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ONCOLOGY: RESEARCH ARTICLE

# Association of fludarabin, cytarabine, and fractioned gemtuzumab followed by hematopoietic stem cell transplantation for first-line refractory acute myeloid leukemia in children: A single-center experience

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## Abstract

**Context:** Acute myeloid leukemia (AML) is a rare disease in children, with only 50% to 60% event-free survival. Among patients with AML, 10% do not respond to first-line chemotherapy. There is no recommendation concerning second-line treatments. Gemtuzumab ozogamicin (GO) is a monoclonal antibody targeting CD33, linked to calicheamicin. We report the efficacy and tolerance of a salvage regimen of fludarabin, cytarabine, and GO (FLA-GO) in patients refractory to first-line treatment.

**Methods:** Eight patients (median age 14.5 years), who had more than 2% minimal residual disease (MRD) by flow cytometry (MRD flow), received gemtuzumab 3 mg/m$^2$ on days 1, 4, 7, associated with cytarabine 2000 mg/m$^2$ and fludarabin 30 mg/m$^2$ on days 1 to 5.

**Results:** Six patients achieved complete remission (CR) (blast count morphology $\leq 5 \times 10^{-2}$, CR-MRD flow $< 1 \times 10^{-3}$ for four patients). Five patients received a second course. We observed 11 episodes of febrile neutropenia, including 6 septicemias without complication. There was no fungal infection or toxic death. Two patients received granulocyte colony stimulating factor. One patient had partial platelet recovery; one, prolonged pancytopenia. All patients received hematopoietic stem cell transplantation (HSCT). We observed five mild-to-severe sinusoidal obstruction syndromes during HSCT procedures, particularly in patients who did not receive defibrotide prophylaxis. At the date of last contact (median follow-up: 58 months; range: 22-78), six patients were in continuous CR with negative MRD. Two patients died of post-HSCT relapse.

**Conclusion:** FLA-GO is a good salvage regimen for pediatric refractory AML, with significant but acceptable toxicity. HSCT is mandatory to achieve sustained CR in these patients.

KEYWORDS

gemtuzumab, refractory AML, salvage therapy, AML: molecular diagnosis and therapy, pediatric hematology/oncology, minimal residual disease

## 1 | INTRODUCTION

Acute myeloid leukemia (AML) is a rare disease in children, with only 50% to 60% event-free survival (EFS). Until 2018, first-line therapy in France was applied according to the ELAM02 protocol: patients received cytarabine and mitoxantrone for induction, and high-dose

Abbreviations: AML, acute myeloid leukemia; BU, busulfan; CR, complete remission; CRp, CR with incomplete platelet recovery; CY, cyclophosphamide; EFS, event-free survival; FLA-GO, fludarabin, cytarabine, and GO; FLT3-ITD, Fms-like tyrosine kinase 3-internal tandem duplication; G-CSF, granulocyte colony stimulating factor; GO, gemtuzumab ozogamicin; HLA, human leucocyte antigen; HSCT, hematopoietic stem cell transplantation; LSC, leukemia stem cells; MRD, minimal residual disease; SOS, sinusoidal obstruction syndrome; TKI, tyrosine kinase inhibitor.

  

cytarabine and amsacrine as consolidation. However, under this regimen, 10% of patients did not respond and were considered refractory.[1] There are currently no recommendations concerning second-line treatment, and few therapeutic options are available. Moreover, the small number of patients in the pediatric population renders prospective studies difficult to launch.

Gemtuzumab ozogamicin (GO) is a monoclonal antibody targeting CD33, linked to calicheamicin, a cytotoxic agent. After binding CD33 receptor, the complex is internalized; calicheamicin is released intracellularly and cleaves double-strand DNA. GO was first tested in adults, under inconstant schedules and therapeutic associations. It was proven to be safe in children, with the same side effects than in adults.[2] Its major side effects are myelosuppression with prolonged thrombocytopenia (due to alteration of $CD33^+$ platelet precursors) and sinusoidal obstruction syndrome (SOS), particularly in patients with a history of hematopoietic stem cell transplantation (HSCT).[3] The use of GO was reported in monotherapy or in association with conventional chemotherapy: it is often fractioned to avoid major toxicity.[4,5] The first study in France reporting the use of GO for pediatric refractory or relapsed AML highlighted very heterogeneous practices between centers, which varied in terms of doses, association to chemotherapy, and efficacy.[3] Most salvage regimens only achieved a 30% to 50% success rate.[6]

