AML Genomics for the Clinician

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Acute myeloid leukemia (AML) is a heterogeneous disease, characterized by frequent resistance to available chemotherapeutic agents. The basic therapy for patients with AML has changed little over the past 30 years. Improvements in outcome in recent decades in younger adult cohorts have generally been ascribed to better supportive care (ie, transfusion and antimicrobial therapy); older adults with AML continue to fare poorly. The explosion of new knowledge regarding the AML genome has yet to be translated into therapeutic benefit, but analysis of specific molecular features in AML samples has enabled the field to more accurately parse out prognosis and assign appropriate therapies (eg, chemotherapy vs allogeneic stem cell transplantation) for groups of patients. Cytogenetic analysis, whether by metaphase or interphase analysis, has been the main tool used to divide patients into varying prognostic subsets, but it has been modified in recent years to include assessment of mutations in a small number of genes. In the past several years, new technologies have provided strategies to interrogate individual cancer genomes in a broad and in-depth fashion. The present article discusses the potential of these new technologies, particularly gene panel and whole-exome or whole-genome sequencing, to improve diagnosis, prognosis, and therapeutic outcome in AML.

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When a patient with known or suspected acute leukemia is seen in the clinic, in addition to examination of the peripheral blood film and bone marrow aspirate smear, it has been standard practice for the past 30 years to also send an aliquot of cells to a flow cytometry laboratory (to objectively define lineage [ie, acute lymphoblastic leukemia (ALL) vs acute myeloid leukemia [AML]], as well as to assess for cytogenetic aberrations. The assessment of gross chromosomal abnormalities is usually made by analysis of at least 20 metaphases obtained on a stimulated sample of bone marrow or peripheral blood cells. Alternatively, a sample of blood or marrow can be analyzed with a panel of fluorescent in situ hybridization (FISH) probes. FISH can detect specific abnormalities, but some rearrangements are cryptic and others are not routinely interrogated because their frequency in the AML population is low. The routine use of cytogenetic analysis at the time of AML diagnosis has allowed patients of all ages to be partitioned into distinct prognostic subgroups.

About 15% of patients have so-called “favorable” prognosis (60%-70% long-term disease-free survival) AML.

This group is characterized by aberrations in the heterodimeric core-binding factor (CBF) transcription factor manifested cytogenetically as either inversion of chromosome 16 or the t(8;21) translocation. In inv(16), a fusion protein, created via juxtaposition of the smooth muscle myosin heavy chain gene to CBFB, leads to impaired hematopoietic differentiation. Analogously, the t(8;21) cytogenetic abnormality results in the RUNXI-RUNXIT1 fusion, with RUNXI encoding the CBFA component of the CBF heterodimer. About 15% of patients are found to have adverse cytogenetics (<15% long-term disease-free survival), which encompasses those with complex abnormalities (>3 individual abnormalities) or those with abnormalities in the 3q26 (EVI1) locus, loss of the long arm of chromosome 7, monosomy 7, loss of the long arm of chromosome 5, or monosomy 5. A recently delineated, even more adverse prognostic group, the so-called “monosomal karyotype,” encompasses those whose blasts have 1 monosomy and 1 structural abnormality or ≥2 monosomies. The vast difference in outcome in these 2 disparate subgroups has resulted in a therapeutic strategy of consolidating those who obtain remission with chemotherapy for patients with inv(16) or t(8;21); those with adverse cytogenetics are more appropriately allocated to allogeneic stem cell transplant in first complete remission (recommended, even if the only available product is cord blood or a haploidentical graft).

Patients with neither favorable nor adverse cytogenetics comprise the so-called intermediate-risk group. Most cases harbor no structural abnormalities on cytogenetic analysis, but the intermediate group also includes patients with...
other abnormalities, including but not limited to trisomy 8 and abnormalities of chromosome 9 and chromosome 11q23. Controversy about optimal consolidation strategies for the intermediate group has been resolved to a certain degree by the assessment of specific mutations in a subset of genes (Table 1).

