and intravenous immunoglobulin 400 mg/kg once per 3 weeks were commenced. Although, CMV PCR was found to be negative after three weeks of ganciclovir, diarrhea, abdominal discomfort and weight loss continued. Colonoscopy was repeated and showed newly developed dirty white colored plaques disseminated to all colon while regression of the ulcerations in the distal rectum. Several biopsies were obtained for histology and HSV, CMV, candida, TBC and C. difficile PCR. The PCR studies were negative except for C. difficile. So, oral metronidazole and vancomycin treatment was given for 3 weeks. However, clinic of the patient did not change even control C. difficile PCR negative. Therefore 3rd colonoscopy was performed (Fig. 1). Infectious stool studies were negative. The PCR studies were all negative. Histological examination of biopsies revealed severe active chronic colitis with extensive ulcerations and some necrosis. There were no microscopic findings suggestive of GVHD. Treatment was initiated with oral steroid therapy (2 mg/kg/day prednisolone), oral mesalazine (1500 mg/day) and oral budesonide (9 mg/day). After these treatments, the clinical symptoms disappeared and colonoscopic findings decreased gradually.

**Discussion:** Diarrhea is a major cause of morbidity among recipients of Auto-SCT. The cause is often multifactorial. The use of antineoplastic agents, antibiotics,

radiotherapy, alterations in diet and various pathogens have all been implicated. Autoimmune inflammatory bowel disease, CMV colitis and concomitant infection with other pathogens such as Clostridium difficile should be considered in the differential diagnosis.

**Keywords:** Stem cell transplantation, colitis

Severe deep ulcerations disseminated into the all colon



PS-104

Abstract:0254

### PRETRANSPLANT SERUM FERRITIN LEVELS MIGHT BE ASSOCIATED WITH INFERIOR SURVIVAL IN PATIENTS WITH IRON OVERLOAD THAT UNDERWENT ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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**Objectives:** Iron overload increases the risk of infections, veno-occlusive disease and hepatic dysfunction in post-transplant period. Our objective was to investigate the association of pre-transplant ferritin levels with complications and survival after allogeneic hematopoietic stem cell transplantation (alloHSCT)

**Materials-Methods:** We retrospectively analysed data of 361 patients that underwent allogeneic HSCT into two groups: patients with a serum ferritin level  $\geq 500$  ng/ml (Group 2), and patients with  $< 500$  ng/ml (Group 1) obtained until 3 months prior to transplantation.

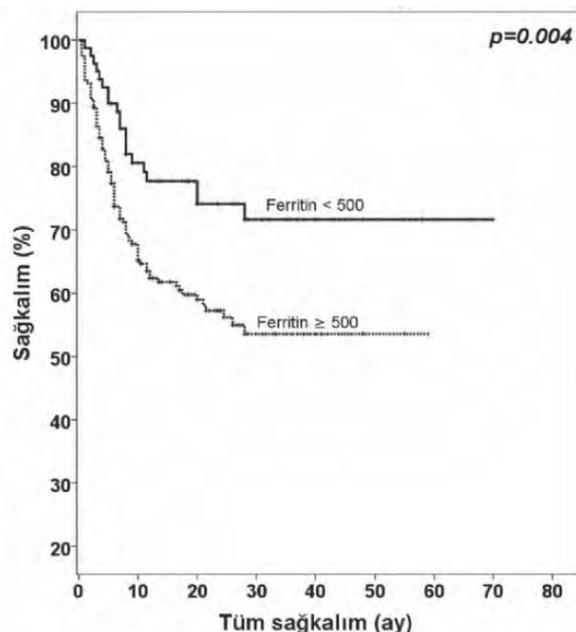
**Results:** A total of 316 patients were included in the study. Of the patients; 136 were acute myeloid leukemia (AML, 43.0%), 82 were acute lymphoblastic leukemia (ALL, 25.9%), 32 aplastic anemia (AA; 9.8%) and others (MDS, CML) şeklindeydi. 83 patients (%26.2) were in Group 1 and 233 patients (%73.8) in Group 2. The mortality rates were; in Group 1, 21 of 83 patients (25.3%) died during follow-up and in Group 2, 92 of 133 patients (69.1%) died ( $p < 0.001$ ). While median overall survival (OS) was found 20 months (min-max, 1-70 months) in Group 1, it was found 10.5 months (min-max, 0.5-76

months) in Group 2. The difference was statistically significant ( $p=0.004$ ). For disease-free survival (DFS); it was found median 18 months (min-max, 1-69 months) in Group 1 and 10 months (min-max, 1-67 months) in Group 2 ( $p=0.038$ ). Mortalite oranları; Grup 1'de 21 hasta (21/83; %25.3) iken, Grup 2'de 92 hasta (92/133; %69.1) şeklinde bulundu ( $p<0,001$ ).

Conclusion: Pre-transplant elevated serum ferritin levels might be associated with inferior survival in alloHST recipients with iron overload.

**Keywords:** alloHST, ferritin

Figure 1



Kaplan-Meier curve for overall survival (OS) analysis

PS-105

Abstract:0264

**DURABLE REMISSION IN ADULT HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS AFTER BONE MARROW TRANSPLANTATION WITH A NONMYELOABLATIVE REGIMEN INCLUDING ALEMTUZUMAB, FLUDARABINE AND MELPHALAN**

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Introduction: Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening immunodeficiency characterized by severe systemic hyper-inflammatory response to infectious or other triggers of the immune system.

Case: A 37-year-old woman presented with prolonged fever, hepatosplenomegaly, pancytopenia, hypogammaglobulinemia, high ferritin level, hypertriglyceridemia and liver dysfunction. All classes of immunoglobulin (Ig) were decreased. Bone marrow biopsy showed a hypercellular marrow with no evidence of malignancy. On bone marrow aspirate, numerous monocytes and marked hemophagocytosis without any evidence of immature cells were noted. Cytogenetic analysis showed no abnormalities. She was diagnosed as HLH. A molecular screening for PRF1, MUNC13-4, and STX11 mutations was negative. Screening studies for an underlying malignancy or an infectious organism remained negative. Final diagnosis was secondary HLH in the presence of COVID. HLH-2004 protocol was initiated along with IVIG 0.5 g/kg IV every 4 weeks. After initial therapy consisting of 8 weeks etoposide, dexamethasone and cyclosporine, partial response was achieved and continuation therapy was begun. Eleven months after initial diagnosis, she was in stable disease status with normal blood cell count but with high serum ferritin levels and mild organomegaly. At that time, she underwent bone marrow stem cell transplant from a 6/6 HLA-matched 29 year old female sibling donor. RIC regimen consisting of alemtuzumab, fludarabine and melphalan was used. Ursodeoxycholic acid and intravenous defibrotide were used for VOD prophylaxis. Major early transplant-related complications were CMV infection on day 10 and Pneumocystis jirovecii pneumonia (PJP) on day 17. VOD, acute GVHD and chronic GVHD were not encountered during follow-up. Patient had 100% donor cells in the T cell compartment by day +28. She subsequently developed stable mixed chimerism (75% donor cells on 3th month, 55% on 6th month, 74% on 9th month, 72.5% on 12th month, 71% on 15th month) without requiring interventions. Full donor T cell chimerism was attained on the 18th and the 24th month. At last follow-up 26 months after AHST, the patient is alive and in remission of her underlying disease with ongoing full-donor chimerism.

Conclusion: Several studies revealed that RIC regimen including alemtuzumab, fludarabine and melphalan dramatically improved survival in HLH. Our patient experienced CMV and PJP infections at an earlier posttransplant period as compared to patients having received myeloablative conditioning regimens. Due to development of mixed chimerism, the patient was monitored closely and no additional interventions including DLI or second stem cell infusion were needed. Eventually, she developed full donor chimerism. Clinical experience in HLH after RIC regimen remains relatively short, and further follow-up is needed to confirm that patients will maintain sufficient donor chimerism to sustain disease remission.

**Keywords:** Adult hemophagocytic lymphohistiocytosis, stable mixed chimerism

PS-106

Abstract:0265

**PURE RED CELL APLASIA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION WITH MAJOR ABO MISMATCH AND REDUCED INTENSITY FLUDARABINE-BASED CONDITIONING**

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**Introduction:** Pure red cell aplasia (PRCA), a recognized complication of major ABO-incompatible AHSCT, is characterized by anemia, reticulocytopenia, and absence of erythroblasts from otherwise normal bone marrow.

**Case:** A 55 year old male was diagnosed with accelerated phase chronic myelogenous leukemia (CML) in July 2007. He was first treated with hydroxyurea and cytosine arabinoside, followed by imatinib. Although complete hematologic response was obtained, no partial cytogenetic response was achieved after 6 months. Imatinib was switched to nilotinib. Bcr-Abl mutations were not detected. No HLA matched related donor was available, so an unrelated donor search was initiated. No cytogenetic response was obtained after 12 months under nilotinib and dasatinib was started. Yet by 6th month of dasatinib treatment, no partial cytogenetic response was obtained. Dasatinib was stopped and hydroxyurea was started. He underwent peripheral blood stem cell transplantation from HLA-A antigen mismatched 50 year old unrelated female in October 2014. At time of transplantation, he was at accelerated phase, had 100% Ph chromosome and bcr-abl transcript level of 33%. There was major ABO mismatch between donor (A Rh+) and recipient (O Rh-) with the patient having an isoagglutinin anti-A titer of 1/64. Because of old recipient age and high EBMT risk score of 5, RIC regimen including fludarabine, busulfan and ATG-F was used to decrease AHSCT-related toxicities. Combination of cyclosporine and a short-course of methotrexate was given for GVHD prophylaxis. He became dependent on erythrocyte transfusions after day 18. Full donor chimerism was attained on day 28 and 5% Ph chromosome was detected on cytogenetic analysis. On follow up, reticulocyte count failed to recover. Bone marrow aspiration on day +43 showed sufficient megakaryopoiesis and granulopoiesis, yet <1% erythroid precursors. Biochemical analysis showed mild increase in LDH. Direct antiglobulin test was negative. The isoagglutinin anti-A titer (IgG) was >1/1024. PCR for parvovirus B19 was negative. PRCA was diagnosed. 1 mg/kg/day corticosteroid treatment was started. Simultaneously, double-filtration plasmapheresis (DFPP) was performed every other day. After a total of 10 sessions of DFPP, isoagglutinin anti-A titer (IgG) decreased to 1/32 and gradual corticosteroid taper was initiated. 15 units of red blood cell transfusions in total has been transfused. At last follow-up 90 days after AHSCT, he remains transfusion independent.

**Conclusion:** There has been no consensus over the best treatment option for PRCA after major ABO-incompatible HSCT because of the limited number of patients in most studies and absence of prospective studies. Also, there is no recommendation whether any treatment should be given to PRCA patients in this clinical setting. We conclude that an adequate dose of steroids

and repeated DFPP can be the first line of therapy for PRCA after ABO-mismatched AHSCT.

**Keywords:** major ABO mismatch, pure red cell aplasia

## Acute Myeloid Leukemia

PS-107

Abstract:0125

**DIAGNOSTIC CHARACTERIZATION OF ACUTE PROMYELOCYTIC LEUKEMIA AND TARGETED THERAPY IN THIS LEUKEMIA**

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This type of acute myeloid leukemia is characterized either by abnormal promyelocytes with distinctive large granules and multiple Auer rods (faggots or sultan bodies cells) or, less commonly, by atypical hypogranular or microgranular cells with bilobed or multilobed nuclei. These cells contain procoagulant material which, when released into the circulation, causes disseminated intravascular coagulation (DIC). Excessive bleeding due to DIC is common. The microgranular variant of APL may be mistaken with Acute monocytic leukemia (AMOL). In the microgranular variant M3, only occasional cells have granules visible by light microscopy, so, morphology and immunophenotyping is suggested for diagnosis of this variant M3. The cytogenetic documents of t(15;17), and FISH is required for confirmation of diagnosis. The translocation t(15;17) (q22;q21) or t(15;17) (q22,q11-12) is the genetic hallmark of APL, resulting in the PML-RARa fusion protein. PML-RARa Recruits histone deacetylases (HDAC) and represses target genes of wildtype RARa leading to a block of myeloid differentiation. The Retinoic Acid Receptor  $\alpha$  (RAR $\alpha$ ) Chimeric Proteins PML-, PLZF-, NPM-, and NuMA-RARa\_ Have Distinct Intracellular Localization Patterns. NPM-RARa\_ is predominantly nuclear whereas NuMA-RARa\_ is predominantly cytoplasmic. Typical immunophenotypic findings in APL: CD34. HLA-DR and Tdt usually is negative and CD2 is expression in a minority, CD13++ CD33+++ This leukemia with t(15;17) has a good prognosis, because it is sensitive to retinoid and chemotherapy, whereas PLZF-RARa Consistent with this, APL patients bearing the t(11;17)(q23;q21) respond poorly to ATRA treatment. All-trans retinoic acid (ATRA) as a highly effective therapy in acute promyelocytic leukemia (APL). The patients receiving ATRA followed by chemotherapy did significantly better compared with patients treated with ATRA alone or chemotherapy alone. With this combination the CR rate range from 90 to 96 percent. Treatment options for patients with relapsed disease include arsenic trioxide and allogeneic stem cell transplant. Alternative RARa Fusion Genes RARa, retinoic acid receptor  $\alpha$ , PML. promyelocytic leukemia. PLZF. promyelocytic leukemia zinc finger;, NPM. nucleophosmin; NuMA. nuclear apparatus;. STAT5b. single transduction and transcription factor 5b. fusion gene karyotype response to ATRA PML-RARa t(15;17) yes PLZF-RARa t(11;17)(q23 No NPM-RARa t(5;17) yes NuMA-RARa t(11;17)(q13; yes STAT5b-RARa t(17;17) No

**Keywords:** Leukemia

PS-108

Abstract:0127

### THErapy RELATED ACUTE PROMYELOCYTIC LEUKEMIA WITH MITOXANTRONE IN MULTIPLE SCLEROSIS

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Introduction: Mitoxantrone is a DNA-topoisomerase 2 inhibitor used for treatment of relapsing-remitting or progressive multiple sclerosis (MS). An increasing proportion of acute promyelocytic leukemia cases are therapy-related (t-APL), which develop following exposure to radiotherapy and/or chemotherapeutic agents that target DNA topoisomerase II, particularly mitoxantrone and epirubicin. We present here one patient with MS who developed t-APL after use of mitoxantrone.

#### Laboratory findings of the patient

	Value of the patient	Normal range
Haemoglobin	11 g/dl	13.6-17.2 g/dl
Haematocrit	32.3%	39.5-50.3%
White blood cell count	1600/mm <sup>3</sup>	4300-10300/mm <sup>3</sup>
Neutrophil count	700/mm <sup>3</sup>	2100-6100/mm <sup>3</sup>
Platelet count	19000 µ/L	150000-450000 µ/L
Level of serum urea	27mg/dl	1-20 mg/dl
Level of serum creatinine	0.6 mg/dl	0.51-0.95 mg/dl
Level of serum sodium	137 mEq/L	136-146 mEq/L
Level of serum potassium	3.7 mEq/L	3.5-5.1 mEq/L
Level of serum üric acide	7.7 mg/dl	3.5-7.5 mg/dl
Level of serum total protein	6.7 mg/dl	6.6-8.8 mg/dl
Level of serum of albumine	4.1 mg/dl	3.5-5.2 mg/dl
Aspartate aminotransferase	26 U/L	1-35 U/L
Alanine aminotransferase	22 U/L	1-35 U/L
Gamma glutamyl transferase	39 U/L	1-38 U/L
Lactate dehydrogenase	395 U/L	1-248 U/L

Case: A 42 years old female patient was admitted to our clinic with fever, fatigue, nausea. His past medical history included of multiple sclerosis for 20 years. Patient received interferon. The patient received eight cycles of mitoxantrone one and a half years ago due to non-response to interferon. On evaluation of his routine laboratory tests, she was found to have haemoglobin of 11 g/dl, haematocrit of 32.3%, white blood cell (WBC) count of 1.600/mm<sup>3</sup>, neutrophil count of 700/ mm<sup>3</sup> and platelet count of 19000 µ/l. Level of serum urea was 27 mg/dl, creatinine 0.6 mg/dl, sodium 137 mEq/L, potassium 3.7 mEq/L, uric acid 7.7 mg/dl, total protein 6.7 mg/dl, albumin 4.1 mg/dl, aspartate aminotransferase 26 UI/L, alanine aminotransferase 22 UI/L, gamma glutamyl transferase 39 U/L, lactate dehydrogenase 395 UI/L (Table I). Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were within the reference range. A peripheral blood smear showed a large number of blasts (90%) with abundant cytoplasmic granules. Bone marrow aspirate demonstrated a markedly hypercellular marrow with 20% blast cells and 80% abnormal promyelocytes. Flow cytometry study demonstrated these cells to be

phenotypically positive for CD13, CD33, myeloperoxidase (MPO), and negative for HLA-DR. PML/RAR-alpha fusion gene rearrangement was confirmed by fluorescence in situ hybridization and molecular analysis. The patient was diagnosed as having t-APL, and remission induction chemotherapy with doxorubicin (45 mg/m<sup>2</sup> for 3 days), cytarabine (200 mg/ m<sup>2</sup> for 7 days) and all-trans retinoic acid (45 mg/m<sup>2</sup> ) was administered. A bone marrow study performed on day +28 showed complete morphologic, cytogenetic and molecular remission. Consolidation therapy could not be given to patients because of fungal pneumonia. She was died because of pulmoner aspergillosis after 40 days remission induction treatment.

Conclusion: Acute promyelocytic leukemia with the translocation t(15;17) was over-represented in the MS population in comparison with cancer patients also treated with mitoxantrone. The timing of this complication, risk, mortality and relationship to exposure remain uncertain. Haematological monitoring should continue for at least 5 years after the last dose of mitoxantrone.