We report herein the use of GO in a comprehensive cancer center for patients refractory to first-line induction and consolidation according to the ELAM02 protocol. All patients received the same salvage therapy of fludarabin, cytarabine, and GO (FLA-GO) fractioned in three doses of 3 mg/m$^2$, followed by HSCT, a regimen described as showing the best efficacy and tolerance.[7]

## 2 | METHODS

We retrospectively analyzed the records of patients treated in a comprehensive cancer center (IHOPe, Lyon, France) who received FLA-GO regimen for a refractory AML. According to the ELN 2017 recommendations, complete remission (CR) was defined by a cytological blast count inferior to 5%, and CR-MRD flow when CR was confirmed by minimal residual disease (MRD) inferior to $1 \times 10^{-3}$ in flow cytometry.[8] CRp was defined as a CR with incomplete platelet recovery.

MRD multiparametric flow cytometry, based on an eight-color panel, was used: CD13, CD33, CD177, CD7, CD19, CD56, CD34, CD38, HLA-DR (where HLA is human leucocyte antigen), CD123, CD90, CD45-RA, CLL1/TIM3/CD97. The strategy selected for MRD flow was based on the analysis of both leukemia-associated immunophenotype and most immature $CD34^+$ $CD38^-$ leukemia stem cells (LSC).[9,10] FACS CANTO II flow cytometer (Becton Dickinson, San Jose, CA, USA) was used for acquisition of samples and analysis was made using DIVA software (Becton Dickinson).

### 2.1 | Patients

Eight patients (four males, four females) received FLA-GO treatment in the center between 2013 and 2018. Median (range) age at diagnosis was 14.5 years (11.1-17.5). All patients had received first-line treatment according to the ELAM02 protocol (Table 1): induction included cytarabine 1400 mg/m$^2$, mitoxantrone 60 mg/m$^2$, and at least one intrathecal injection of methotrexate, cytarabine, and hydrocortisone.[1] The eight patients had refractory disease after induction, with a median (range) residual blast count of 30% (2-90). They received first consolidation with high-dose cytarabine (18 g/m$^2$) and amsacrine (300 mg/m$^2$), and presented with a median residual blast count of 15% (3-90) after this second treatment course and before GO. The median (range) CD33 expression on blast cells prior to GO treatment was superior to 50% (50%-99%) for all patients (Supporting Information 1).

### 2.2 | Salvage regimen

Patients received the FLA-GO course as follows (Table 2): gemtuzumab 3 mg/m$^2$ over 2 hours on days 1, 4, and 7, associated with cytarabine 2000 mg/m$^2$ over 3 h on days 1 to 5, and fludarabin 30 mg/m$^2$ over 30 min on days 1 to 5.[7]

## 3 | RESULTS

### 3.1 | Efficacy

Of the eight patients treated, five achieved CR with a blast count $\leq 5 \times 10^{-2}$, of whom four had MRD flow $<10^{-3}$. All of them received a second course (with one to three doses of GO 3 mg/m$^2$) to reinforce CR and allow HSCT organization (Table 3).

One patient did not have the criteria for CR due to a lack of hematological recovery, but had MRD $<10^{-2}$ (disease control).

One patient had a residual blast count of 60% and received asparaginase and methotrexate chemotherapy without efficacy. Another patient displayed partial failure with blast count decreasing from 15% to 6% and received subsequent clofarabin, daunoxome, cytarabine chemotherapy, according to a phase I study,[11] associated with ponatinib because of Fms-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD), allowing to finally achieve CR and undergo HSCT (Supporting Information 2).

### 3.2 | Tolerance

Immediate tolerance was acceptable. One moderate allergy occurred after infusion of GO, which resolved after administration of antihistaminic and steroids. Further infusions were well tolerated under premedication. None of the patients experienced SOS following GO. One patient complained of myalgia which was treated using stage 2 painkillers.

