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Genome Sequencing as an Alternative to Cytogenetic Analysis in Myeloid Cancers

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Abstract

BACKGROUND—Genomic analysis is essential for risk stratification in patients with acute myeloid leukemia (AML) or myelodysplastic syndromes (MDS). Whole-genome sequencing is a potential replacement for conventional cytogenetic and sequencing approaches, but its accuracy, feasibility, and clinical utility have not been demonstrated.

METHODS—We used a streamlined whole-genome sequencing approach to obtain genomic profiles for 263 patients with myeloid cancers, including 235 patients who had undergone successful cytogenetic analysis. We adapted sample preparation, sequencing, and analysis to detect mutations for risk stratification using existing European Leukemia Network (ELN) guidelines and to minimize turnaround time. We analyzed the performance of whole-genome sequencing by comparing our results with findings from cytogenetic analysis and targeted sequencing.

RESULTS—Whole-genome sequencing detected all 40 recurrent translocations and 91 copy-number alterations that had been identified by cytogenetic analysis. In addition, we identified new clinically reportable genomic events in 40 of 235 patients (17.0%). Prospective sequencing of samples obtained from 117 consecutive patients was performed in a median of 5 days and provided new genetic information in 29 patients (24.8%), which changed the risk category for 19 patients (16.2%). Standard AML risk groups, as defined by sequencing results instead of cytogenetic analysis, correlated with clinical outcomes. Whole-genome sequencing was also used to stratify patients who had inconclusive results by cytogenetic analysis into risk groups in which clinical outcomes were measurably different.

CONCLUSIONS—In our study, we found that whole-genome sequencing provided rapid and accurate genomic profiling in patients with AML or MDS. Such sequencing also provided a greater diagnostic yield than conventional cytogenetic analysis and more efficient risk stratification on the basis of standard risk categories. (Funded by the Siteman Cancer Research Fund and others.)

GENETIC PROFILING IS A ROUTINE COMponent of the diagnostic workup for an increasing number of cancers and is used to predict clinical outcomes and responses to targeted therapies. Mutations that are clinically actionable for any individual type of cancer typically span a

wide range of genomic events, including chromosomal rearrangements, gene amplifications and deletions, and single-nucleotide changes. The diversity of these findings necessitates the use of multiple platforms to obtain the genetic information needed for clinical management. Whole-genome sequencing is an unbiased method of detecting all types of mutations[1] and could potentially be used to replace current testing algorithms. Such sequencing can also be performed on a limited amount of DNA and can identify genomic changes that may be cryptic in other types of analyses.[2] These features of whole-genome sequencing suggest that it could improve genomic profiling in patients with cancer.

Genomic abnormalities are particularly important for diagnostic classification and risk assessment in patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). Recurrent chromosomal abnormalities are the basis for the AML genomic classification system of the World Health Organization, and the association of these alterations and certain genetic mutations with clinical outcomes[3-7] has led to the development of algorithms for genetic risk stratification in patients with AML.[3,8] Similar studies involving patients with MDS have resulted in the cytogenetic component of the International Prognostic Scoring System–Revised (IPSS-R) in such patients.[9] Although advances in sequencing technology have improved the ability to identify genetic mutations, the detection of chromosomal rearrangements is primarily performed through conventional metaphase cytogenetic analysis (i.e., karyotyping). The latter approach is effective but has several limitations, including the need to obtain viable cells, low sensitivity, and limited resolution. Fluorescence in situ hybridization (FISH) and targeted sequencing assays that use DNA, RNA, or both are also used, but these methods are informative only in the regions selected for analysis and may provide incomplete information regarding identified chromosomal rearrangements. As a result, conventional cytogenetic analysis remains an essential component of the diagnostic workup for patients with AML or MDS.[3,8]

The importance of genetic profiling in such patients and the variety of clinically relevant mutation types suggest that whole-genome sequencing could be used in place of standard testing approaches. Although the high cost of sequencing and complex, time-consuming analysis methods have historically restricted such sequencing to research studies,[10-16] recent advances have made this analysis simpler to perform, faster, and less expensive. In this study, we developed a streamlined approach to whole-genome sequencing for genomic profiling of patients with AML or MDS and applied it to diagnostic clinical samples in real time to evaluate its feasibility, accuracy, and utility in the clinical setting.

METHODS

PATIENTS

This study was approved by the institutional review board at Washington University in St. Louis and was conducted in accordance with the provisions of the Declaration of Helsinki. We obtained all the samples that were included in this study from patients with a known or suspected diagnosis of AML or MDS who were seen at the Washington University School of Medicine. All the patients provided written informed consent for genomic sequencing studies. Retrospective samples were obtained from cryopreserved diagnostic bone marrow or peripheral-blood specimens. Prospective samples were obtained from fresh bone marrow

aspirate or peripheral-blood specimens collected from consecutive, unselected patients for whom clinical cytogenetic analysis by means of karyotyping had been requested from May 2019 through February 2020.