**Keywords:** Acute myeloid leukemia, multiple sclerosis

PS-109

Abstract:0167

### CASE STUDY OF PROGRESSION TO ACUTE MYELOID LEUKEMIA IN PRIMARY MYELOFIBROSIS UNDER JAK2 INHIBITOR (RUXOLITINIB) TREATMENT

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Introduction: Primary myelofibrosis (PMF) belongs to a group of diseases called Philadelphia chromosome-negative myeloproliferative neoplasms and is characterized by clonal myeloproliferation, atypical megakaryocytic hyperplasia and build-up of fibrosis in the bone marrow. JAK2V617F mutation is found in 50-60% of patients. PMF is incurable with conventional therapy and its only curative therapy is allogeneic stem cell transplantation. Ruxolitinib (Rx) which is a potent inhibitor of both JAK1 and JAK2 is a new drug effective in controlling constitutional symptoms in PMF. In this study, we aim to present the case of a PMF patient transforming acute myeloid leukemia (AML) in 6th month of Rx treatment.

Case: Physical examination revealed massive splenomegaly and hepatomegaly in the 53-year-old female patient who admitted to our clinics with fatigue and abdominal distention. Lab studies revealed a leukocyte (WBC) 11500/uL, hemoglobin 12 gr/dL, thrombocyte 270000/uL, LDH 717 u/L. Immature erythroid and myeloid cells, tear-drop cells were seen in peripheral blood smear (PBS). Abdominal usg revealed spleen was enlarged to 17, liver to 20 cm. Bone marrow biopsy showed hypoplastic bone marrow elements, megakaryocytic hyperplasia and positivity (3+) of reticular fiber staining. JAK2 mutation was 78% positive. Patient was diagnosed as PMF according to the 2008 WHO criteria and DIPSS was calculated as 1. Patient was started on hydroxyurea (HU) and interferon (IF) was added in the 4th year. HU, IF were discontinued the patient who had no improvement in blood count and had an increase in constitutional symptoms in the 7th year and Rx 10 mg/day was started. It was increase to 30 mg/day in the 2th month. In the 6th month of the treatment, spleen was enlarged to 31 cm size and WBC 202000/uL, 20% blasts

in PBS was diagnosed as AML according to WHO criteria. Patient was started on idarubicin and Ara-C as induction therapy. On the 20th day of treatment, WBC did rise up to 102000/uL and 44% blasts in PBS was detected. The patient was started on FLAG-IDA (fludarabine, idarubicin, high dose Ara-C). On the 11th day of FLAG-IDA, spleen size reduced by 20%, constitutional symptoms relieved. Treatment is still continued.

Discussion: The most feared complication of PMF is the transformation to AML and transformation rates reaches 21% high-risk patients. In a phase II study, Rx was used on 38 patients with AML. Three of 18 patients with postmyeloproliferative neoplasm AML showed a significant response; 2 patients had complete remission, 1 had remission with insufficient improvement in blood count. In our case, constitutional symptoms didn't improve enough with Rx treatment and transformation to AML is detected. This is the first reported case that Rx was used but still progressed to AML in literature. Although Rx is considered to be an effective antileukemic drug, patients with PMF who are started on it should be monitored closely due to the risk of transformation to AML.

**Keywords:** Ruxolitinib, acute myeloid leukemia

PS-110

Abstract:0173

### AN IMPORTANT PROGNOSTIC FACTOR IN ACUTE LEUKEMIA: INFECTION

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Aim: One of the important reason that increase the morbidity and mortality in cancer patients is infectious diseases. Neutropenia due to bone marrow infiltration and chemotherapy induced bone marrow suppression will increase the infection rate in acute leukemia patients. In this study we aim to investigate the association between infection and mortality rate in acute leukemia.

Method: Total of 103 including 70 male and 33 female acute leukemia (ALL and AML) patients charts were investigated retrospectively. Patients whose clinical presentation, imaging studies and positive culture is consistent with infection was included in the study. Patients were divided into two groups according to their age; patients that older than 65 and younger than 65. The differences between groups and survival analyses were assessed by chi square and Kaplan Meier.

Results: The median age of patients was 56. Out of 103 patients 39 of them were older than 65 years (37.9%). Median survival rate was found 9 months in 72 patients (69.9%). Infection rate was found 38.5% in patients older than 65 and 57.8% in patients younger than 65. Infection rate was found lower in older patients which is statistically significant ( $p=0.06$ ). The mortality rate higher in patients whose cultures were positive ( $p=0.01$ ).

Discussion: The lower infection rate among older acute leukemia patients might be due to lower doses of chemotherapeutic agents and better supportive treatment. One never forget that infection is one of the major reason of morbidity and mortality in acute leukemia.

**Keywords:** Acute leukemia, infection

PS-111

Abstract:0185

### ACUTE PROMYELOCYTIC LEUKEMIA, EXPERIENCE OF THE MILITARY HOSPITAL MOHAMMED V, RABAT, MOROCCO

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Acute promyelocytic leukemia (APL) is a rare, aggressive, subtype of acute myeloid leukemia (AML) with distinct clinical, hematologic, cytogenetic and molecular features. This study is a retrospective review of 9 adult patients (5 male, 4 female), mean age of 39.4 (18-70 years), with APL referred to our department from January 2007 to January 2015. Following induction therapy, 5 patients achieved complete remission and have been followed in remission, one patient died of bleeding during induction therapy and one died of severe sepsis during re-induction therapy. 2 of 9 patients died before any treatment. One patient developed retinoic acid syndrome. There was no case of relapse. APL is one of the most curable forms of hematologic malignancies in adults if quick diagnosis, efficient treatment and supportive care system are performed. The prognosis also depends on use of All Trans Retinoic Acid, one of the most important therapy option in the treatment. Developing countries may have difficulties to obtain this drug and this may lead to an increase in the mortality rates.

**Keywords:** Acute promyelocytic leukemia, local experience

PS-112

Abstract:0190

### EVALUATION OF CYTOKINES AND SOLUBLE ADHESION MOLECULES IN PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA: THE ROLE OF TNF-ALPHA AND FLT3-ITD

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Objectives: Acute myeloid leukemia (AML) cells are highly resistant to therapy. The presumed molecular basis of this resistance is the effect of tumor necrosis factor alpha (TNF- $\alpha$ ) and other cytokines on endothelial adhesion molecule expression. The aim of this study was to test the hypothesis that cytokines and soluble adhesion molecules correlate in AML.

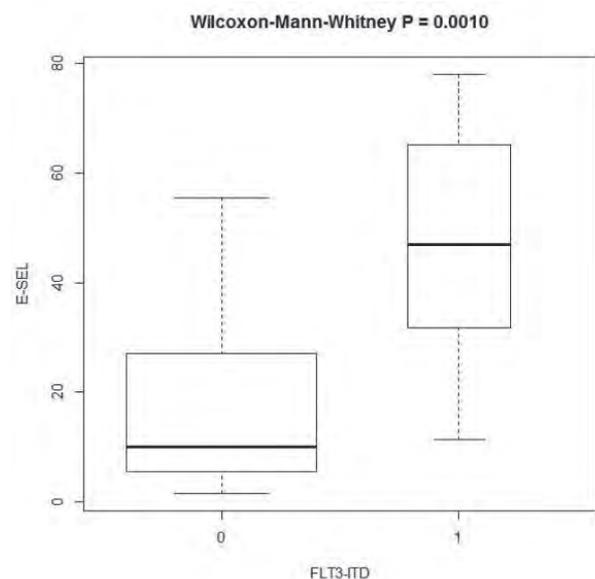
Patients and Methods: A total of 53 newly diagnosed AML patients, 20 males and 33 females, mean age 53.1  $\pm$  13.4, median 56.4 years, were studied. Only patients eligible for induction chemotherapy were included. According to cytogenetic and molecular genetic evaluation, 11 patients were classified as low risk, 9 as intermediate-1

risk, 13 intermediate-2 risk and 20 as high risk. The FLT3-ITD mutation was present in 12 cases. There was previous hematologic disorder that progressed to AML in 21 patients; 17 from myelodysplastic syndrome, 2 from chronic myelomonocytic leukemia, 1 from primary myelofibrosis and 1 from unclassifiable myeloproliferation. All analytes were measured by biochip array technology (Randox Laboratories Ltd., Crumlin, UK). This technology is used to perform simultaneous quantitative detection of multiple analytes from a single sample. We evaluated serum levels of the following 16 cytokines and 4 soluble adhesion molecules: interleukins (IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13), vascular endothelial growth factor (VEGF), TNF- $\alpha$ , interferon-gamma (IFN- $\gamma$ ), epidermal growth factor (EGF), monocyte chemoattractant protein-1 (MCP-1), E-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Age, leukocyte count, secondary AML, CRP, FLT3-ITD and remission were variables. Statistical analysis was performed in R version 3.1.2.

Results: VCAM-1 correlated with ICAM-1 ( $p < 0.0001$ ), E-selectin ( $p < 0.0001$ ), leukocyte count ( $p = 0.0005$ ) and TNF- $\alpha$  ( $p = 0.0035$ ). E-selectin correlated with leukocyte count ( $p < 0.0001$ ), P-selectin ( $p = 0.0032$ ) and MCP-1 ( $p = 0.0119$ ). CRP correlated with IL-6 ( $p < 0.0001$ ), leukocyte count negatively correlated with IL-7 ( $p = 0.0318$ ). FLT3-ITD was associated with higher E-selectin ( $p = 0.0010$ , Fig.1) and lower IL-7 ( $p = 0.0252$ ). Secondary AML patients were older. Failure of induction therapy was associated with not significantly higher CRP and lower P-selectin. Leukocyte count ( $p < 0.0001$ ), FLT3-ITD ( $p = 0.0017$ ) and secondary AML ( $p = 0.0439$ ) influenced the principal component.

Conclusions: Leukemic cells can modulate the micro-environment. Cytokine, adhesion molecule levels and leukocyte count correlate in AML. Understanding these mechanisms may form the basis of novel therapeutic approaches.

#### Association of FLT3-ITD and E-selectin



E-selectin was higher in FLT3-ITD positive cases.

Acknowledgement: The work was supported by a specific research project "Analysis of defined prognostic factors in acute myeloid leukemia" and by a long-term organisation development plan 1011.

**Keywords:** Cytokines, adhesion molecules

PS-113

Abstract:0197

#### VISUAL TOXICITY DUE TO CYCLOSPORIN AFTER ALLOJENIC TRANSPLANTATION IN ACUTE PANMYELOBLASTIC LEUKEMIA WITH MYELOFIBROSIS PATIENTS

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Acute panmyeloblastic leukemia with myelofibrosis (APMF) is a rare form of acute myeloid leukemia and is characterized by acute panmyeloid proliferation with increased blasts and accompanying fibrosis of the bone marrow that does not meet the criteria for AML with myelodysplasia related changes. 1

APMF is classified under acute myeloid leukemia not otherwise specified by WHO 2008. This entity is distinct and needs to be distinguished from acute megakaryoblastic leukemia (AML-M7), myelodysplastic syndrome - refractory anemia with excess blast II (MDS-RAEB-II) with fibrosis, primary myelofibrosis (PMF) and AML with myelodysplasia related changes. The clinical course of this entity is rapidly progressive and fatal, therefore, it is essential to be aware of this entity and distinguish it from its mimickers. Though it may be extremely difficult to differentiate APMF from its mimickers in some cases, detailed clinical history and hematological work up can be helpful in such cases. Many consider, as evidenced by many published articles, that APMF is a variant of MDS. Since the outcome of these patients is poor therefore it is important to aggressively manage these patients with timely diagnosis as it can reduce morbidity and prolong life. In this case report other differential diagnosis considered are highlighted.

Case: A 29 year old female patient referenced on weakness. Laboratory results showed pancytopenia is found to be present. The organomegaly not detected. PY in anisocytosis, tears cells, myeloid progenitor cells are rarely found to be present. flow cytometry is CD2,3,5,7,45 (+) MPO was determined to negative. Dysplasia of megakaryocytes in the bone marrow, 10% CD34 + mononuclear cells were detected grade 3 fibrosis. Panmyelosis was diagnosed with Acute myelofibrosis. Allogenic Bone Marrow Transplantation made the patients had significant visual toxicity of cyclosporin after 20 days. Drug was discontinued and was converted to tacrolimus, greatly decreased the toxicity after 3 mounts.

Conclusion: In patients taking cyclosporine should be noted that the severe toxicity.

**Keywords:** Cyclosporine toxicity, acute panmyelosis with myelofibrosis

PS-114

Abstract:0198

**AZACITIDIN EFFICACY ON HIGH RISK MYELODISPLASTIC SYNDROME AND ACUTE MYELOID LEUKEMIA WITH MULTINEAGE DYSPLASIA; SINGLE CENTER EXPERIENCE**

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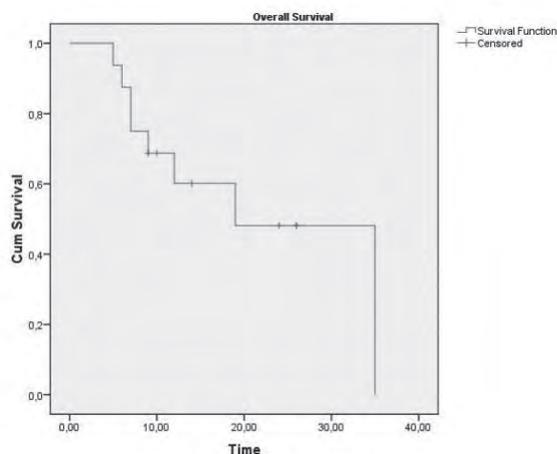
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**Introduction:** Myelodysplastic syndrome is a clonal hematopoietic stem cell disorder characterized by ineffective hematopoiesis and a high incidence of progression to acute myeloid leukemia. Acute myeloid leukemia (AML) with multilineage dysplasia which is defined according to WHO classification system is usually associated with older age and unfavorable cytogenetic findings. Azacitidine is currently a preferred agent for patients with high risk-MDS and the patients with AML that have features similar to MDS. In the present study, we reported the results of 16 patients with high risk-MDS and AML with multilineage dysplasia that were treated with azacitidine.

**Material And Method:** This was a retrospective review of the patients with high risk-MDS and AML with multilineage dysplasia treated at the Hospital of Namık Kemal University with azacitidine. Disease status was defined by the WHO classification system. Clinical and laboratory features of the patients were recorded at the diagnosis. The IPSS were used to evaluate the risk of the patients with MDS. Azacitidine was administrated subcutaneously at a dose of 50-75mg/m<sup>2</sup> daily for the first 7 days of each 28-day cycle. Treatment was to be continued until disease progression or unacceptable toxicity was observed. Bone marrow aspiration and biopsies were performed at a minimum of every 4 months or as required. Response to azacitidine was evaluated according to the International Working Group 2006 criteria. Descriptive statistics were used for baseline characteristics and responses and Kaplan-Meier method was used to evaluate survival.

Kaplan-Meier curve for overall survival of the patients



**Results:** Between January 2012 and December 2014, 16 patients with high risk MDS and AML with multilineage dysplasia were identified who were treated with

azacitidine. Baseline characteristics of these patients are shown in Table 1. Three patient (%18,8) had a complete remission, 5 patients (%31,3) had a partial remission and 3 patients (%18,8) had hematologic improvement. Overall response rate was %69,7. Median time to response was 12 weeks. Estimated median progression free survival and overall survival were 17 months and 19 months (Figure 1), respectively.

**Discussion:** We observed high response rate to azacitidine in our patients in spite of that 7 of them were AML. Our result shows that azacitidin is effective in AML with multilineage dysplasia as well as high risk MDS.

**Keywords:** Acute myeloid leukemia, azacitidine

The baseline characteristics of the patients

Age (years), median (range)	73 (55-82)
Sex (f/m), n	8/8
Hgb, gr/dl, mean±SD	9,1±1,69
Leucocytes,µl, mean±SD	4430±2501
Platelets, µl, mean±SD	114750±91106
Creatinin mg/dl, mean±SD	0,97±38
Albumin gr/dl, mean±SD	3,92±0,53
LDH U/L mean±SD	545±566
Cycles number, median (range)	6 (2-14)
Time to response (weeks) median (range)	12 (4-19)
IPSS (for MDS patients), n	3
Intermediate-2	6
High	
WHO Classification	4
RAEB 1	5
RAEB-2	
AML with MLD	7

PS-115

Abstract:0200

**EVALUATION OF SERUM CYTOKINE AND ADHESION MOLECULE PROFILE IN NEWLY DIAGNOSED ACUTE LEUKEMIAS USING BIOCHIP ARRAY TECHNOLOGY**

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**Background:** Altered levels of cytokines and adhesion molecules have been found in many pathological states and have been linked to many diseases including acute leukemias, both myeloid (AML) and lymphoblastic (ALL). The cytokine system constitutes an interacting functional network where the contribution from single cytokines is modulated by the levels of other cytokines. It may therefore be more relevant to look at the total serum profile of cytokines and adhesion molecules. Biochip array technology enables simultaneous detection of multiple cytokines and adhesion molecules in a single sample and provides valuable information relating to each tested analyte and possible associations between analytes in each sample.

**Objectives:** To compare serum levels of 17 cytokines and 5 adhesion molecules in patients with newly diagnosed AML and ALL using the innovative biochip array technology. This generates a patient profile, which is relevant when investigating interacting functional networks. **Methods:** A total of 15 newly diagnosed AML patients (median age 51, range 24 – 61 years, 8 males and 7 females) and 15 newly diagnosed ALL patients (median age 46, range 24 – 63 years, 11 males and 4 females) were studied. We evaluated circulating levels of the following 22 analytes: interleukins (IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-23), vascular endothelial growth factor (VEGF), tumor necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-gamma), epidermal growth factor (EGF), monocyte chemoattractant protein-1 (MCP-1), E-selectin, L-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1). All analytes were measured by biochip array technology using chemiluminescent sandwich immunoassays applied to the Evidence Investigator analyzer (Randox). We analyzed serum samples at the diagnosis of AML and ALL. Probability values ( $p < 0.05$ ) were considered statistically significant.

