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# Genomic testing in myeloid malignancy

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## Abstract

Clinical genetic testing in the myeloid malignancies is undergoing a rapid transition from the era of cytogenetics and single-gene testing to an era dominated by next-generation sequencing (NGS). This transition promises to better reveal the genetic alterations underlying disease, but there are distinct risks and benefits associated with different NGS testing platforms. NGS offers the potential benefit of being able to survey alterations across a wider set of genes, but analytic and clinical challenges associated with incidental findings, germ line variation, turnaround time, and limits of detection must be addressed. Additionally, transcriptome-based testing may offer several distinct benefits beyond traditional DNA-based methods. In addition to testing at disease diagnosis, research indicates potential benefits of genetic testing both prior to disease onset and at remission. In this review, we discuss the transition from the era of cytogenetics and single-gene tests to the era of NGS panels and genome-wide sequencing—highlighting both the potential and drawbacks of these novel technologies.

**KEYWORDS**

clinical testing, leukemia, myeloid malignancy, next-generation sequencing, RNA-seq

## 1 | INTRODUCTION

The myeloid malignancies are a group of related disorders characterized by defective hematopoiesis originating from a hematopoietic stem/progenitor cell and showing myeloid differentiation. These disorders include acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), and myeloproliferative neoplasms (MPN).[1,2] With the rapid adoption of next-generation sequencing (NGS) approaches in clinical laboratories, the scope, timing, and eligibility for genetic testing are also rapidly changing. The World Health Organization (WHO) and European Leukemia Network (ELN) have both recently reclassified AMLs,[3,4] adding new disease subtypes and stratification criteria incorporating more molecular alterations than previous schemas. The incorporation of additional markers is likely to increase, as the mutational, epigenomic, and expression landscape of myeloid malignancies is better explored.[5,6]

Cytogenetic testing is the cornerstone of risk stratification in the myeloid malignancies.[7] In AML, patients are largely stratified to favorable or adverse risk status according to the presence or absence of recurrent cytogenetic changes such as the core binding factor fusions typifying low-risk AML, and the many rearrangements involving *KMT2A* (*MLL*) often found in high-risk disease.[8] In recent years, clinical guidelines have been expanded to incorporate other gene variants, as evidence has accumulated for their prognostic or predictive impact. Mutations in *FLT3*, *NPM1*, *CEBPA*, and *KIT* are included in the National Comprehensive Cancer Network (NCCN) risk stratification,[9] while the most recent ELN guidelines now also consider mutations in *TP53*, *RUNX1*, and *ASXL1*.[4] Additional testing may also be useful for splicing modifiers such as *SF3B1* as well as for predictive markers such as *IDH1* and *IDH2*, where novel therapeutic agents may be indicated.[10]

Complicating the testing landscape, potentially preleukemic states such as clonal hematopoiesis of indeterminate potential (CHIP) (also referred to as age-related clonal hematopoiesis), idiopathic cytopenia of undetermined significance (ICUS), and clonal cytopenias of undetermined significance (CCUS) have also been

  

recently described.[11-15] Pediatric AMLs have a different mutational landscape than adult leukemias, and so also require a different clinical approach.[16] As well as screening patients at diagnosis, there are potential benefits to testing patients at remission and/or relapse. Whether widespread screening prior to overt disease has clinical utility and how such a program would be implemented remain open questions.

When the number of genes required for clinical assessment was small, single-gene tests (using Sanger sequencing or PCR-based assays) were common and were typically coupled with fluorescence in-situ hybridization (FISH) probes to detect recurrent structural rearrangements. However, in the last 5 years, NGS-based gene panel testing has become widespread. The increase in the number of potential markers coupled with the rapid drop of sequencing costs has led to the increasing feasibility of wider genomic testing for myeloid malignancies. This raises several important questions including whether wider genomic testing is justified and which technologies should be used for screening patients.

