Leveraging Cancer Genome Information in Hematologic Malignancies

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ABSTRACT

The use of candidate gene and genome-wide discovery studies in the last several years has led to an expansion of our knowledge of the spectrum of recurrent, somatic disease alleles, which contribute to the pathogenesis of hematologic malignancies. Notably, these studies have also begun to fundamentally change our ability to develop informative prognostic schema that inform outcome and therapeutic response, yielding substantive insights into mechanisms of hematopoietic transformation in different tissue compartments. Although these studies have already had important biologic and translational impact, significant challenges remain in systematically applying these findings to clinical decision making and in implementing new technologies for genetic analysis into clinical practice to inform real-time decision making. Here, we review recent major genetic advances in myeloid and lymphoid malignancies, the impact of these findings on prognostic models, our understanding of disease initiation and evolution, and the implication of genomic discoveries on clinical decision making. Finally, we discuss general concepts in genetic modeling and the current state-of-the-art technology used in genetic investigation.

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INTRODUCTION

Our understanding of the biology, therapy, and prognostication of hematologic malignancies, including leukemias, lymphoma, and multiple myeloma (MM), has a long and informative history that has had a major impact on clinical oncology. Hematologic neoplasms were the first to be shown to be responsive to cytotoxic chemotherapy, to be characterized by recurrent genomic alterations including chromosomal translocations, and to be effectively treated with molecularly targeted therapy. Moreover, studies in acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL) have shown that clonogenic cytogenetic abnormalities inform prognosis and can guide therapeutic interventions.

More recently, genomic studies including candidate gene resequencing, copy number profiling, and whole-genome/exome sequencing have led to the identification of recurrent mutations and amplifications/deletions in a spectrum of hematologic malignancies. In addition, several of these newly identified genetic abnormalities have been shown to have prognostic importance in the clinical context. Here, we review the prognostic relevance of novel disease alleles in hematologic malignancies and discuss how genetic data can be used to inform outcome and therapy in leukemia, lymphoma, and MM.

MYELOID MALIGNANCIES

AML

The genetic basis of AML has been investigated in a series of large-scale genomic profiling studies. The prognostication of AML has historically been based on cytogenetic data, which can separate patients into favorable, intermediate, and unfavorable risk disease. Patients with inversion 16 (inv16) and t(8;21) are classified as having good risk disease, whereas patients with monosomy of chromosome 5/7 or with complex karyotypes are considered to have poor risk disease.¹ Notably, this classification has influenced therapeutic decisions, as patients with favorable chromosomal alterations are treated with induction/consolidation chemotherapy alone and patients with unfavorable chromosomal alterations are offered allogeneic stem-cell transplantation as part of their therapy.² As such, cytogenetic profiling has become the standard of care for all patients with AML, and chromosomal alterations are used to guide postinduction therapeutic decisions in the clinical context.

Patients with AML having intermediate-risk cytogenetics, including those with a normal karyotype, have variable outcomes. Schlenk et al³ reported that patients with normal karyotype AML with *CEBPA* mutations, or with *NPM1* mutations without co-occurring *FLT3*-ITD mutations, had improved

Fig 1. Risk profile for de novo acute myeloid leukemia based on large-scale mutational studies from Eastern Cooperative Oncology Group E1900⁹ study for patients (A) without *FLT3*-ITD and (B) with *FLT3*-ITD mutations.

relapse-free survival relative to other genotypes. Patients with *FLT3*-ITD, or lacking mutations in *NPM1*, *FLT3*, and *CEBPA*, had a comparatively worsened outcome. More importantly, they also demonstrated that allogeneic stem-cell transplantation improved outcomes in patients with *FLT3*-ITD mutations and in those patients without mutations in *NPM1*, *FLT3*, and *CEBPA*. This study represented the first demonstration of how mutational profiling can inform AML biology and prognosis and led to the incorporation of testing for *CEBPA*, *NPM1*, and *FLT3*-ITD into the routine clinical care for patients with AML who are less than 60 to 65 years of age.