Although subject to ongoing change and reassessment, the current recommended panel of genes to be assessed for mutations at the time of diagnosis includes determination of whether there is a mutation in NPM1, KIT, FMS-related tyrosine kinase 3 (FLT3), or CEBPA.1 This recommendation has the strongest support for younger patients with normal cytogenetics (although this information is not predictable at initial presentation). The FLT3 gene encodes a transmembrane tyrosine kinase in which mutations have been detected in ~30% of patients with AML.8,9 About two thirds of these mutations represent a duplication (ranging from 1 to >100 amino acids) in the juxtamembrane domain, the so-called “length” of the internal tandem duplication (ITD) mutation. This change causes autodimerization of the growth factor and activation without need for binding to the cognate ligand (FLT3 ligand). In general, the presence of such a mutation confers an adverse prognosis within the normal cytogenetic and probably other cytogenetic subgroups.10 Loss of the unmutated FLT3 allele and/or variability in mutant clone size can affect the “allelic ratio” (the proportion of FLT3-ITD vs FLT3 wild-type alleles in a tumor sample). Some studies have reported that a higher FLT3-ITD allelic ratio is associated with worse prognosis, although this claim remains controversial.11 About one third of FLT3 mutations, or 5% to 10% of patients with AML overall, have an autoactivating point mutation in the tyrosine kinase domain. The prognostic impact of this lesion is less clear12,13 and is not currently used to make therapeutic decisions. Nucleophosmin 1 (NPM1) is a shuttling protein that moves substrates between the nucleus and cytoplasm.14 Point mutations in this gene cause mislocation of the protein to the cytoplasm, and in the absence of concurrent FLT3-ITD are associated with a favorable prognosis, particularly in patients with normal cytogenetics.15,16 Mutation of both alleles encoding the CEBPA transcription factor in the setting of a normal karyotype confers a favorable prognosis, but this is only present in ~5% of AML cases.17,18

The assessment of FLT3-ITD, NPM1, and CEBPA genotypes in AML not only refines prognosis19 but is also used to determine optimal consolidation therapy. Specifically, those with normal karyotype AML who have mutations in NPM1 but do not have a FLT3-ITD mutation do relatively well and are optimally managed with consolidation chemotherapy alone.20 Patients with any other combination of FLT3-ITD and NPM1 mutations (eg, FLT3-ITD/NPM1 wild type; FLT3-ITD/NPM1 mutant, or FLT3 wild type/NPM1 wild type) should undergo allogeneic stem cell transplantation in first remission if a suitable donor exists. Patients with mutations of both CEBPA alleles do well enough that intensive consolidation therapy, which carries a lower risk of morbidity/mortality than allogeneic transplantation, should be used.

Although patients with inv(16) or t(8;21) are in the favorable group, the data suggest that those who have an associated activating mutation in KIT (encoding the c-kit tyrosine kinase) have an inferior prognosis.21,22 It has been proposed that the 25% of patients with CBF cytogenetics whose blasts also harbor a KIT mutation (who have a <30% likelihood of disease-free survival) should undergo allogeneic transplantation.23 However, this notion is based more on the inferior prognosis than on prospectively derived clinical data demonstrating improved outcomes with transplantation. Because the clinician might not know at the time of diagnosis whether the patient has a CBF cytogenetic abnormality, it is prudent to routinely request assays for FLT3, NPM1, CEBPA, and KIT mutational profiling at the time of diagnosis.

In addition to their prognostic impact, several of these mutations could identify patients eligible for specific

### Table 1. Risk Stratification of Patient With De Novo Acute Myeloid Leukemia

<table>
<thead>
<tr>
<th>Risk Category</th>
<th>Cytogenetic</th>
<th>Genetic</th>
<th>Prevalence (%)</th>
<th>4-Year Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very high</td>
<td>Monosomal karyotype*</td>
<td>NPM1&lt;sup&gt;WT&lt;/sup&gt;/CEBPA&lt;sup&gt;WT&lt;/sup&gt;</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>High</td>
<td>Complex&lt;sup&gt;+&lt;/sup&gt; or unfavorable&lt;sup&gt;+&lt;/sup&gt;</td>
<td>c-KIT&lt;sup&gt;Mut&lt;/sup&gt;</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Normal, and: inv(16) or t(8;21), and: NPM1&lt;sup&gt;Mut&lt;/sup&gt;/FLT3&lt;sup&gt;WT&lt;/sup&gt;</td>
<td>25</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal, and: Normal, and: CEBPA biallelic</td>
<td>5</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Favorable</td>
<td>t(15;17)</td>
<td>c-KIT&lt;sup&gt;WT&lt;/sup&gt;</td>
<td>10</td>
<td>65</td>
</tr>
<tr>
<td>Very favorable</td>
<td></td>
<td></td>
<td>12</td>
<td>85</td>
</tr>
</tbody>
</table>