After the 13 courses, 11 episodes of febrile neutropenia were observed, among which 6 were documented with septicemia, with no complication after antibiotic treatment. None of the patients needed intensive care. There was no fungal infection or toxic death.

**TABLE 1** First-line treatment according to ELAM02 protocol

| **Induction** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Day** | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| Cytarabine continuous injection 200 mg/m$^2$/d | X | X | X | X | X | X | X |
| Mitoxantrone 12 mg/m$^2$ | X | X | X | X | X | | |
| Intrathecal methotrexate, dexamethasone, cytarabine | X | | | (X)[a] | | | (X)[b] |
| **Consolidation 1** | | | | | | | |
| **Day** | **1** | | | **2** | | | **3** |
| Cytarabine 3 g/m$^2$/12 h | XX | | | XX | | | XX |
| Amsacrine 100 mg/m$^2$/d | X | | | X | | | X |

[a]If central nervous system involvement.
[b]If M4, M5, leucocytes ≥50 G/L.

**TABLE 2** Treatment protocol

| **Day** | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
|---|---|---|---|---|---|---|---|
| Gemtuzumab 3 mg/m$^2$ | x | | | x | | | x |
| Cytarabine 2000 mg/m$^2$ | x | x | x | x | x | | |
| Fludarabin 30 mg/m$^2$ | x | x | x | x | x | | |

### 3.3 | Hematological recovery

Two patients received granulocyte colony stimulating factor (G-CSF) because of prolonged neutropenia and/or septicemia. Neutrophil recovery (count > 0.5G/L) and platelet recovery (count > 100 G/L) occurred at median day 34.

Two patients were in CRp, and the following course started before their platelet count achieved 100 G/L. One of them did not show hematological recovery after FLA-GO, displaying prolonged neutropenia and thrombocytopenia despite G-CSF and transfusions. HSCT conditioning started at day 70 without hematological recovery, with MRD detectable at 0.3%.

One patient had prolonged pancytopenia due to treatment failure and persistent blast infiltration.

The other five patients had complete hematological recovery before day 40.

### 3.4 | Outcome

The eight patients received HSCT: three from sibling donors, four from bone marrow unrelated donors, and one from an unrelated cord blood. AML status before conditioning was: CR-MRD flow $<10^{-4}$ for three patients, $<10^{-3}$ for two patients, $\leq 10^{-2}$ for two patients, and partial response (15%) for one patient with refractory disease. The median (range) time between the last dose of GO and the beginning of conditioning was 44 days (26-73).

Six of the patients received myeloablative conditioning with busulfan (BU) and cyclophosphamide (CY). Two patients received reinforced conditioning: clofarabin, cytarabin, BU, and CY for the refractory AML patient with persistent blastosis (15%), and total body irradiation, fludarabin, CY, and rituximab for anti-HLA allo-immunization for the other patient. Prevention of graft versus host disease was managed using cyclosporine, and anti-lymphocyte globulin for patients receiving nonfamilial HSCT. Two patients with FLT3-ITD received ponatinib as maintenance therapy during six months (starting at day 30 post-HSCT).

Five patients received SOS prophylaxis with defibrotide and ursodesoxycholic acid from days 1 to 21. Three presented mild-to-moderate SOS according to the European Society for Blood and Marrow Transplant criteria.[12] One had cerebral bleeding under defibrotide, leading to treatment interruption. Three patients only received ursodesoxycolic acid prophylaxis: one presented with moderate SOS and received defibrotide for 8 days, and one had very severe SOS, requiring hemodialysis and intensive care during 10 days. There was no toxic death during HSCT procedure.

At the date of last contact, six patients were in continuous CR, with a median follow-up of 58 months (22-78). All of them had complete chimerism, with MRD-flow undetectable at last marrow evaluation. Two patients presented post-HSCT relapse, after 1 and 5 months respectively, including the refractory patient who had not achieved CR after FLA-GO or at the time of HSCT (Figure 1).