CONVENTIONAL CYTOGENETIC AND MOLECULAR ANALYSIS

All cytogenetic and FISH analyses were performed in the Cytogenomics and Molecular Pathology Laboratory at the Washington University School of Medicine according to standard clinical protocols. We obtained data regarding genetic mutations as part of standard diagnostic testing using polymerase-chain-reaction (PCR)–based assays for the internal tandem duplication mutation in *FLT3* (*FLT3*-ITD) and the *NPM1c* mutation, a laboratory-developed clinical sequencing assay, or both. (Details regarding the genetic mutations is provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.) Cytogenetic and molecular results were used to assign patients to established European Leukemia Network (ELN) or IPSS-R risk categories.[3,9]

GENOME SEQUENCING

We processed samples and performed sequencing to a target coverage depth of 60× in the Clinical Sequencing Laboratory at the McDonnell Genome Institute of Washington University in St. Louis, a laboratory that has been licensed according to the Clinical Laboratory Improvement Amendments of 1988. This analysis involved the identification of mutations in 40 genes,[17] genomewide copy-number alterations greater than 5 Mbp, and structural variants matching 612 recurrent structural alterations in myeloid cancers. (Details regarding genetic identification and structural variants are provided in Tables S1 and S2 in the Supplementary Appendix.) We used the results of whole-genome sequencing to assign patients to a genetic risk group through the same classification systems that are used for conventional analyses.

CONFIRMATORY STUDIES

We used FISH, PCR, chromosomal microarray analyses, and RNA-sequencing data to confirm findings on whole-genome sequencing that had not been detected by cytogenetic analysis. We used standard protocols to perform chromosomal microarray analysis in the Washington University Cytogenetics Core. In the PCR-confirmation analyses, we used primers designed to detect structural variant breakpoints. The methods that were used in RNA sequencing for structural variants have been reported previously.[11]

STATISTICAL ANALYSIS

In the time-to-event survival analysis involving study patients with AML, we used death as the end point for the Kaplan–Meier analysis or Cox proportional-hazards regression to test for equal survival across genetic risk groups. Additional details regarding the methods that were used in the statistical analysis are described in the Supplementary Appendix.

RESULTS

STREAMLINED APPROACh TO WhOLE-GENOME SEQUENCING

We developed a streamlined approach to whole-genome sequencing (ChromoSeq) that was designed to provide comprehensive genomic profiling of clinically relevant mutations in samples obtained from patients with AML or MDS, while minimizing the turnaround time and technical complexity (Fig. 1A). In this approach, we used scalable methods of sample preparation that can be performed by a single technician in less than 8 hours with commercially available reagents, followed by standard high-throughput sequencing. Automated tumor-only variant analysis detected mutations in selected genes, copy-number alterations of more than 5 Mbp, and recurrent structural variants[18,19] (Tables S1 and S2). We then summarized these findings in a concise clinical report (Fig. S1A and S1B).

We performed a head-to-head comparison of this approach with conventional cytogenetic analysis and targeted sequencing using 235 samples obtained from patients with a known or suspected hematologic cancer who had undergone successful cytogenetic analysis (Table 1, Fig. 1B, and Table S3). This sequencing analysis yielded a mean genome coverage of 50×; a mean of 5.1 clinically relevant mutations (range, 0 to 20) were detected per patient across all variant types (Fig. S1C and S1D). The sensitivity of whole-genome sequencing for recurrent translocations that had been reported on cytogenetic analysis was 100% (40 of 40 samples) (Fig. 2A).

Whole-genome sequencing identified cytogenetically cryptic structural variants in 13 patients, including complex or cryptic chromosomal translocations involving the inv(16)(p13.1q22) fusion gene *CBFB–MYH11* in 2 patients, the t(7;21)(p22;q22) fusion gene *USP42–RUNX1* in 1 patient, and 10 rearrangements involving *KMT2A*, all of which were verified with the use of orthogonal methods (Fig. 2B and Fig. S2A, Table S4, and Section 1.5 in the Supplementary Appendix). Whole-genome sequencing detected 100% (91 of 91) of the clonal copy-number alterations that had been detected on cytogenetic analysis among the 143 patients in whom conclusive and unambiguous results had been identified by karyotyping (Fig. 2A). In addition, sequencing identified 21 new copy-number alterations in 14 of these patients, 12 of which were confirmed by other methods (Fig. 2C, Table S5, and Fig. S2B). The remaining 9 new copy-number alterations showed altered coverage patterns on whole-genome sequencing but could not be confirmed by orthogonal methods because of their small size, low abundance, or both (Fig. 2C, Fig. S2C, and Table S5). Whole-genome sequencing also provided definitive identification of copy-number alterations in an additional 13 patients with ambiguous or inconclusive results by cytogenetic analysis (Table S5). When we combined these results with the findings in 14 patients who had conclusive results by cytogenetic analysis and newly identified copy-number alterations, plus the findings in 13 patients who were identified as having new structural variants, we determined that 40 of 235 patients (17.0%) had results that had not been detected by conventional cytogenetic analysis.

In a comparison of genetic mutations that were identified on whole-genome sequencing with those that were identified on high-coverage (>500×) targeted clinical sequencing involving 102 patients, we found sensitivities of 84.6% for single-nucleotide variants and 91.5% for

insertion–deletion (indel) mutations, along with a positive predictive value of more than 99% for variants with a minimum variant allele fraction of 5% (Fig. 2A and Table S6). Similar performance was observed when considering only mutations in genes necessary for risk stratification in patients with AML, including a combined sensitivity of 87.5% for single-nucleotide variants and indels in *ASXL1*, *CEBPA*, *FLT3*, *NPM1*, *RUNX1*, and *TP53* (Fig. S2D and S2E). False negatives occurred either because the variants were in subclones or were at low coverage positions on whole-genome sequencing (Fig. S2F and S2G); such variants were more readily detected with higher coverage sequencing (Fig. S2H).[20]

CLINICAL FEASIBILITY AND DIAGNOSTIC YIELD

We evaluated the feasibility of using whole-genome sequencing for routine clinical testing by prospectively sequencing samples obtained from 117 consecutive patients (Table S7). For this cohort, whole-genome sequencing was performed in weekly batches with a median batch size of 4 (range, 1 to 11) with the use of bone marrow aspirate samples submitted for karyotyping and FISH studies from April 2019 through February 2020. The median total processing time was 5.1 days, which included 2 days for library preparation, 2 days for sequencing, and less than 1 day for analysis (Fig. 3A). The shortest times were about 3 days (approximately 78 hours), when clinical laboratory staffing allowed samples to be sequenced in dedicated sequencing runs immediately after library generation. Sequencing was successful in all the samples, and only 5 samples (4.3%) had less than 25× genome coverage in a single assay run. Seven samples required manual review of the automated copy-number alteration calls, with the remaining 110 samples (94.0%) needing no additional interventions to finalize the sequencing report.

