Targeted therapy for fusion-driven high-risk acute leukemia

Yana Pikman1 and Kimberly Stegmaier1,2

Division of Hematology/Oncology, Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston Children’s Hospital, Boston, MA; and2Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA

Despite continued progress in drug development for acute leukemias, outcomes for patients with some subtypes have not changed significantly in the last decade. Recurrent chromosomal translocations have long been recognized as driver events in leukemia, and many of these oncogenic fusions portend high-risk disease. Improved understanding of the molecular underpinnings of these fusions, coupled with novel chemistry approaches, now provide new opportunity for therapeutic inroads into the treatment of leukemia driven by these fusions. (Blood. 2018;132(12):1241-1247)

Introduction

In 2017, 4 new acute myeloid leukemia (AML) therapies were approved by the Food and Drug Administration: gemtuzumab ozogamicin, midostaurin, CPX-351, and enasidenib. Despite this progress, AML remains difficult to treat, with overall 5-year survival for adults with AML static at 24%. Moreover, for children with AML, long-term survival remains behind most other pediatric cancers. More progress has been made in the treatment of acute lymphoblastic leukemia (ALL), the most common cause of cancer in children, where long-term survival is closer to 90%, and the impact of advances in Chimeric Antigen Receptor T-cell therapy is yet to be fully realized. Nevertheless, ALL still accounts for the second leading cause of pediatric cancer-associated mortality, and outcomes for adults with ALL remain far inferior to that of children. Chromosomal translocations, leading to the expression of aberrant fusion oncoproteins, are a recurrent event in both AML and ALL. These fusion oncoproteins involve a diversity of protein classes, including kinases and transcription factors. This Spotlight highlights the role for targeting leukemia fusions, and their interacting partners, in the treatment of acute leukemias.

Direct targeting of fusion oncoproteins

Chromosomal translocations can result in a variety of fusions with differing functions and ease of targetability. A subset of fusions involves kinases that are readily druggable. Some examples include BCR-ABL1, the most comprehensively studied; fusions of nucleoporins NUP98 or NUP214 with ABL1 kinase; and fusions resulting in the activation of JAK kinase signaling pathways in ALL. A second major class of fusions involves transcription factors. These fusions tend to be more difficult to directly target than the kinases due to the challenges in targeting transcription factors (eg, nuclear location, protein-DNA interaction, disordered nature of the fusion protein, and lack of a stable binding pocket). Some fusions involving transcription factors, such as RUNX1-RUNX1T1 (AML1-ETO) in AML and ETV6-RUNX1 (TEL-AML1) in ALL, are associated with a good prognosis, whereas others, such as CBFA2T3-GLIS2 in pediatric AML2 and KMT2A (mixed lineage leukemia [MLL]) in pediatric ALL,3,4 are associated with a poor prognosis.

Targeting kinase fusions

In 1973, Rowley described the components of the Philadelphia chromosome in cells from 9 patients with chronic myelogenous leukemia (CML). This translocation between chromosomes 9 and 22 results in the fusion protein BCR-ABL1, leading to aberrant activation of the ABL1 kinase. Drucker and colleagues described a first targeted inhibitor of BCR-ABL1, Gleevec (imatinib). This discovery, and ultimately the successful clinical development of imatinib, transformed the leukemia field with the idea that driver kinases can be inhibited with therapeutic success. For treatment of CML, imatinib resulted in a major cytogenetic response in 87% of patients and complete cytogenetic response in 76%, with an 8-year overall survival rate of 85%. The success of imatinib spurred the development of the next generation of tyrosine kinase inhibitors for treatment of CML. Dasatinib, nilotinib, and ponatinib have been shown to be effective for imatinib-resistant CML. The success of imatinib in CML has also inspired similar treatment paradigms in other diseases. For example, patients with ALL harboring the BCR-ABL1 translocation have, until very recently, been treated with chemotherapy followed by allogeneic stem cell transplantation. The Children’s Oncology Group study AALL0031 revealed that the addition of imatinib to an aggressive chemotherapy regimen improved survival in pediatric patients with BCR-ABL1+ Ph+ ALL and avoided the toxicity of stem cell transplantation; a finding also demonstrated in a European clinical trial for Ph+ ALL. Dasatinib and ponatinib are currently being integrated into clinical trials for adult Ph+ ALL and may be more effective than imatinib in combination with chemotherapy. These studies have changed the paradigm for treatment of this high-risk ALL subtype and provided support for the testing of other targeted therapies in acute leukemia.

