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What do functional genomics tell us about pathogenesis of AML?

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ABSTRACT

While molecular genetic abnormalities can tell us much about the pathogenesis of acute myeloid leukemia (AML), these molecular genetics do not always explain drug resistance or sensitivity, leaving room for other mechanisms of tumor pathogenesis outside of genetic events. The Beat AML 1.0 project was a multicenter project to sequence and functionally query AML samples against over 120 drugs. The results have helped form disease models on how mutations affect disease phenotype and drug sensitivity and have assisted in identifying gene signature profiles that may facilitate selecting the most effective treatment options. However, there are factors outside of genetic abnormalities that affect disease pathogenesis. For example, tumor-associated macrophages in the tumor microenvironment play a role in pathogenesis and represent therapeutic targets.

Introduction

Beat AML 1.0 was developed from an agreement 6 years ago between Oregon Health & Science University and the Leukemia and Lymphoma Society to develop a consortium of 11 academic medical center partners, which included The University of Texas Southwestern Medical Center, Stanford University Medical Center, the University of Utah, the University of Colorado Anschutz Medical Campus, University of Florida Health, Temple Health Fox Chase Cancer Center, University of Miami, The Ohio State University Wexner Medical Center, The University of Kansas Medical Center, and the National Health, Lung, and Blood Institute. The goal was to accrue 900 AML patient samples for preclinical work to perform exome and RNA sequencing, ex vivo drug sensitivity studies, and immune microenvironment work. This data would all be amalgamated with computational biology to make predictions about biomarkers of drug response, drug sensitivity, drug resistance, and parlay that information into clinical trials.

Beat AML 1.0

The first stage of Beat AML 1.0 is complete, with 950 unique AML patient samples, over a thousand functional drug screens have been performed (including 99 unique pharmaceutical partner drugs and 243 unique nonproprietary drugs, as well as 123 drug combinations), and over 37,000 clinical labs have been collected from this patient cohort. The majority (672) of this dataset was published in *Nature* in 2018 [1], while Zhang et al. [2] discussed results from 122 of the samples from patients who were treated with the FLT3 inhibitor crenolanib. The genetics of these patients was followed both before and after treatment to help understand the clinical evolution of disease as a mechanism of resistance. A final wave of 156 patient samples will be reported in a subsequent publication as a validation cohort.

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In the first cohort [1], there were 11 novel gene mutations (with 1% cohort frequency) that were bona fide somatic mutations that have not been reported in any other AML or other malignancy cohort. These mutations could be potential novel drivers of AML. When compared with the most frequently mutated genes in The Cancer Genome Atlas (TCGA) project, there were generally similar frequencies, with a few exceptions (eg, *SRSF2* was more frequent in the Beat AML 1.0 cohort, regardless of de novo disease status). However, when comparing long tails, or those mutations with less than 2% cohort frequency, there was a large diversity of rare variants. This diversity should be noted in the context of clinical trials that are oriented around one frequently mutated gene, while these rare variants have unclear roles in pathogenesis, drug response, and drug resistance.

A network-based stratification of these mutations, which is essentially a semi-supervised clustering of mutant genes according to protein–protein interaction networks and pathways, showed 14 distinct clusters. The most prominent in each network include *ASXL1*, *JAK2*, *SRFS2*, *IDH1/2*, *FLT3/NPM/DNMT3A*, *TP53*, *SF3B1*, *STAG2*, *N/KRAS*, *PTPN11*, *DNMT3A*, *FLT3/NPM*, and *CEBPA/GATA2*. This potentially augments the scheme of Papaemmanuil et al. [3] and could enrich our understanding of mutant clusters of AML.

In a parallel study, a similar sequencing approach was completed for Philadelphia-negative neutrophilic leukemia (CNL), atypical chronic myeloid leukemia (CML), myelodysplastic syndromes (MDS), and chronic myelomonocytic leukemia (CMML) [4]. The prominent mutant genes in these diseases showed that many diverse disease phenotypes arise from near identical genetic drivers. This leads to the conclusion that the specific mutations present and the order of acquisition must impact the eventual disease phenotype and potentially treatment efficacy. For example, if an epigenetic mutation (eg, *ASXL1*) occurs first with signaling mutations (eg, *CSF3R)* second, tyrosine kinase inhibitors (TKIs) may be less effective while epigenetic drugs may be more effective. Conversely, if a signaling mutation occurs before an epigenetic mutation, TKIs may be more effective with earlier intervention.

The Beat AML 1.0 samples were also put through RNA sequencing, with gene expression patterns that clustered around some cytogenetic and genetic events. Furthermore, ex vivo drug sensitivity profiling created individualized drug sensitivity profiles. Unsupervised clustering confirms drugs with similar targets co-segregate and shows correlations with clinical, genetic, and cytogenetic disease features. With this data, we can begin to ask some questions about drug response as it correlates with different parameters. A large number of drugs were tested in both de novo and MDS-transformed AML samples, and while most drugs were more effective in de novo AML samples, some, especially the histone deacetylase (HDAC) inhibitor panobinostat, were more effective in the MDS-transformed AML samples.