## 2 | IS THERE A PLACE FOR WIDER PANELS OR GENOME-WIDE TESTING IN THE MANAGEMENT OF MYELOID MALIGNANCY?

Recent studies suggest that wider genomic testing may benefit patients with myeloid malignancies. Large gene panels are capable of assessing hundreds of targets simultaneously, while genome-wide approaches such as whole-exome sequencing (WES) or whole-genome sequencing (WGS) can assess thousands. An increased number of recurrent genetic aberrations associated with prognostic relevance have been identified, and the number of therapies directed toward specific gene aberrations is increasing; for example, targeted therapies directed toward *FLT3* and *IDH1*/*IDH2* have recently received FDA approval.[10] At present, there are only a handful of genes that have direct impact on the clinical management of AML, although wider gene panels in MDS are useful for diagnostic purposes.

Many clinical laboratories have adopted panels that query many more genes in the anticipation of the utility of these genes for current and future clinical trials, and for facilitating research projects. The Foundation Medicine FoundationOne Heme panel targets over 400 genes using a hybrid capture methodology to report on both gene mutations, gene fusions, copy number alterations (CNAs), tumor mutation burden, and microsatellite instability.[17] While only subsets of the genes on the panel may have current clinical relevance for a given disease, detection of mutations in additional genes on the panel may still be useful due to the "long tail" of rare variant frequencies. For example, variants in the splicing genes *SF3B1*, *SRSF2*, and *U2AF1* are common in MDS, whereas *ZRSR2* variants are rare, but equally important for diagnostic purposes if present. Additionally, because many structural variants (SVs) may be the result of complex rearrangements (eg, gene fusions involving one known and one novel gene, or three-way fusions), previously undescribed variants may still be clinically actionable, but not detectable with standard clinical testing. Smaller, more targeted panels, such as the Karyogene test from the Wellcome Trust Sanger Institute[18] (49 genes, as well as gene fusion and CNA targets), and the Illumina TruSight Myeloid panel (54 genes) are also in wide use and offer a different trade-off between cost and comprehensiveness than larger panels.

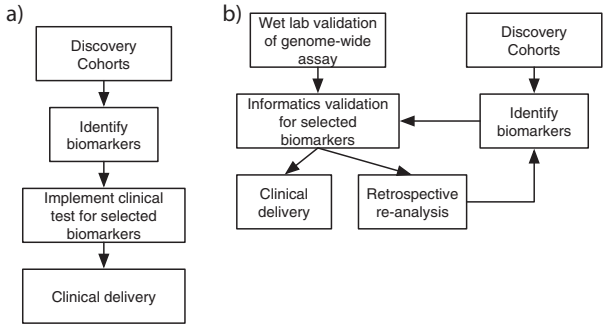


**FIGURE 1** Comparison of traditional (A) and iterative (B) strategies for clinical assay development

The traditional paradigm in clinical genetics (Figure 1A) suggests the development and widespread implementation of targeted clinical assays only after the identification and demonstration utility for selected biomarkers. As NGS costs drop, and more biomarkers requiring genome-wide information are identified, an iterative approach (Figure 1B) suggests itself; once genome-wide assays (ie, WES and WGS) are validated in a clinical laboratory, they can be used for both current clinical needs and to facilitate retrospective reanalysis, allowing for the identification of new biomarkers. These new biomarkers (along with biomarkers discovered in orthogonal patient cohorts) can be incorporated into the clinical assay after confirmation using a prospective approach. This strategy offers the benefit of reduced assay development costs and a step toward broader precision medicine approaches in the myeloid malignancies.[19] For both targeted and genome-wide approaches, there are several trade-offs to be considered in determining which testing platforms and technologies should be used.