This set of consecutive patients was also evaluated to estimate the diagnostic yield from whole-genome sequencing as compared with testing with cytogenetic analysis and targeted sequencing. This analysis was performed separately in samples obtained from patients with AML and in those obtained from patients with MDS. (Seven patients with other diagnoses were excluded from this analysis.) In the AML samples, the comparisons included clinical results from a standard FISH panel[3,21] along with cytogenetic analysis and targeted sequencing to provide a realistic estimate of the expected yield of whole-genome sequencing. In this prospective cohort, results from conventional cytogenetic analysis and FISH assays in the 68 patients with AML resulted in the diagnosis of acute promyelocytic leukemia with the fusion gene PML–RARA in 5 patients and in the assignment of 27 patients to the adverse-risk group, 10 to the intermediate-risk group, and 19 to the favorable-risk group on the basis of established guidelines[3,8]; 7 patients had unsuccessful or inconclusive results on cytogenetic analysis and could not be assigned to a risk group (Table S7). Four patients were assigned to risk groups solely on the basis of positive FISH results for either *PML–RARA* (1 patient) or del(5q) (3 patients) (Fig. 3B).

Whole-genome sequencing that was performed on samples obtained from the same cohort identified new abnormalities that were not present in the karyotype analysis or reported by FISH in 17 of 68 patients (25%). These abnormalities included cryptic or complex chromosomal rearrangements in 5 patients, new copy-number alterations that resulted in a complex karyotype in 4 patients, and identification of either a normal karyotype (in 4

patients) or 1 or 2 cytogenetic abnormalities in patients with inconclusive or unsuccessful results by cytogenetic analysis (in 4 patients) (Table S8). Using data only from whole-genome sequencing and a PCR assay for *FLT3*-ITD, we reclassified 10 of 68 patients (15%) without acute promyelocytic leukemia to a risk group that differed from the one that was based on conventional testing (Fig. S3A). A similar yield was observed for the 42 prospective patients with MDS; of these patients, 12 (29%) had inconclusive results on cytogenetic analysis or new findings on whole-genome sequencing and 9 (21%) were assigned to a new IPSS-R risk category (Fig. S3B and Tables S7 and S8). These findings bring the combined number of patients with a reclassified risk-group assignment to 19 of all 117 patients (16.2%) who were included in this prospective cohort.

PREDICTIVE VALUE USING EXISTING GENETIC-RISK CATEGORIES

We next asked whether whole-genome sequencing could be used in place of cytogenetic analysis to predict clinical outcomes using existing genetic risk groups. To avoid the confounding effect of hematopoietic stem-cell transplantation on outcome, we focused our analysis on 71 patients with AML who did not undergo this procedure, including 41 prospective and 30 retrospective patients; 58 patients (82%) received intensive induction chemotherapy, whereas the remaining 13 were treated with hypomethylating agents. These patients were assigned to a genetic risk group on the basis of whole-genome sequencing alone or conventional testing (the combined results of cytogenetic analysis, clinical FISH results, and targeted sequencing). The *FLT3*-ITD mutational status that was based on a PCR assay was used in both these classifications.

Risk-group assignments that were based on conventional testing were in agreement with the results from whole-genome sequencing for 63 of 71 patients (89%); 8 patients were reassigned to a different risk category, including 5 who had new adverse-risk findings that were identified by whole-genome sequencing (Table S9 and Fig. S4A). Risk groups that were defined according to the two methods had the expected associations with overall survival (adjusted P=0.09 by log-rank test in groups identified by conventional testing; adjusted P=0.01 by log-rank test in groups identified by whole-genome sequencing) (Fig. 4A and 4B). Whole-genome sequencing provided slightly better identification of patients with adverse risk and poor outcomes than conventional testing, with a hazard ratio for death of 0.32 (95% confidence interval [CI], 0.11 to 0.92) on age-adjusted Cox regression analysis, as compared with a hazard ratio of 0.66 (95% CI, 0.17 to 1.05) by conventional risk-group analysis. Similar results were observed in a larger cohort of 101 patients who were treated with either consolidation chemotherapy or stem-cell transplantation (Table S9 and Fig. S4B and S4C).

We reasoned that whole-genome sequencing could have the greatest benefit for patients for whom cytogenetic results are unavailable at diagnosis, which occurs in up to 20% of patients with AML.[7,22-24] Thus, we used whole-genome sequencing to evaluate 27 patients with AML who were not treated with stem-cell transplantation (of whom 22 received standard induction chemotherapy), who could not be assigned to a risk group at the time of diagnosis because of unsuccessful cytogenetic analysis (in 6 patients), inconclusive results (in 13), or unknown results (in 8), and who had no reports of risk-defining events by FISH (Table S10).