We can also correlate drug sensitivity and resistance data with mutational events. Unsurprisingly, *FLT3* mutations confer greater sensitivity to FLT3 inhibitors, while wild-type *FLT3* specimens still have correlations between drug sensitivity or resistance and mutational subsets of disease (eg, samples with *NRAS* mutation with wild-type *FLT3* are more sensitive to selumetinib, trametinib, and CI-1040 but more resistant to pazopanib and vemurafenib).

We also examined gene expression as it relates to drug sensitivity [1]. For example, there is a 17-gene signature that significantly differentiates ibrutinib-sensitive vs -resistant cases. To find these gene signatures, we performed a supervised analysis with the 20% most- and least-sensitive cases to any drug to find significant signatures for approximately two-thirds of the 120 drugs on the panel. Using a novel visualization developed by our group (which we denote as a “Buzzsaw plot”), we correlated both gene mutations and gene expression with drug sensitivity and resistance (Fig. 1). These patterns often overlap with the gene expression signatures. All of this data is available through the Vizome portal (www.vizome.org), which is a user-friendly point-and-click portal that allows users to visualize and query the data set without needing command-line programming skills.

What genetic abnormalities don't tell us about AML pathogenesis

Approximately 25% of AML and CLL patient samples show very good sensitivity to CSF-1 receptor (CSF1R) by siRNA and highly selective small molecules [5,6]. It was initially thought that this sensitivity related to the genetics of tumor pathogenesis, but AML specimens do not have mutations in *CSF1R* and it is not overexpressed in AML tumors. Therefore, we looked for clinical or genomic subsets that correlate with *CSF1R* sensitivity. While there were no genetic subsets that co-segregate cleanly with *CSF1R* inhibitor sensitivity, there are markers that correlate better with resistance, especially worse prognostic factors, p53 mutations, and 17p involvement, but none of these correlates completely. Furthermore, those patients who have samples resistant to CSF1R inhibitors in vitro have inferior outcomes compared to those who are sensitive or intermediate.

While the CLL cells do not express CSF1R, a subset of CD14-positive monocyte-macrophage cells do [5]. In AML, mass cytometry (CyTOF) was used to examine AML patient samples against an antibody panel to identify and cluster high-expressing CSF1R cells, but only 1% of patient samples express CSF1R. These specimens have higher CSF1R inhibitor sensitivity. When compared phenotypically to healthy donor specimens, the CSF1R-hi cells have a dramatic difference in expression of markers such as HLA-DR, CD33, CD16, and CD8, among others. This could represent macrophages that are reprogrammed in the case of AML or it could indicate that the macrophages are tumor derived.

To determine whether CSF1R-positive cells in AML are clonally derived, we isolated the CSF1R-positive cells from samples in which the somatic mutations were known. We then sequenced the CSF1R on our positive and negative fractions and found that the mutation was identifiable in both the CSF1R-positive and -negative fractions. Because the CSF1R-positive population is so rare, it is unclear whether these cells are clonally derived or whether they are a healthy infiltrating population of tumor-associated macrophages.

In some cases, the pathogenesis of AML depends on paracrine production of hepatocyte growth factor (HGF) by supportive cells in the microenvironment. The HGF interacts with c-MET in the AML blast, favoring leukemic clone expansion by supporting CSF1R-positive macrophage-like cells that act as nurse-like cells in the tumor microenvironment [7]. Treatment with CSF1R inhibitors deprives the AML cells of this paracrine signal from the supportive cell, leading to cell death of the AML clone (Fig. 2). These tumor-

