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Molecular pathogenesis of AML: Translating insights to the clinic

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Abstract

Specific combinations of mutations, including FLT3 and IDH1/IDH2/TET2, frequently co-occur in acute myeloid leukemia (AML) and are associated with poor prognosis. These mutation combinations can be modeled in mice to provide a more genetically accurate model of AML. Within these models, stem cells may be different depending on how experiments are conducted and based on context. No one mutation can turn on a gene; rather the perfect storm of the right genes in the right cell is necessary to produce AML. Furthermore, this understanding is therapeutically relevant. Rapid and accurate targeted DNA sequencing will identify mutations of prognostic and therapeutic significance and will guide treatment choices in the future.

Keywords

Acute myeloid leukemia; AML; DNA sequencing; FLT3; TET2; murine models

Introduction

Our rapidly increased understanding of the pathogenesis of acute myeloid leukemia (AML) is being used to improve prognostication and clinical care. However, there are significant real-world challenges to using molecular testing in the clinical context. Over the last few years, there have been a large number of investigators in the field who have led to the discovery of a plethora of new mutations in AML, myeloproliferative neoplasms (MPN), and myelodysplastic syndromes (MDS). Both myeloid and lymphoid leukemias have benefited more greatly from gene discovery, exome and genome sequencing, and candidate gene sequencing than other tumors. For example, although ovarian cancer has been fully sequenced, genome/exome sequencing did not lead to new insights into the biology; there were no novel highly recurrent mutations; and there were no new drug targets. However, in leukemia, genomic discoveries have impacted clinical care. New mutations explain biology, new potential putative drug targets have been discovered, and these mutations occur frequently enough in patients that they can be feasibly tested for. While these discoveries are great news, they also present challenges as to how to use this information for patients. The

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fundamental challenge is how to translate these exciting discoveries to actual use in the clinical setting.

Clinical significance of mutations in AML

A number of novel mutations have been discovered in AML patients, though the clinical and biologic relevance of these novel disease alleles has not been fully delineated. Whole genome sequencing led to the identification of novel recurrent disease alleles *IDH1* mutations [1] and *DNMT3A* mutations [2]. Exome and targeted resequencing studies identified novel disease alleles in AML, MDS, and MPNs. These include *TET2* [3,4], *IDH2* [5,6], *ASXL1* [7], cohesion mutation [8,9], and spliceosome components [10].

The ECOG 1900 trial elucidated the frequency of gene mutations in a cohort of 502 AML patients. There are no mutations in AML that occur in the majority of patients, while some solid tumors, like pancreatic cancer, have dominant driving mutations. Mutation analysis in ECOG 1900 also showed that AML is not one disease. Studies in breast cancer or glioblastoma with gene expression profiling and mutational analysis result in 4 or 5 distinct but robust clusters. AML has 16–18 biologically distinct subgroups [11,12], so these subtypes must be split from a prognostic and mechanistic standpoint as well as from a therapeutic standpoint.

Importantly, this data can be used not just to describe the genetic heterogeneity, but also for prognostication. Small sets of genes have been grouped into a revised AML risk stratification based on integrated mutational profiling (Table 1). While this is still a framework for mutational data that is being used to impact and predict outcome, it can be based on targeted sequencing of just 8–10 genes. The challenge is to find what targeted genes will be of clinical and therapeutic utility to avoid requiring the sequencing of a patient's entire genome or exome.

Patients with intermediate-risk cytogenetics can be stratified by overall survival based on *NPM1* and *IDH* mutations (more favorable survival). We demonstrated that mutations in epigenetic modifiers, found in 40% of patients, portend 12% overall survival rate. New strategies need to be developed for the patients in the lowest overall survival group based on integrated mutational profiling: Should these patients receive allografts or should new trial designs be developed based on disease pathogenesis?

As disease pathogenesis and the mechanics of these mutations become more clear, patients will more easily be classified based on different types of mutations (Table 2). For example, there are hydroxylation pathway mutations (*TET2* or *IDH1/2* mutations), epigenetic modifications (*DNMT3* and *ASXL1* mutations and *MLL* alterations), class I alterations (*FLT3*, *N/KRas*, and *Kit* mutations), and class II alterations (*CEBPA*, *NPM1*, and *RUNX1* mutations and *CBF* translocations). Other novel pathways will continue to emerge that include spliceosome mutations and the recently identified mutations in cohesion pathway genes, which are important in nucleosome positioning and other aspects of higher order DNA structure. These organized sets of mutations fit together both functionally and genetically. An important goal moving forward will be to understand how these types of mutations contribute to leukemia and figure out what subsets of AML patients have those mutations. Human genetic and functional studies are needed to delineate different mutational classes and their role in leukemic transformation and in response to therapy.

There is a lack of faithful models of adverse risk subsets of AML based on cooperation between known co-occurring disease alleles other than in *MLL*-positive AML (*FLT3*-ITD + *MLL* fusions/*MLL*-PTD). There are few murine or xenograft models of poor-risk, multiple-hit genotypes of AML that are seen commonly in the clinic. All commonly available cell

lines represent only 40% of the genetic diversity of adults with AML. Development of such models and cell lines is of biologic and therapeutic relevance to test novel therapies and to understand mechanisms of resistance. If the models do not reflect the genetics of actual patients, then research results will not mirror what is seen in the clinical setting.