The mean age at diagnosis in this cohort was similar to that of patients with defined cytogenetic risk (60.8 years and 54.7 years, respectively), and the median overall survival was 11.2 months (95% CI, 5.6 to 38.8) (Fig. 4C). In this cohort, whole-genome sequencing identified risk-defining chromosomal abnormalities in 4 patients, including *KMT2A* and *RUNX1–RUNXT1* rearrangements in 1 patient each or a complex karyotype in 2 patients; the remaining 23 patients had either a normal karyotype or one or two abnormalities and were assigned to a risk category on the basis of mutations identified by whole-genome sequencing (Table S10 and Fig. S4D).

Survival analysis of these patients showed that risk predictions that were based on whole-genome sequencing also correlated with outcomes, with significantly longer overall survival in 21 patients with intermediate or favorable risk (median survival, 20.5 months; 95% CI, 5.6 to 38.8) than in 6 patients with adverse risk (median survival, 3.3 months; 95% CI, 1.7 to 18.9; adjusted P = 0.03 by log-rank test) (Fig. 4D); hazard ratio of 0.29 (95% CI, 0.09 to 0.94) by age-adjusted Cox regression analysis. This survival difference was superior to that resulting from the assignment of patients to risk groups on the basis of gene mutations alone (Fig. S4E) and was maintained when 11 additional patients with inconclusive results on cytogenetic analysis who underwent allogeneic stem-cell transplantation were included in this cohort (total of 38 patients) (Table S10 and Fig. S4F).

DISCUSSION

In this study, we demonstrated the clinical utility of whole-genome sequencing for the genomic evaluation of patients with AML or MDS. Results from 263 patients showed that such sequencing was equivalent to or better than conventional testing, both in analytical performance and clinical applicability. Whole-genome sequencing detected 100% of the clinically significant abnormalities that had been identified by cytogenetic analysis and clinical FISH assays. In addition, sequencing provided new genetic information in 25% of patients, more than half of whom would have been assigned to a different genetic risk category with results from conventional testing. In practice, the diagnostic yield of whole-genome sequencing will depend on laboratory-specific karyotyping practices and the use of FISH or other ancillary testing; some rapid diagnostic assays may still be required for urgent treatment decisions (e.g., FISH or quantitative PCR for *PML–RARA* rearrangements and PCR for *FLT3*-ITD mutations). However, our study shows that whole-genome sequencing can provide definitive results for clinically relevant genomic events with the use of a single test. Prospective real-time sequencing of samples obtained from consecutive patients showed that such sequencing yields complete genomic information in a clinically relevant timeframe. This speed was made possible by faster laboratory methods and automated data analysis that focused on clinically relevant mutations, which allowed us to generate reports in as little as 3 days. We also found that such results can be used for risk predictions with existing, clinically validated risk-stratification systems. Although larger studies involving more patients will be required to firmly establish the clinical performance of whole-genome sequencing, our proof-of-concept study shows that this method has the potential to add prognostic value by expanding risk stratification to more patients, especially for those with inconclusive results on cytogenetic analysis, where whole-genome sequencing could have an immediate effect on treatment decisions.

We found that the logistical barriers for the genomic profiling of tumors by whole-genome sequencing can essentially be eliminated with the approaches described here. However, an additional (and major) barrier to implementation has been cost. Unlike other clinical assays in which technical labor is a substantial expense, the cost of whole-genome sequencing is driven nearly entirely by the sequencing itself. As a result, the continued decrease in the price of sequencing[25] now makes the costs associated with this method similar to those of current testing platforms, which are estimated to range from $1,000 to $2,000 per patient.[26] (Details regarding cost comparisons are provided in the Supplementary Appendix.) The current cost of reagents, technical labor, and analysis for whole-genome sequencing with the approach described here is approximately $1,900 on the basis of a list price of approximately $11 to generate 1 billion base pairs of sequence data. In high-volume laboratories where sequencing costs are lower (approximately $7 per Gbp),[25] the cost would be about $1,300. Although the actual charge for clinical whole-genome sequencing will probably be higher owing to the additional costs associated with clinical-laboratory implementation, these calculations suggest that this method is likely to reach price parity with standard testing when sequencing falls below $5 per Gbp. Since sequencing data can also provide additional genetic information that is often obtained by means of other genetic assays (e.g., pharmacogenetic testing or HLA typing), price parity for some patients will come even sooner.