## 3 | HOW SHOULD PATIENTS BE SCREENED?

Clinically relevant molecular alterations can be broken down into several categories. At present, comprehensive assessment of myeloid malignancies requires detection of large SVs, CNAs, single nucleotide variants (SNV), and insertion/deletion (indel) alterations. Many academic and commercial clinical laboratories have recently adopted gene panel testing (reviewed in[20]). Most panels include (at a minimum) all the genes necessary for stratifying patients according to standard clinical criteria for myeloid malignancies, but often extend far beyond those guidelines, assessing mutations in more than 100 genes. The strength of gene panels is in their lower cost (compared to multiple single-gene tests,

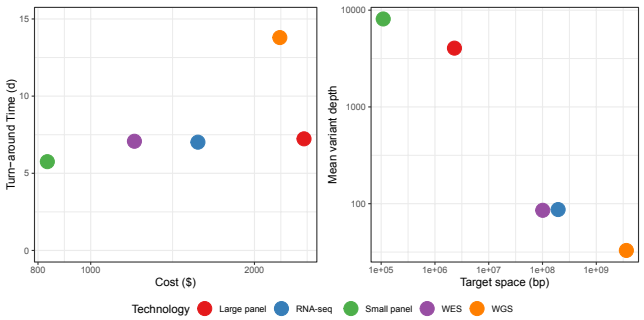


**FIGURE 2** Attributes of select next-generation sequencing (NGS) technology platforms. The left panel compares the estimated cost and turnaround time for clinical myeloid assays, while the right panel compares the potential target space against mean variant depth. Values are illustrative for the purposes of comparison and represent typical approaches used

WES, or WGS), the limitation of the assay to only preselected targets (limiting the risk of incidental findings), and the ability to achieve very high sequence coverage depth over the selected targets (Figure 2). The incorporation of unique molecular identifiers (UMIs) to library preparation protocols increases the sensitivity of mutation detection by tagging individual molecules, allowing for the discrimination of sequenced reads originating from different original molecules.[21] This should permit applications such as minimal residual disease (MRD) detection and analysis of circulating cell-free tumor DNA. Hybrid capture panels have further increased the utility of gene panels, allowing the detection of both DNA-based and RNA-based alterations in a single assay. By designing capture probes for genes with multiple known fusion partners (eg, *KMT2A*), gene panels incorporating RNA-based targets can target fusions and CNA targets, along with SNV and short indel alterations. In principle, a benefit of panel-based testing is the ability to iteratively redesign and add additional targets as new genes are added to the testing menu. In practice, panel redesign can be costly and time-consuming; multiple design and validation iterations are often required to address sequencing coverage issues, and analyses that rely on batch-scale properties of the panel (such as CNA detection) can require a large number of samples to be processed with a consistent panel design and/or the inclusion of control samples to achieve a high accuracy.[22]

Whole-exome sequencing offers the benefits of complete coverage of most annotated coding exons in the genome. In clinical settings where findings in novel genes may be useful (for example, in clinical trials for personalized medicine approaches), WES may be an appropriate choice. Additionally, WES does not suffer nearly as much from the redesign and revalidation issues that gene panels do. In practice, coverage of particular coding exons in WES library kits varies widely—for example, coverage of *CEBPA* is quite poor in standard exome capture kits. These issues have resulted in the adoption of "augmented" exome kits,[23] which add additional probes to address low-coverage regions. For routine clinical testing, where only alterations in genes with established prognostic or predictive relevance are considered, whole-exome sequencing results in reduced coverage (and hence poorer sensitivity) than gene panels for similar sequencing effort.

As the cost of sequencing drops, it is tempting to consider bypassing the target design and validation cycles required for panels and WES by moving directly to WGS. Compared to WES, WGS offers coverage of the bulk of the genome—allowing for much improved detection of structural variation, copy number changes, and alterations in noncoding regions. It also allows interrogation of the noncoding genome, which may be the next avenue for biomarker discovery. While the cost of WGS is dropping, the bioinformatic challenges associated with analysis and storage of WGS data still impose significant challenges in scale for most clinical laboratories. For example, Griffith et al[24] recommend WGS at a depth of 200-300× for comprehensive analysis, which corresponds to approximately 600-900 Gb of sequence data. Additionally, to realize continued drops in sequencing costs, large investments in new or upgraded sequencing machines may be required and often can only be justified if the scale of testing at a given laboratory is also dramatically increased. Crucially, the interpretation challenge for WGS data is vast—there are many potential types of variant to be detected, each of which requires analytic sensitivity and specificity metrics to be established. As with WES, WGS may only be suitable in contexts where it has the potential to provide actionable information (eg, in clinical trials for personalized medicine approaches or trials studying genome biomarker approaches). Alternatively, low-coverage WGS may be sufficient for the detection of large structural variants,[25] which may be a useful complement to existing assays.

