IN THE SPOTLIGHT

Bedside to Bench and Back: Identifying a New Clinically Relevant Driver in Pediatric Acute Myeloid Leukemia

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Summary: In this issue of *Blood Cancer Discovery*, Umeda and colleagues identify and comprehensively analyze a novel recurrent *UBTF* mutation (tandem duplications) in pediatric acute myeloid leukemia. Acute myeloid leukemia cases with *UBTF* tandem duplications display distinctive biologic features, including association with *FLT3*-ITD and *WT1* mutations and high-risk disease, and appear to represent a new genetic subtype of acute myeloid leukemia.

See related article by Umeda et al., p. 194 (7).

Acute myeloid leukemia (AML) is a highly heterogeneous disease, with a broad range of clinical presentations and highly variable outcomes. This diversity in clinical behavior reflects the underlying complexity of its pathogenesis, which in turn relates in large part to the unique portfolio of genetic events in each case. AML is currently classified into distinct disease groups based on the presence of specific mutations or chromosomal alterations, which tend to be mutually exclusive of one another (1). In the current WHO Classification of Myeloid Neoplasms, this predominantly genetically based classification is overlaid by aspects of the disease ontogeny, such as prior history of cytotoxic chemotherapy or evolution from an antecedent myelodysplastic syndrome (2). Importantly, the unique AML subtypes in the current WHO classification represent prognostically relevant groups (3) and are used to guide the therapeutic approach, such as the choice of upfront therapy and the decision as to whether to proceed to bone marrow transplantation. While historic classifications of AML relied on conventional karyotype, the current era of comprehensive genetic analysis of AML includes next-generation sequencing (NGS) panels that interrogate a large number of genes known to be drivers of AML as well as NGS-based panels to detect cryptic gene fusions. These new technologies have validated existing AML disease categories originally established by conventional karyotyping and have revealed new AML genetic subtypes, initially heralded by the identification of AML with *NPM1* mutation as a novel mutationally defined subtype (4).

Despite the current widespread use of detailed genetic interrogation of individual AML cases in clinical practice (and availability of many new therapies, including those that target specific genetic drivers), many challenges remain in the care of patients with AML. Some cases lack known distinct genetic or ontologic classes, and are classified as "AML, not otherwise specified" (AML, NOS) in the current WHO Classification of Myeloid Neoplasms and comprise up to 44% of pediatric patients with AML (5). In a comprehensive genomic analysis of 1,540 patients with AML, 15% either lacked driver mutations or had no class-defining genetic lesions (1) and a similar proportion of pediatric AML cases do not have a clear defining genetic driver (6). These AML cases that fail to segregate into an established disease category are frustrating for both diagnosticians and clinicians due to uncertainty regarding optimal clinical management, and often a lack of targetable genetic drivers. While rates of clinical remission in both adult and pediatric AML are high, low-level measurable residual disease (MRD) often persists and drives an all-too-common disease relapse, occurring in up to 40% of pediatric patients with AML. Treatment approaches in AML must thus balance the need to provide intensive therapy to eradicate MRD, while avoiding adverse effects of aggressive therapy that may not be needed for the more treatment-responsive disease subtypes. Thus, a desirable goal in advancing AML diagnosis is the identification of new genetic drivers that could help dictate more rational and patient-specific therapeutic approaches.

In this issue of *Blood Cancer Discovery*, Umeda and colleagues studied a series of relapsed pediatric AML cases using intensive genomic analysis and identified a previously rarely described genomic alteration, *UBTF* tandem duplications (*UBTF-TD*), in a large subset (8.8%) of cases (7). Through interrogation of larger *de novo* pediatric and adult AML cohorts, they identified *UBTF-TD* in 4% of pediatric AMLs. AML with *UBTF-TD* appears to represent a novel AML subtype present predominantly in pediatric patients that demonstrates aggressive clinical behavior.

The UBTF (upstream binding transcription factor) protein influences the epigenetics of ribosomal DNA and RNA transcription. It has been described to be mutated in myeloid malignancies but is not generally included in the targeted NGS panels used in clinical practice to evaluate AML and

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other myeloid neoplasms. Moreover, the TDs in the *UBTF* gene found by the authors involved either in-frame 3′ insertions [internal tandem duplications (ITDs), similar to those commonly found in the *FLT3* gene in AML] or in-frame duplications [partial tandem duplications (PTDs), similar to those found in the *KMT2A* gene in AML and myelodysplastic syndromes]. ITDs and PTDs can be notoriously difficult to detect by NGS and often require dedicated informatics pipelines or fragment analysis assays. Standard NGS pipelines in fact can typically detect TDs only when captured as insertions during alignment, thus the longer duplications will be missed or their allelic frequency largely underestimated. Even more specialized algorithms like CICERO that are designed to improve recognition of structural variants may miss some noncanonical TDs, like some of those seen in *UBTF*. When the authors found *UBTF-TDs* in the initial cohort and recognized the limitations in the informatic methods used for structural variant detection, they performed an integrated analysis using a combination of CICERO, RNAindel, and a novel soft-clip read–based approach that led to the identification of several additional cases initially missed.