**Results:** Comparing cytokine and adhesion molecules levels in newly diagnosed AML and ALL patients, we found significant increase in AML in serum IL-4 ( $4.71 \pm 2.69$  ng/L vs.  $1.10 \pm 1.08$  ng/L;  $p < 0.0001$ ), IL-2 ( $10.69 \pm 8.55$  ng/L vs.  $4.03 \pm 2.15$  ng/L;  $p < 0.01$ ), IL-3 ( $18.84 \pm 21.63$  ng/L vs.  $7.34 \pm 3.41$  ng/L;  $p < 0.05$ ) and significant decrease in serum VEGF ( $63.93 \pm 67.85$  ng/L vs.  $139.33 \pm 133.47$  ng/L;  $p < 0.05$ ), VCAM-1 ( $716.22 \pm 364.38$  mcg/L vs.  $1078.54 \pm 456.96$  mcg/L;  $p < 0.05$ ). No significant differences were found in the levels of other evaluated cytokines and adhesion molecules.

**Conclusion:** Our results indicate that serum profile of cytokines and adhesion molecules differs in newly diagnosed AML and ALL patients. We found significant differences in serum IL-4, IL-2, IL-3, VEGF and VCAM-1. Further studies are needed to establish if these alterations could be used as a clinically relevant biomarker for acute leukemias.

The work was supported by a long-term organization development plan 1011 (FMHS).

**Keywords:** Cytokines, acute leukemia

PS-116

Abstract:0201

### FREQUENCY AND PROGNOSIS OF NFM1 AND FLT3 MUTATIONS IN ACUTE MYELOBLASTIC LEUKEMIA IN TURKEY

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**Introduction:** Cytogenetics properties is known as one of the the most important pronostic marker in acute myeloblastic leukemia (AML). Almost half of the patients with AML have normal cytogenetics findings. This subgroup shows quite heterogenous prognosis. Recently, several studies reported that FLT3 and NFM1 mutation status have significantly impact on prognosis in patients with intermediate cytogenetics risk. However the

frequency of FLT3 and NFM1 mutation and the impact of these mutations on prognosis did not documented yet in Turkish population with AML.

**Methods:** This retrospective study included newly diagnosed, adult AML patients with normal karyotype between 2012-2014. Patients with low risk molecular marker including inv16, t(15;17), and t(8;21) were excluded. Thirtysix patients (19 male/17 female; median age 45 (19-58)years ) were enroled the analysis. FLT3-ITD, FLT3-D835 and NFM1 were tested by PCR Induction regimen was Idarubicine and ARA-C (3+7). Response assessment was performed at day 28 of induction chemotherapy.

**Results:** FLT3-ITD, FLT3-D835 and NFM1 positivities were found in 8 (22,2%), 1(2,8%) and 10 (27,8%) patients, consecutively. One patient had both FLT3-ITD and NFM1 mutations. FLT3 was more frequent in female patients than male (41,2% vs 5,2%;  $p=0,01$ ). NFM1 frequency was not different between two gender. Both FLT3 and NFM1 frequency were not different among FAB subgroups. Median white blood cells was higher in FLT3-ITD mutation positive groups ( $p<0,05$ ), and median age, ECOG status, baseline hemoglobin, platelet count, and percentage of blast in bone marrow at the time of diagnosis were not statistically different between FLT3-ITD or NFM1 mutation positive and negative patients. Induction failure was more frequent in patients with FLT3-ITD mutations (50% vs 3,5%;  $p<0,001$ ), but it was not different between NFM1 positive and negative groups. At the end of the follow-up (median 15,5 (2-31) months) overall survival (OS) and leukemia free survival (LFS) were 68% (median survival time was not reached) and 60,6% (median 8 (1,9-14,4) months), respectively. RFS probability was significantly lower in FLT3-ITD mutation positive group than FLT3-ITD negative group (12,5% vs 80%;  $p<0,001$ ). OS probability was lower in FLT3-ITD positive patients (48,6% vs 87%;  $p>0,05$ ). LFS higher in NFM1 positive group than negative group (70% vs 44,2), but this finding was not statistically significant, and OS was not different between NFM1 positive and negative groups (63% vs 60%) ( $p>0,05$ ).

**Discussion:** Frequency of FLT3-ITD and NFM1 mutations in patients with cytogenetically normal AML patients had reported 25-30% and 47-52,9%, respectively. In the present study, the NFM1 frequency was lower than literature in patients with normal Karyotype AML. The prevalence of FLT3-ITD mutation in females was more frequent in our series. FLT3-ITD mutation were related high leucocyte count and poor prognosis like as previously published data.

**Keywords:** FLT3 mutation, NFM1 mutations

PS-117

Abstract:020

### AN ATYPICAL PRESENTATION OF ACUTE MONOBLASTIC LEUKEMIA MIMICKING HEMOPHAGOCYTIC SYNDROME

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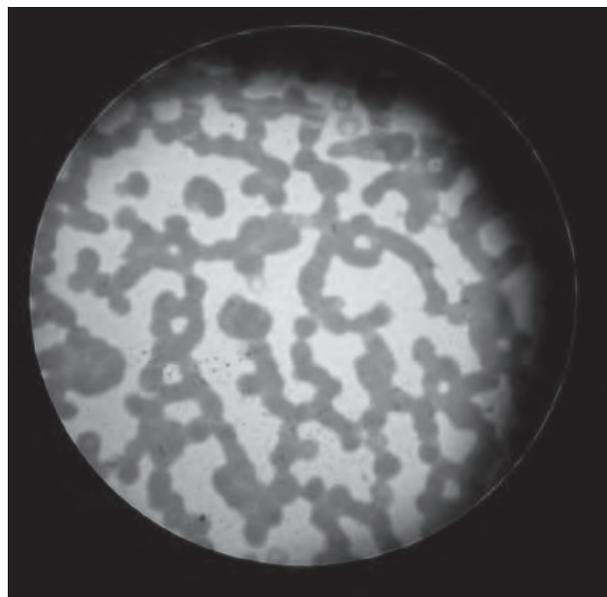
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A 49 year-old male patient applied to a state hospital with two month symptoms of fever and common arthralgia. His standard tube agglutination test for Brucellosis

was positive. He was started on combined therapy consisting of oral rifampicin and doxycycline. He was admitted to our clinic because of ongoing fever. Initial laboratory test results were shown on Table-1. Initial physical examination findings were papillary atrophy of the tongue. He had no peripheral lymphadenopathy or organomegaly. Blood pressure was 112/80 mm Hg, pulse 84 beats per minute, and temperature 37.8°C. We detected anisocytosis, poikilocytosis and giant platelets on his peripheral blood smear. His standard tube agglutination and Rose Bengal spot test for Brucellosis were negative. Pancytopenia of the patient was thought to be due to Brucellosis and antibiotherapy. He refused the procedure of bone marrow biopsy despite strongly recommended. He was called for control visits. He didn't come for control visit because he felt better. After two months with no symptoms, he started to complain with shortness of breath and fever. He was admitted to emergency service with these complaints and also newly started lethargy. Cerebral CT scan showed no sign of hemorrhage. His laboratory results on this second admission was shown on Table-1 also. He had multiorgan failure. His viral hepatitis markers and EBV serology were negative. He was suspected to have hemophagocytic syndrome according to the proposed HLH diagnostic criteria in 2009 (Table-2). Numerous characteristic monoblastic cells were observed on his peripheral blood smear (Figure). Bone marrow aspiration and biopsy was performed to patient. There were no sign of hemophagocytosis on bone marrow. Flow cytometry of bone marrow and peripheral blood showed that blastic cells had CD14,CD33, CD38 and CD4 positivity but no CD25. Acute monoblastic leukemia diagnosis was made and he started on cytarabin and idarubicin protocol. He died on the first day of chemotherapy.

**Keywords:** Acute monoblastic leukemia, hemophagocytic syndrome

Characteristic monoblastic cells on peripheral smear



**Laboratory results of the patient**

	1 <sup>st</sup> admission	2 <sup>nd</sup> admission
WBC (X10 <sup>9</sup> /L)	1.1	11.3
Hemoglobin (g/dl)	9.8	11.8
Platelet count (X10 <sup>9</sup> /L)	64	17
Ferritin (ng/ml)	1650	16662
Vitamin B12 (pg/ml)	219	286
ESR (mm/h)	21	42
LDH (U/ml)	260	1917
CRP (mg/l)	36	140
Total bilirubin (mg/dl)	1.1	4.1
Direct bilirubin (mg/dl)	0.6	2.9
Serum Creatinin (mg/dl)	0.7	3.6
ALT (U/l)	41	65
AST (U/l)	35	60
Triglyceride(mg/dl)	-	232
Fibrinogen (mg/dl)	-	920
INR	1.1	1.2

results both at first and second admissions

**Proposed HLH diagnostic criteria in 2009**

1. Molecular diagnosis of hemophagocytic lymphohistiocytosis (HLH) or X-linked lymphoproliferative syndrome (XLP).
2. Or at least 3 of 4:
a. Fever
b. Splenomegaly
c. Cytopenias (minimum 2 cell lines reduced)
d. Hepatitis
3. And at least 1 of 4:
a. Hemophagocytosis
b. ↑ Ferritin
c. ↑ sIL2R $\alpha$ (age based)
d. Absent or very decreased NK function
4. Other results supportive of HLH diagnosis:
a. Hypertriglyceridemia
b. Hypofibrinogenemia
c. Hyponatremia

PS-118

Abstract:0224

**THE EXPERIENCE OF ONE ROMANIAN CENTER OF HEMATOLOGY IN THE TREATMENT WITH HYPOMETHILATING AGENTS**

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Introduction: The clinical studies demonstrated that treatment with hypomethylating agents (5-azacytidine and decitabine) in intermediate/high risk MDS resulted in complete cytogenetic responses even in cases with a complex karyotype. On the other hand, for patients with AML who do not qualify for aggressive chemotherapy and allogeneic medullary transplantation, treatment with hypomethylating agents leads to transfusional independence and increase in quality of life.

**Materials-Methods:** We present the evolution under treatment with hypomethylating agents in 12 patients, diagnosed with intermediate/high risk MDS and AML in our Department between 2009-2015. There were 8 men and 4 women, with ages between 56-84 years, 7 of them diagnosed with intermediate/high risk MDS, and 5 diagnosed with AML, unfit to chemotherapy. 9 patients (6 patients with MDS and 3 patients with AML) received treatment with 5-azacytidine and 3 patients, one man with AML post MDS and 2 women with AML de novo, received Decitabine. Cytogenetic exam was performed in all cases and an abnormal karyotype was obtained in 2 cases, both with MDS, one patient with a complex karyotype, including del (5)(q32;qter) and one with 12 monosomy. The selected schedules were: 5-Aza 75 mg/m<sup>2</sup>/d, for 7 days, repeated every 28 days and Decitabine 20 mg/m<sup>2</sup>/d, for 5 days, repeated every 28 days.

**Results:** All patients had a good tolerance to therapy, without significant adverse events. The overall response to 5-Aza was heterogeneous, with no significant differences regarding blast percentage, with one complete response in the case with 12 monosomy. Unfortunately in the 3 patients with AML treated with Decitabine, there was a delay in the time of treatment initiation due to administrative and financial issues, and they died due to disease progression. There were no side effects.

**Conclusions:** The presented data indicate similar results to that in the literature. The most important effect of treatment was on the quality of life by the reduction in the transfusional demand. The hypomethylating agents are a less toxic alternative to classical cytotoxic/antimetabolites agents.

**Aknowledgements:** This presentation has been elaborated and written by Daniela Georgescu, MD and third year PhD student since 2011 at UMF Carol Davila under coordination of Prof. Dr. Anca Lupu, MD, PhD. This paper is supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/159/1.5/S/137390/

**Keywords:** Hypomethylating, agents

PS-119

Abstract:0231

### **CEREBRAL VENOUS SINUS THROMBOSIS IN ACUTE PROMYELOCYTIC LEUKEMIA (APL): A POSSIBLE ASSOCIATION TO ATRA**

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**Objectives:** Bleeding is the usual manifestation of APL-related coagulopathy and is associated with significant morbidity and mortality. Thrombotic complications are less common, mostly reported following all-trans retinoic acid (ATRA) treatment. Cerebral venous sinus thrombosis is very rarely encountered.

**Case:** A 29-year-old female patient was admitted to emergency department with complaints of headache, epistaxis and multiple echymosis. Complete blood count revealed WBC 7.5 x 10<sup>9</sup>, Hb 9.6 gr/dl and platelet count 42 x 10<sup>9</sup>. In the peripheral smear, atypical

promyelocytes with prominent and dense auer rods were observed. Flow cytometric analysis of the bone marrow aspirate revealed 85% blast cells which stained positive for CD45, CD117, CD33, MPO, CD64; and negative for CD15, CD13, CD34, HLADR. Bone marrow biopsy and aspiration were consistent with APL. Molecular analysis confirmed the presence of PML-RAR- $\alpha$  fusion. Coagulation parameters were in normal ranges. Patient was started ATRA 45mg/m<sup>2</sup> daily and idarubicin 9 mg/m<sup>2</sup> (Days 1,3,5) on the day of admission. Three days later, the patient suddenly developed headache, diplopia and abducens nerve paralysis. She was found to have papilledema and increased intracranial pressure. Antiedema therapy was immediately started. Since the cranial MRI findings could not rule out leptomeningeal infiltration, high dose cytosine arabinoside (Ara-C) was also added to the protocol and intrathecal treatment (Ara-C, methotrexate and dexamethasone) was given for a total of 4 times during hospitalization. Each time a sample of cerebrospinal fluid was analyzed but cytologic involvement could not be demonstrated. During follow-up, there was minimal neurological improvement. However, papilledema and increased intracranial pressure findings continued. A second MRI demonstrated subacute thrombus in the left transverse and sigmoid sinuses. She was started low molecular weight heparin. After the induction therapy, the patient reported that her headache was under control during the two-week abstinence from ATRA. Since her symptoms reoccurred when ATRA was restarted, ATRA was permenately omitted from the consolidation chemotherapy.

**Discussion:** There are quite a low number of APL cases with sinus venous thrombosis in the literature. The etiology of thrombosis in APL is difficult to distinguish whether is related to the primary disease itself or the treatment. Thrombosis in APL can occur as a component of the disseminated intravascular coagulopathy. Increased intracranial pressure is a well-known side effect of ATRA. However, ATRA has also been associated with thrombosis in some reports. Therapy-related thrombosis is not limited to ATRA but also reported with anthracyclines. In APL, it must be kept in mind that life threatening thrombosis may also be encountered either as a result of the disease itself or the treatment. Demonstration of the exact etiologic factor of thrombosis requires further investigation.

**Keywords:** Acute Promyelocytic Leukemia, Cerebral venous sinus thrombosis

PS-120

Abstract:0262

### **INTENSE MANAGEMENT OF ELDERLY ACUTE MYELOID LEUKEMIA? YES**

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**Aim:** The aim of this study to investigate the affect of low dose treatment and standart treatment on survival in elderly acute myeloid leukemia (AML) patients.

**Methods:** This is a retrospective study including 24 AML patients at age 65 and older who presented o hematology clinic between 2009 and 2014. There were 11 female (45.8 %). Patients who treated with 10mg/

m2 cytosine arabinoside were considered as nonintensive chemotherapy group (Arm A), whereas patients who treated with 7+3 or 5+2 induction treatment following by 2-4 consolidation treatment were considered as intensive chemotherapy group (Arm B).

Results: The median age of patients was 74 (min:65, max:86), the median survival time was 9 months. While there were 11 (45.8%) patients in Arm B, 13 patients in Arm A. During the management period 41,7% had an clinical infection and the cultures were positive in 29.6% of them. No association was found between clinical and microbiological infection and survival. Whereas the median survival time was 7 months in arm A, it was 13 months in arm B. The association of treatment with survival was found statistically significant ( $p=0,001$ ).

Conclusion: Physicians might prefer nonintensive treatment for elderly AML patients due to low survival rates in patients who are treated with intensive treatment. Based on our findings; intense treatment should be tried in elderly patients who has good performance status.

**Keywords:** Acute myeloid leukemia, elderly

PS-121

Abstract:0263

### **BLURRINESS DIFFERENTIAL DIAGNOSIS: DON'T FORGET ACUTE PROMYELOCYTIC LEUKEMIA!**

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Aim: The aim of this study is to investigate the presenting symptoms and hematologic parameters of acute promyelocytic leukemia and its association with prognosis. Methods: Fourteen patients with acute promyelocytic leukemia who followed up in hematology clinic between 2009 and 2014 were included in this retrospective study.

Results: The median age of patients were 45 (min:19, max:86), and median survival time was 17.7 months. The level of hemoglobin, leukocyte and trombocyte were 8.8g/dl, 4450mmol/L and 28500/mm<sup>3</sup> respectively at the time of diagnosis. Out of 14 patients, 5 of them had low INR level (35,7%) and 8 of them has low fibrinogen level (57.2%). 6 patients had low, 5 had medium and 3 had high risk. Eight (57.1%) had remission. Three patients were died without remission and three patients (21,4%) relapsed. Two patients presented with blurriness and intraretinal hemorrhage was seen during ophthalmic examination. Blurriness, diplopia and intraretinal hemorrhage occurred in one patient during the second day of treatment. The presenting symptoms were fever in four patients (28,5%), fatigue and ecchymoses in seven patients (50%) and epistaxis in one patient (7.1%).

Conclusion: Clinicians should be aware that acute promyelocytic leukemia patients might present not only with fever, fatigue or bleeding symptoms but also blurriness.

**Keywords:** Acute promyelocytic leukemia, blurriness

PS-122

Abstract:0271

### **UNEXPECTED ADVERSE EFFECT OF CLOFARABIN: BRONZE HYPERPIGMENTATION**

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Introduction: Skin toxicity in acute myeloid leukemia (AML) is a condition that with a lot of chemotherapy agents. Clofarabine is a second-generation purine nucleoside analogs used in the treatment of AML. Diarrhea, liver toxicity, skin toxicity, hand-foot syndrome, mucous membrane involvement, renal failure, bone marrow suppression may be listed as a side effect of Clofarabine. We describe a case of a 74-year-old male with refractory acute myeloid leukemia with skin toxicity as bronze hyperpigmentation, diarrhea, hyperbilirubinemia and liver toxicity associated with clofarabine treatment.

bronze hyperpigmentation



Case: In 2013, 74-year-old male patient was evaluated because of fatigue and bisitopenia and diagnosed as AML M4. Patient's hemoglobin (Hgb): 8.4 mg/L white blood cell (WBC): 2010 K/L neutrophils: 1370 K/L platelets (Pit): 20000 K/ul and in the bone marrow biopsy had a blastic cell growth that showing staining diffuser with MPO and 50% with CD34 and CD117. Performance status of patient is one. Citarabine and idarubicin were performed with induction chemotherapy and then citarabine was performed three times. Allogeneic transplantation could not be performed because there is no HLA-matched donor. However 11 months later revealed a recurrence. Salvage chemotherapy for patient was started with clofarabine 40 mg/m and sitozinarabinozid 2 g/m. The time of recurrence the patient's Hgb:12.4 mg/L, WBC:2440 K/L, neutrophils:390 K/L, Plt:20000 K/ul and the bone marrow biopsy had a blastic cell growth.