RNA-seq offers additional benefits beyond DNA-based assays, while also being capable of detecting many of the same types of alterations. There are many recurrent gene fusions in the myeloid malignancies, and known fusion genes often have many partners.[8] This makes the design of cDNA capture probes for gene panels difficult (though some capture technologies allow for probe designs where only one fusion partner is known[26]), while RNA-seq allows for unbiased detection of a broad range of gene fusion events. RNA-seq also allows for the incorporation of any of a multitude of published expression-based prognostic signatures. For example, the LSC17 gene expression signature[27] can identify patients with higher and lower risk in AML. In Mendelian disorders, RNA-seq has proved a useful method to identify novel disease-associated genes, through the use of outlier expression, monoallelic expression, and splicing analyses.[28,29] Translation of RNA-seq into the clinic is challenging due to both inter- and intralaboratory technical variation in establishing appropriate thresholds.[30]

Detection of molecular alterations by gene panel, WES, WGS, or RNA-seq all offer the benefits of base-level resolution, can potentially be used to determine clonal status in AML and may be more reproducible than standard cytogenetic testing. While these methods can provide results for many genes, the interpretation of these tests can be very complicated—mutations in many genes are likely to be reported as variants of uncertain significance (VUS), and variants

in most genes on larger panels are not currently clinically actionable. One solution for this challenge is to adopt informatic pipelines that allow for querying only a limited set of genes for a given patient—this limits the challenge of incidental or uncertain findings, while simplifying wet laboratory processes and allowing for additional queries without resequencing patient material.

There are several other potential technologies for clinical screening in the myeloid malignancies. For detection of CNAs, DNA microarrays and multiplex ligation-dependent probe amplification can be used. RNA microarrays are still widely used for global gene expression profiling, and reduced representation gene expression analysis platforms such as RT-PCR or NanoString can inexpensively and sensitively quantify gene expression for a limited set of targets. Similar to the trade-off between gene panels and WGS, reduced representation CNA and gene expression assays require redesign and revalidation as the knowledge of disease pathogenesis grows, compared to more comprehensive platforms. Looking forward, long-read sequencing technologies, exemplified by Nanopore and PacBio, can be used for improved detection of transcript isoforms and structural variants. All these technologies offer different trade-offs between cost, turnaround time, and the class and scope of molecular alterations they are able to recover.

Turnaround time for clinical assays needs to be minimized. For selected disease conditions and molecular alterations, a greatly reduced turnaround time can alter treatment courses. While gene panel testing can often take 2-3 weeks, oftentimes clinical questions revolve around single genes. *FLT3* testing, for example, offers clinically actionable information (the presence of internal tandem duplication or tyrosine kinase domain mutations can be targeted with FDA-approved drugs) and should be obtained as rapidly as possible following initial diagnosis. It will likely be the case that focussed panels (or single-gene tests) with rapid turnaround time (<48 hours) will need to be performed in addition to larger gene panels.[31] For MRD applications, rapid turnaround assays like digital droplet polymerase chain reaction (ddPCR) can also be useful when the allele fraction of specific mutations needs to be tracked.

The maturation of NGS-related technologies raises the question of how (or indeed whether) standard cytogenetic and FISH assays should be maintained as part of diagnostic clinical testing. Cytogenetic testing has the advantage of a long history of diagnostic importance in the myeloid malignancies, single-cell resolution, and potentially faster turnaround time compared to NGS assays. However, cytogenetic methods are prone to interobserver disagreement and can be challenging to interpret. Additionally, many cryptic gene fusions may not be detected by either cytogenetics or FISH. "Complex" cytogenetics can mask a large array of different molecular alterations. For example, clinical guidelines consider all patients with more than three cytogenetic events to be "complex" karyotype, with an associated adverse risk. However, using RNA-seq the specific gene fusion events can be independently evaluated—in some cases leading to a revised risk stratification. For example, in our own data (T.R.D. and A.K., unpublished data) we observed an AML case initially classed as "complex," where RNA-seq revealed a *CBFB-MYH11* (inv(16)) gene fusion event and gene expression and outcomes consistent with other core binding factor AMLs.