Until recently, the spectrum of somatic disease alleles that contribute to AML had not been systematically evaluated. Recent efforts have sought to define the spectrum of somatic mutations in patients with AML. Ley et al⁴ first reported the results of whole-genome sequencing of a sample from a patient with cancer, specifically reporting the genome of a patient who presented with normal karyotype AML. This work revealed *NPM1* and *FLT3*-ITD mutations, as well as eight novel somatic mutations, thus providing proof of principle that whole-genome sequencing can be used to identify somatic mutations in patients with AML. Subsequent whole-genome/exome sequencing studies identified recurrent *IDH1* mutations in AML,⁵ followed by the discovery of recurrent mutations in *DNMT3A* in AML.^{6,7} Welch et al⁸ reported whole-genome sequencing of 24 patients with AML, including both patients with acute promyelocytic leukemia (APL) and non-APL AML. Of note, compared with solid tumors, the mutational burden in AML is significantly less, with a median of 10 somatic mutations per AML genome that result in missense, frameshift, or nonsense changes in the coding sequence of known genes.

In addition, recent studies have used candidate gene sequencing approaches to identify novel, recurrent somatic mutations in AML. *ASXL1*, an epigenetic regulator, is mutated in 5% to 15% of patients with AML.^{9,10} On the basis of the identification of *IDH1* mutations in

AML, *IDH2* mutations were identified through candidate gene sequencing and metabolic profiling.¹¹ Collectively, *IDH1* and *IDH2* mutations occur at a frequency of 15% to 30% of patients with AML. *TET2* mutations occur in approximately 10% of patients with AML.¹² Indeed, data from whole-genome sequencing in AML suggest there are at least 23 genes that are recurrently mutated in AML; these include genes with specific “hotspots” targeted by mutations (eg, *C-KIT*, *FLT3*) as well as tumor suppressors (eg, *TET2*, *ASXL1*), which are inactivated by nonsense/frameshift alleles.

The primary challenge, then, is to determine how these data can be used to inform outcome in AML, guide the use of existing therapies, and inform the development of novel therapeutic approaches for patients with AML. In an effort to determine whether a larger set of mutant disease alleles can inform outcome in AML, Patel et al⁹ performed mutational profiling of 18 genes from a single phase III trial cohort of patients with AML younger than 60 years. These data allowed the identification of genetically distinct subsets of patients with AML, based on mutational status, with dramatically different prognoses (summarized in Fig 1). These data suggest that the mutational status of nine recurrently mutated genes can be used to improve prognostication in AML. We contend that clinical trials should aim to identify molecularly defined patients at high risk of relapse, such that these patients are offered novel therapies early in their disease course to improve outcomes in AML.

In addition, mutational profiling in AML may also inform the use of existing therapies based on genetic data. The Eastern Cooperative Oncology Group E1900 trial demonstrated that induction therapy that included 90 mg/m² of daunorubicin per day was superior to treatment with induction therapy, which included a conventional dose of 45 mg/m² per day. Post hoc analysis of this trial cohort found that high-dose daunorubicin markedly improved outcomes for patients with *DNMT3A* mutations, *MLL* fusions, or *NPM1* mutations

and that patients' wild type for these three genes did not benefit from higher dose induction chemotherapy. Although these findings need to be further validated in a prospective clinical trial, these data suggest that specific, genetically defined subsets of patients with AML benefit from dose-intense induction chemotherapy and demonstrate how profiling of large, phase III AML trials can inform prognosis and therapeutic decisions with near-term clinical relevance.

Myelodysplastic Syndromes

Several recent candidate gene and whole-exome approaches have yielded new insights into myelodysplastic syndrome (MDS). These include *EZH2* mutations^{13,14} and mutations in the spliceosome machinery.¹⁵⁻¹⁷ However, the challenge has been to delineate how this knowledge can be used to inform the care of patients with MDS. In a seminal article, Bejar et al¹⁸ performed extensive mutational profiling of a large cohort of patients with MDS and found that mutations in five genes, specifically *ASXL1*, *EZH2*, *TP53*, *ETV6*, and *RUNX1*, predicted for adverse outcome in MDS. More recently, they extended their genetic studies to patients with lower risk MDS and found that mutations in the same genes (with the exception of *ETV6*) were associated with independent, adverse, prognostic relevance in lower risk MDS.¹⁹ Consequently, there are now clinical tests for mutations in these specific genes available for clinicians and patients with MDS.