## 4 | DISCUSSION

We report eight pediatric patients treated with FLA-GO followed by HSCT for refractory AML after induction and consolidation according to the ELAM02 protocol. Five of them achieved CR due to this regimen and one achieved disease control. Six are in continuous CR after HSCT. This regimen seems to constitute an interesting salvage therapy, with better efficacy than other reported protocols.

We chose to adapt an FLA regimen with GO without using anthracyclines since the patients had already received anthracyclines during previous treatment courses and were supposed to then undergo HSCT: the aim was to limit cardiac toxicity before conditioning. We chose to

**TABLE 3** Patient characteristics, treatments, and outcomes

| Sex age | AML characteristics | MRD before GO (%) | % CD33 on blasts before GO | Complications | MRD after GO (%) | Supplementary course | Complications | HSCT | Conditioning | Defibrotide | SOS (EBMT criteria) | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M 14 | M5, no cytogenetic or molecular alteration | 15 | 50 | FN | <0.1 | FLA + GO (3 × 3 mg/m$^2$) | FN | Unrelated 10/10 | BU CY | Preventive 21 days | 0 | CR 6.5 years post-HSCT |
| M 15 | M2, FLT3-ITD | 90 | 99 | Septicemia *Pseudomonas aerugi-nosa*,pancytopeni | 60 | Methotrexate asparaginase | | Unrelated 9/10 | Clofarabin cytarabine BU CY | Preventive 31 days | Moderate | Relapse at D30 post-HSCT, died |
| F 14 | M1, FLT3-ITD | 15 | 75 | | 6 | Clofarabin, aracytine, daunoxome, ponatinib | | Sibling | BU CY | Preventive 22 days | 0 | Maintenance ponatinib 6 months. CR 5.5 years post-HSCT |
| F 15 | M2, MLL rearrangement, FLT3-ITD, IDH2 mutation | 80 | 95 | Septicemia *Granulicatella adia-cens*,myalgia, G-CSF | <0.1 | FLA + GO (2 × 3 mg/m$^2$) | FN allergic reaction | Double-cord 4/6 + 5/6 | Fludarabin CY TBI | Preventive 21 days | Moderate, cerebral bleeding under defibrotide | CR 5 years post-HSCT |
| M 17 | M1 t(6;9) | 2 | 50 | Septicemia *Staphylococcus epidermidis* | 0.08 | Fludarabin + GO (2 × 3 mg/m$^2$) | | Unrelated 10/10 | BU CY | Preventive 18 days | Mild | CR 4.5 years post-HSCT |
| M 11 | M4eo, FLT3-ITD | 3 | 85 | FN G-CSF, prolonged pancytopenia | 0.5 | No | | Sibling | BU CY | Curative 8 days | Moderate | Maintenance ponatinib 6 months. CR 3.7 years post-HSCT |
| F 11 | M4, MLL duplication | 15 | 74 | Septicemia *Enterococcus fecalis* + *Staphylococcus epidermidis*.CRp | 1 | FLA + GO (2 × 3 mg/m$^2$) | Septicemia *Staphylococ-cus haemolyticus* | Unrelated 9/10 | BU CY | No | 0 | Relapse 5 months post-HSCT, died |
| F 16 | M4, IDH2 mutation | 3 | 51 | Septicemia *Streptococcus mitis* | 0.03 | FLA + GO (1 × 3 mg/m$^2$) | FN | Sibling | BU CY | Curative 21 days | Very severe, hemodialy-sis | CR 1.8 year post-HSCT |

Abbreviations: AML, acute myeloblastic leukemia; BU, busulfan; CNS, central nervous system; CR, complete remission; CY, cyclophosphamide; F, female; FN, febrile neutropenia; FLT3-ITD, Fms-like tyrosine kinase 3 internal tandem duplication; GO, gemtuzumab ozogamicin; HSCT, hematopoietic stem cell transplantation; IDH, isocitrate dehydrogenase 2; M, male; MLL, mixed-lineage leukemia; MRD, minimal residual disease (blast count on bone marrow); SOS, sinusoidal obstruction syndrome; TBI, total body irradiation.