As the importance of testing at multiple timepoints (ie, diagnosis, remission, and relapse) increases, sequencing-based assays should be able to scale more effectively than cytogenetic assays and provide higher sensitivity to events at low clonal frequencies (excluding CNAs, which are difficult to detect in low-frequency clones). In particular, it is likely to become increasingly important to determine which molecular alterations are occurring within the same disease clone—methods that rely on using both cytogenetic and sequencing-based assays will not be able to address this challenge as effectively as comprehensive sequencing-based methods. Single-cell methods for assaying either DNA or RNA are currently being used to show the heterogeneity within patient samples.[32-34] At present, these methods are too time-consuming and costly for routine clinical application. As single-cell genomic methods mature, and the impact of clonal heterogeneity on disease outcomes becomes more clear, these methods will likely have a large impact on clinical decision-making.

Laboratories must consider the risks of incidental findings with the use of large panels in testing myeloid malignancies. There are several germ line mutations which may predispose toward myeloid malignancies,[35,36] and sequencing panels that include these genes will identify both somatic and germ line alterations. For example, Drazer et al[37] confirmed several cases of germ line variants in *DDX41*, *GATA2*, and *TP53* identified by tumor gene panel sequencing. Additionally, the risk of discovering germ line variants that are unrelated or only peripherally related to hematologic malignancies is also problematic. For instance, variants in *EZH2* are associated with Weaver syndrome,[38] and mutations in a range of genes are associated with RASopathies such as Noonan syndrome.[39] As panel sizes increase, so does the risk of detecting incidental findings unrelated to the clinical questions at hand.

The main choice facing most clinical laboratories is which mix of technologies provides the best analytic validity and clinical utility for their budgets and patient populations. In all cases, patients should be screened in clinically accredited laboratories,[40,41] which have validated assay performance. At present, the combination of focussed gene panel testing with cytogenetic testing is the best option for laboratories seeking to meet current diagnostic needs, while we expect RNA-based and genome-wide testing modalities to become more relevant in the future.

## 4 | WHAT IS THE POTENTIAL VALUE ADDED BY GENOME-WIDE TESTING?

Beyond assessment of standard molecular alterations, genomic testing offers several additional benefits. For example, genome- or transcriptome-wide tests such as WGS and RNA-seq can also be used to infer human leukocyte antigen (HLA) genotypes—this may be useful for immunogenomics approaches and identifying HLA genotypes before transplant. Additionally, genome-wide approaches

can identify cases where the HLA loci are themselves altered by the disease. For example, Christopher et al[42] used RNA-seq to identify AML patients with the downregulation of HLA loci genes at relapse after transplantation, suggesting that dysregulation of immune function was involved in this relapse and potentially reversible.

While the prognostic significance of some gene fusions is well-established (the *PML-RARA*, *RUNX1-RUNX1T1*, *MLLT3-KMT2A*, and *CBFB-MYH11* fusions all correspond to distinct WHO subtypes of AML), the landscape of fusions in cancer is very complicated.[43,44] The prognostic relevance of cryptic or novel gene fusions is difficult to interpret, especially when those fusions are accompanied by additional somatic mutations. In some cases, these fusions occur in patients with complex cytogenetics, where the prognosis is already adverse; still, the presence of gene fusions in disease-related genes can serve to provide novel hypotheses for personalized medicine approaches and guide patients to targeted therapies.