*Two monosomies or one monosomy plus balanced translocation.
†More than 3 abnormalities.
‡3-7, 7q-, -5, 5q-, 3q, or t(6;9).
clinical trials. For example, referral to a trial in which a FLT3 inhibitor is being studied might be appropriate for patients with relapsed FLT3-ITD AML. Sorafenib and sunitinib, approved for use in renal carcinoma and hepatocellular carcinoma on the basis of their ability to inhibit vascular endothelial growth factor receptor, are relatively potent FLT3 inhibitors.24,25 Off-label use of these agents alone or in combination with hypomethylating agents in relapsed FLT3-ITD AML has been reported.26 Midostaurin,27 lestaurtinib,28 and quizartinib29 are FLT3 inhibitors in development that have some clinical activity in mutant FLT3 AML. Quizartinib is a potent and selective agent that is being compared with chemotherapy in patients with advanced mutant FLT3 AML. Crenolanib30 also inhibits the FLT3 tyrosine kinase domain point mutation, which is a reported mechanism underlying acquired quizartinib resistance.31 KIT also represents a therapeutic target; early clinical data suggest that adding dasatinib to standard chemotherapy might yield a clinical benefit.32 Mutations in the IDH1 and IDH2 genes encode enzymes that produce a novel reaction product (2-hydroxyglutarate)33 which affects DNA methylation and gene transcription. The prognostic significance of IDH1 and IDH2 mutations in AML is variable, but early clinical results with a specific IDH2 inhibitor suggest that relapsed patients with a mutation in that gene might be good candidates for a clinical trial with this agent. RAS mutations, seen in ~20% of patients with AML,34 promote aberrant mitogenic signaling through the mitogen-activated protein kinase pathway. These mitogen-activated protein kinase inhibitors such as trametinib have shown promise in patients with these mutations.35 Finally, mutations in the genes encoding members of the RNA splicing machinery may also be valid therapeutic targets in the near future.

GENE PANEL TESTING

As mentioned earlier, the standard diagnostic evaluation of patients with AML currently includes some level of genomic characterization, consisting of chromosomal karyotype (complemented by FISH analysis, as indicated) and mutational profiling for a small number of abnormalities (eg, PML/RARA fusion; NPM1 or FLT3 insertions; point mutations in CEBPA and KIT). The wealth of new genetic findings in AML (as described by Mazzarella et al in this issue of the Journal) has not yet been incorporated into standard risk stratification or treatment algorithms. Routine clinical testing for these lesions cannot be recommended currently, but there is little doubt that this will change in the near future. If there is compelling evidence that some of this information is potentially actionable, there will likely be demand for broader genetic testing in advance of established clinical guidelines. Actionable results may include mutations that re-classify patients into an adverse prognostic category (and, therefore, prompt referral for allogeneic transplantation) or results that identify vulnerabilities for targeted therapy or establish eligibility for clinical trials.

Traditional testing methods (eg, Sanger sequencing, real-time polymerase chain reaction) do not scale efficiently with increasing numbers of genetic lesions queried. Next-generation sequencing (NGS) approaches offer potential advantages in cost and turnaround time. NGS of a group of target genes (a “panel”) begins with preparation of template DNA extracted from tumor tissue (eg, bone marrow aspirate). Whole-genome sequencing (WGS) studies have demonstrated that the majority of bone marrow cells are part of the malignant clone in AML,36,37 obviating the need to separate tumor cells from residual normal cells (ie, by flow sorting or magnetic bead purification). The genomic regions selected for sequencing can be amplified from genomic DNA by polymerase chain reaction (eg, by parallel reactions in microfluidic devices) or by hybridization capture using probes (“baits”) complementary to the region targeted for sequencing (as described by Mardis in this issue of the Journal). Barcoded adapters, specific to individual patients, can be annealed to the sequencing libraries so that several samples can be pooled (for more efficient use of sequencing capacity) and later deconvoluted to provide patient-specific results. Once a sequence is produced, a variety of computational tools are available to perform alignment to a reference genome, call variants that are present in the tumor, and exclude known inherited polymorphisms.