Myeloproliferative Neoplasms

Significant strides in the genetic understanding of myeloproliferative neoplasms (MPN) have occurred in the past several years. In 2005, several groups identified somatic, recurrent *JAK2V617F* mutations in the majority of patients with polycythemia vera, essential thrombocythemia, and primary myelofibrosis.²⁰ Subsequently, mutations in *JAK2* exon 12, in the thrombopoietin receptor *MPL*,²¹ and in the negative regulator of the JAK-STAT pathway *LNK*²² have been identified in *JAK2V617F*-negative patients. Taken together, these data underscore the central role of JAK-STAT signaling in patients with MPN and led to the development and approval of JAK inhibitors for the treatment of primary myelofibrosis.²³

More recently, mutations outside the JAK-STAT pathway have been identified in patients with MPN. In some cases, these mutations are most common in patients with MPN who transform to AML.²⁴ Specifically, mutations in *TET2*,²⁵ *IDH1/2*, *RUNX1*, *TP53*,²⁵ *WT1*, *CBL*, and *NRAS* occur most commonly in post-MPN patients with AML. More recently, the acquisition of *SRSF2* mutations has been identified at the time of leukemic transformation.²⁶ Importantly, the frequency and spectrum of mutation in post-MPN AML differs from that found in de novo AML, indicating that transformation from MPN to AML has a distinct molecular pathophysiology (Fig 2).

In contrast to AML and MDS, where molecular studies of large, well-annotated clinical cohorts have identified specific mutations with robust prognostic relevance, the role of specific disease alleles in predicting outcome and response to therapy in patients with MPN has not been well delineated.

Disease Evolution and Clonality

The molecular basis for relapse in myeloid malignancies has not been conclusively delineated; however, recent work by Ding et al²⁷ used whole-genome sequencing of eight patients with AML at initial diagnosis and at relapse to study clonal evolution with AML therapy.

In all patients studied, a dominant clone was present at the time of diagnosis and relapse. At the time of relapse, in five cases a relapsed subclone emerged as the dominant clone, and this relapsed clone was characterized by the acquisition of additional mutations not present at diagnosis before antileukemic therapy. In the other three patients, the dominant clone at the time of diagnosis remained dominant at the time of relapse, but had acquired additional mutations not present before therapy. Further work in this area to define the spectrum of genetic and epigenetic alterations acquired at the time of relapse will allow for a better understanding of the molecular basis of therapeutic relapse in myeloid malignancies and may inform the development of novel therapeutic interventions to prevent or to treat relapsed leukemia.

LYMPHOID MALIGNANCIES

Acute Lymphoblastic Leukemia

Acute lymphoblastic leukemia (ALL) is the most common malignancy seen in children; please see the accompanying review on pediatric malignancies. Copy-number analysis using SNP arrays²⁸ led to the identification of deletions, chromosomal translocations, and loss-of-function mutations targeting *PAX5*, a transcription factor required for normal B-cell development. This same study also identified deletions in *IKZF1*, *IKZF3*, *E2A*, *EBF1*, and *LEF1*, suggesting that B-cell ALL (B-ALL) is commonly characterized by loss-of-function somatic alterations in members of the B-cell differentiation pathway. Of note, *IKZF1* deletions cause loss of the DNA zinc-finger binding domain, resulting in a dominant-negative protein, and are most common in *BCR-ABL* rearranged B-ALL, where they mark a subset of patients with B-ALL with poor prognosis.²⁹

The presence of *BCR-ABL* fusions and deletions in *IKZF1* in high-risk, pediatric ALL led investigators to hypothesize that high risk B-ALL is characterized by co-occurring mutations that activate tyrosine kinase signaling with mutations in B-cell differentiation pathways. Indeed, gene expression profiling led to the identification of a subset of patients with B-ALL having so-called Ph-like ALL, which was characterized by a gene expression signature similar to that observed in *BCR-ABL* rearranged B-ALL and by a poor overall outcome.³⁰ Importantly, subsequent studies have identified a high frequency of activating mutations in tyrosine kinase signaling pathways in Ph-like B-ALL. These include, notably, mutations in *TP53/RB*, *JAK/STAT*, lymphoid development pathways, and *RAS* signaling pathways. The *JAK2R683* mutation was initially identified in patients with B-ALL associated with Down syndrome.³¹ *JAK1* mutations have been described in adult B-ALL.³²