Additionally, recent studies have raised the possibility of "genome as a biomarker" approaches—approaches that consider characteristics of the genome as a whole (rather than point alterations). Tumor mutation burden (ie, the number of observed somatic alterations observed in a patient) is thought to be an indicator for response to immunooncology drugs.[45] Mutational signature analysis[46] can also reveal underlying mutation-generating processes. While the genomes of patients with myeloid malignancies have fewer somatic alterations than other cancers, these approaches may still yield benefits for patients as these phenomena are better described. Additional genomic biomarkers such as signatures of structural variation[47] and microsatellite instability (MSI) have also been described as potential biomarkers, although the presence of MSI in leukemia may be rare.[48]

## 5 | WHAT ARE THE SPECIFIC ADVANTAGES OF A TRANSCRIPTOME-WIDE ASSAY?

There are many potential applications and benefits of adding clinical RNA-seq testing.[30,49] Early microarray papers were used to discriminate AML from ALL,[50] and other groups have published either single-gene or multigene prognostic expression-based assays for predicting outcome, relapse probability, or other features.[51,52] In our own laboratory, we recently sought to compare WGS, WES, and RNA-seq for potential application as platforms for clinical diagnostics in AML and MDS (T.R.D. and A.K., unpublished data). We found that RNA-seq offered the most substantial clinical benefits, due to the ability to identify expressed gene fusions, SNV, and short indel variants, as well as whole-transcriptome expression information. We observed an increase in variant calling sensitivity (for a set of 44 myeloid-related genes) in RNA-seq, due to consistent coverage problems in standard exome capture kits, and were able to restratify several patients by detecting novel gene fusion events. While genome-wide SNV detection using RNA-seq would not be expected to have the same sensitivity as deep WGS data (due to varying levels of gene expression, or nonexpressed mutations), RNA-seq variant calling on a limited set of targets can usefully augment DNA-based variant calling or indeed serve as a stand-alone assay for myeloid-relevant genes. Additionally, by applying gene expression signature analysis, we were able to restratify patients from the ELN intermediate-risk group into better- and worse-risk subsets. Altogether, the RNA-seq assay provided more clinically actionable information than either the WGS or WES approaches. Our laboratory has also seen that expression information is useful in inferring the mechanisms of lenalidomide resistance.[53]

We are also at the beginning of understanding the role of noncoding RNAs in myeloid malignancy. Newer RNA-seq protocols will be able to provide useful information on lncRNAs and other RNA species. MicroRNAs have many demonstrated roles in AML pathogenesis,[54] and miRNA expression signatures have also been used to predict event-free survival in pediatric AMLs.[55] Recent work has identified the recurrence of common splicing mutations in myeloid malignancies. Inference of the particular downstream splicing changes induced by these alterations is likely to be important for identifying precision therapies.[56] RNA-seq can also be used to infer "splicing signatures", which can group patients into groups with differing patterns of splice-site usage, without relying on detection of a canonical splicing mutation.[57]

The epigenetic landscape of myeloid malignancies may also have useful information for clinical management. For example, classification of cancer types based on profiling of differentially methylated regions of DNA from cell-free DNA (cfDNA) from plasma has recently been demonstrated.[58] Myeloid malignancies are also often affected by mutations in epigenetic modifiers such as *DNMT3A* and *TET2*, and hypomethylating agents are standard of care in subsets of patients. The mechanisms of resistance to treatment with azacitidine may also involve distinct epigenetic cell states (Kieran O'Neill and Aly Karsan, unpublished data), and prediction of resistance to this drug may require clinical assessment of epigenetic states.

## 6 | ADDITIONAL TESTING CONSIDERATIONS

There are several additional considerations for laboratories incorporating genome-scale diagnostics. Laboratories must carefully consider how to handle potential germ line changes discovered through testing, requiring collaboration between diagnosticians and local genetic counselors to be established in order to effectively triage and manage these findings.[59] This issue is complicated by the fact that several genes may be targeted by both germ line and somatic mutations,[37] so simply separating genes into familial and diagnostic panels may not suffice. Genome-wide screening increases this challenge substantially.

