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.

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. 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. 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. 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 al reported that patients with normal karyotype AML with CEBPA mutations, or with NPM1 mutations without co-occurring FLT3-ITD mutations, had improved
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 who are less than 60 to 65 years of age. Recent efforts have sought to define the spectrum of somatic mutations in patients with AML who are less than 60 to 65 years of age. Recent efforts have sought to define the spectrum of somatic mutations in patients with AML who are less than 60 to 65 years of age. Recent efforts have sought to define the spectrum of somatic mutations in patients with AML who are less than 60 to 65 years of age. Recent efforts have sought to define the spectrum of somatic mutations in patients with AML who are less than 60 to 65 years of age. Recent efforts have sought to define the spectrum of somatic mutations in patients with AML who are less than 60 to 65 years of age. Recent efforts have sought to define the spectrum of somatic mutations in patients with AML who are less than 60 to 65 years of age. Recent efforts have sought to define the spectrum of somatic mutations in patients with AML who are less than 60 to 65 years of age.

Fig 1. Risk profile for de novo acute myeloid leukemia based on large-scale mutational studies from Eastern Cooperative Oncology Group E1900 study for patients (A) without FLT3-ITD and (B) with FLT3-ITD 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 and mutations in the spliceosome machinery. 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 al 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. 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 thrombocytopenia, and primary myelofibrosis. Subsequently, mutations in JAK2 exon 12, in the thrombopoietin receptor MPL, and in the negative regulator of the JAK-STAT pathway LNK were 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.

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. Specifically, mutations in TET2, IDH1/2, RUNX1, TP53, 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. 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 al 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.

### 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 arrays 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.

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. 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. JAK1 mutations have been described in adult B-ALL.

More recently, Roberts et al 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.
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 al\(^ {42}\) 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.\(^ {43}\) 

Several studies have investigated clonal evolution from the time of initial ALL diagnosis to relapse to identify factors that may portend relapse. Yang et al\(^ {45}\) 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 included common mutations in members of the polycomb repressive complex 2 (PRC2), specifically EZH2 and SUZ12, which are commonly mutated in MDS and MPN.

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**Fig 2.** Comparison of the relative frequency of mutations (A) in de novo acute myeloid leukemia (AML),\(^ {19}\) 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.\(^ {19,26}\)
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, ETF6, and genes involved in B-cell development were the genes most affected by these 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 DORPRET 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. Fabbi 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 in 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 LKHL6 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 Fabbi 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.55 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 κB (NF-κB) 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 DTX1.Mutations and CNAs in the NF-κB pathway were also identified. Finally, mutations in genes involved in chromatin remodeling, such as MLL2, EP300, and CREBBP, among others, were identified.58 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-κB pathway, have been identified in the activated B-cell (ABC) subtype of DLBCL.61
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 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. 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 (10%) and genes in the canonical and non-canonical NF-kB pathway, most notably TRAF3. 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.

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. Del(17p13) and TP53 mutations also occur more frequently in more advanced stages of disease. 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.

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. 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. 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. Notably, BRAF mutations (V600E, K601N) were observed in 4% of patients in recent genetic analysis of MM. Because the BRAFV600E 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, can be targeted with potent, specific small molecule therapeutics.
Leveraging Cancer Genome Information in Hematologic Malignancies

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 hematologic 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

Manuscript writing: All authors
Final approval of manuscript: All authors

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