Clinical relevance

Even if we use better, more relevant models to test novel therapies, it is not clear how best to implement these in the clinical setting. Most trials have been done in relapsed/refractory disease or in elderly patients not fit to receive chemotherapy. Most drugs have had a low complete remission (CR) rate in 3rd or 4th line, and these drugs are never moved to use in upfront trials. In fact, the only approved drugs for AML in the last decade are hypomethylating agents and gemtuzumab. No drug has shown additive benefit to induction chemotherapy sufficient to change the standard of care in AML in more than 40 years. However, some of the drugs being tested, while not active in 3rd or 4th line patients or in addition to induction chemotherapy, may have activity in other settings or in only certain AML subtypes. One question is whether it is time to start rethinking clinical trials in AML. When looking at overall survival, 40% of AML patients have only a 12% 3-year overall survival rating. This means that 88% of AML patients with high risk genetic lesions will relapse and die within 3 years. Why are these patients only receiving novel agents after they relapse and are refractory to everything else?

A novel approach to clinical trials in AML would include mutational profiling to allow us to identify the 40% of high-risk AML patients with an overall survival rate of 10% or less, or those for whom the current standard of care is not sufficient, and conduct trials in this subset. Only those with poor genetic risk and those not fit enough or without a donor for allograft would be eligible for these trials. Such trials could either add novel agents in consolidation or add a maintenance regimen to prevent relapse. Given the 10% overall survival rate in this patient group, the event rate will be high, allowing researchers to see a clinical signal in 50 patient cohorts by powering the trials to ascertain a reduction in the relapse rate to 50%–60%. Epigenetic/stem cell targeted therapies and other approaches are ready to be tested in this context, and they may be more active in this setting rather than in those patients who have already relapsed.

How do we implement this in the clinical setting? First, there is a need for robust sequencing platforms for rapid, accurate mutational profiling. A subset of these genes is composed of large tumor suppressors in which nonsense/frameshift mutations are clinically relevant. For genes like DNMT3A, TET2, or ASXL1 that are as large as 5 kilobases in coding with high GC content, the cull of mutation versus wild-type has profound prognostic relevance yet is difficult to ascertain with certainty. The ability to get high quality coverage for an entire coding region is as important as cost or throughput; and rapid, accurate analysis is as important as sequencing technology. Furthermore, sensitivity is an issue: It is not clear if rare (1%–5%) subclones with good/poor prognosis mutations have prognostic relevance. For example, if a patient has a core binding factor leukemia with 2% DNMT3A mutation, it is unclear whether that clone is going to relapse and whether that patient should be treated.

Worthwhile targeted leukemia mutational screening must use a robust, clinically tractable platform for rapid, accurate mutation detection. Our first version includes 32 genes: 18 genes from the AML study plus 14 additional genes recently identified in AML, MPN, and MDS. It includes 600 genes for patients with all hematologic malignancies. We use PCR amplification and sequencing using RainDance and HiSeq/MiSeq. This system allows for rapid (15–20 minutes) analysis of raw sequence data for known/predicted pathogenic variants as well as copy number data, deletions, amplifications, fusions, and mutations. The

aim of the targeted screening is to provide actionable information by the time of hospital discharge for AML patients. One critical aspect of useful screening is high coverage of genes in the sequencing panel. At a minimum, 250x coverage is needed to enable identification of real mutations with a rapid analytic pipeline.

Conclusion

Genetic studies of leukemia patients can identify mutations that point to novel pathways involved in the pathogenesis of different malignancies. Novel disease alleles can be used to improve prognostic and therapeutic decisions in cancer patients. Now is the time to figure out how to use this data in a targeted DNA sequencing approach for AML patients. Exome and genome sequencing is the answer for all cancer patients, but specific clinical utility must be demonstrated. In many cases, targeted DNA sequencing may guide the use of existing therapies and lead to the development of novel therapies.

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Table 1

Revised AML risk stratification based on integrated mutational profiling.

Cytogenetic Classification	Mutations		Overall Risk Profile
Favorable	Any		Favorable
Normal karyotype or intermediate-risk cytogenetic lesions	<i>FLT3</i> -ITD-negative	Mutant <i>NPM1</i> and <i>IDH1</i> or <i>IDH2</i>	
	<i>FLT3</i> -ITD-negative	Wild-type <i>ASXL1</i> , <i>MLL-PTD</i> , <i>PHF6</i> , and <i>TET2</i>	Intermediate
	<i>FLT3</i> -ITD-negative or positive	Mutant <i>CEBPA</i>	
	<i>FLT3</i> -ITD-positive	Wild-type <i>MLL-PTD</i> , <i>TET2</i> , and <i>DNMT3A</i> and trisomy 8–negative	
	<i>FLT3</i> -ITD-negative	Mutant <i>TET2</i> , <i>MLL-PTD</i> , <i>ASXL1</i> , or <i>PHF6</i>	Unfavorable
	<i>FLT3</i> -ITD-positive	Mutant <i>TET2</i> , <i>MLL-PTD</i> , <i>DNMT3A</i> , or trisomy 8, without mutant <i>CEBPA</i>	
Unfavorable	Any		

Table 2

Classes of mutations in AML.

Classes of mutations	Genes involved
Hydroxylation pathway mutations	TET2 or IDH1/2 mutations
Epigenetic modifications	DNMT3 and ASXL1 mutations and MLL alterations
Class I alterations	FLT3, N/KRas, and Kit mutations
Class II alterations	CEBPA, NPM1, and RUNX1 mutations and CBF translocations
Novel pathways	spliceosome and cohesion pathway genes