Whether or not to include matched normal samples is also an important question. For most clinical testing applications in myeloid malignancies, only a diagnostic specimen (peripheral blood or bone

marrow) is available. Matched normal tissue can be difficult to come by, since buccal swabs and saliva may be contaminated with blood cells. Hair follicles or cultured marrow fibroblasts may offer alternative sources of uncontaminated normal tissue or skin samples obtained at remission.[24] Sequencing matched normal tissue can double the reagent and consumable costs for a given test, so development of methods that can obviate the need for matched normals have the potential for substantially reducing testing costs. For many variants, distinguishing between germ line and somatic variants based on population frequency observations and variant allele fraction (VAF) can be effective. For example, Garofalo et al[60] observed that germ line false-positive variants (ie, germ line alterations incorrectly inferred to be somatic alterations) could be limited using large germ line variant databases and expert review. However, this is not a foolproof solution by any means, especially in populations underrepresented in genetic databases. In a large cohort (1566 patients screened using a 341-gene panel), Schrader et al[61] observed variants of uncertain significance in nearly all patients, with presumed pathogenic germ line variants in 15.7% of the cohort. In another study, Jones et al[62] observed a large number of variants that could not be correctly identified as germ line in origin when comparing matched and unmatched approaches.

As panel sizes increase, the interpretation burden placed on clinical laboratories grows. In large panels, many variants with uncertain significance may be uncovered, which may cause unintended stress for patients and clinicians. Additionally, combinatorial effects between most variants are not understood. Therefore, the bottleneck for most clinical laboratories is in variant interpretation and compute infrastructure, rather than the wet laboratory aspects of sequencing.[63]

Current stratification models in AML and MDS generally rely on straightforward decision trees for defining patient risk status. As the number of clinical and mutational features that are known to be relevant to disease stratification increases, these simple decision trees may no longer suffice. As examples of potential revised approaches, Gerstung el al[64] observed that combining mutations and expression in MDS leads to better outcome predictions in MDS, and combining many clinical and molecular markers using a knowledge bank approach[65] can provide additional insight. These approaches offer the benefit of allowing for more sophisticated integration of multiple data types. Additional multiomic prediction methods are likely to leverage deep learning methods to infer the likely disease course and risk status for patients.[66] However, the outcomes to these models are probabilistic in nature, and often difficult to interpret, and thus may not be initially welcomed by a clinical community used to more straightforward algorithms. As well, while probabilistic models can incorporate information from many variables, these models require data from many patients (on the order of thousands to tens of thousands) to achieve good accuracy. As the number of variables in any given model increases, so do the potential combinations of molecular alterations. Incorporating genetic alterations across many genes and clinical variables will require a paradigm shift in disease stratification.

Additional pragmatic considerations in testing include the eligibility of molecular testing for insurance coverage or reimbursement, which varies widely between jurisdictions. As well, whether or not a patient is initially admitted to a hospital associated with an academic cancer center will influence the type of testing available, raising issues of equity.

## 7 | WHEN SHOULD SCREENING OCCUR?

In clinical settings, mutational screening in myeloid malignancies is usually performed only at initial disease diagnosis (or upon suspicion of the presence of myeloid malignancy). Emerging evidence shows that testing at remission, or following relapse, might offer substantial benefits in treating patients (Figure 3). For example, testing at remission can identify the degree to which the patient has cleared disease clones, and which mutations are present in any remaining clones. In AML, this approach has been shown to be a useful predictor of relapse—patients with detectable mutations are at much higher risk of relapse.[67,68] Similarly, MDS patients with mutations persisting after transplantation are at a higher risk of progression.[69] Screening at remission can be accomplished by targeted MRD assays (ie, targeting specific known mutations at diagnosis), using error-corrected sequencing methods to detect mutations at very low (ie, <1%) VAFs. However, using wider panels at remission may identify novel mutations emerging or expanding after therapy, as has been observed in deeply sequenced AML genomes at relapse.[24] While these recent studies highlight the value of MRD detection, it remains the case that many patients with no detectable mutation burden will still experience poor outcomes. In this latter situation, transcriptomic or epigenomic assays may be of value in the future as we better understand the implications of these findings and approaches are standardized.