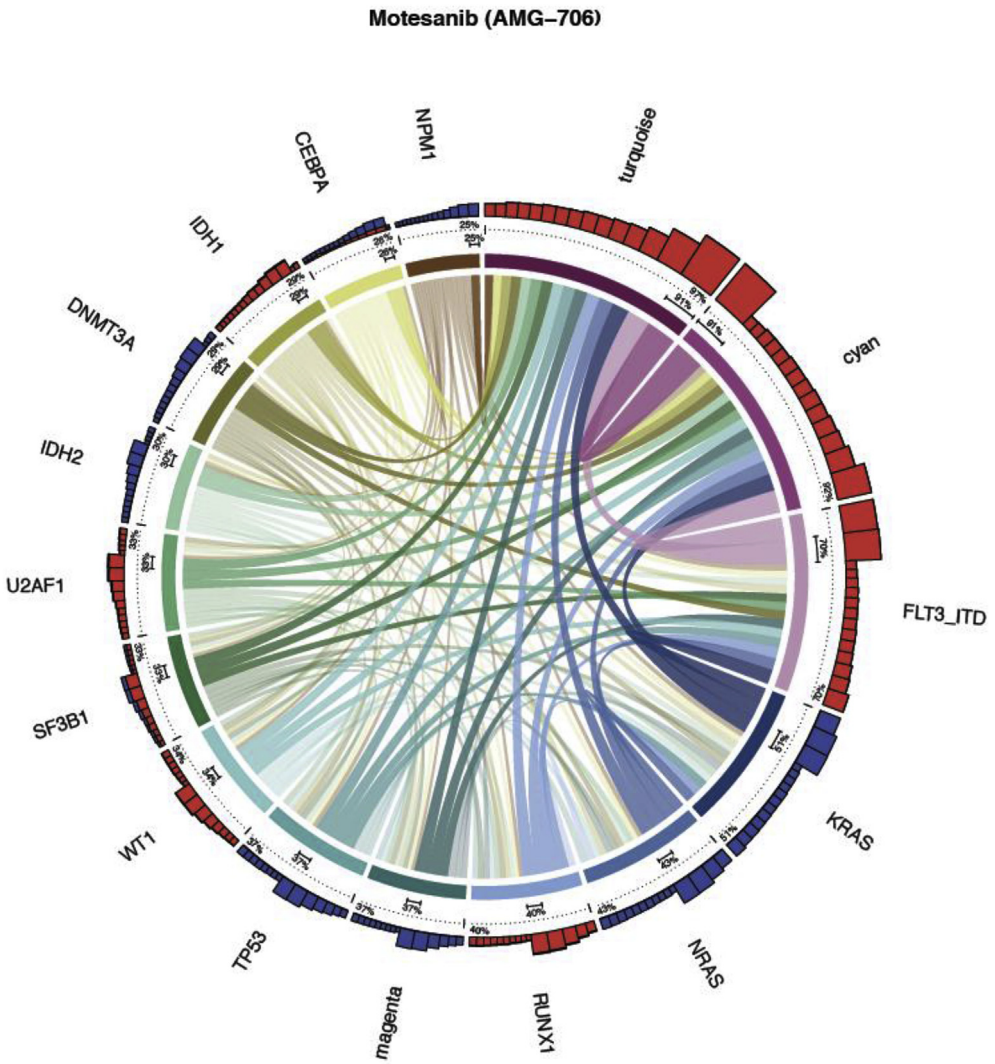

Fig. 1. Buzzsaw plot of motesanib.
Legend: An example of a buzzsaw plot, which visually correlates both gene mutations and gene expression with drug sensitivity and resistance.

associated macrophages seem to be important in the development of AML in a quarter of cases and are more important in either early stage AML or in AML that develops with better prognostic markers, like those with p53 mutations. These tumor-associated macrophages could be targetable in a substantial proportion of AML patients.

Conclusion

AML Beat 1.0 is the largest functional genomic data set on primary tumors to date. The Vizome data visualization/exploration tool facilitates broad access and leveraging of data by the community. This data can inform disease pathogenesis and biomarkers and combinations for clinical development. It is hoped this will lead to novel hypotheses and important observations that can be tested both in the labs and clinically. While the AML Beat 1.0 project is finished, the utility of this data is just beginning to be realized.

Disclosure

Dr. Tyner disclosed the following:
Consulting fees: Vivid Biosciences.
Contracted research: Agios, Aptose, AstraZeneca, Gilead, Genentech, Incyte, Janssen, Seattle Genetics, Syros, Takeda.
Ownership interest: Vivid Bioscience.
Bottomly, Wilmot, and McWeeney have nothing to disclose.

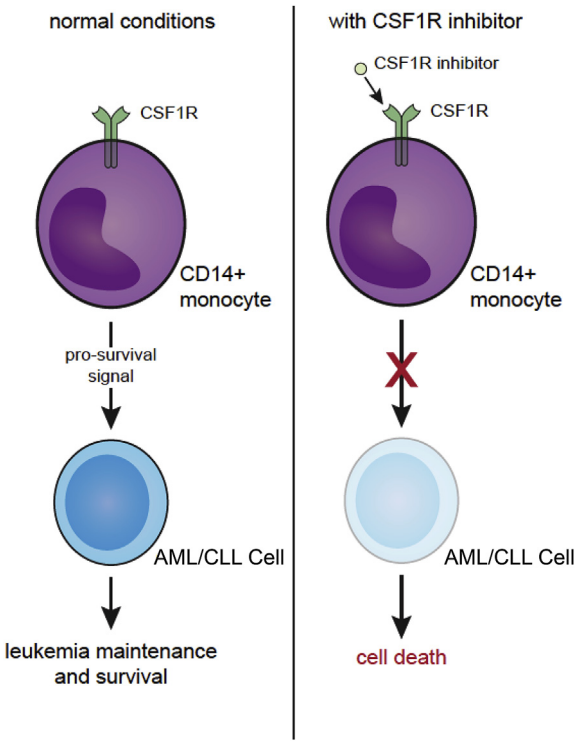

Fig. 2. Pathogenesis of AML.
Legend. Under normal conditions, supportive cells in the tumor microenvironment affect proliferation of the AML blast through paracrine signaling and production of HGF, which interacts with cMET on the AML cell. This signaling and interaction can be inhibited by CSF1R inhibitors, effectively leading to AML cell death.

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