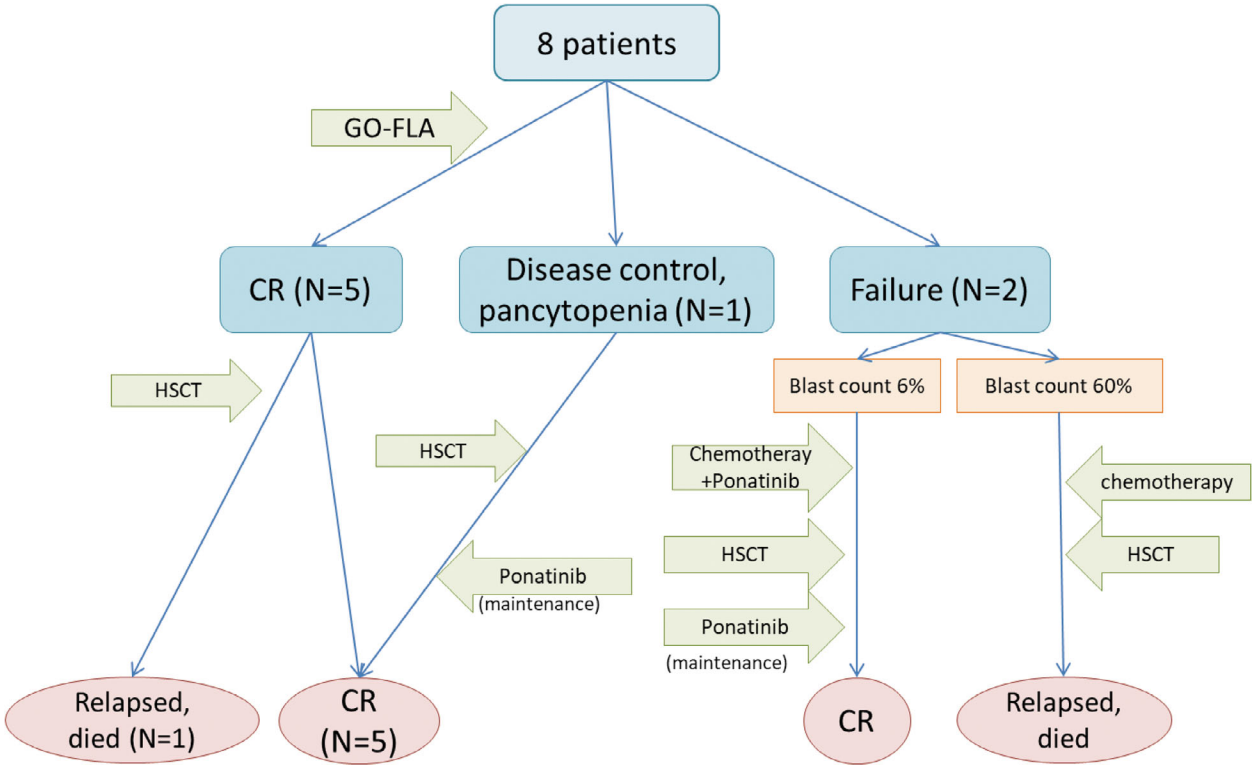


**FIGURE 1** Outcome of patients after FLA-GO course

give G-CSF only to patients with significant toxicity, based on a randomized study showing an increased incidence of relapse in children with AML with G-CSF receptor isoform IV overexpression.[13]

For patients who responded to the regimen but needed another course of chemotherapy due to HSCT organization, the same regimen was given. The first patient received three other doses of GO, and the other patients received only one or two doses, depending on their MRD flow and previous tolerance to GO. This choice was done with the aim of limiting GO toxicity and risk of SOS because they underwent HSCT as soon as possible after neutrophil recovery to maintain high-dose intensity. The results herein suggest that when two or more courses are needed, reducing the number of GO doses might help limit the risk of post-HSCT SOS.