Implementing whole-genome sequencing for clinical testing can provide a unified, stable, and extensible platform that minimizes laboratory-specific bias and that can be standardized throughout the world. Although our study focused on myeloid cancers, many of the advantages of whole-genome sequencing that we observed will directly apply to patients with other cancers. Whole-genome sequencing can be performed on DNA from tissue biopsy samples of solid tumors, which are often insufficient for standard molecular assays and difficult to culture for cytogenetic studies. The benefits could be even greater for these cancer types, in which whole-genome sequencing could be used to rapidly survey the entire genome for an expanding number of key mutations and structural alterations with only a small amount of DNA. Such an approach would simplify genomic testing for these patients and probably increase the yield of clinically relevant findings, which may ultimately improve the precision of approaches for treating many patients with cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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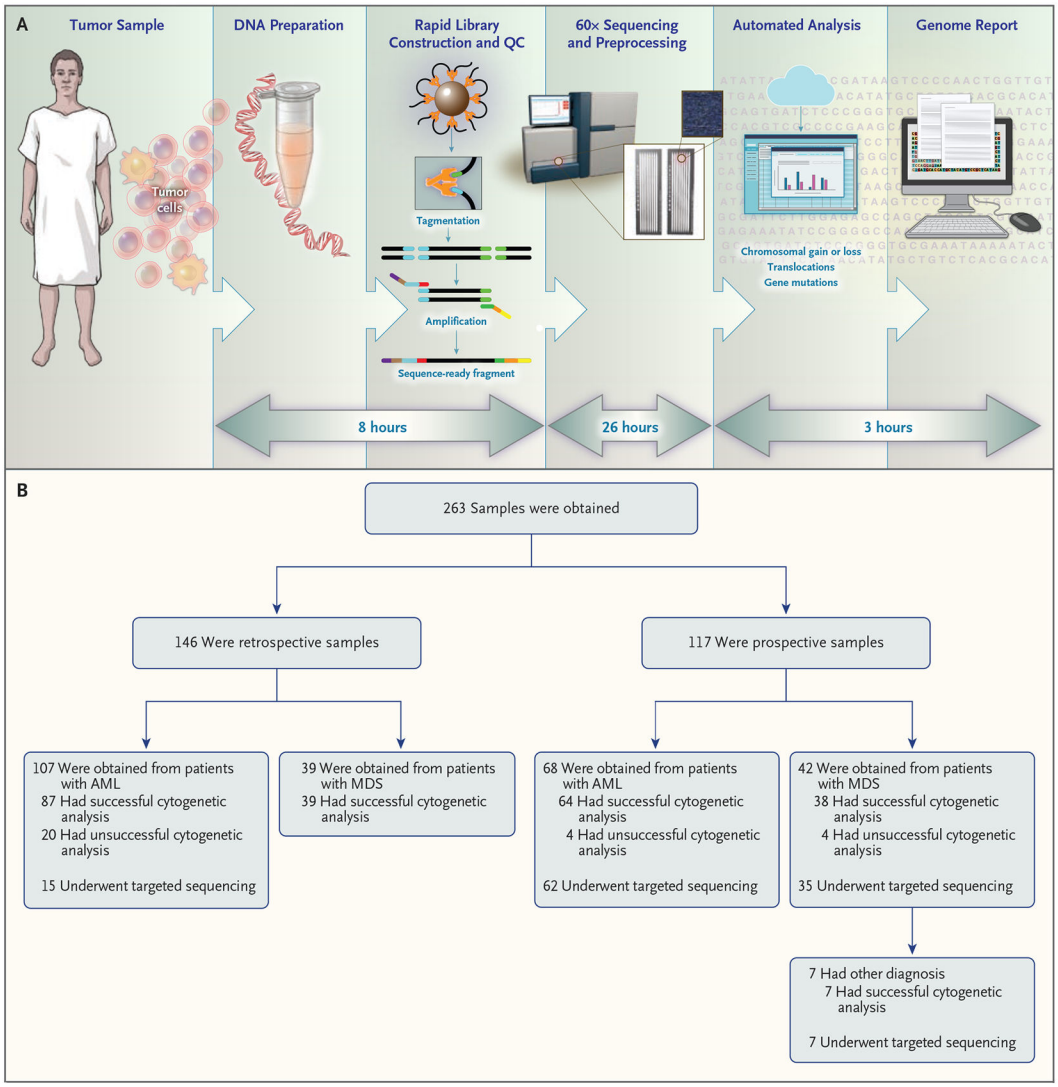

Figure 1. Timeline of Whole-Genome Sequencing (WGS) Process and Study Design.

Panel A shows the workflow and approximate processing time for each step of the rapid WGS method used for samples obtained from the study patients. As the first step in library construction, unfragmented DNA is cleaved and tagged for analysis in a process called tagmentation. Examples of the reports that were generated by this process are provided in Figures S1A and S1B in the Supplementary Appendix. Panel B shows the design of the study involving both retrospective and prospective cohorts of patients with acute myeloid leukemia (AML) or myelodysplastic syndromes (MDS). The retrospective cohort included 146 samples obtained from individual patients selected to represent a broad range of cytogenetic and molecular features of AML and MDS. The prospective cohort included 117 unselected, consecutive samples obtained from patients with a known or suspected myeloid cancer for whom cytogenetic testing was requested at the study center. Seven of these patients ultimately received a diagnosis other than AML or MDS. QC denotes quality control.

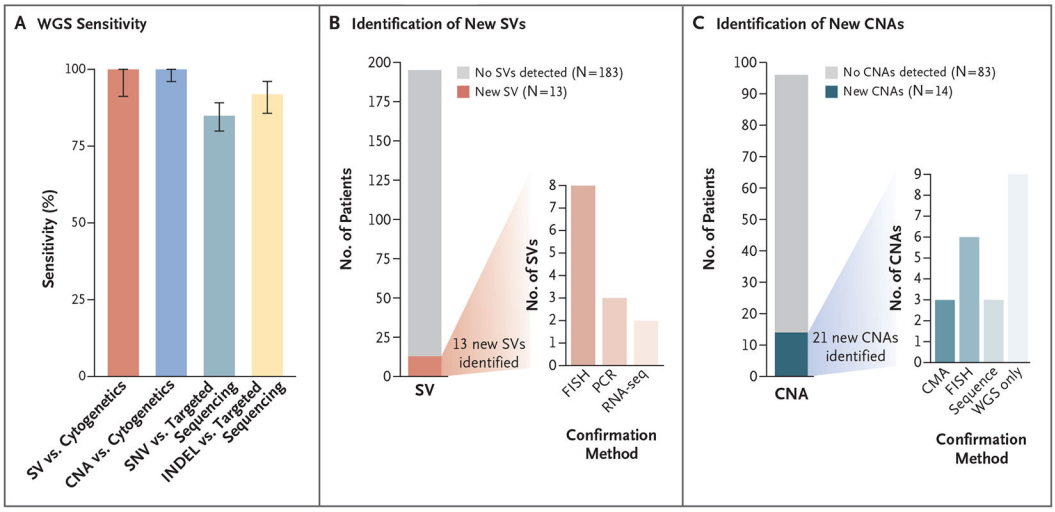

Figure 2. A Comparison of WGS with Conventional Cytogenetic Analysis and Targeted Gene Sequencing.