Diarrhoea, hyperbilirubinemia and liver dysfunction was occurred on the fifth day. The patient's indirect/total bilirubine: 2,2/5,9 mg/dl, aspartate transaminase (AST): 64 U/I, alanine transaminase (ALT): 96 U/I. Bone marrow response was evaluated and found to be in hematologic remission. He was discharged twenty-fourth day. When the patient's second course to start, renal function, coagulation parameters, liver function tests and bilirubine levels were normal. At the same time, the patient's Hgb: 8,3 mg/L, WBC: 13020 K/L, neutrophils: 10930 K/L, Plt: 82000 K/ul. At the fourth day of second course, diarrhea and liver dysfunction was occurred, as AST:425 U/I, ALT: 625 U/I. At the fifth day had fever and seventh day hyperbilirubinemia was developed, measured as direct/total bilirubine: 1,9/3,2 mg/dl. At the same time, the patient's total body developed bronze pigmentation. The patient continued to fever on the ninth day, aspergillus infection was detected in thorax tomography. On the twelfth day, the patient was exitus due to septic shock.

**Discussion:** The side effects of clofarabine in the second course may be more aggressive than the first course. The bronze hyperpigmentation with clofarabine should also be meant to keep in mind. Rashes with clofarabine are described but has not been reported in the literature bronze hyperpigmentation.

**Keywords:** Clofarabine, bronze hyperpigmentation

PS-123

Abstract:0274

### AN ACUTE PROMYELOCYTIC LEUKEMIA CASE PRESENTED WITH GRANULOCYTIC SARCOMA RECURRENCE

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**Introduction:** Granulocytic sarcomas (GS) are malign cells located extramedullary which are composed of immature myeloid cells. Granulocytis sarcomas are mostly related with acute myelocytic leukemia, but they can be also related with myeloproliferative disorders or myelodysplastic syndrome. (1-3) Most involved areas in granulocytic sarcomas are bone, soft tissue, periost, lymph nodes and skin. Central nervous system involvement is rare, they are limited with a few case report in literature. (1)

**Case:** A 64 year old male patient who is diagnosed as acute promyelocytic leukemia 5 year ago with promyelocytes in blood smear, bone marrow and PML-RARα t(15;17) gene expression is our case. He had ATRA and idarubicin induction, afterwards ATRA and antracycline consolidation treatment thrice. And then he had a maintenance treatment with ATRA, 6-mercaptopurine and methotrexate during 2 years. First 2 years with 3 months periods, after 2 years with 6 months periods he was followed up for residual disease. His follow up was made with QT-PCR. Our patient admitted to emergency service with a complaint of headache. In complete blood count; we detected hb 13.1 gr/dl, wbc 13800/mm<sup>3</sup>, platelets 98000/mm<sup>3</sup>. Intracranial mass was detected with cranial magnetic resonance imaging. A biopsy was performed from intracranial mass and pathology specimens revealed a tumor which stains strongly positive with MPO, weakly positive with CD117, CD 68, and negative with CD38, Desmin, SMA, S-100, EMA, vimentin

immunohistochemically. Diagnosis was established as myeloid sarcoma. In bone marrow examination a blast population was detected in a ratio of %41. In flow cytometry CD33, CD117, CD13, CD15 was strong positive whereas CD34 and HLA-DR was negative. And also with detection of PML-RARα gene expression our patient was evaluated as APL recurrence. We planned his treatment as fludarabine 30 mg/m<sup>2</sup>/5days IV, cytosine arabinoside 2 g/m<sup>2</sup>/gun/5days IV, ATRA 45 mg/m<sup>2</sup>/day PO, intrathecal metotrexate 12mg, intrathecal cytosine arabinoside 100mg. Intrathecal treatments were performed once per 3 days until no blast had been detected in cerebrospinal fluid. After third intrathecal treatment there was no blast in cerebrospinal fluid and we continued with maintenance treatment during 6 periods. After 1 month there was no mass in cranial MRI, bone marrow morphology and flow cytometry evaluation was normal.

**Discussion:** We have shown in this case, our treatment protocol can be used as a successful induction treatment with no neurological complications in a rare central nervous system recurrence of APL which have high risk of failure in treatment.

**Keywords:** Granulocytic sarcoma

PS-124

Abstract:0276

### MYELOID SARCOMA OF THE TEMPORAL BONE: A CASE REPORT

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**Introduction:** A myeloid sarcoma is a tumor mass consisting of myeloid blasts with or without maturation occurring at an anatomical site other than the bone marrow. The tumor can involve any part of the body, but commonly involved sites include subperiosteal bone structures of the skull, paranasal sinuses, sternum, ribs, vertebrae, pelvis, lymph nodes and skin. The neoplasm usually occurs in patients with acute myeloid leukemia, myelodysplastic or myeloproliferative disorder. In the head and neck, myeloid sarcoma has been reported to occur in the maxilla, soft palate, nasopharynx, lip, salivary glands, mandible, and temporal bone. These patients often complain of auricular pain. Cranial neuropathies especially of facial nerve could be due to leukemic infiltration. Myeloid sarcoma has been described in association with a variety of chromosomal abnormalities. Chromosome 8 abnormalities are the most common genetic aberration in myeloid sarcoma. Myeloid sarcoma is frequently mistaken for non-Hodgkin lymphoma, small round cell tumor (neuroblastoma, rhabdomyosarcoma, Ewing sarcoma/PNET, and medulloblastoma), and undifferentiated carcinoma. The diagnosis is easily missed when immunohistochemistry is not used.

**Case:** A 4 year -old girl with previously diagnosed acute myeloid leukemia related with myelodysplastic syndrome associated with trisomy 8 and 17 presented with auricular pain and peripheral fascial paralysis. She had undergone left and right mastoidectomy one and two years ago respectively, and the diagnosis was cholesteatoma. After one year following the operation cranial MRI revealed a mass located in left temporal bone, infiltrating the cellulæ and partly extending to middle ear. A biopsy of the left middle ear revealed neoplastic infiltration of blastoid cells. An immunohistochemical panel including CD43, lysozyme, myeloperoxidase, CD68, CD117, CD3

and CD20 was used. Neoplastic cells showed immunexpression with Myeloperoxidase and the final diagnosis was myeloid sarcoma.

Conclusion: Myeloid sarcoma, a form of extramedullary deposition of leukemic cells, has been found more commonly in myeloid leukemia. In patients of acute myeloid leukemia, metastasis to temporal bone should be considered when peripheral fascial paralysis is present. The correct diagnosis of myeloid sarcoma is important for adequate therapy, which is often delayed because of a high misdiagnosis rate. This case report highlights the importance of complete hematopathologic workup for the pathological specimen obtained by surgical intervention for mastoiditis and otitis media in leukemia patients.

**Keywords:** myeloid sarcoma, acute myeloid leukemia

## Chronic Myeloid Leukemia

PS-125

Abstract:0117

### IMPACT OF HEALTH CARE FINANCE ON HEALTH RELATED QUALITY OF LIFE (HRQOL) OF CHRONIC MYELOID LEUKAEMIA (CML) PATIENTS. A SINGLE INSTITUTE STUDY

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Background: Data on the effects of health finance on HRQOL can contribute to further help improve outcome of CML patients receiving long-term chronic therapy with Imatinib. The main objective of this study is to quantify (HRQOL) and relation to health finance in patients with (CML) treated with Imatinib.

Methods: Between March and October 2013, Patients were identified through hospital medical records and were categorized into two groups according to their finance either health insurance or government reimbursement. Eligibility criteria included: patients with CML started Imatinib as first-line therapy for at least 2 years. Patients had to be at least in complete cytogenetic response at time of study entry.

Functional Assessment of Cancer Therapy-Leukemia (FACT-Leu) was used to assess patient's HRQOL using set of questions (FACT-General or FACT-G), as well as a leukemia-specific subscale. The FACT-G items are divided into four primary domains: Physical Well-being, Social Well-being, Emotional Well-being, and Functional Well-being. The Leukemia subscale is a 17-item scale designed to assess patient concerns relating to leukemia. Independent T test was used.

Results: 60 patients were included in study 31 patient treated by health insurance (group 1), 29 patients treated by government reimbursement (group 2). The median age of patient were 49 years in both groups. Compliance (defined as taking > 90% of prescribed dose) was significantly better in health insurance group.

The poor adherence in group 2 was mainly due to irregular prescription refill rather than patient choice in contrast to group 2 in which the patients forgets or choose not to take the drug. The poor adherence also was reflected on development of side effects patients in group 2 experienced less side effects which was statistically significant in comparison to group 1. There was a significant difference between two groups (p.value < 0.05) as regard

Social Well-being, FACT-Leukemia Trial Outcome Index, FACT-Leukemia total score, FACT-G total score & leukemia subscale in favor of health insurance due to regular drug delivery & less paper work required. Higher education & absence of comorbid conditions was associated with better HRQOL in both groups.

Conclusion: maintaining regular health finance is essential to improve CML patient's response rate and improve their quality of life.

**Keywords:** Chronic myeloid leukaemia, HRQOL

PS-126

Abstract:0146

### XANTHELASMA PALPEBRARUM: A NEW SIDE EFFECT OF NILOTINIB

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Introduction: Chronicmyeloid leukemia (CML) is a chronic myeloproliferative disease characterized by tyrosine kinase activity caused by translocation between chromosomes 9 and 22. BCR-ABL tyrosine kinase inhibitors (TKIs) are highly effective in the treatment of CML. Imatinib mesylate was the first of this novel family of drugs. The appearance of imatinib resistances and intolerance led to the introduction of second-generation TKIs, such as dasatinib and nilotinib. Metabolic disturbances such as dyslipidemia and cutaneous adverse effects are the most common non-hematological adverse reactions of the TKIs. We present two cases of unusual xanthelasma palpebrarum induced by nilotinib.

Case-1: A 40-year-old woman was referred to investigate the etiology of leukocytosis. Physical examination was unremarkable. Laboratory results are in Table-1. She was diagnosed with chronic phase, positive BCR-ABL CML. She was started on imatinib 400 mg orally once daily. Because of haematological toxicity at month 4 imatinib discontinued, nilotinib 400 mg twice daily started. Fifteen months after starting nilotinib she presented with yellowish plaques around eyelids. On physical examination she had xanthelasma palpebrarum. Her baseline serum lipid profile was normal, but showed markedly elevation 15th months of nilotinib therapy (Table-1). She had been counseled to have strict low cholesterol diet and advised for regular exercise and was started on atorvastatin 20 mg once daily and continued on nilotinib 400 mg twice daily.

Case-2: A 29-year-old woman was referred to investigate the etiology of leukocytosis. Physical examination was unremarkable. Laboratory results are in Table-1. She was diagnosed with chronic phase, positive BCR-ABL CML. She was started on imatinib 400 mg orally once daily. Because of haematological toxicity at month 4 imatinib discontinued, nilotinib 400 mg twice daily started. Six months after starting nilotinib she presented with yellowish plaques around eyelids. On physical examination she had xanthelasma palpebrarum. Her serum lipid profile was normal at baseline and when she admitted with xanthelasma palpebrarum (Table-1). Therefore, she was not started on statin treatment. She continued on nilotinib 400 mg twice daily.

Discussion: Nilotinib has been demonstrated to cause metabolic disturbances such as dyslipidemia and

cutaneous adverse effects such as pruritis, non-specific rashes, dry skin and alopecia. Case 1 showed markedly elevated lipid profile so xanthelasma palpebrarum can be associated with dyslipidemia. Case 2 had xanthelasma palpebrarum although normal lipid profile. This suggests that xanthelasma palpebrarum can be a different cutaneous side effect of nilotinib.

**Conclusion:** To our knowledge, there have been no other reports of xanthelasma palpebrarum developing in patients treated with nilotinib. However, further studies that include a larger patient cohort to confirm xanthelasma palpebrarum as a side effect of nilotinib.

**Keywords:** Nilotinib, xanthelasma palpebrarum

View of the eye lids of patients

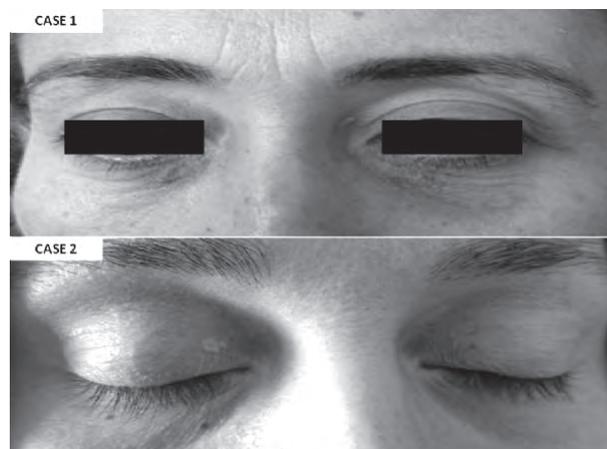


Table 1. Laboratory results

Laboratory results	Case 1	Case 2
Haemoglobin (11,7-17 g/dl)	11.8	13,3
Leukocyte (4,6 – 10,2 ×103/uL)	62,30	26,30
Neutrophil (2,0 – 7,0 ×103/uL)	58,2	21,2
Platelets (142,0 – 424,0 K/ uL)	349	233
BCR/ABL (breakpoint cluster region-Abelson)	90.1%	92%
Jak2 mutation	Negative	Negative
Total cholesterol (130,0 - 200,0 mg/dL)	300	160
LDL- cholesterol (60,0 - 130,0 mg/dL)	220	100
Triglyceride (50,0 - 150,0 mg/dL)	180	90

PS-127

Abstract:0175

**UPFRONT GENERIC IMATINIB FORMULATIONS OFFER A NON-INFERIOR EFFICACY AND SAFETY PROFILE WHEN COMPARED WITH THE ORIGINAL IMATINIB**

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**Background:** Tyrosine kinase inhibitors are the mainstay of treatment of chronic myeloid leukemia (CML). Recently, four different generic Imatinib formulations were approved for the frontline treatment of CML in Turkey. All of these commercially available generic Imatinibs are

cheaper than the original one. The average cost benefit is 130 dollars per box. Our aim was to investigate and compare the efficacy of commercially available generic Imatinibs, which were used as initial frontline therapy in a newly diagnosed chronic phase CML (CP-CML) setting.

**Methods:** 35 patients who were diagnosed as CP-CML between July 2011 and Mar 2013 were included in the study. 14 patients received generic Imatinib and 21 patients received original Imatinib at a starting dose of 400 mg. Patients' demographics, risk scores, side effects and imatinib response were recorded retrospectively. Hematologic, cytogenetic and molecular responses were compared among generic and original TKI groups.

**Results:** Median age of all patients was 52 (18-81). There were no significant difference among age, gender and Eutos risk scores between generic (Group A) and original (Group B) Imatinib groups. Median follow up time was 13.5 (10-33) months in group A and 26 (10-36) in group B (p = 0.013). All patients were able to achieve a complete hematologic response at 3rd month. Complete cytogenetic response rates at 6th month were 57.1% and 52.6% for Group A and B, respectively (p = 0.530). Major molecular response rates at 6th month were 35.7% and 31.6% for Group A and B, respectively (p = 0.721). 2 patients in Group A and 3 patients in Group B were switched to 2nd generation TKI due to resistance (p = 0.679). Combined hematological and non-hematological adverse event rates were similar in both groups (28.6% and 33.3% for Group A and B, respectively; p = 0.543).

**Conclusions:** There is very limited and conflicting data regarding the efficacy and tolerability of generic Imatinib formulations. Among our patient cohort, at a reasonably long term follow-up, generic formulations were not inferior to the original Imatinib regarding the efficacy and tolerability

**Keywords:** Imatinib, Generic

PS-128

Abstract:0193

**DOES ADVERSE EVENTS DUE TO TIROSINE KINASE INHIBITOR'S MEAN GOOD EFFICACY IN CRONIC MYELOID LEUKEMIA PATIENTS?**

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**Aim:** We aim to investigate comorbid conditions, medications and drug interactions with tyrosine kinase inhibitors(TKI) and their effect on survival in chronic myeloid lymphma (CML) patients. **Methods:** We reviewed all 30 chronic myeloid leukemia patients treated at our institution with imatinib as initial therapy.

**Results:** Median age was 57.5 years, median follow up was 42 months. Median major molecular response (MMR) and complete hematologic response (CHR) time was 8.5 months and 58 days respectively. Patients with MMR and CHR have longer survival time (p=0.024, p=0<0.001). Patients with comorbidities and taking additional medications has more adverse effects due to TKI (p=0.025, p=0.026). No association between comorbidity, medication use and survival has been found. It has been shown that CML patients with adverse effect due to TKI has longer survival time (p=0.02).

Conclusion: Drug interactions is an unwanted situation that limits the medication use. This study shows that occurrence of side effects due to imatinib in CML patients is an indicator of imatinib efficacy. Instead of cessation or dose reduction of this medication, the strategies to manage these side effects should be improved.

**Keywords:** Chronic myeloid leukemia, adverse effect

PS-129

Abstract:0209

### IS AZACITIDINE EFFECTIVE IN MYELOID BLASTIC PHASE OF CML?

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Introduction: Even in the tyrosine kinase inhibitors (TKI) era, the prognosis of patients with chronic myeloid leukaemia (CML) in myeloid blast crisis remains dismal with few patients surviving longer than 6 months. Here we report a case treated with 5-azacytidine (AZA) for myeloid blast crisis of CML.