Gene panel testing, by design, looks “under the lamp-post” at genes known to be recurrently mutated in cancer. This approach has its advantages (eg, deeper coverage at targeted sites, simpler analytical requirements) and disadvantages (eg, mutations outside the target space are missed). The selection of genes to include in a panel is subjective. Parameters to consider when prioritizing genes for inclusion in a panel may include: the prognostic value of mutations in the gene, the predictive value of mutations for response to specific therapies, and previous knowledge of how often the gene is mutated in AML (because genes that are more frequently mutated make more efficient use of sequencing capacity). It is important for clinicians to know which genes are included in the panel they request (and which genes are not), and whether all exons (for all transcripts) are targeted (including untranslated regions and splice sites) or only mutation “hotspots.”

The initial steps of sample processing (ie, genomic DNA extraction, library preparation) can be performed in a single day. Sequence production requires 1 to 7 days (depending on the platform used). Alignment, variant calling, interpretation, and reporting if performed by using an automated pipeline requires an additional day, making it feasible for a high-volume testing facility to move from a tissue specimen to a clinical report in <1 week. A variety of commercial and academic centers currently offer gene panel testing with turnaround times approaching this target (Table 2).
DNA-based panel testing, as currently deployed in most settings, excels at detection of single nucleotide variants in most targeted regions. Notable exceptions are regions of high GC content or secondary structure (eg, in \textit{CEBPA}). Small insertion/deletion (indel) events (< 25 bp) are efficiently detected, but larger indels are plagued by a high false-positive rate, requiring validation using other methods. If the target enrichment strategy is sufficiently dense, it is possible to identify focal copy number abnormalities (eg, microdeletions/amplifications), but larger structural variants and rearrangements require other strategies for detection (as discussed later).

\textbf{RETURN OF RESULTS}

The analysis and interpretation of sequencing results are currently the most challenging and time-consuming steps in clinical gene panel testing. Ideally, a clinical report should clearly communicate the “bottom line” results, while retaining more granular information in the body of the report. Results may first be simply dichotomized into “mutation not detected” versus “mutation(s) detected.” In the case of the former finding, these results may be sufficient or may prompt additional testing. When mutations are detected, further explanation is required, because mutations in various genes (and different alleles within the same gene) have different biological and clinical implications.

Mutations currently integrated in prognostic algorithms (eg, \textit{FLT3-ITD}, cytoplasmic nucleophosmin) have been vetted in thousands of patients from independent cohorts and can be reported with little additional annotation. For all other mutations, additional supporting evidence is required to build a case for reporting them as potentially significant. Strong evidence (at least for a role in the biology of the tumor) is provided when the specific variant has been previously reported in multiple cases of AML or other cancers. Novel truncating mutations (eg, nonsense, frameshift) in known tumor suppressor genes that are recurrently inactivated in cancer are likely to be biologically significant. The frequency of mutations in genes recurrently mutated in human cancers can be queried in a variety of databases (eg, COSMIC, TumorPortal). Computational predictions (eg, mutations at evolutionarily conserved residues, mutations with predicted translational consequences) and preclinical evidence of functional consequences are less reliable sources of information. Links to supporting literature should be provided in all cases. Some analytical pipelines run automated queries linking mutations to relevant clinical trials or potential targeted therapies,\textsuperscript{10} which can be helpful to clinicians. When compelling evidence of biological or clinical significance is lacking, mutations should be reported as “variants of unknown significance.”

NGS also provides a quantitative measurement of mutant allele abundance, often expressed as the variant allele fraction (VAF), which is the number of sequencing reads supporting the variant allele divided by the total sequencing reads mapped to that position. For a heterozygous mutation in a diploid region of the genome that is present in 100% of tumor cells, the VAF is approximately equal to 50%. Mutations in the first population of cells that become clonally dominant in AML (the “founding” clone) often have VAFs approaching 50%. Subclones frequently emerge from the founding clone during disease progression or in response to selective pressure induced by therapy.\textsuperscript{38,39} Mutations in emerging subclones will have lower VAFs, on average, than mutations in the founding clone from the same patient. In chronic lymphocytic leukemia, the presence of subclonal mutations is predictive of shorter time to disease progression.\textsuperscript{41} The clinical significance of founding clone versus subclone mutations in AML has not been established. Nevertheless, this information should be included in reports and may be useful in the future to assess response to therapy and to detect minimal residual disease. A recent study demonstrated that the clonal architecture of AML as defined by WGS of bone marrow cells is recapitulated in the