Panel A shows the sensitivity of WGS for the detection of recurrent structural variants (SVs) and copy-number alterations (CNAs) as compared with conventional cytogenetic analysis and for the detection of single-nucleotide variants (SNVs) and insertion–deletions (INDELs) as compared with high-coverage targeted gene sequencing. I bars denote 95% confidence intervals. Panel B shows the identification and confirmation by WGS of 13 new recurrent SVs that were not detected by conventional cytogenetic analysis, as supported by orthogonal methods, including fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR) with sequencing of SV breakpoints, or detection of fusion transcripts in RNA-sequence (RNA-seq) data. Panel C shows the identification of 21 new CNAs in 14 patients; 12 of these alterations were confirmed by chromosomal microarray (CMA), FISH, or sequence-defined breakpoints. An additional 9 CNAs were identified by WGS only and could not be confirmed by CMA (in 6 patients) or confirmation was not attempted because of the size or abundance of the CNA event (in 3 patients). CNAs were also identified in 13 patients with ambiguous or inconclusive results on cytogenetic analysis. Additional details regarding these comparisons are provided in Tables S4 and S5 and Figure S2C in the Supplementary Appendix.

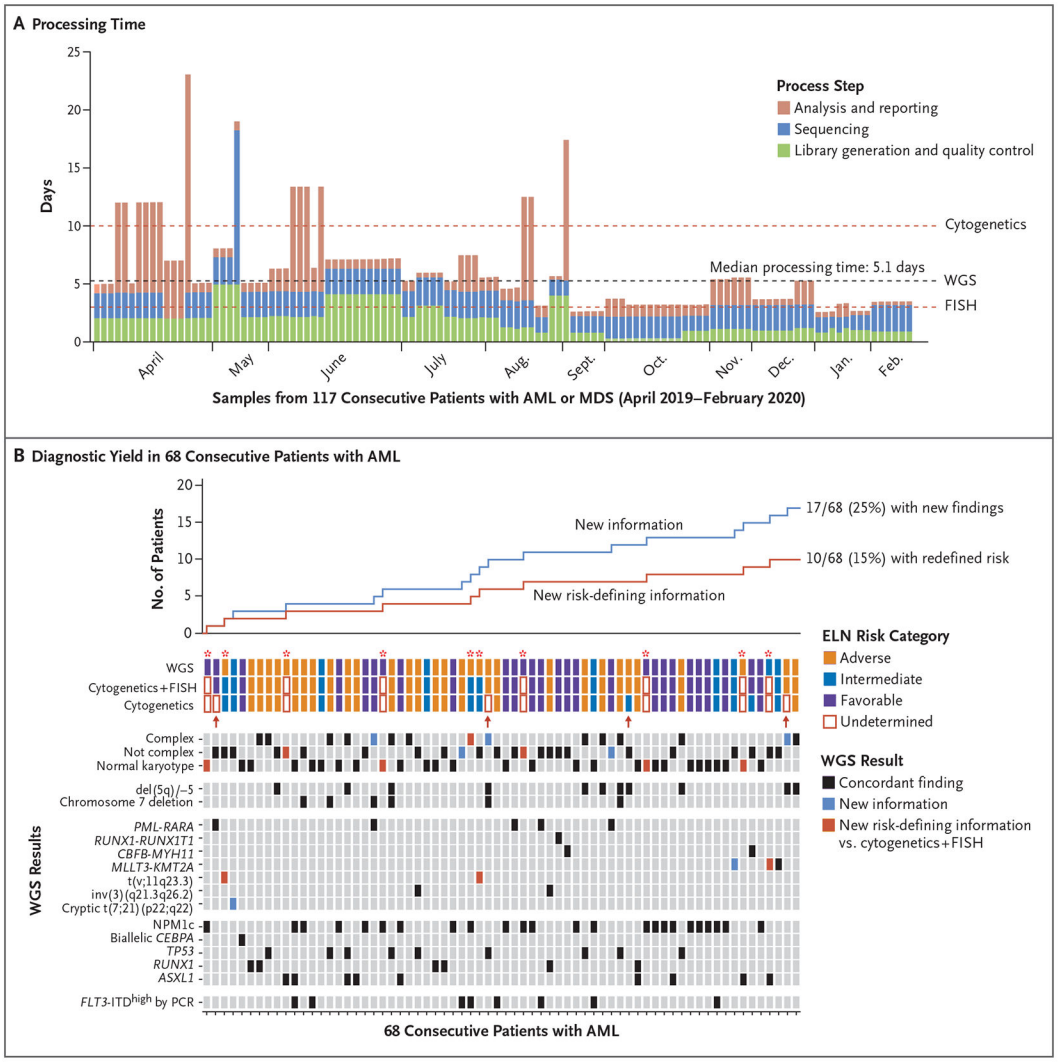

Figure 3 (facing page). Clinical Feasibility and Diagnostic Yield of WGS-Based Genomic Profiling in 117 Consecutive Patients.