Case: 52 years old male was diagnosed as CML chronic phase in 2011 and treated with imatinib mesylate since the time of diagnosis. In March 2013, patient was diagnosed as CML blastic phase. Laboratory tests were as: WBC: 293000 /mm<sup>3</sup>, Hb: 9.5 gr/dL, Plt: 48000 /mm<sup>3</sup>, creatinine: 0.7 mg/dL, LDH: 1440 IU/L. 80% of the cells were myeloblasts on peripheral smear. The patient was in myeloid blastic crisis and 3+7 remission induction chemotherapy with dasatinib was admitted. But he was refractory to treatment and FLAG regimen was performed and dasatinib was continued. However, patient was refractory to this salvage regimen as well. At this period, dasatinib was continued and AZA was started. At the beginning of AZA, hemogram was as: WBC: 24190 /mm<sup>3</sup>, Hb: 7.1 gr/dL, Plt: 119000 /mm<sup>3</sup>. After 4 courses of AZA, complete remission (CR) was achieved; WBC: 3200 /mm<sup>3</sup>, neut.: 1660 /mm<sup>3</sup>, Hb: 9.6 gr/dL, Plt: 228000 /mm<sup>3</sup> and <5% blasts in bone marrow aspiration. At this period (January, 2014), allogeneic bone marrow transplantation was performed from HLA-matched sibling donor. The patient is still alive with CR and under dasatinib treatment.

Discussion: Blast crisis of CML, occasionally of sudden onset, is an ominous clinical event that is difficult to treat. The aim of initial management is to revert to chronic phase with plans to proceed with allogeneic hematopoietic cell transplantation in chronic phase, if possible. Myeloid blast crisis, which occurs in approximately 70% of cases, does not respond well to standard induction regimens for AML, although responses to TKIs, alone or in combination with chemotherapy, have been noted. The preferred initial treatment is the use of a TKI (with or without chemotherapy) followed by an allogeneic HCT for eligible patients. Recent data showing that epigenetic anomalies are associated with CML progression provide a rationale for reassessing the use of demethylating agents in myeloid blastic phase of CML. Experimental data suggest that epigenetic dysregulation is a feature of CML stem cells and hypermethylation of several targets, particularly tumour suppressor genes, plays an important role in disease progression and leukaemic transformation. Thus it is possible that the association of AZA and TKI may have an additive effect by targeting several

oncogenic mechanisms within the blastic component of the disease, as shown in our patient.

**Keywords:** Azacitidine, CML

PS-130

Abstract:0255

### A CASE OF CHRONIC MYELOID LEUKEMIA WITH ABL1 AND FOXP1 GENE DELETION

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Objective: Chronic myeloid leukemia (CML) is characterized by BCR-ABL1 fusion transcript. Deletion of ABL1 is observed in 15% of the patients. FOXP1 gene belongs to the forkhead box (FOX) transcription factor family. This gene may act as a tumor suppressor as it is lost in several tumor types. FOXP1 deletions were identified in some solid tumors and acute myeloid leukemia (AML) and translocations were identified in acute lymphoblastic leukemia (ALL). We report a CML patient with complex karyotype, and also ABL1 and FOXP1 gene deletions.

Methods: Karyotyping, fluorescence in situ hybridization (FISH) and Quantitative Real Time PCR (QRT-PCR) techniques were used to confirm CML diagnosis in a 38 years old male patient. FISH was performed using LSI BCR/ABL tricolor, dual fusion translocation probe. p210 and p190 fusion transcripts of BCR-ABL fusion gene were analyzed with QRT-PCR. ABL1 kinase domain mutation analysis was performed by Sanger sequencing. Array comparative genomic hybridization (aCGH) was performed by using Roche NimbleGen Human CGH ISCA 630K Array.

Results: Karyotype and FISH analysis results of the patient were reported as 46,XY,t(9;22)(q34;q11.2),ins(10)(10;3)(p11.2;p13p25)15/46,XY,t(9;22)(q34;q11.2)1 (Fig. 1A), and nuc ish(AS1x1),(ABL1,BCR)x2(ABL1 con BCR) x195/100. QRT-PCR was unable to detect the fusion transcript. Imatinib mesylate (IM) was administered to the patient, however, a resistance to the drug was observed one month later. At the second referral, 7% atypical fusion signal was detected by FISH, and QRT-PCR for p190 fusion transcript was weak positive. We did not detect ABL1 kinase domain mutations. aCGH analysis revealed a 1.2Mb deletion at chromosome 9q34.11-q34.12 involving an unusual breakage region in the ABL1 gene and another deletion of 4.9Mb at 3p12.3-p14.1 region (Fig. 1B). FOXP1 was one of the 28 genes deleted in the second region.

Discussion: The current patient has a complex karyotype and FISH analysis revealed an unusual signal pattern. We did not detect the fusion transcript by QRT-PCR. To illuminate other abnormalities we performed aCGH as the patient developed resistance to IM treatment. aCGH analysis identified a 1.2Mb deletion in ABL1 gene which was known to be related with resistance to treatment. In this context our finding might be one of the explanations of treatment resistance in our patient. Other additional molecular defect, a tumor suppressor gene inactivation, was also identified in our patient. Detecting FOXP1 deletion suggests that follow-up of the patient will be more complex than classic CML, since FOXP1 deletion might also negatively

affect cellular control mechanisms in addition to ABL1. In conclusion FOXP1 deletion together with ABL1 deletion might renders additional risk to our patient as unexpected genetic abnormalities, and during follow-up of the patient, transformation to AML should always be kept in mind.

**Keywords:** FOXP1, ABL1

**Karyogram and aCGH result of the patient**



A) 46,XY,t(9;22)(q34;q11.2),ins(10)(10;3)(p11.2;p13p25)15/46,XY,t(9;22)(q34;q11.2)1 B) 4.9Mb deletion at 3p12.3-p14.1 region

PS-131

Abstract:0272

### NILOTINIB INDUCED CHOLESTATIC HEPATITIS A CASE REPORT

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**Introduction:** Nilotinib has a relatively favorable safety profile and shows activity in cases of CML resistant to treatment with imatinib, another tyrosine kinase inhibitor currently used as a first-line treatment (2). nilotinib may cause side effects including: Headache, stomach pain, constipation, diarrhea, weight changes, weakness, nausea and vomiting. Increased blood glucose and high blood pressure may also occur. Serious side effects include QT prolongation, fainting, seizures, fever associated with reduced white blood cells, reduced platelets and red blood cell counts, infection, bleeding in the brain, reduced liver function, pancreatitis, and increased or reduced thyroid function(4). We report a rare case of cholestatic hepatitis in patient taking nilotinib.

**Case report:** We here report a case of 70 year old female patient diagnosed as CML Philedelphia Chromosome + (Ph +) eight years ago. She was first treated with imatinib and at the third month considered susceptible to treatment. In the first year of the treatment PCR was examined at intervals of three months and we examined PCR for six month intervals after the first year of treatment. At eighteenth month major molecular response was achieved. On the 7th year of the imatinib treatment PCR resulted as t(9;22) 1.1% positive and bone marrow biopsy showed t(9;22) 14% by fluorescent in situ hybridization(FISH) method. She was considered to be resistant to imatinib

and nilotinib treatment started. After 3 months t(9;22) was 0,003% positive. In the ninth month of the treatment laboratory results were aspartate aminotrasferase 506 U/L, alanine aminotrasferase 570 U/L, gamma glutamyl transferase 182 U/L, Total bilirubin 9.2 mg/dl, direct bilirubin 2,23 mg/dl, HbsAg non reactive, anti HBs non reactive, anti HbcIgM non reactive, HAV IgM non reactive, anti HCV non reactive, lactate dehydrogenase 227 U/L, direct coombs negative, indirect coombs negative. (table 1) She was hospitalised for etiological reseach of acute hepatitis. Abdominal usg showed thickened and edematous gallbladder wall (9 mm). Also common bile duct was dilated (12 mm) and no stone found on the MR cholangiography. Endoscopic retrograde cholangiopancreatography performed and no additional information about cholestasis etiology achived. Nilotinib treatment is stopped and her liver functicon tests improved. On her last visit she was also t (9;22) negative.

**Discussion:** This effect is explained by suppressive effect of nilotinib on hepatic oxidative/nitrosative stress cascades and neutrophil accumulation in the liver. Our patient has hepatic cholestasis which is nilotinib dose-dependant. Clinicians must be aware that nilotinib may be toxic for liver. We conclude that nilotinib could cause hepatotoxicity-cholestatic hepatitis and patients taking nilotinib need to be closely monitored, dose reduction or discontinuation of treatment can be the treatment of choice.

**Keywords:** Nilotinib, Hepatitis

PS-132

Abstract:0278

### GENERIC IMATINIB MESYLATE IS AS EFFECTIVE AS ORIGINAL GLIVEC IN THE MANAGEMENT OF CML

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Unsustainable drug prices in chronic myeloid leukemia (CML) and cancer may be causing harm to patients. Advocating for lower drug prices is a necessity to save the lives of patients who cannot afford them (Experts in CML. Blood. 2013;121(22):4439-4442). The patent date of imatinib mesylate in USA has just expired in January 2015. Patent expiration dates for imatinib may be different in different countries/regions. In Turkey, generic imatinib preparations are currently present. The only concern for generic imatinib is its efficacy over the original drug, Glivec or Gleevec. The aim of this multicenter study is to assess the efficacy of generic imatinib over Glivec in terms of hematological, cytogenetic, and molecular responses in CML. In this study, the retrospective data of 120 Turkish CML patients receiving imatinib from six different CML centers across Turkey were analyzed (68 females, 56.7%; 52 males, 43.3%). The mean age was 53 years (21-81). The most frequent ECOG performance status was "0" (65%). The distribution of genders between centers was similar. At the study onset, 86.7% of the patients (n=104) were using original molecules, and 13.3% of the patients (n=16) were using generic molecules. Original (86.7%),

16 generic imatinib mesylate. The patients were evaluated at 4 different time points for change of medication and efficacy. The mean period between each evaluation was 9 months. Initial evaluation showed that a patient who was using only original molecule, switched to second generation tyrosine kinase inhibitor (TKI) treatment. In this period, hematological response (HR) was observed in 99.2% of the patients, cytogenetic response (CR) was observed in 88.7% of the patients (47 of 53), and molecular response (MR) was observed in 75% of the patients. For each evaluation, the ratio of drugs that were preferred by the clinicians is shown in Figure 1. According to Figure 1, clinicians had a tendency to prefer generic molecules in each sequent visit, and this change rate was significant ( $p < 0.001$ ). The rate of using generic molecules was 13.3% in the initial evaluation, 20.8% in the second visit, 41.7% in the third visit, and 43.3% in the fourth visit. The rates of switching from original molecule to generic molecule, from original molecule to second generation TKI, and from generic molecule to second generation TKI are shown in Table 1. Accordingly, 11 patients, who were using original molecules during all cohort, switched to second generation TKI. On the other hand, only 1 patient, who was using generic molecules, switched to second generation TKI. Response to treatment is shown in Figure 2. Therefore, we did not find any significant difference in HR, CR, and MR for original and generic drugs in each visit. Based on this data, generic imatinib mesylate is as effective as original Glivec in the management of CML.

**Keywords:** CML

Figure 1. The ratio of drugs that were preferred by the clinicians for each evaluation.

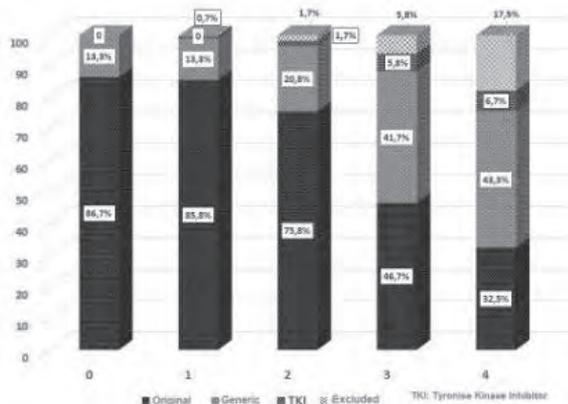


Table 1. The rates of switching from original molecule to generic molecule, from original molecule to second generation TKI, and from generic molecule to second generation TKI.

	Original- Generic		Original- Second generation TKI		Generic- Second generation TKI	
	n	%	n	%	n	%
First Visit	1/103	1,0	-	-	-	-
Second Visit	12/103	11,6	1/103	1,0	1/17	5,9
Third Visit	28/91	30,8	5/91	5,5	-	-
Fourth Visit	28/56	50,0	5/56	8,9	-	-

## Other

PS-133

Abstract:0101

### EXTREME LEUCOCYTOSIS IN PNH: NOT ALWAYS LEUKEMIC TRANSFORMATION

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**Introduction:** Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired hematopoietic stem cell disorder. Eculizumab, the only monoclonal antibody that blocks terminal complement binding to C5 is the only approved therapy for PNH. Although it is known that the patients under therapy are prone to infections with encapsulated organisms, there is no evidence of other serious infections associated with eculizumab. Herein, we report a case of thrombophlebitis presented with leukemoid reaction (LR) soon after eculizumab infusion for PNH.

**Case Report:** 82-years old male patient who had a history of myelodysplastic syndrome (MDS), admitted to our hematology department with increasing anemic symptoms. Flow cytometric analysis revealed a large PNH clone. Administration of prednisone (1mg/kg/d) treatment did not improve transfusion dependency and eculizumab treatment was considered. Only 4 days after the first infusion, he referred to our emergency department with complaint of fever, left foot swelling and pain. Left lower leg skin was hot, tender and swollen. The laboratory results revealed a sudden extreme leucocytosis (WBC:128,400/mm<sup>3</sup>) suggesting a leukemic transformation at first sight but there was no significant decrease in other series (Table 1). His peripheral blood smear revealed left-shift with myelocytes, metamyelocytes, and rare myeloblasts was found compatible with LR. Left lower extremity venous doppler USG showed chronic thrombus and thrombophlebitis. Ampicillin-sulbactam and enoxaparin treatment started and due to improvement in clinical and laboratory signs, he received second infusion of previously postponed eculizumab. 4 days after second infusion his complaints at his left leg worsened with elevated acute phase reactants. Piperacillin-tazobactam treatment started and thrombophlebitis regressed under therapy. He is still on eculizumab therapy.

**Discussion:** The occurrence of persistent neutrophilic leukocytosis above 50,000cells/mm<sup>3</sup> for reasons other than leukemia is defined as leukemoid reaction (LR). Although sudden increase in leukocyte count above 100.000cells/mm<sup>3</sup> in a patient with MDS brings leukemic transformation into mind, it can be associated with other situations. While no relationship of eculizumab with serious infections is reported other than encapsulated bacterial infections, it should be kept in mind that PNH itself increases tendency to thrombosis. This may cause serious infections which may be presented with LR mimicking leukemia, especially in elderly patients.

**Conclusion:** It should be kept in mind that in PNH patients, sudden onset of extreme leukocytosis may also

depend on factors other than leukemic transfusion such as thrombophlebitis. In elderly PNH patients like ours, who is under high dose steroid therapy, considering prophylactic anticoagulant and antibiotic therapy with eculizumab treatment may prevent these complications.

**Keywords:** Paroxysmal nocturnal hemoglobinuria, Leukemoid reaction

Table 1

	Before First Eculizumab Regimen	At presentation in emergency department	Before discharge (Day 12)
WBC	8.020/mm <sup>3</sup>	128.400/mm <sup>3</sup>	8.700/mm <sup>3</sup>
Hemoglobin	8,3gr/dL	10,2gr/dL	9,9gr/dL
Platelet	266000/mm <sup>3</sup>	115000/mm <sup>3</sup>	65000/mm <sup>3</sup>
RPI	1,71%	0.7%	0,589%
CRP	0,10mg/dL	36mg/dL	2,5mg/dL
ESR	14mm/h	119mm/h	43mm/h

Laboratory results (WBC: White blood cell, RPI: Reticulocyte production index, CRP: C reactive protein, ESR: Eritrocyte sedimentation rate)

PS-134

Abstract:0120

### OCHROBACTRUM ANTHROPI BACTEREMIA

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**Introduction:** Ochrobacterum anthropi is a non-lactose fermenting Gram-negative, oxidase-positive, urease positive, non-lactose fermenting, motile bacillus formerly known as Achromobacter Centres for Disease Control and Prevention (CDC) group Vd. It is available in hospitals, environmental water sources, swimming pools, dialysis liquids, normal saline and antiseptic solutions.

Of the clinically relevant species, O. anthropi is increasingly recognized as a potentially problematic, opportunistic and nosocomial pathogen. Infection is commonly seen in immune-compromised patients, such as those with debilitating illnesses or malignancy. Most cases of human disease that have been reported occur due to this pathogen and are associated with central venous catheter line infection. The organism is usually resistant to B-lactam antibiotics, yet susceptible to gentamicin, ciprofloxacin, trimethoprim/sulphamethoxazole and carbapenems.

**Case Report:** A 75 years old female who was diagnosed with Acute Myeloid Leukemia sub type of M5 (FAB Classification) two months ago, started Azacitidine therapy (75 mg/m<sup>2</sup>/day for 7 days every 4 weeks) after the diagnosis. Her past medical history is not significant, except the obesity. Due to vascular access problem, the patient has a central venous catheter. During the second course of Azacitidine therapy, on day 3, the patient has a fever and chills. On physical examination, her axillary body temperature is 38,9. The central venous catheter is clean and no pain on the palpation has been observed. Concurrent blood cultures were sent at this time from 2 different sites (peripheral vein, right internal jugular vein per catheter). Upon obtaining the cultures, we started intravenous piperacillin tazobactam monotherapy at a dose of 4 x 4,5 g/day. Despite the therapy, the fever continued for 72 hours and we added Teicoplanin to Piperacillin Tazobactam.

All 2 sites of blood cultures were positive for Ochrobacterum anthropi. The antibiotic susceptibility testing showed that O. Anthropi was sensitive to imipenem, meropenem, amikacin, ciprofloxacin and trimethoprim-sulfamethoxazole, and resistant to ceftazidime, piperacillin-tazobactam, Colistin, Ceftriaxone, Aztreonam and Cefepime.