The data in this study point to *UBTF-TD* being a disease driver and defining a specific new AML subgroup. Indeed, the features of these cases are characteristic of a subgroup-defining genetic lesion in myeloid disease classification: (i) mutual exclusivity with other genetically defined AML subgroups; (ii) a particular comutation pattern, with frequent accompanying *WT1* and *FLT3*-ITD mutations, similar to *NUP98* rearranged AML; (iii) occurrence at a high variant allele fraction (VAF) and presence at the time of initial diagnosis with retention at relapse; (iv) association with a simple karyotype (most commonly normal karyotype or trisomy 8); and (v) clinical features of young age (typically adolescence, with a median age of 13.4 years) and poor response to conventional AML therapy, with high rates of MRD and relapse and shortened overall survival compared with other AML subtypes. Interestingly, although a distinct genetic event, *UBTF-TD* shares several features with *NUP98::NSD1* and *NPM1*-mutated AML subtypes. On transcriptomic analysis, the authors found shared common expression of *HOX* gene cluster among these subtypes. Comutations of *FLT3*-ITD were common to all three genetic drivers, and like *NUP98::NSD1* AML, *WT1* comutations as well as PRDM16 overexpression were also common in *UBTF-TD* AML. Of note, *NPM1*-mutated AML has a more favorable prognosis than the other two entities, which the authors hypothesized may be due to its lower overall expression of PRDM16, a poor prognostic indicator in pediatric AML. The authors conducted *in vitro* studies and found that UBTF-TD expression in hematopoietic stem cells enhanced proliferation and engendered a transcriptional profile similar to AML, supporting its role as an AML driver.

AML with *UBTF-TD* is an aggressive disease, as suggested by its enrichment in the relapse cohort compared with the primary AML cohorts investigated by the authors, as well as by outcome analysis: the patients had an overall 5-year survival of 44%, which was significantly shorter than AML patients in the same cohorts lacking *UBTF-TD*. Although the associated *FLT3*-ITD and *WT1* mutations also confer aggressive behavior to AML in general, the adverse prognosis of *UBTF-TD* appeared to be independent of these mutations, as it conferred significantly poorer survival (and higher rates of MRD positivity after induction) compared with *FLT3-ITD* or *WT1*-mutated AML cases lacking the *UBTF-TD*.

Umeda and colleagues' study advances our understanding of AML by disclosing a novel genetic subtype with unique biology and poor outcome, until now hidden among the "black-box" group of AML-NOS. The authors' approach of moving from discovery in a cohort of relapsed AML to detailed genetic characterization (including developing a new informatics pipeline to facilitate identification of the often-elusive *UBTF-TD*) and then validating the influence of *UBTF-TD* on patient outcome in larger AML cohorts illustrates how applying genomic interrogation of patient databases can advance the care of patients with AML. The study also illustrates the challenges of diagnosing and caring for patients with AML in an era of evolving technology, in that the *UBTF-TD* is not easily identifiable by commonly available targeted molecular assays and NGS panels, and would require refining existing genetic testing. More broadly, optimally predicting clinical behavior and selecting therapy in AML may ultimately require the evaluation of additional parameters not currently included in standard AML diagnostic workup, such as comprehensive assessment for germline mutations that predispose to myeloid malignancies and evaluation of miRNA, methylation profile, transcriptome, proteomic profile, and leukemia cell phenotype ("stemness"; refs. 8, 9). In addition, long read sequencing, optical genome mapping, and the use of multiple specialized algorithms will lead to unveiling currently cryptic or elusive structural variants that may be driving events in the progressively shrinking cohort of AML-NOS (10). While increasing the complexity of diagnostic testing poses significant challenges in implementation, these could bear great potential benefits to patients with AML. Therapeutic approaches can be better adapted to the individual patient, with the potential to more effectively eradicate the disease while avoiding undesirable effects of excessively aggressive therapy when not warranted. Umeda and colleagues' study takes us further towards this goal.

Authors' Disclosures

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