More recently, Roberts et al³³ performed whole-genome and transcriptome sequencing of 15 cases of Ph-like B-ALL. In the majority of these cases, they identified mutations or translocations in tyrosine kinase signaling pathway effectors, most commonly in the JAK-STAT signaling pathway. However, these alterations occurred through a variety of genetic alterations, including activating point mutations in oncogenes, loss-of-function mutations in negative regulators, and translocations of specific signaling effectors. As such, developing clinical-grade tests to identify the spectrum of targetable alterations in B-ALL will require a multimodal approach that identifies mutations, deletions, and translocations that are amenable to specific molecularly targeted therapies.

Fig 2. Comparison of the relative frequency of mutations (A) in de novo acute myeloid leukemia (AML),⁹ post-myeloproliferative neoplasm (MPN) AML,²⁶ and myelodysplastic syndrome (MDS)^{18,19} from three candidate gene sequencing studies. Mutation frequency (B) *FLT3-ITD*, (C) *DNMT3A*, (D) *TET2*, and (E) *TP53* in de novo and post-MPN AML.^{9,26}

The majority of T-ALL cases are characterized by activating mutations in *NOTCH1*.³⁴ Other recently described mutations in T-ALL include *FBXW7* (a ubiquitin ligase involved in regulating Notch turnover),^{35,36} *IL7R*,³⁷ *PHF6*,³⁸ *WT1*,³⁹ *PTPN2*,⁴⁰ and *FLT3-ITD*.⁴¹

Studies by Coustan-Smith et al⁴² have identified a specific subset of T-ALL, known as early T-cell precursor ALL, which is associated with a particularly poor prognosis and often characterized by mutations commonly seen in myeloid malignancies, including *IDH* and *ETV6* mutations.⁴³ Recent whole-genome sequencing efforts⁴⁴ in early T-cell precursor ALL led to the identification of mutations in *GATA3*, *IKZF1*, *ETV6*, *BRAF*, *IL7R*, *JAK1*, and *JAK3*. Notably, this

included common mutations in members of the polycomb repressive complex 2 (PRC2), specifically *EZH2* and *SUZ12*, which are commonly mutated in MDS and MPN.

Disease Evolution and Clonality

Several studies have investigated clonal evolution from the time of initial ALL diagnosis to relapse to identify factors that may portend relapse. Yang et al⁴⁵ used single-nucleotide polymorphism (SNP) arrays in 20 paired diagnosis/relapse pediatric B-ALL samples to address this question. Many copy number alterations (CNAs) identified at diagnosis were present at relapse. *EBF1* and *IKZF1* deletions occurred

frequently in paired diagnosis and relapse samples. Deletions of these genes were also found at relapse only in a subset of cases, and the overall frequency of these deletions, as well as deletions in *CDKN2A*, were found at a higher frequency in these diagnosis/relapse pairs than had been previously reported, thus potentially implicating them in the development of relapse. Another study of 61 diagnosis/relapse pediatric B-ALL and T-ALL pairs using SNP arrays demonstrated a significant increase in CNAs in B-ALL relapse samples, but not in relapsed T-ALL relapse samples.⁴⁶ This included gain of new CNAs and loss of CNAs present at diagnosis. *CDKN2A/B*, *ETV6*, and genes involved in B-cell development were the genes most affected by new alterations at relapse. The finding that the relapse leukemic population shares some genetic features with the diagnosis leukemic population, together with the lack of a unifying set of genomic alterations at relapse, suggests that divergent ALL subclones are present at diagnosis and that minor subclones in the diagnostic sample can manifest as the dominant clone at relapse.