After the culture results, we changed the piperacillin tazobactam to meropenem 3x500 mg/day and stopped the teicoplanin. The patient became afebrile within 24 hours and repeated blood cultures are negative.

**Discussion:** So while dealing with immune-compromised debilitated hosts, we should not miss out the unusual pathogens like Ochrobacterum, which are generally nosocomially acquired pathogen, affecting immunocompromised individuals. Although this organism seems to be of relatively low virulence, it can produce clinically significant fatal infections in debilitated patients. So, it is very important to follow infection control guidelines to control such opportunistic pathogens in the hospital environment.

**Keywords:** Ochrobacterum anthropi, bacteremia

PS-135

Abstract:0126

### AN AFIBRINOGENEMIA CASE WHO HAD THROMBOSIS AND INTOLERANCE TO EXOGENOUS FIBRINOGEN TREATMENT

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**Introduction:** Congenital afibrinogenemia is characterized by deficiency of fibrinogen which manifests with massive hemorrhage. Interestingly in these cases thrombotic events could be seen. Herein, we aimed to report an afibrinogenemia case who had thromboembolic event and had intolerance to exogenous fibrinogen treatment.

**Case Report:** 39 years old woman had diagnosed as afibrinogenemia in childhood and she was taking exogenous fibrinogen treatment. She applied to hospital in 2002 with excessive menstrual bleeding which was controlled with transamine tablets. Since then she had experienced hypermenorrhoea only one time till year 2005. In 2005, she fell down stairs because of decreased vision. Eye examination was normal so she was consulted to neurology. MR was performed and cerebrovascular event was revealed. She was hospitalized and oral contraceptive drug was stopped which was given because of ovarian cyst. Acetylsalicylic acid 100 mg/day was given. Hereditary thrombophilic tests revealed factor 5 leiden, MTHFR and Prothrombin 20210 A normal. Anticardiolipin IgG, antiphospholipid antibody IgG and IgM was negative. She had experienced excessive menstrual bleeding in the second menstrual cycle after the discontinuation of oral contraceptive drug. Therefore, progesterone releasing implant was inserted subcutaneously in order to prevent menstrual cycles. In 2006, she still had limited vision without any improvement. In 2007, she had improvement in vision. Also, she experienced allergic reaction which was aggregated after exogenous fibrinogen treatment. So, cryoprecipitate was given instead of exogenous fibrinogen. In 2008 and 2015, her progesterone implant had changed two times under cryoprecipitate infusion.

Discussion: Herein we reported an afibrinogenemia case with cerebrovascular event. In literature there are a few cases that reported thrombotic events in afibrinogenemic cases. Some of these cases had hereditary thrombotic risk factors. In our patient we did not detect any hereditary risk factors. In one of the reports in literature oral contraceptive drugs were shown as the reason of thrombotic event. Likewise our case was using oral contraceptive drug during the cerebrovascular event. Most interestingly although the mechanism is not yet clear some studies suggested there is a relationship between fibrinogen activation and thrombin inhibition. So our patients' thrombotic event could be explained by afibrinogenemia and oral contraceptive treatment. In literature there are reports that compared the efficiency of cryoprecipitate and exogenous fibrinogen concentrate treatment. We experienced no problem with managing hemostasis with cryoprecipitate instead of exogenous fibrinogen concentrate. To conclude, afibrinogenemia manifests with bleeding however thrombotic risk in these cases should be kept in mind and in case of fibrinogen concentrate intolerance replacement with cryoprecipitate is effective.

**Keywords:** Afibrinogenemia, thrombosis

PS-136

Abstract:0141

#### **THROMBOCYTOPENIC PNH PATIENT TREATED SUCCESSFULLY WITH TERMINAL COMPLEMENT INHIBITOR ECULIZUMAB**

Yasin Kalpakçı, Funda Ceran, Abdullah Agit, Ahmet Kürşad Güneş, Gülten Korkmaz, Aysun Gönderen, Ömer Önder Savaş, Hacer Berna Afacan Öztürk, Simten Dağdaş, Gülsüm Özet  
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**Introduction:** PNH (Paroxysmal Nocturnal Hemoglobinuria) is hematopoietic stem cell disorder that is consequence of a specific mutation in PIGA gene. Subsequent complement activation leads to complement-mediated hemolysis and release of free hemoglobin that causes major and life threatening complication of PNH, atypical venous thromboembolism (VTE). Thrombocytopenia founds in important part of PNH patients and these are more likely to have VTE. Thrombocytopenia may indicate underlying MDS or AA but also complement-mediated platelet consumption as a new definition. A novel monoclonal antibody Eculizumab inhibits not only complement-mediated hemolysis but also the complement-mediated platelet consumption as the purpose of this case report.

**Case:** 66 years old male patient firstly diagnosed AA in 2004 and treated successfully with immunosuppressive agents. 10 years later in October 2014 he consulted with hemolytic anemia concomitant thrombocytopenia (18 x 103/μl). Serum LDH enzyme level and reticulocyte count was substantially increased. In flowcytometric analysis 85% PNH clone detected in granulocytes with FLAER. To reveal underlying AA or MDS, we performed bone marrow sampling. Bone marrow was hypercellular with normal maturation. Due to intensive erythrocyte transfusion requirement, Eculizumab therapy has started at the dose of 600 mg first 5 weeks and after 900 mg every 2 weeks. Although 5 weeks passed after starting the treatment and he no longer needs to have erythrocyte transfusion with almost nearly normalized serum LDH level, thrombocytopenia was persisting. Because of bone marrow cellularity

in AA can be quite patchy, we performed second bone marrow sampling and the result is the same. After 6 weeks past from starting the therapy, platelet count normalized in a few days (156 x 103 μl).

**Discussion:** Thrombocytopenia founds in PNH 25-50 % patients. The efficacy of Eculizumab in thrombocytopenic patients has only been reported as an abstract in 2009 ASH Annual Meeting Abstract. 195 PNH patients, 49 of whom were thrombocytopenic, from the SHEPHERD, TRIUMF and Phase 2 Eculizumab trials treated with Eculizumab. Following terminal complement inhibition, platelet counts were significantly increased (normal level in 33% and >80 x103 μl in 36% patients). By the way of these results, they have defined complement-mediated platelet consumption which was not previously reported in literature. The case we offer in this report also supports this definition. Together with normal bone marrow and increased reticulocyte counts, how else can be explained the increment in platelets by inhibition of terminal complements. In thrombocytopenic PNH, after bone marrow sampling to reveal underlying AA or MDS, we suggest that terminal complement inhibition by Eculizumab can also inhibit the consumption of platelets and ameliorate thrombocytopenia.

**Keywords:** PNH, Thrombocytopenia

PS-137

Abstract:0149

#### **A PEDIATRIC CASE WITH BLASTIC PLASMACYTOID CELL NEOPLASM**

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**Background:** Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare hematologic malignancy derived from plasmacytoid dendritic cells (PDCs). The disease may occur at any age, but most patients are elderly men who present with skin lesions and/or involved lymph nodes, spleen, and bone marrow. BPDCN is very rare in children. We present a boy diagnosed with BPDCN. Case: A 12-year-old boy presented with a hyperemic firm mass on the right thigh which had developed after a trauma and had progressed within 8 months. A biopsy revealed Blastic plasmacytoid dendritic cell neoplasm (BPDCN) (CD 56+ and CD 123+). He was referred to us for further investigation and treatment. Blood laboratory tests were within normal ranges. No atypical cells were detected in the bone marrow aspiration, there were few focal areas with cells positive for CD123 and CD 56 by immunohistochemistry in bone marrow biopsy. CSF cytology was negative. PET-CT revealed multiple bone involvement in the extremities. BFM-ALLIC 2009 High risk protocol was initiated, with remission in bone marrow by the end of first month and remarkable regression of bone involvement at the end of first month, with complete response at the end of second month. High risk chemotherapy and stem cell transplantation was planned, but had no matched-family donor, so chemotherapy was continued as per protocol. An unrelated donor is found recently and is planned to undergo HDCT+transplantation.

Conclusion: BPDCN are very rare neoplasms in children with bad prognosis. They usually present with cutaneous involvement; bone marrow and bone involvement is common. Due to its rarity, treatment is not standard. In the literature, there are cases treated by ALL or by AML protocols and/or bone marrow/stem cell transplantation. In patients presenting with cutaneous lesions with hyperemia and swelling BPDCN should be considered in the differential diagnosis. Aggressive treatment such as high risk ALL protocols and SCT may lead to better survival.

**Keywords:** Blastic plasmacytoid dendritic cell neoplasm, children

PS-138

Abstract:0171

### MEDIASTINAL T-LYMPHOBLASTIC LYMPHOMA RELAPSED WITH HYPEREOSINOPHILIA

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Introduction: Hyper eosinophilia, either clonal or reactive, has been described in association with multiple hematological malignancies but it is vary rare with lymphoblastic lymphomas. A case of a patient presenting with hyper eosinophilia that evolved into T-cell lymphoblastic lymphoma relapse was reported.

Case: A 19 year-old male patient presented to Ege University Hematology Department with shortness of breath in June 2014. Thorax computerized tomography (CT) revealed a 14x11 cm mediastinal mass and pleural effusion. Biopsy from mediastinal mass was performed and CD01a, CD3, CD10 positive T-lymphoblastic lymphoma/leukemia was reported. Bone marrow biopsy was normocellular so the patient was considered as lymphoblastic lymphoma (LL). Three courses CODOX-M (Cyclophosphamide, vincristine, doxorubicin, high-dose methotrexate) altering with IVAC (Etoposide, cytarabine, ifosfamide, methotrexate) chemotherapy regimens were administered. Mass and effusion was totally resolved. 20 days after the last chemotherapy regimen hyper eosinophilia (3.27x103µL, 30.9%) was detected in routine control without any symptom. The patient was hospitalized with fewer 1 week later. Eosinophil count was progressed to 10.6 x103µL, 50.6% and shortness of breath was started. Massive pleural and pericardial effusion and 10 cm mediastinal mass were detected in thorax CT. Cytological examination of pericardial fluid has revealed presence of lymphoblastic cells. Chemoterapy was immediately started and at5th day of treatment all signs and symptoms of lymphoma disappeared. The patients has been still under treatment and stem cell transplantation from HLA full-matched sibling donor was planned.

Discussion: Although the association of ALL with hyper eosinophilia is well recognized, only few cases of underlying LL has previously been reported. Association of hyper eosinophilia and LL depends on t(5;14)(q31;q32) and IL3 hypersecretion in reported cases. The cytogenetic results of our patient are pending. Clonal hyper eosinophilia in LL may be a poor prognostic factor because the few cases which were reported are relapsed in a short time. In cases with hyper eosinophilia as early sign should be evaluated more carefully and urgently.

**Keywords:** Hyper eosinophilia, Lymphoblastic Lymphoma

PS-139

Abstract:0172

### WE WOULD NOT OVERLOOK THE ENDOCRINE DISORDERS IN ANEMIA

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Hematopoiesis is the formation of blood cellular components. The effect of growth factors and hormones (erythropoetin, thyroid ve corticosteroid hormones) are important in producing of erythrocytes. We would like to present a panhypopituitarism with hypoproliferative anemia case to point out endocrine disorders in differential diagnosis of anemia.

A 36-year-old man without any significant past medical history presented to the hematology outpatient clinic with generalized waekness for a long time. Further questioning revealed a remote history of head trauma and scalp fracture which was occurred around 5 years ago. Laboratory findings revealed anemia with 7.2 g/dl hemoglobine level and reticulocytopenia. Bone marrow biopsy was performed to evaluate his anemia and 30% of bone marrow cellularity was found. Endocrinology consultation was requested due to abnormal thyroid function test. Patient's panel of anterior hypophysis hormones was detected as the following; FSH=0.572 mIU/ml, LH=0.100 mIU/ml, Prolactin= 28.26 ng/ml, testosterone=0.025 ng/ml, cortisol=1.97 mcg/dl, GH= 0.281 ng/ml, ILGF-1=25 ng/ml, TSH=4.15 uIU/ml, free-T3=1.32 ng/dl, FT4= 0.48 ng/dl. Cranial and hypophysis MRI scan was carried out due to panhypopituitarism pre-diagnosis. MRI revealed variation of hypothalamo-hypophysis anatomy and posteriorly located infundibulum. Bilaterally small testicals was found during testicular ultrasonography.

As a conclusion, we would like to emphasize that endocrine disorders needs to be considered in diagnosis of anemia since the nonspecific symptoms such as weakness, fatigue might be seen in both condition.

**Keywords:** Anemia, hypopituitarism

PS-140

Abstract:0177

### DETECTION OF BRAF V600E MUTATION IN HAIRY CELL LEUKEMIA

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Objective: Hairy cell leukemia (HCL) is an indolent disease with distinct immunophenotypic features which is characterized by pancytopenia, splenomegaly, infiltration of bone marrow, spleen and liver with hairy shaped leukemic B-cells. BRAF V600E mutation, detected previously in some malignancies, has been found to be disease-defining in HCL. BRAF, a serine-threonine kinase, is a member of RAS-RAF-MEK-ERK (MAPK) signaling pathway. This pathway is responsible from cell survival, proliferation and differentiation. It has been shown that activating BRAF V600E mutation leads to uncontrolled activation of MEK-ERK pathway which leads to neoplastic transformation in HCL. In this study, we aim to take an inventory of HCL patients who are newly diagnosed or at follow-up

after conventional therapies and investigate the presence of *BRAF* V600E mutation in these patients with RT-PCR method.

**Material-Method:** *BRAF* V600E mutation was analyzed in peripheral blood in 22 patients (10 newly-diagnosed, 12 in remission) from Istanbul University Istanbul Faculty of Medicine, Division of Hematology Outpatient Service and 10 control patients with other B-cell chronic lymphoproliferative diseases.

**Patients**

NEWLY DIAGNOSED PATIENTS										
AGE	SEX	SYMPTOMS	LABORATORY FINDINGS	LDH	LDH	LDH	LDH	TREATMENT	SIDE EFFECTS	LAST STATUS
39	M	TRAP +	+	+	+	+	+	Cladribine	Neutropenia, fever	Remission
75	M	TRAP Anemia +	+	+	+	+	+	Cladribine	UTI	Remission
72	F	TRAP Anemia +	+	+	+	+	+	Cladribine	Neutropenia, fever, arthralgia	Remission
45	M	TRAP Anemia +	+	+	+	+	+	Cladribine	Neutropenia, leukopenia, fever	Remission
52	F	TRAP Anemia +	+	+	+	+	+	Cladribine	Neutropenia, leukopenia, fever	Remission
32	M	TRAP Anemia +	+	+	+	+	+	Cladribine	Neutropenia, fever	Remission
52	F	TRAP Anemia +	+	+	+	+	+	Cladribine	Neutropenia, fever	Remission
38	M	TRAP Anemia +	+	+	+	+	+	Cladribine	Fever	Remission
44	M	TRAP Anemia +	+	+	+	+	+	Cladribine	Neutropenia, fever	Remission
48	F	TRAP Anemia +	+	+	+	+	+	Cladribine	Neutropenia, fever	Remission
45	F	TRAP Anemia +	+	+	+	+	+	Cladribine	Neutropenia, fever	Remission
PATIENTS IN REMISSION										
84	M	TRAP +	+	+	+	+	+	Cladribine		Remission
67	M	TRAP +	+	+	+	+	+	Cladribine	Subconjunctival, PRN, DC/Cladribine	Remission, T-cell CLL
64	M	TRAP +	+	+	+	+	+	Cladribine		Remission
55	M	TRAP +	+	+	+	+	+	Cladribine		Remission
46	M	TRAP +	+	+	+	+	+	Cladribine		Remission
49	M	TRAP +	+	+	+	+	+	Cladribine		Remission
30	M	TRAP +	+	+	+	+	+	Cladribine + splenectomy		Remission
47	M	TRAP +	+	+	+	+	+	Zidovudine		Remission
42	M	TRAP +	+	+	+	+	+	Cladribine, 4 cycles rituximab		Partial remission
38	M	TRAP Anemia +	+	+	+	+	+	Zidovudine		Remission
55	M	TRAP +	+	+	+	+	+	Cladribine		Remission, leukopenia, splenectomy
35	M	TRAP Anemia +, Lysis, neutropenia, hyperleukemia	+	+	+	+	+	RT + Zidovudine		Remission
45	M	TRAP +	+	+	+	+	+	Zidovudine		Remission

**Information of the patients**

**Findings:** *BRAF* V600E mutation was not detected in any of the patients in the control group. Median age of HCL patients was 45 (38-73). %82 were male, %18 were female. Patient information are given in Table 1 and 2. Analysis of the newly diagnosed patients revealed the mutation in 8 of 10 patients. One of the patients whom the mutation was found negative, didn't achieve remission with cladribine, died due to prolonged febrile neutropenia and fungal infection. The other patient with leukocytosis presented signs compatible with HCL, received cladribine treatment uneventfully. Then the patient was lost to follow-up. All patients received cladribine as first-line treatment. Seven patients received broad-spectrum antibiotics in the neutropenic period. Two patients died because of infections. Mutation was found in four of the 12 patients in the remission group. All patients received cladribine as first line except the oldest patient (splenectomy, interferon- $\alpha$  and two cycles of cladribine consequently). One patient received 4 cycles of rituximab. One patient developed T-cell CLL during follow-up. 4 of 12 patients had received second cycle cladribine after relapse. One of the patients whom the mutation was positive, had recurrence during follow-up, another patient was in partial remission, eventually relapsed. Mutation was detected positive in two patients in complete remission. These patients were thought not to achieve molecular remission.

**Conclusion:** Detection of *BRAF* V600E mutation could be helpful for diagnosis of HCL. When the mutation is found negative, other B-cell chronic lymphoproliferative diseases should be considered. Usefulness of investigation of the mutation in treated patients remains an area to be elicited.