Panel A shows the time it took to process samples obtained from 117 consecutive patients with AML or MDS by means of WGS from April 2019 through February 2020. The median processing time for all study patients is indicated by the dashed horizontal black line. The height of each bar shows the total time in days for processing, starting from construction of the sequencing library and ending with completion of the automated final report for an individual patient sample. The duration of each individual step (as obtained from time stamps recorded in the information management system of the clinical laboratory) is indicated by the shaded bar segments and includes the duration of library generation and quality assessment, sequencing, and analysis and reporting. These times reflect the processing time plus waiting time before the next step. Longer turnaround times occurred because of delays between steps, rather than longer processing times. The dashed horizontal red lines show the recommended maximum turnaround time for FISH testing and conventional cytogenetic analysis, according to published recommendations,[21] although shorter turnaround times occur in many laboratories. Panel B shows the yield of new WGS

findings in samples obtained from 68 unselected, consecutive patients with AML. The top panel shows the cumulative number of patients with new genomic findings that were identified by WGS, as compared with conventional cytogenetic analysis or FISH, performed at the time of diagnosis, along with the cumulative number of patients with new events that changed the category of genetic risk group on the basis of established European Leukemia Network (ELN) guidelines.[3] FISH testing included assays for *PML–RARA*, *CBFB–MYH11*, *RUNX1–RUNX1T1*, del(5q), and chromosome 7 deletion, according to recommendations[3,21]; all testing was performed in samples obtained from 60 of 68 patients (88%), and subgroups of these assays were performed for the remaining patients. The results of ELN assignments to a genetic risk group by WGS, conventional cytogenetic analysis with FISH, and cytogenetic analysis alone are shown in the middle panel. The red asterisk indicates that the patient's risk group was reclassified according to the WGS results, and the red arrow indicates that the conventional risk-group assignment was based on FISH results alone. Genomic events that were detected by WGS are shown in the bottom panel and are labeled as concordant with cytogenetic analysis, FISH, or target sequencing (in black), new findings made by WGS (in blue), and new findings that resulted in a change in the ELN genetic risk group (in red). The status regarding internal tandem duplication in *FLT3* (*FLT3*-ITD) and the allele ratio as determined by PCR were used for both conventional and WGS-based risk stratifications.

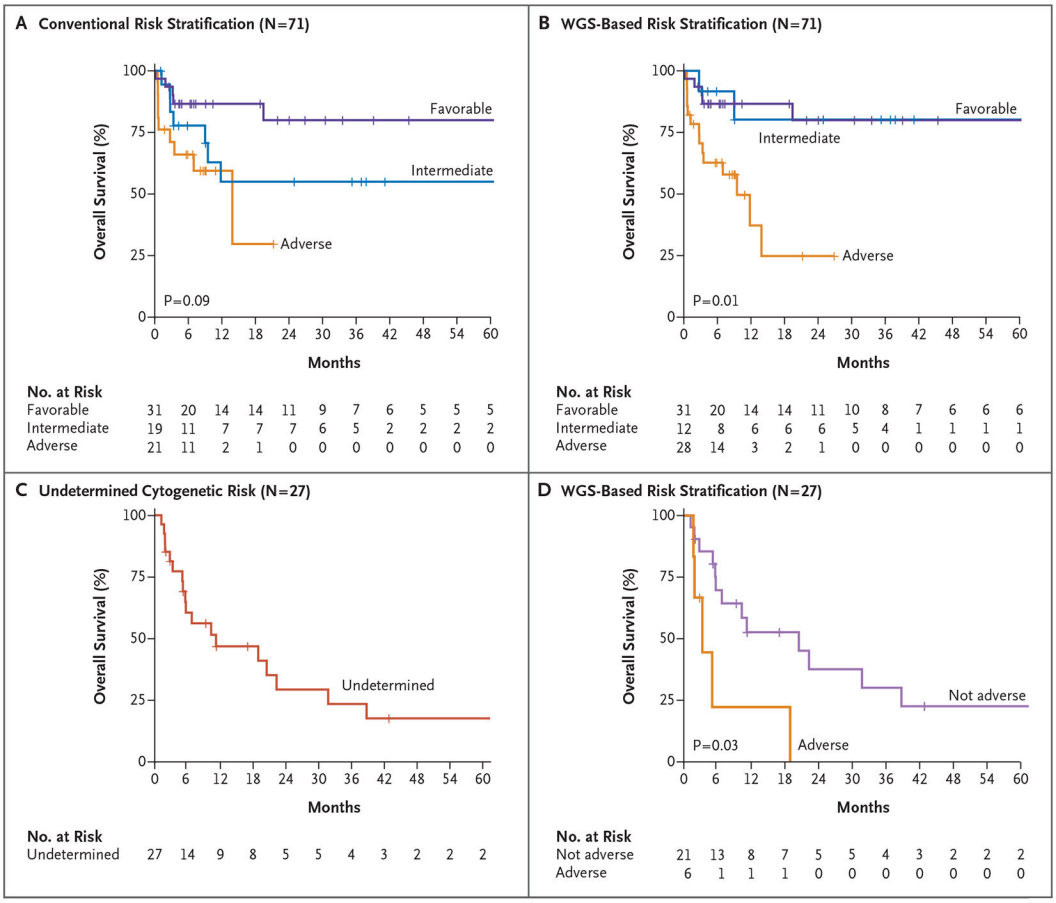

Figure 4. Risk Assessment by WGS in Patients with AML, According to Existing Genetic Risk Groups.