Therapeutic Implications

The most successful therapeutic innovation in ALL has been the successful targeting of tyrosine kinases. A number of studies have demonstrated improved outcomes in adults with BCR-ABL-positive B-ALL when a tyrosine kinase inhibitor is incorporated in a treatment regimen (reviewed elsewhere). In the pediatric population, the use of imatinib with intensive chemotherapy has also been demonstrated to result in a significant improvement in overall survival compared with historical controls.⁴⁷ Notably, patients who went on to receive allogeneic stem-cell transplantation followed by maintenance imatinib had similar outcomes to patients treated with imatinib and chemotherapy.⁴⁸ Other genetic lesions involved in the pathogenesis of ALL may also be targetable, including inhibition of the DOT1L kinase in MLL-positive ALL,⁴⁹ targeting of the *NOTCH1* pathway in T-ALL,⁵⁰ and JAK kinase inhibition in patients with ALL having mutations in the JAK-STAT signaling pathway. This will require the use of validated diagnostic assays to identify these lesions and demonstration of target inhibition with clinically active compounds against these targets in the clinical setting.

Chronic Lymphocytic Leukemia

Recent investigative efforts in chronic lymphocytic leukemia (CLL) have used genome and exome sequencing to delineate the spectrum of mutant disease alleles, which occur in CLL. Fabbri et al⁵¹ performed whole-exome sequencing and SNP analysis in five patients with CLL. They identified recurrent somatic mutations in *NOTCH1*, identical to those seen in T-ALL, as well as somatic mutations in *TGM7*, *BIRC3*, and *PLEKHG5*. Whole-genome sequencing of four cases of CLL revealed recurrent mutations in *NOTCH1*, *XPO1*, *MYD88*, and *KLHL6*. *NOTCH1* and *XPO1* mutations were associated with nonmutated immunoglobulin status, whereas *MYD88* and *KLHL6* were associated with mutated immunoglobulin status.⁵² Most recently, two studies using whole-exome sequencing to profile large CLL cohorts identified recurrent mutations in the splicing factor *SF3B1*. This was also found in whole-exome and whole-genome sequencing of 88 patients with CLL.⁵³ Exome sequencing also identified mutations in *TP53*, *ATM*, *MYD88*, *NOTCH1*, *ZMYM3*, *MAPK1*, *FBXW7*, and *DDX3X* in CLL.^{53,54} However, the prognostic relevance of specific mutant disease alleles in CLL has only recently been investigated. Work by Fabbri et al⁵¹ demonstrated that in CLL, *NOTCH1*

mutations are associated with a shorter time to progression requiring therapy and a shorter overall survival. *SF3B1* mutations have been associated with more rapid disease progression and a decrease in overall survival⁵⁴ and are associated with del 11q, a known poor prognostic marker.⁵³ It will be important to delineate whether specific mutations in CLL predict the need for therapy, influence the response to CLL therapeutics, or influence overall outcome in large, homogeneously treated cohorts.

Transformation to diffuse large B-cell lymphoma (DLBCL), known as Richter transformation, remains a significant clinical problem in CLL and in other low-grade lymphoid malignancies. Rossi et al⁵⁵ identified frequent mutations of *TP53*, deletion of chromosome 17p13, and *c-MYC* amplification and translocations in patients who transformed to large-cell lymphoma. There were notable differences in the spectrum of genetic events between DLBCL derived from CLL and de novo DLBCL. For example, *BCL6* and *BCL2* translocations, as well as mutations targeting *EZH2* and genes of the nuclear factor κ B (NF- κ B) pathways were largely absent in CLL transformed to DLBCL, although these are common genetic alterations in de novo DLBCL. Of additional note, although most cases of transformed disease were clonally related to the precursor CLL, approximately 20% of DLBCLs were not clonally related, potentially implicating the presence of multiple precursor clones. Finally, *NOTCH1* mutations are enriched in patients with CLL who transform to DLBCL and in patients with CLL having chemotherapy-refractory disease.⁵¹ The biologic contribution of these mutations to the process of transformation remains an open area of investigation.

Lymphoma

Recent studies have identified a spectrum of disease alleles in non-Hodgkin lymphoma (NHL). Whole-exome sequencing in splenic marginal zone lymphoma (SMZL) identified recurrent mutations in *NOTCH2* (SMZL),^{56,57} as well as mutations in *NOTCH1* and in Notch pathway members *SPEN* and *DTEX1*. Mutations and CNAs in the NF- κ B pathway were also identified. Finally, mutations in genes involved in chromatin remodeling, such as *MLL2*, *EP300*, and *CREBBP*, among others, were identified.⁵⁶ Thus these analyses have revealed the involvement of specific pathways in the pathogenesis of SMZL. Of note, *NOTCH2* mutations are associated with an increased risk of relapse and death in SMZL.⁵⁸ Whether similar genomic alterations are seen in other low-grade lymphoid neoplasms remains to be delineated.