**Keywords:** Leukemia, *BRAF*

PS-141

Abstract:0178

**EVALUATION OF ADULT PATIENTS WITH LYMPHADENOPATHY IN TERMS OF EPIDEMIOLOGY, CLINICAL, LABORATORY AND ETIOLOGY**

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**Aim:** In this study, we aimed to investigate parameters that predict benign or malign etiology in patients who underwent lymph node biopsy.

**Materials-Methods:** Total of 150 patients (female: 73; male:77) were included to the study who admitted to the hospital and underwent lymph node biopsy between January 2011 and April 2012. Epidemiological characteristics, clinical and laboratory findings and pathology results were evaluated using the computer program.

**Results:** Anemia, leukopenia, thrombocytopenia and elevation of the LDH level was significantly correlated with malignancy. Rate of benign results was higher in cervical, submandibular, axilla and inguinal lymphadenopathy region. And rate of malign results was higher in intra-abdominal and supraclavicular lymph nodes. Also rate of malignancy was higher in patients who has splenomegaly and whose lymph node index <2. Sedimentation and CRP levels and hepatomegaly were not associated with the malignancy.

**Conclusions:** Cytopenias (anemia, leukopenia, thrombocytopenia), high lactate dehydrogenase levels, the presence of splenomegaly, lymph node index <2, the presence of intra-abdominal and supraclavicular lymphadenopathy may predict malignant etiology in patients with lymphadenopathy.

**Keywords:** Lymphadenopathy, etiology

PS-142

Abstract:0180

**INFLUENCE OF PROGNOSTIC FACTORS ON OVERALL SURVIVAL IN MYELOYDYSPLASTIC SYNDROMES**

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**Background:** Accurate prediction of a patient's prognosis is useful to define the risk posed by the disease. Age, gender, peripheral blood cytopenias, proportion of bone marrow (BM) blasts, performance status, comorbidities, transfusion dependence, specific karyotype abnormalities and molecular biomarkers can refine the prediction of prognosis in MDS. **Aim:** to assess the influence of the some prognostic factors like age, gender, cytopenias, BM blast percentage, transfusion dependence, ferritin, hemoglobin (Hb), lactate dehydrogenase (LDH), albumin and specific karyotype abnormalities in myelodysplastic syndromes on overall survival (OS).

**Patients and Methods:** we retrospectively analyzed the cohort of 108 patients diagnosed between 1.1.2011 and 31.12.2013 at the University Clinic of Hematology, Ss Cyril and Methodius University, Skopje, Macedonia. They were evaluated for clinical and hematologic features at diagnosis and at leukemic transformation. **Results:** in the study group 62 were man and 46 women. Male to female ratio was 1.35 to 1. The differences in OS between men and women were significant (p =.03015). The mean age at diagnosis was 66,6 years. According to the age OS was 16,4 months. FAB subtypes influenced OS significantly (p =.03015). OS inversely correlated with BM blast percentage (p=.02327). Cytopenias had no impact on OS (p=.33755). Hb as a whole and groups with different levels of Hb had no influence on OS (p =.12142) and (p=.07535), respectively. The group with ferritin <500  $\mu$ g/L had better OS (p=.04720). Transfusion dependence, LDH and

albumin had no impact on OS. Leukemic transformation was noticed in 10 (9,3%) patients. Mortality was 36,1%.

Conclusion: gender, FAB subtypes, BM blast percentage and the serum levels of ferritin had an influence on OS, while age, hemoglobin level, transfusion dependence, LDH and albumin had no impact on OS.

**Keywords:** Myelodysplastic syndromes, prognostic factors

PS-143

Abstract:0192

### WHAT SHOULD BE THE RIGHT TIME FOR THE CHELATION THERAPY IN MYELODYSPLASTIC SYNDROME?

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**Aim:** The aim of this study to determine the association of ferritin level and survival in myelodysplastic syndrome patients.

**Methods:** Forty patients with MDS, comprising 14 females (35%) and 26 males (65%), who were followed up in our hematology clinic between 2009 and 2014 were included in the study. At the time of diagnosis, hemogram parameters, ferritin levels were noted. Survival estimates of the patients were calculated starting from the diagnosis date until death due to disease or any other cause.

**Results:** The mortality rate of the patient group was 55%, and median survival was 18 months. Median ferritin level was 277,74 ng/ml (min:13,9ng/ml max:2000ng/ml). There were 28 patients (70%) with ferritin level less than 500 ng/ml. Mortality was found to be higher in patients with ferritin levels>500 ng/ml (p=0.025).

**Discussion:** What remains unknown is whether lowering serum ferritin levels with chelation therapy will improve morbidity and mortality in MDS. Our study demonstrated that mortality increases with ferritin level >500ng/ml as opposed to previous studies. In light of these findings, we suggest that chelation treatment should be started when the ferritin level exceeds 500ng/ml.

**Keywords:** Myelodysplastic syndrome, ferritin

PS-144

Abstract:0206

### DOES AGE PLAY A ROLE IN PERIPHERAL NEUROPATHY IN MULTIPLE MYELOMA PATIENTS?

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**Aim:** Peripheral neuropathy (PN) is a common side effect of cancer therapy that can have a profound impact on quality of life and survivorship. The aim of this study is to evaluate the association between age and peripheral neuropathy and identify the risk factors for peripheral neuropathy in multiple myeloma patients.

**Methods:** We conducted a retrospective review of 92 multiple myeloma patients (53 males, 39 females) who

presented to hematology clinic between 2009 and 2014. Their gender, performance status, chemotherapeutic regimens, diabetes status, treatment cessation rate and electromyography results were noted. The association between age, gender, chemotherapeutic agents, presence of diabetes, treatment cessation rate and neuropathy was analyzed with chi square statistical analysis.

**Results:** The mean age of patients was 64±11.6. Patients were divided according to their age in two groups to those older than 65 years (group I, n=46) and those younger than 64 years (group II, n=46). Peripheral neuropathy rate which is confirmed with electromyography was found 27.2%. The rate of neuropathy was 26.1% in group 1 and 28.3% in group 2. No significant association was found between age and peripheral neuropathy (p=1.00). The treatment cessation rate was 14.1% (n=13) among the patients. No association was found between treatment cessation rate and neuropathy.

**Conclusion:** This is one of the first, large sample size studies evaluating the association between age and peripheral neuropathy which is confirmed with electromyography in multiple myeloma patients. The association between age and neuropathy was found statistically insignificant.

**Keywords:** Multiple Myeloma, Neuropathy

PS-145

Abstract:0208

### URINARY INCONTINENCE: AN INDEPENDENT RISK FACTOR FOR QUALITY OF LIFE IN CANCER PATIENTS

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**Aim:** The International Continence Society defines urinary incontinence as 'involuntary loss of urine that is a social or hygienic problem'. Urinary incontinence impacts the lives of older individuals and it is considered one of the most important and recurrent geriatric syndromes. The aim of this study is to determine the prevalence of urinary incontinence in cancer patients and to evaluate its association with age and quality of life.

**Method:** 133 patients with cancer were assessed at hematology/oncology outpatient clinic. The validated form of the Turkish version of the International Consultation on Incontinence Questionnaire-Short Form was used to evaluate urinary incontinence and quality of life (QOL). Descriptive statistics were used. The association between urinary incontinence and age, gender and quality of life were evaluated with chi square.

**Results:** A total of 133 patients including 84 male and 49 female were evaluated. The mean age of patients is 62.5±12.3. While 45.9% of patients are older than 65, 54.1% of them are less than 64. The rate of urinary incontinence was found 40.6% (n=54). The association between urinary incontinence and age, quality of life has been shown statistically significant with chi square (P<0.001, P<0.001 respectively). The mean of ICI-Q and QOL score is 7.6±3.1 and 3.2±1.7 respectively. The most common type of urinary incontinence is urge incontinence

following by stress, mix and overflow (12.8%, 12%, 11.3% and 4.5% respectively).

Conclusion: Our results suggest that urinary incontinence is a significant problem which is under diagnosed and undertreated in cancer patients. It inversely affects the quality of life. While focusing on cancer and chemotherapy this important problem should not be underestimated. This leaves incontinent patients with unresolved physical, functional, and psychological morbidity, and diminished quality of life. The study suggests that awareness and education regarding incontinence should be increased among cancer patients and screening of UI is an important part of their assessment.

**Keywords:** Cancer, Urinary Incontinence

PS-146

Abstract:0210

### HOW SAFE IS DABIGATRAN IN ELDERLY?

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Objective: To report a case of dabigatran induced gastrointestinal bleeding without any obvious source and emphasize the risk of drug interaction in an elderly patient.

Case: An 87-year-old woman admitted to the hospital with three days history of melena. Patients' past medical history is significant for hypertension, nonvalvular atrial fibrillation and recently diagnosed diabetes mellitus. Her medications were perindopril/indapamid, nifedipine, furosemide, repaglinide and dabigatran. Two months prior to admission she was switched from warfarin to dabigatran 110 mg twice daily due to poor controlled INR. Laboratory tests were normal except elevated urea nitrogen and creatinine, 82 mg/dL and 1.8 mg/dL respectively. Routine coagulation assays were prolonged prothrombin time 25 seconds (range 12-18); INR was 2.0; activated partial thromboplastin time was 53.3 seconds (range 25-40). Her oral intake was stopped and intravenous fluids and proton pump inhibitor infusion was initiated. She was transfused 3 packed red blood cells. Her vitals stabilized. Endoscopy and colonoscopy was performed and no pathology was identified. Bleeding has been stopped. Patient was discharged home after 3 days admission in stable condition.

Discussion: Our case suggest that drug interactions may increase the risk of bleeding additional to old age in patients who are treated with dabigatran. This case highlights the bleeding risk with dabigatran in an elderly patient even in recommended doses. Larger prospective, well controlled studies evaluating dabigatran's safety in elderly should be considered. Clinicians should use these medications with caution in geriatric population particularly over 85 years old.

**Keywords:** Dabigatran, Elderly

PS-147

Abstract:0238

### HANNOVER BONE MARROW CLASSIFICATION OF CHRONIC MYELOPROLIFERATIVE DISORDERS AND THE 2008 EUROPEAN CLINICAL, MOLECULAR AND PATHOLOGICAL (2008 ECMP) CRITERIA FOR CLASSIFICATION AND STAGING OF MYELOPROLIFERATIVE NEOPLASMS

J. J. Michiels

*Jan Jacques Michiels*

Bone marrow histology is pathognomonic clue for hematopathologists to accurately distinguish the BCR/ABL negative chronic Myeloproliferative Disorders (CMPD) Essential thrombocythemia (ET) and polycythemia vera (PV) from BCR/ABL positive chronic myeloid leukemia (CML) and ET, and from thrombocythemia associated with myelodysplastic syndromes in RARS-T and 5q-minus syndrome. The 2008 European Clinical Molecular and Pathological (2008 ECMP) classifications distinguish three distinct clonal myeloproliferative neoplasms (MPN) of JAK2V617F mutated ET, JAK2 wild type MPL mutated ET and JAK2/MPL wild type ET. The 2008 ECMP criteria could delineate three prefibrotic stages JAK2V617F mutated ET as normocellular ET, ET with features of early PV (prodromal PV), and ET with hypercellular megakaryocytic granulocytic myeloproliferation (EMGM) and 6 clinical stages of PV, which have important prognostic and therapeutic implications. Spontaneous EEC and low serum erythropoietin (EPO) levels are highly specific for JAK2V617F mutated ET, prodromal PV, masked PV and classical PV. The quantitation of JAK2V617F mutation allele burden plays a key-role in the diagnostic work-up and staging of ET, PV and MF patients. The JAK2V617F mutation allele burden in heterozygous mutated ET is low but high in combined heterozygous - homozygous or homozygous mutated PV and EMGM. The combined use of JAK2V617F mutation load, spleen size are of major prognostic significance in terms of critical care medicine on top of pre-treatment bone marrow histopathology. This has important therapeutic implications for the first, second and third line treatment options in prodromal, classical and masked PV.

JAK2 wild type ET carrying the MPL515 mutation is a separate and distinct MPN entity without features of PV in blood (normal serum EPO) and bone marrow at diagnosis and during follow-up. JAK2/MPL wild type ET is associated with primary megakaryocytic granulocytic myeloproliferation (PMGM) and appears to be the third distinct MPN entity first defined as chronic granulocytic megakaryocytic myeloproliferation in the 1990 Hannover Bone Marrow classification of CMPD. Myelofibrosis (MF) is not a disease but a secondary response to cytokines released from the clonal granulocytic and megakaryocytic proliferative cells in MPNs of various molecular etiology. Large Prospective Unmet Need (PUN) studies of treated and newly diagnosed MPN patients are warranted to delineate the natural history and outcome of MPN of various molecular etiology during long-term or life long follow-up.

**Keywords:** MPL mutation

PS-148

Abstract:0240

### INCREASED ERYTHROCYTES ON TOP OF BONE MARROW HISTOLOGY, LOW SERUM EPO LEVEL AND JAK2 MUTATION SCREENING DISCRIMINATES JAK2V617F MUTATED ESSENTIAL THROMBOCYTHEMIA

J. J. Michiels

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Bone marrow histology differentiates essential thrombocythemia (ET), polycythemia vera (PV) and primary megakaryocytic granulocytic myeloproliferation (PMGM) from all variants of primary or secondary erythrocytosis and reactive thrombocytosis with a sensitivity and specificity of 100%. Bone marrow morphology, serum EPO level and JAK2 allele mutation load cannot discriminate between ET and prodromal PV versus classical PV. A typical JAK2 mutated MPN bone marrow histology with erythrocytes above  $5.8 \times 10^{12}/L$  in males and above  $5.5 \times 10^{12}/L$  in females (normal cut-off value is  $5.5 \times 10^{12}/L$ ) separates PV from ET and prodromal PV obviating the need of red cell mass (RCM) measurement. Erythrocytes remain increased at values above  $6 \times 10^{12}/L$  in PV in complete hematological remission by phlebotomy but hemoglobin and hematocrit values normalize as the consequence of micro-erythrocytosis due to iron deficiency. The combination of increased RCM, increased plasma volume, and normal or low erythrocyte counts is characteristic for Inapparent PV (IPV) due to significant splenomegaly as the cause of increased RCM in the absence of hyper-volumemic symptoms. Six sequential phenotypes of JAK2 mutated MPNs include: JAK2V617F positive heterozygous ET, prodromal PV, hetero/homozygous mutated PV, masked PV, PV-MF and IVP. JAK2 exon 12 JAK2 mutated MPN usually present as idiopathic erythrocythemia or early stage PV. JAK2 wild MPL515 mutated ET is a distinct clonal MPN entity in which the megakaryocytes are larger and giant with hyperlobulated staghorn-like nuclei without features of PV in blood and bone marrow. Bone marrow histology in CALR mutated ET and MF revealed a typical PMGM picture showing dysmorphic immature megakaryocytes with cloud-like nuclei, which are not seen in MPL515 mutated ET and also not in JAK2V627F mutated ET, prodromal PV and PV.

**Keywords:** JAK2

PS-149

Abstract:0241

### BIOLOGY DIAGNOSIS AND CLASSIFICATION OF MPD

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According to strict morphological, biochemical, cytogenetic and molecular criteria including the Philadelphia (Ph) chromosome and bcr/abl fusion gene and protein, chronic myeloid leukemia is a malignant disease with an obligate transition into acute leukemia, whereas essential thrombocythemia (ET), polycythemia vera (PV) and agnogenic myeloid metaplasia (AMM) form the Ph-chromosome and bcr/abl negative chronic myeloproliferative disorder (MPD) featured by a benign proliferation of the 3 hematopoietic cell lines. Increase and clustering of abnormal enlarged megakaryocytes together with no or various degrees of increased erythropoiesis and/or granulopoiesis in bone marrow biopsy specimens appears to be a pathognomonic clue to the diagnosis of prefibrotic MPD

when WHO bone marrow features are applied. On top of established WHO bone marrow features in combination with JAK2V617F mutation screening, and laboratory features including endogenous elyroid colony (EEC) formation and serum erythropoietin (EPO) levels we propose updated European clinical, molecular and pathological (ECMP) criteria for the differential diagnosis of true ET, PV and chronic idiopathic myelofibrosis (CIMF) or AMM. The ECMP criteria reduced the platelet count of  $600 \times 10^9/l$  to the upper limit of normal ( $>400 \times 10^9/l$ ) as inclusion criterion for the diagnosis of thrombocythemia in various MPDs. When WHO and ECMP criteria are applied, PVSG-defined ET includes three distinct entities: true ET, early PV mimicking ET, and thrombocythemia associated with early CIMF-0 mimicking ET. Compared to characteristic bone marrow features pathognomonic for MPD, spontaneous EEC and low serum EPO levels are not sensitive enough as isolated markers for the diagnosis and differential diagnosis of prefibrotic ET, PV and CIMF-0. Bone marrow histology assessment remains the gold standard criterion for the diagnosis and staging of the MPDs. PVSG-defined ET and the presence of the JAK2V617F mutation, EEC, PRV-1 overexpression and low serum EPO levels is consistent with early PV ("forme fruste" PV) when ECMP criteria are applied. The combination of JAK2V617F mutation and increased hematocrit ( $>0.051$  males and  $>0.48$  females) is consistent with the diagnosis PV (specificity 100%, sensitivity 95%) without the need of red cell mass measurement. About half of the ET and CIMF patients are JAK2V617F positive (sensitivity 50%). The degree of JAK2V617F positivity of granulocytes is related to disease stage: heterozygous in true ET and early PV and mixed hetero/homozygous to homozygous in overt and advanced PV and CIMF-1 to 3. The proposed ECMP criteria for the differential diagnosis of ET, PV, CIMF in patients with JAK2V617F positive and JAK2 wild type MPD should be evaluated in prospective management studies in search for the most relevant prognostic factors of therapeutic significance.