Recent research demonstrating the existence of preleukemic syndromes like CHIP also suggests potential benefits to screening tests prior to disease onset. However, the benefits of this type of testing are unclear at present—detection of a mutation at low VAF in a CHIP-related gene (eg, *DNMT3A*, *TET2*, and *ASXL1*) is not sufficient

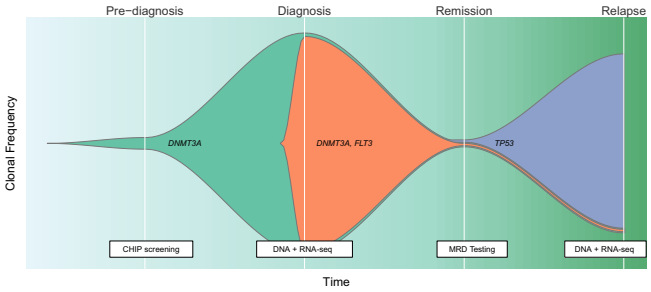


**FIGURE 3** Disease progression and molecular testing in acute myeloid leukemia (AML). In this plot, clonal evolution for a stereotypical AML case is presented as clonal frequencies through time, with principal somatic mutations for each clone indicated. At each significant timepoint, the type of molecular testing recommended is shown in the lower text boxes

to distinguish between CHIP and low-grade MDS. Interestingly, early detection of CHIP may predict risk of developing cardiovascular disease,[70] with potentially actionable consequences. As well, *PPM1D* mutations are common in solid tumor patients who have received platinum therapy and appear to predispose to a secondary hematological malignancy.[71] Screening for these mutations may also be of clinical benefit. Further research is needed to better define the risks and benefits of broad-based screening for clonal hematopoiesis.

Typically patients are only screened when there is a suspected myeloid malignancy or upon morphological evaluation of bone marrow specimens. If more patients are screened prior to disease onset, there is a risk of identifying MDS- or AML-related mutations in cases that are not going to develop into frank malignancy—this can incur substantial costs to the healthcare system and significant stress for patients. On the other hand, some proportion of these cases may benefit from early mutation identification and monitoring—the trade-off between disease prevalence, and the positive and negative predictive value of screening prior to disease onset are currently unclear. Large studies have established that patients with CHIP are at a much higher risk of developing a hematological malignancy.[13] Additional studies with longer follow-up periods are required to establish the proportion of patients with mutations in different CHIP genes who go on to develop a hematological malignancy. When patients present with clear signs of MDS or AML, diagnostic testing is clearly indicated and cost-effective.[72] Genomic testing can help establish a definitive diagnosis, potentially identifying alternative hematological malignancies.

## 8 | CONCLUSIONS

The potential amount of molecular data that can now be routinely acquired for a given patient presenting with or suspected of having a myeloid malignancy has increased enormously with the availability of NGS-based approaches. Research studies have elaborated the landscape of molecular alterations in these diseases, revealing both recurrent and rare mutations, and the fact that most diseases are made up of several competing clones. However, the clinical actionability for most of these alterations is limited to alterations in a few genes that define molecular risk categories or with targeted therapies available. Moreover, for any single patient, the additional utility of genomic information is uncertain, compared to traditional screening methods.[73,74]

However, genomic screening tests have substantial benefits in identifying novel risk genes, cryptic gene fusions, and the clonal composition of samples, among other benefits. More clinical trials are adopting “basket” or “umbrella” approaches, which mean more patients may become eligible for trials, but only if they receive testing that is comprehensive enough to identify a broad range of molecular alterations. Additionally, genome-wide screens allow for retrospective reanalysis, allowing for the development of novel hypotheses for new research projects. Due to exciting developments showing how the whole genome, transcriptome, or epigenome can serve as biomarkers for underlying pathogenic processes, genome-wide screening may also become more widely used.

The availability of complex NGS data sets also serves as a harbinger of a likely change in patient stratification algorithms—from straightforward decision trees to probabilistic models incorporating many variables. Altogether, the union of sensitive molecular screening with novel informatics algorithms promises to improve our ability to understand a given patient's disease, to determine appropriate treatment courses, and ultimately to improve outcomes in the myeloid malignancies.

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## CONFLICT OF INTERESTS

None.

## AUTHORS CONTRIBUTION

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