Whole-exome sequencing of DLBCL led to the identification of recurrent mutations in *CREBBP* and *EP300*, a histone and nonhistone acetyltransferase respectively, in DLBCL.⁵⁸ Exome and transcriptome sequencing studies in DLBCL and follicular lymphoma (FL) have also revealed recurrent mutations in *MEF2B*, which cooperates with *EP300* and *CREBBP* in histone modification.⁵⁹ These investigators also found recurrent mutations in an H3K4-specific histone methyltransferase, *MLL2*, which occur in almost 90% of patients with FL. Of note, activating mutations in the PRC2 member *EZH2* have been identified in FL and DLBCL,⁵⁹ which is in direct contrast to the loss-of-function *EZH2* mutations seen in myeloid malignancies (Fig 3). Mutations in several genes involved in immune recognition by T cells, such as *B2M*, *CD58*, and *TNFSF9*, have been identified in B-cell ALL.⁶⁰ Finally, mutations in *CARD11*, a component of the NF- κ B pathway, have been identified in the activated B-cell (ABC) subtype of DLBCL.⁶¹ The

Fig 3. Mutations in genes involved in H3K27me3 histone mark are found in various myeloid and lymphoid diseases.

prognostic and therapeutic relevance of specific mutations in different lymphomas remains to be delineated.

The recent genetic findings detailed previously may help to guide therapy in specific contexts. The presence of *NOTCH* mutations and aberrations of the NF- κ B pathway offer potential therapeutic targets. As well, the mutations in *MLL2*, *CREBBP*, and *EP300* suggest a possible role for epigenetic therapies in DLBCL and FL. Most notably, *EZH2* mutations, which result in increased H3K27 trimethylation, have been validated as a target in recent preclinical studies.⁶² Finally, mutations in regulators of the JAK/STAT pathway, including *SOCS1*⁶³ (Fig 4), have led to the investigation of JAK inhibitors in both NHL and Hodgkin lymphoma.⁶⁴

Plasma Cell Malignancies

Plasma cell malignancies encompass a spectrum of clinical entities, which include monoclonal gammopathy of unknown significance, smoldering multiple myeloma, MM, and plasma cell leukemia. Although the role of specific translocations and CNAs in plasma cell malignancies has been known for some time, the role of specific somatic mutations in these diseases has been investigated much more recently. Recent insights into the pathogenesis of MM have been

Fig 4. Mutations in various genes in the JAK/STAT pathway (MPL, CRLF2, JAK1, JAK3, JAK2, LNK, SOCS1, STAT3) implicated in hematologic malignancies.

provided by somatic mutational profiling studies. The largest investigation of somatic mutations to date in MM used whole-genome sequencing and whole-exome sequencing to characterize 38 cases of MM.⁶⁵ Mutations were identified in well-known oncogenes and tumor suppressors, including *NRAS*, *KRAS*, and *TP53*, as well as a host of less well-described alleles. However, the frequency of each novel mutation was low (< 10%), suggesting that MM is a genetically heterogeneous disease with many different mutations contributing to disease pathogenesis in different patients. Other studies have identified mutations in *UTX*⁶⁶ (10%) and genes in the canonical and non-canonical NF- κ B pathway, most notably *TRAF3*.⁶⁷ Mutations in genes, in addition to *UTX*, involved in *HOXA9* regulation, and in the coagulation cascade, were significantly enriched in patients with MM using pathway-specific analyses.⁶⁵