\begin{table}[h]
\centering
\caption{Gene Panel Testing for Acute Myeloid Leukemia*}
\begin{tabular}{llcc}
\hline
\textbf{Entity} & \textbf{Assay} & \textbf{Gene Number} & \textbf{Turnaround (days)} \\
\hline
NeoGenomics Laboratories & NeoTYPE AML & 8 & 7 \\
Washington University Genomics and Pathology Services & Comprehensive Cancer Gene Set & 40 & NA \\
Caris Life Science & Molecular Intelligence & 47 & 7-14 \\
Foundation Medicine & FoundationOne Heme & 405 & > 14 \\
Genoptix, Inc. & AML Molecular Profile & 9 & 7-10 \\
Genection & MyAML & 193 & NA \\
Knight Diagnostic Laboratory & GeneTrails AML/MDS & 43 & 10-14 \\
\hline
\end{tabular}
\end{table}

*A list of representative entities is provided. The list is not intended to be exhaustive. Information provided is from publically accessible Web sites.

MDS, myelodysplastic syndrome; NA, not available.
peripheral blood, raising the possibility that serial measurements using peripheral blood samples may be an adequate surrogate to monitor disease status in the bone marrow.

A comprehensive report from gene panel testing should also include elements of assay performance. Each testing laboratory must empirically determine a limit of detection for mutations (eg, VAF ≥ 5%) at a minimum depth of sequencing (eg, 200-fold coverage). Targeted regions that fail to meet the coverage threshold (eg, typically due to low abundance or poor-quality template DNA) should be listed in the body of the report so that the clinician can appropriately interpret “negative” results. The report should also clearly indicate whether the assay has been validated for detections of indels, structural variants, and rearrangements. It may seem trivial to demand this level of housekeeping detail for genetic testing when similar disclosures do not accompany other laboratory tests (eg, serum chemistries). However, clinical NGS testing is an area of active development, with many alternative strategies for sample processing, sequence production, and variant identification, interpretation, and reporting. Until protocols and procedures have been standardized and endorsed by regulatory agencies, this type of supporting information is essential to correctly interpret and compare results generated by different testing entities.

DOCTOR, SHOULD I GET MY GENOME SEQUENCED?

Given the rapid pace of discovery in cancer genomics, it is understandable that patients and their physicians are motivated to obtain more comprehensive analysis of the leukemia genome and use this information for clinical decision making. As mentioned earlier, even large gene panels will fail to reliably detect some abnormalities within the targeted region and are completely uninformative for lesions outside the target. Hybridization capture can be used to enrich and interrogate a much broader repertoire of open reading frames, but even under optimal conditions, the entire coding region of the genome (whole-exome sequencing [WES]) is not adequately sampled with existing commercially available reagents. Even WGS is not exhaustive. WGS provides more comprehensive detection of mutations in coding and noncoding regions, copy number alterations, and clonal architecture, but deconvolution of structural variants remains challenging, and even single nucleotide variant/indel detection remains difficult in some regions of the genome (particularly areas with highly repetitive sequence). Transcriptome sequencing (“RNA-seq”) is optimal for detection of rearrangements that result in expressed fusion genes and can provide information about mutant allele expression, alternative splicing, or loss of expression due to nonsense-mediated decay. One strategy that is being tested in a clinical setting combines WGS, WES, and RNA-seq to take advantage of the complementary strengths of each platform. None of these techniques provides information about epigenetic alterations (eg, DNA methylation, histone modification), but there are robust tools to evaluate these abnormalities as well (as described by Mardis in this issue of the Journal).

Comprehensive genome-wide analysis in a clinical setting remains a research endeavor, currently feasible in a finite number of academic centers. There are anecdotal instances in which this analysis has yielded actionable results that were missed by routine diagnostics, providing proof-of-concept that these tools can improve on the existing armamentarium of tests. The labor and supply costs required to support these assays have decreased substantially, but the economics are not yet favorable for routine implementation in the clinic. Interpretation and reporting results will remain considerable challenges in the near term, as described earlier. Confidence in the interpretation of biological significance and clinical implications of genomic data falls rapidly as the scope broadens from mutational hotspots to large gene panels, WES, and WGS. Even for gene panel testing, a recent survey of academic physicians at one of our institutions noted that a substantial minority (28%) had low confidence in their ability to interpret test results, highlighting the need for physician education in clinical genomics.

UNIQUE CHALLENGES RAISED BY LARGE-SCALE CLINICAL SEQUENCING

Centers that are contemplating ambitious sequencing programs (ie, large gene panel, WES, WGS) in the clinical realm must confront a number of key issues, including the following.