**Keywords:** myeloproliferative disorders

PS-150

Abstract:0242

### ERYTHROMELALGIC MICROVASCULAR DISTURBANCES, MAJOR THROMBOSIS AND HEMORRHAGIC MANIFESTATIONS OF THROMBOCYTHEMIA IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA: PERSONAL EXPERIENCES AND THERAPEUTIC IMPLICATIONS 1974-2014

J. J. Michiels

*Jan Jacques Michiels*

Aspirin responsive erythromelalgic and migraine-like microvascular ischemic attacks (MIAs) are the main presenting symptoms of thrombocythemia in patients with essential thrombocythemia (ET) and polycythemia vera (PV) at platelet counts above  $400 \times 10^9/L$ . If left untreated thrombocythemia patients are at high risk of both microvascular ischemic peripheral, cerebral and ocular ischemic manifestations and major arterial thrombosis. The high thrombotic risk in thrombocythemia decreases to less than 2% per 100 patient-years by primary prevention with low dose aspirin. Such low thrombotic risk by primary prevention with low dose aspirin of microvascular thrombosis applies for low, intermediate and high MPN disease burden, is not age dependent. Both microvascular

and major thrombosis do recur when not on low dose aspirin during follow-up. Von Willebrand factor (VWF) mediated platelet thrombi formation, as well as increased proteolysis of the VWF multimers in one and the same patient do occur simultaneously or in sequence leading to the paradoxical occurrence of thrombosis and bleeding at platelet counts above  $1000 \times 10^9/L$  due to an acquired von Willebrand disease type 2A. The Bergamo criteria for high thrombotic risk as the indication for hydroxyurea treatment on MPN disease are by the International Prognostic Score of Thrombosis in ET (IPSET). This illogically leads to significant overtreatment with hydroxyurea simple because a significant number of so-called high thrombotic risk ET and PV do in fact have low to intermediate myeloneoproliferative (MNP) disease burden. According to the world wide used IPSET guidelines in ET, PV and MNP review papers there is a global overtreatment of ET and PV patients with hydroxyurea. Activated leukocytes are innocent bystanders in promoting the risk of platelet-mediated microvascular ischemic and thrombotic complications in JAK2V617F mutated ET and PV. MNP disease burden in patients with JAK2V617F positive ET and PV is related to JAK2 allele burden and associated with leukocytosis, thrombocytosis, constitutional symptoms and splenomegaly. Increased platelet count (thrombocytopenia) and leukocytes (leukocytopenia) above  $15 \times 10^9/l$  is best treated by low dose pegylated interferon (PegasisR) 45 ug once per week or once per two week in correcting leukocytes to normal and platelet to near normal or normal to postpone the use of hydroxyurea as long as possible. This is associated with reduction of thrombosis risk to near zero, with reduction of enlarged spleen sizes, and above all pegylated interferon prevents splenomegaly in early and intermediate stage PV patients during very long-term follow-up.

**Keywords:** MPN

PS-151

Abstract:0243

**INCREASED ERYTHROCYTE COUNTS ON TOP OF BONE MARROW HISTOLOGY, LOW SERUM EPO LEVEL AND JAK2 MUTATION SCREENING DISCRIMINATES JAK2V617F MUTATED ESSENTIAL THROMBOCYTHEMIA FROM POLYCYTHEMIA VERA**

J. J. Michiels

*Jan Jacques Michiels*

Bone marrow histology differentiates essential thrombocythemia (ET) and polycythemia vera (PV) from all variants of primary or secondary erythrocytosis and reactive thrombocytosis with a sensitivity and specificity of 100%. Bone marrow morphology, serum EPO level and JAK2 allele mutation load are pathognomonic for trilinear myeloproliferative neoplasm (MPN) but cannot discriminate between ET and prodromal PV versus idiopathic erythrocythemia (IE) and classical PV. The present study demonstrates that a typical JAK2 mutated MPN bone marrow histology with erythrocytes above  $5.8 \times 10^{12}/L$  in males and above  $5.5 \times 10^{12}/l$  in females (normal cut-off value is  $5.5 \times 10^{12}/L$ ) separates IE and PV from ET and prodromal PV obviating the need of red cell mass (RCM) measurement. Erythrocyte counts remain increased at values above  $6 \times 10^{12}/L$  in PV in complete hematological remission by phlebotomy alone, but hemoglobin and hematocrit values normalize as the consequence of micro-erythrocytosis due to iron deficiency. Normal to low erythrocyte counts and increased blood volume is

characteristic for so-called Inapparent PV (IPV) due to significant splenomegaly with hypersplenism in the absence of hypervolumic symptoms and the presence of constitutional symptoms related to splenomegaly. The 2015 WHO-CMP classification distinguishes six sequential phenotypes or stages within the JAK2 mutated trilinear MPN: JAK2V617F-positive heterozygous ET, prodromal PV, hetero/homozygous mutated PV, masked PV, and advanced PV with significant splenomegaly and myelofibrosis or inapparent PV (IVP).

**Keywords:** ET

PS-152

Abstract:0244

**THE 1990 HANNOVER BONE MARROW CLASSIFICATION OF MYELOPROLIFERATIVE DISORDERS AND THE EUROPEAN CLINICAL, MOLECULAR AND PATHOLOGICAL (2008 ECMP) CRITERIA FOR CLASSIFICATION AND STAGING OF MYELOPROLIFERATIVE NEOPLASMS:**

J. J. Michiels

*Jan Jacques Michiels*

Bone marrow biopsy (BMB) is pathognomonic clue to accurately distinguish the BCR/ABL negative chronic Myeloproliferative Disorders (CMPD) Essential thrombocythemia (ET) and polycythemia vera (PV) from BCR/ABL positive chronic myeloid leukemia (CML) and ET, and from thrombocythemia associated with myelodysplastic syndromes in RARS-T and 5q-minus syndrome. The modification of PVSG into the 2008 WHO, and the European Clinical Molecular and Pathological (2008 ECMP) classifications agree upon the diagnostic criteria for JAK2 mutated PV. The 2008 ECMP criteria distinguish three distinct clonal myeloproliferative neoplasms (MPN) of JAK2V617F mutated ET, JAK2 wild type MPL mutated ET and JAK2/MPL wild type ET. The 2008 ECMP criteria could delineate three pre-fibrotic stages JAK2V617F mutated ET as normocellular ET, ET with features of early PV (prodromal PV), and ET with hypercellular megakaryocytic granulocytic myeloproliferation (ET.MGM) and 6 clinical PV stages of important prognostic and therapeutic implication. Spontaneous EEC, low serum erythropoietin (EPO) levels and JAK2 mutations are highly specific for ET, prodromal PV, masked PV and classical PV, but are normal in JAK2 wild type ET and MF. The quantitation of JAK2V617F mutation allele burden plays a key-role in the diagnostic work-up and staging of ET, PV and MF patients. The JAK2V617F mutation allele burden in heterozygous mutated ET is low but higher and high in combined heterozygous - homozygous or homozygous mutated PV and ET.MGM, which is of major prognostic significance on top of pre-treatment bone marrow histopathology. This has important therapeutic implications for the first, second and third line treatment options in prodromal, classical and advanced (masked) PV. JAK2 wild type ET carrying the MPL515 mutation is a separate and distinct MPN entity without features of PV at diagnosis and during follow-up. JAK2/MPL wild type ET associated with primary megakaryocytic granulocytic myeloproliferation (PMGM) is the third distinct MPN entity first defined as chronic granulocytic megakaryocytic myeloproliferation in the 1990 Hannover Bone Marrow classification of CMPD. Myelofibrosis (MF) is not a disease but a secondary response to cytokines released from the clonal granulocytic and megakaryocytic proliferative cells in MPNs of various molecular etiology.

**Keywords:** BMB

PS-153

Abstract:0268

**CYTOGENETIC FOLLOW-UP OF A MYELODYSPLASTIC SYNDROME PATIENT WITH 46,XX,DEL(5)(Q13),DEL(11)(Q23) KARYOTYPE**

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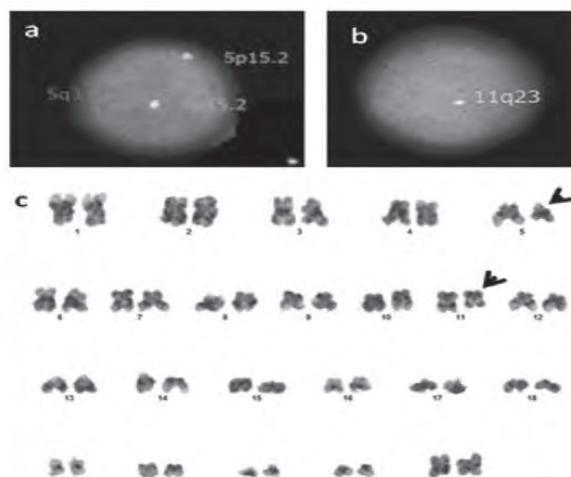
**Objectives:** Interstitial and terminal deletions of the long arm of chromosome 11 are one of the recurrent cytogenetic abnormalities in Myelodysplastic Syndromes (MDS) (1). Although prognostic risk group of the patient with this finding as a sole anomaly is good, it shows variation according to presence with another chromosomal abnormality (>3 abnormalities), and whether the MDS is de novo or secondary. Large deletions including the 11q23 region are commonly reported in acute myeloid leukemia (AML) (2). We aim to report the results of cytogenetic follow-up of a MDS patient with 46,XX,del(5)(q13),del(11)(q23).

**Methods:** A bone marrow sample of 67 years old female patient with pancytopenia and suspicion of MDS was sent to the cytogenetic laboratory of our department. Stimulated and unstimulated, short and long term cultures were set up using appropriate media. Interphase nuclei and metaphase spreads obtained from unstimulated cultures were used for florescent in situ hybridization (FISH) analysis. Five probes (Vysis, IL) were used; LSI EGR1/D5S23, D5S721, LSI D7S486/CEP7, LSI D20S108, CEPD8Z1, LSI MLL (11q23). The patient was started azacitidine 75 mg/m<sup>2</sup> s.c. 7 days every 28 days.

**Results:** We observed del(5)(q31) in 40% of the evaluated cells by FISH analysis. Chromosome abnormalities were detected both on chromosome 5q and chromosome 11q by cytogenetic analysis. It was reported as 46,XX,del(5)(q13),del(11)(q23). We performed FISH to investigate of 11q23 rearrangements. 40% single MLL signal was observed. The clinical and cytogenetic results of patient during follow-up are depicted in the Table.

**Conclusion:** Deletion of chromosome 11q as a sole or with a non-complex karyotype is infrequent in MDS patients. It recently grouped as the cytogenetic very good-risk group according to the IPSS-R (3). Deletion of chromosome 5 especially region of q13 is frequently observed with the deletion of 11q23 region (1). We also were detected the both deletion in our patient without another chromosome abnormality. The patient was accepted in RAEB-2 group as clinically. During the follow-up, we did not observe cytogenetic respond or additional another abnormality. Although, the rate of transformation to AML is 35% in patients with RAEB-2 within two years (4), it was not occurred current patient up to the present. As a consequently, we did not observed any additional prognostic effect of 11(q23) deletion with 5(q13) deletion as a permanent cytogenetic abnormality in current patient.

**Keywords:** Deletion 11(q23), MDS



FISH images of the patient showed deletion on 5q31 (a) and deletion 11q23 (b) regions. Karyotype of the patient showed break points regions of deleted chromosomes at 5(q13) and at 11(q23) (c).

	May 2013	September 2013	May 2014	January 2015
Karyotype	46,XX,del(5)(q13),del(11)(q23)	-	46,XX,del(5)(q13),del(11)(q23)	-
FISH	nuc ish (MLL,EGR1) x180/200	nuc ish (EGR1x1) 30/200, (MLLx1) 20/200	nuc ish (MLL,EGR1) x1 100/200	nuc ish (EGR1x1) 80/200, (MLLx1) 96/200
Hb(g/dL)	9.77	10.2	7.9	7.05
WBC	3 000/μL	3 500/μL	3 000/μL	3 220/μL
Platelet	89.000/μL	60 700/μL	76 000/μL	47 800/μL
Blast ratio (%)	12	10	5	-
Treatment (Azacitidine)	-	After cycle 4	After cycle 7	After cycle 11

The cytogenetic and clinical results of the patient during follow-up.

PS-154

Abstract:0279

**A RARE TYPE OF LEUKEMIA: AGGRESSIVE NATURAL KILLER CELL LEUKEMIA**

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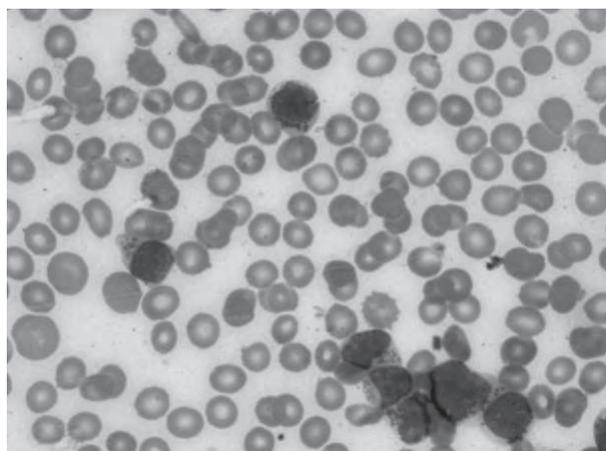
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**Introduction:** Aggressive natural killer cell leukemia (ANKL) is a subgroup of large granular leukemias (LGLs) that has very poor prognosis. ANKL is a disease that gets its roots from neoplastic NK cells. The disease is comparatively common in the far east Asian population. The rate on men and women is about equal. The disease is seen in adults over 40 years of age. Patient has systematic symptoms like fever, night perspiration, weight loss, liver dysfunction, hepatosplenomegaly and sometimes lymphadenopathies can be detected. Serious anemia and thrombocytopenia are common due to bone marrow involvement

or hemophagocytosis. The clinical course is very severe regardless of treatment; therefore most of the patients are lost within weeks or days. ANKL cells are morphologically larger than normal LGLs. There are large asurophilic granules in the pale basophilic cytoplasm. The nucleus contains an immature chromatin pattern. Hemophagocytosis is common. In immunological studies the surface CD3 – CD2 + and CD 56+. CD16 and cytoplasmic CD3s are positive in most cases. The CD122 positive and CD 25 negative status shows that ANKL cells have gotten their root from cytotoxic NK cells. Tumor cells can be EBV positive. Here we report a patient with ANKL, which is very rarely seen in our country.

Case: A 63 year old female patient came to our center with complaints of redness in her right eye, weakness and fever. During her examination she had bulbar conjunctival bleeding, enlarged liver, spleen and lymph nodes. Hb 11.2 gr/dl, WBC 5,360 k/uL, plt 42 000 K/uL, sedimentation 28 mm/h, CRP 75 mg/l, INR 1.2 were detected. Brusella tests were negative. 70% PNL, 9% monocyte and 18% lymphocyte were present in the peripheral blood smear. A high number of LGLs caught our attention. During her follow-up her anemia and thrombocytopenia intensified, her leucocyte count increased from 5000 to 165000 and LGLs were seen in the formula upto 68% (Figure 1). The patient developed obstructive icterus due to enlarged portal lymph nodes. CD 16 and CD 56 positive were detected from peripheral blood. The bone marrow and lymph node biopsies also showed NK cell infiltration (Figure 2,3,4). EBV serology is compatible with a previous infection. The patient had very poor performance was started on a dose of 1mg/kg/day methyl prednisolone and 10 mg/m2/week of methotrexate. After this treatment, the general state of the patient improved, her leucocyte count went back to 9800, her spleen and lymph nodes reduced, her bilirubin values went down. But the patient developed neutropenic fever and unfortunately passed away at the 39th day of the treatment.

LGLs in peripheral blood smear, stained by HE, X100



Discussion: Patients with ANKL are treated with CHOP, hyper CVAD or third generation anthacycline regimens. However the prognosis is very poor despite chemotherapy. It appears that the resistance to chemotherapy is established through p-glycoprotein which is a product of the mdr-1 gene. Hematopoietic stem cell transplantation (HSCT) seems to be the only treatment that could be

curative in the disease. More research is needed on the treatment methods for patients with aggressive course NK cell leukemia.

**Keywords:** NK

PS-155

Abstract:0280

### CYTOTOXIC EFFECT OF KV1.3 POTASSIUM CHANNEL BLOCKER CLOFAZIMINE ON HEMATOLOGICAL CANCER CELL LINES

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Introduction / Aim: Potassium ion channels play an important role in regulating cell proliferation by maintenance of the membrane potential and subsequent Ca<sup>2+</sup> signaling. Out of 80 known human K-channel genes, only the voltage-gated K-channel Kv1.3 and the calcium-gated K-channel KCa3.1 are expressed in lymphocytes, with expression levels varying greatly depending on maturation and activation status. As indicated by previous studies, Kv1.3 is involved in the pathogenesis of various hematological malignancies.

Aim of this study was a) to compare the expression levels of Kv1.3 in various hematological cancer cell lines and b) to determine the cytotoxic effect of a specific Kv1.3 blocker, clofazimine, on Kv1.3-positive and negative cells.

Materials and Methods: HL60 (acute promyelocytic leukemia), Namalwa and Ramos (Burkitt lymphoma), K562 (chronic myelogenous leukemia), Jurkat (T cell leukemia), Cem (Acute lymphoblastic leukemia) and U266 (multiple myeloma) cell lines were used in this study. Expression level of the Kv1.3 was determined using qRT-PCR. Potassium ion flux was measured using a thallium-sensitive fluorescence-based assay (FluxOR, Invitrogen). Cell viability assessment was performed with Alamar blue assay.

Results: Hematological cancer cell lines showed variable Kv1.3 expression levels as determined by qRT-PCR. Cem had the highest expression whereas HL-60 and K562 showed no significant channel expression. Specific Kv1.3 blocker clofazimine inhibited potassium ion flux in Cem and Jurkat cells. As expected, there was no blocking effect on Kv1.3-negative cell line K562. We also observed a dose-dependent cytotoxic effect on both Cem and Jurkat cells but not on K562.

Conclusion: Potassium ion channel Kv1.3 is highly expressed in majority of the tested hematological cancer cell lines. Specific Kv1.3 blocker clofazimine showed cytotoxic effect on Kv1.3-positive cell lines but not on Kv1.3-negative cell line K562. Collectively, these results suggest that clofazimine exerts its cytotoxic effect mainly through Kv1.3 channel.

**Keywords:** Kv1.3, Clofazimine, hematological malignancies

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May 21 – 23, 2015 • Istanbul, Turkey**

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