Several genetic abnormalities have been associated with disease progression at various stages. *RAS* mutations have been noted in 5% to 7% of monoclonal gammopathy of unknown significance but in 25% to 45% of patients with MM, indicating that acquisition of these mutations may play a role in disease progression.⁶⁸ Del(17p13) and *TP53* mutations also occur more frequently in more advanced stages of disease.⁶⁸ One recent study used whole-genome sequencing on samples from a patient with t(4;14) at initial diagnosis, relapses, and evolution to plasma cell leukemia. Although 15 single-nucleotide variants were common to all phases of disease, several variants were only found at select stages, suggesting variable clonal dominance. Notably, mutations in cell cycle regulators *RB1* (hemizygous) and *ZKSCAN3* were detected at the time of transformation to plasma cell leukemia.⁶⁹

Currently, limited data exist with regard to using genomic data to select specific therapies in MM. Data from several groups has demonstrated that the use of bortezomib-containing regimens in patients with t(4;14) results in a significant improvement in overall survival.⁷⁰ One study demonstrated that the overall survival, time to progression, and complete response rate did not differ between patients with t(4;14) and those with standard cytogenetic profiles when patients were treated with bortezomib.⁷¹ The recently reported HOVON-65/GMMG-HD4 trial demonstrated that the overall survival and progression-free survival in patients with t(4;14) remained inferior to those without t(4;14) despite the use of bortezomib.⁷² Notably, *BRAF* mutations (V600E, K601N) were observed in 4% of patients in recent genetic analysis of MM.⁶⁵ Because the *BRAF*V600E mutation has been validated as a therapeutic target in melanoma, it remains to be seen whether this finding can be translated into improved therapeutic strategy and outcome in the subset of patients with MM having *BRAF* mutations.

IMPLEMENTING GENOMICS IN THE CLINICAL SETTING

Recent genetic findings in a host of hematologic malignancies have uncovered the involvement of a spectrum of somatic disease alleles in the pathogenesis of leukemias, lymphomas, and plasma cell malignancies. These studies have informed mechanisms of hematopoietic transformation and have led to extensive functional studies and the development of novel, genetically accurate models of different hematologic malignancies. In addition, the identification of specific mutations in therapeutically tractable pathways has opened up new opportunities for therapeutic intervention, as many of the acquired mutations, such as *FLT3*-ITD,⁷³ can be targeted with potent, specific small molecule therapeutics.

However, there remain two critical challenges that limit our ability to translate genomic findings to the clinical context in hematologic malignancies. The first limitation relates to the lack of robust prognostic and predictive data for novel disease alleles on clinically annotated, homogeneously treated patient cohorts. Although in specific contexts such as AML, considerable progress has been made in developing robust molecular classifiers, there remains a considerable need for detailed investigation of the relevance of specific disease alleles to outcome, response to therapy, and risk of relapse in the majority of hematologic malignancies. We hope that the increased, widespread use of genomic profiling platforms will empower these studies and encourage the development of large consortia to evaluate novel biomarkers for clinical utility in hematologic malignancies. This will require use of large patient data sets with high-quality material and clinical annotation and validation studies to determine which molecular lesions are of the highest utility in predicting outcome, choosing therapies, and designing clinical trials.

The second, equally important issue relates to the wide spectrum of disease alleles seen in hematologic malignancies. It has been shown that point mutations, tandem duplications, translocations, and deletions/amplifications all have clinical and biologic relevance in hematologic malignancies. We estimate there are at least 580 genes that have been shown to be targeted by somatic alterations in hematologic malignancies. As such, testing for this wide spectrum of disease alleles will require the development of robust, cost-effective genomic platforms that can detect all of the different types of alterations seen in hematologic tumors. Moreover, given the acute nature of many he-

matologic cancers, there will be a pressing need to develop assays that can be performed, analyzed, and reported in a very short time frame, such that clinical decisions can be made within days to a week of diagnosis. As such, there are unique challenges that face the development of clinical-grade genomic assays for hematologic tumors, and these may require dedicated effort to develop platforms to deliver clinically actionable genomic information to patients with hematologic malignancies and their care providers in a short time course.

In many cases, the rapid identification of genetic abnormalities will afford the opportunity to select treatments that are more likely to have efficacy in a given genetic context or to enroll patients on clinical trials that may target the particular mutation or pathway disrupted in their disease. We believe that the use of genomic profiling in prospective clinical trials will greatly empower the development of high-yield clinical trials and inform the development of therapies for molecularly defined subsets of patients with hematologic malignancies.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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