Is This a Somatic Mutation?

When tumor samples are sequenced without a paired nontumor sample from the patient, sequence variants interpreted as somatic mutations cannot be discriminated from inherited polymorphisms with certainty. There is little ambiguity for well-characterized mutations (eg, FLT3-ITD), but even highly recurrent hotspot mutations (eg, DNMT3A R882H) are reported in databases of sequence variants from normal individuals (eg, NHLBI Exome Variant Server). Furthermore, polymorphisms restricted to ethnic minorities and extremely rare (or “private”) variants are underrepresented (or absent) in existing databases. Therefore, databases alone are not sufficient to “filter” all inherited polymorphisms from somatic mutations. For large gene panel testing (and certainly for exome or genome sequencing), a paired sample obtained from tissue that is distinct from the primary site of disease (for hematologic malignancies, a skin biopsy is optimal in most cases) must be sequenced in parallel with the tumor sample to differentiate acquired somatic mutations from inherited polymorphisms. This requirement adds cost, analytical complexity, and imposes additional burdens on patients and busy clinicians.
Is This Mutation a Biological Driver?

Most of the somatic mutations present in a leukemia sample are pre-existing mutations that accumulate in normal hematopoietic stem/progenitor cells in an age-dependent fashion. When one of these cells becomes transformed, the pre-existing mutations are captured and carried forward into the founding clone. Therefore, even definitive evidence suggesting that a mutation is somatically acquired is not sufficient to ascribe biological or clinical significance. As mentioned earlier, specific mutant alleles recurrently identified in AML or other cancers at a frequency higher than expected by chance are likely to be biologically relevant. The clinical utility of individual mutations (ie, their prognostic and/or predictive value) can only be determined through careful clinical investigation in large, uniformly treated patient cohorts. Finally, because protein-coding genes account for <2% of the genome, it is difficult to ascribe biological relevance to the vast majority of somatic mutations identified by WGS. From the Encode Project, we have learned that nearly all of the genome is transcribed. In AML, recurrent somatic mutations have been described in 1 microRNA (miR-142). It is likely that somatic mutations outside of genic regions that affect expression of coding and noncoding RNAs also play a role in AML pathogenesis, but this is an ongoing area of investigation.

What About Inherited Variants?

Sequencing a paired normal sample aids in the identification of somatic mutations (as described earlier) but may also reveal inherited variants that have implications for the health of the patient or family members. Some of these variants may be relevant for AML pathogenesis, but others may affect traits that are unrelated to the reason the test was ordered. Whether a physician has an obligation to identify and report these “incidental” findings is currently a subject of intense debate. The American College of Medical Genetics has argued that physicians have a duty to inform patients when actionable variants are identified and has recently published a set of specific recommendations. Clinicians should also be aware that the genotype of a relatively small number of polymorphic germline variants is sufficient to uniquely identify an individual and that the contribution of DNA from a single individual can be discerned from large aggregated datasets. Therefore, “de-identified” samples (ie, stripped of all personal information) can be potentially re-identified when subjected to large-scale genetic testing with a paired germline sample. If this information is accessed, it could have adverse legal or financial consequences for patients and their families. All of these potential risks must be discussed with patients before testing, and patients must provide written informed consent. The management and follow-up of some results from germline testing will require referral to trained genetic counselors.

CONCLUSIONS

The wealth of new information about the AML genome provides opportunities for translation into the clinic. A number of challenges must first be overcome, including design of assays that interrogate all potentially actionable targets; development of standardized protocols for sequence production, variant calling, interpretation, and reporting; and implementation of thoughtful strategies that integrate information across a variety of domains, including detection of incidental germline findings. Fortunately, the community of AML investigators has a well-established track record as early adopters of new technologies that were subsequently disseminated into other areas of clinical medicine (eg, cytogenetic analysis, FISH, polymerase chain reaction). National cooperative groups are available to validate the clinical utility of potential genetic biomarkers in large patient cohorts. Currently, metaphase cytogenetics, FISH, and mutational profiling of 4 genes (FLT3, NPM1, CEBPA, and KIT) are standard of care. Large gene panel testing and WES/WGS remain research tools, ideally suited for investigation in academic centers. With further development and refinement, it is likely that these technologies in some fashion will be deployed for more routine use in the care of patients with AML.

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REFERENCES