Panel A shows overall survival for 71 patients with AML who were treated with chemotherapy alone after remission, as stratified into established ELN genetic risk groups[3] on the basis of a combination of conventional cytogenetic analysis, FISH, and targeted gene sequencing. Panel B shows the same cohort as in Panel A with risk stratification according to WGS results. The ratio of the mutated *FLT3*-ITD allele to the wild-type allele, as determined by PCR, was used for both the conventional and WGS classifications; the presence or absence of the mutation was used when allele ratios were not available. Panel C shows the clinical outcomes for 27 patients for whom genetic risk could not be determined because of inconclusive, unsuccessful, or unknown results on cytogenetic analysis. The median survival in this cohort was 11.2 months (95% confidence interval [CI], 5.6 to 38.8). Panel D shows the stratification of the cohort in Panel C into established genetic risk groups with the use of WGS results, which predicted shorter overall survival for patients at adverse risk than for those at intermediate or favorable risk (not adverse) (age-adjusted hazard ratio for death for intermediate or favorable risk versus adverse risk, 0.29; 95% CI, 0.09 to 0.94). All P values were calculated with the use of a log-rank test for equal survival among the groups and were adjusted for multiple comparisons.

Table 1.

Characteristics of the Study Patients.*

Characteristic	Retrospective Cohort	Prospective Cohort
All study patients		
No. of patients	146	117
No. of patients with successful cytogenetic analysis†	126	109
Patients with AML		
No. of patients	107	68
Mean age — yr	53.7	60.6
Female sex — no. (%)	47 (44)	30 (44)
ELN genetic risk group — no. of patients‡		
Acute promyelocytic leukemia with t(15;17)(q22;q21)/*PML–RARA*	5	5
Favorable risk	28	19
t(8;21)(q22;q22.1)/*RUNX1–RUNX1T1*	6	1
inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB–MYH11*	11	2
NPM1c without *FLT3*-ITD or with *FLT3*-ITD^{low}§	8	15
Biallelic *CEBPA*	3	1
Intermediate risk	22	10
t(9;11)(p21;q23)/*KMT2A–MLLT3*	1	1
Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}§	11	6
NPM1c with *FLT3*-ITD or *FLT3*-ITD^{high}§	7	3
Other risk not favorable or adverse	3	0
Adverse	20	27
Complex karyotype or mutated *TP53*	13	13
t(v;11q23.3)/*KMT2A* rearranged	3	0
inv(3)(q21.3q26.2) or t(3;3)(q21.3;26.2)/*GATA2–MECOM*	0	2
Chromosome 5 deletion, del(5q), or chromosome 7 deletion	2	3
Wild-type *NPM1* with *FLT3*-ITD or *FLT3*-ITD^{high}§	2	3
Mutated *RUNX1* or *ASXL1*	0	6
Undetermined¶	32	7

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Characteristic	Retrospective Cohort	Prospective Cohort
Patients with MDS		
No. of patients	39	42
Mean age — yr	59.8	68.9
Female sex — no. (%)	17 (44)	12 (29)
IPSS-R risk category — no. of patients‖		
Very good	1	2
Good	11	17
Intermediate	10	3
Poor	4	5
Very poor	13	6
Undetermined¶	0	9
Patients with other hematologic cancer**	NA	7
Acute lymphoblastic leukemia	NA	5
Other form	NA	2

*AML denotes acute myeloid leukemia, MDS myelodysplastic syndromes, and NA not applicable.

†Successful cytogenetic analysis was defined as the analysis of at least three metaphase cells from cultures prepared from tumor specimens with the use of conventional cytogenetic methods.

‡Risk groups for patients with AML according to the European Leukemia Network (ELN) guidelines are listed by their defining features and were assigned on the basis of results from conventional cytogenetic analysis; results from FISH (performed at diagnosis) for *PML–RARA, CBFB–MYH11, RUNX1–RUNX1T1,* del(5q), and chromosome 7; results from polymerase chain reaction (PCR) for *FLT3*-ITD; and results from targeted sequencing. At least 20 metaphase cells were required to identify a normal karyotype. Nine patients with AML were assigned to a risk group on the basis of positive results on FISH for either *PML–RARA* (1 patient), *CBFB–MYH11* (4 patients), or del(5q) (4 patients). Three patients were classified as having intermediate risk on the basis of cytogenetic analysis and testing for *NPM1c* and *FLT3*-ITD alone because targeted gene sequencing was not performed.

§*FLT3*-ITD status was obtained from clinical testing by means of PCR and capillary electrophoresis. High and low allele ratios were determined on the basis of the established cutoff of 0.5 when available (in 7 of 30 patients with positive results for *FLT3*-ITD); otherwise *FLT3*-ITD status was treated as a binary variable.

¶The category of undetermined risk indicates that risk could not be classified because of inconclusive or unsuccessful results on cytogenetic analysis (i.e., no metaphase cells were available for analysis or a normal karyotype was observed in <20 cells), as described previously.

‖Risk categories are those used in the International Prognostic Scoring System–Revised (IPSS-R). The retrospective cohort also included 2 patients with a t(6;9) translocation, which is risk-defining in AML but has no prognostic significance in MDS. Details are provided in Table S3 in the Supplementary Appendix.

**Whole-genome sequencing was performed on samples obtained from consecutive patients, some of whom subsequently received a diagnosis other than AML or MDS, including acute lymphoblastic leukemia, paroxysmal nocturnal hemoglobinuria, and no pathological diagnosis. Three of the patients with acute lymphoblastic leukemia had positive results for *BCR–ABL1*, which was detected by whole-genome sequencing.