Interestingly, the combination of ponatinib with chemotherapy allowed achieving CR for a patient with refractory disease after FLA-GO. Two patients received ponatinib as maintenance therapy for 6 months following HSCT and were in continuous CR with MRD-flow undetectable and complete chimerism at last follow-up. At the time of the study, none of the tyrosine kinase inhibitors (TKI) were approved for refractory AML in children: we chose ponatinib because of promising previous studies, and its off-label availability in France.[14,15] Since then, midostaurin, described as a multitargeted kinase inhibitor, which significantly improves the prognosis of patients with FLT3-ITD, has been approved for adults in combination with chemotherapy.[16] Gilteritinib, a selective FLT3 inhibitor, has also been recently approved in adult patients.[17] Other TKI, such as quizartinib and crenolanib, are currently being tested in children in ongoing phase I and II trials.[18] However, the best TKI for pediatric AML remains to be identified.

This work is observational, single-centered, and retrospective. This allowed the reporting of a homogeneous population, with comparable patients. The first study reporting the use of GO in France highlighted very heterogeneous practices between centers: the 12 patients had received 5 different schemes of administration.[3] Relapsing patients receiving GO were not studied herein, as it seems that prognosis and responses are different between relapse and refractory AML, with poorer prognostic in refractory patients.[19] Although the present study included only eight patients, it is important to note that most salvage therapies only achieve 30% to 50% of overall response, and a median overall survival of 1 year.[19]

Most patients herein experienced grade IV hematological toxicity, but were hospitalized in a protected unit during treatment. Six septicemias were observed, which is not uncommon after intensive chemotherapy associated with GO. Most patients achieved a normal blood count before day 44, and only three patients had incomplete marrow recovery including one refractory patient. One patient did not show hematological recovery and, because MRD was detectable at 0.3%, received HSCT with persistent pancytopenia. This patient achieved CR after HSCT and underwent a maintenance regimen using ponatinib. Overall infectious and hematological toxicity related to this combination of chemotherapy with GO is significant. However, given the very poor prognosis for patients with refractory AML, we considered it acceptable, since all the salvage regimens have shown a high risk of toxicity on previously heavily treated patients.[3,6]

None of the reported patients presented SOS after FLA-GO course, but five had mild to very severe SOS after HSCT. Only one patient had very severe SOS requiring hemodialysis, but showed completely recovery within 1 month. In order to maintain dose intensity, all patients received HSCT as early as possible following GO treatment (median time 44 days), which is a risk factor for SOS. However, pediatric patients often present little comorbidity, and can afford heavy chemotherapies. Moreover, HSCT remains the only curative treatment for refractory AML.[20] The present data are in favor of the use of preventive defibrotide during HSCT procedure in patients who previously received more than 9 mg/m$^2$ of GO, consistent with previous studies showing that defibrotide reduces the severity of SOS.[21]

The first randomized trial testing the addition of GO to first-line chemotherapy in children reported significant improvement of EFS with GO, by reducing the relapse risk,[22] with acceptable cost impact.[23] This improvement was stronger in low- and intermediate-risk patients. The randomized protocol MyeChild has also been launched to evaluate the efficacy and tolerance of GO in combination with conventional chemotherapy for first-line treatment.[24] The results herein show that GO can also be used in combination with chemotherapy in patients with refractory disease, without major toxicity, except for post-HSCT SOS which should be prevented and closely monitored.

Previous studies showed that GO treatment does not show any benefit in patients with low CD33 expression and that it should be used preferentially in patients with high expression of CD33.[25] In the present cohort, all patients had a CD33 expression higher than 50%, including the two patients who failed to achieve CR under GO. CD33 expression in most immature CD34$^+$ CD38$^-$ LSC needs to be evaluated in a larger cohort of patients and correlated to GO efficacy.

Larger multicenter studies are required to homogenize practices and better characterize the regimen's efficacy and toxicity, in order to improve salvage treatment for refractory patients and provide recommendations.

## 5 | CONCLUSION

Association of FLA-GO is a good salvage regimen for pediatric refractory AML, with significant but acceptable toxicity. HSCT is mandatory to achieve sustained CR in such patients. Prospective studies and long-term follow-up would help evaluate efficacy and characterize long-term toxicity, which is currently barely known. Homogenization of practices and sharing of data is desirable, in order to improve patient prognosis.

### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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