The success of targeting kinase fusions has also been realized in other cancers. Fusions involving NTRK genes, encoding for TRK...
proteins, have been described in a number of cancers. Entrectenib, a pan-TRK, ROS1, and ALK inhibitor, had anti-tumor activity in patients with advanced solid tumors characterized by NTRK, ALK, and ROS1 fusions.19 Larotrectinib, a highly selective TRK inhibitor, had an overall response rate of 75% in pediatric and adult patients across 17 distinct NTRK fusion-positive tumor types,20 and a pediatric phase 1/2 trial showed an objective response of 93%.21 Another example is the successful targeting of ALK fusions in cancers, including non–small cell lung cancer, anaplastic large cell lymphoma, inflammatory myofibroblastic tumors, colorectal carcinoma, and others. Crizotinib, ceritinib, and alectinib are Food and Drug Administration–approved ALK inhibitors for treatment of patients with ALK fusion-positive lung cancers.22 These successes raise the possibility for implementation of drugs targeting fusion proteins in other cancers, including acute leukemia.

Philadelphia chromosome–like ALL (Ph-like ALL) describes a disease that shares the gene expression profile and poor outcome of BCR-ABL1 ALL, but does not express BCR-ABL1.23,24 The frequency of this ALL subtype increases with age, accounting for 10% of pediatric B-cell acute lymphoblastic leukemia and for >20% of ALL in adolescents and adults.25 Kinase activating fusions, involving CRLF2 rearrangements, ABL class fusions, JAK2 or EPOR rearrangements, and others, were identified in up to 90% of Ph-like B-cell acute lymphoblastic leukemia.25,26 For a majority of the identified Ph-like fusions, tyrosine kinase inhibitors exist to target their aberrant signaling, and this has been efficacious in preclinical models and published case reports.26-29 The clinical success of targeting BCR-ABL1 in Ph+ ALL, as described above, argues that this should be successful in Ph-like ALL as well.

Several clinical trials are currently integrating inhibition of signaling driven by Ph-like fusions into therapy for Ph-like ALL: St. Jude Children’s Research Hospital (NCT03117751),30 the Children’s Oncology Group (NCT02723994), and the Dana-Farber Cancer Institute ALL Consortium (NCT03020030). The identification of oncogenic fusions, and their possible targeting, does support the need for genomic testing for all patients with a new diagnosis of acute leukemia, including fusion assessment using targeted approaches or genome-scale RNA sequencing.31-33 Kinase inhibition may not be enough for full therapeutic efficacy, however, and the complete loss of the protein may be necessary, raising interest in the exploration of kinase degraders as described below.

Targeting PML-RARA, a paradigm for the successful targeting of transcription factor fusion genes

The success of directly targeting transcription factor fusions is exemplified in the treatment of acute promyelocytic leukemia (APL). APL is defined by PML-RARA, a fusion protein containing the amino terminus of PML, a zinc-binding protein, and most of the retinoic acid receptor α (RARA). RARA is a nuclear receptor that represses transcription of target genes when not bound by its ligand, retinoic acid (RA). Physiologic levels of RA convert this protein to a potent transcriptional activator, resulting in the expression of genes involved in myeloid differentiation.34 The PML-RARA fusion maintains the DNA binding and dimerization domain of the transcription factor PML, with the DNA and retinoid binding domains of RARA also preserved. The fusion remains an inefficient transcriptional activator, even in the presence of RA. In addition, the fusion forms heterodimers with PML, and this leads to dysregulation of the TP53 pathway.34 All-trans retinoic acid (ATRA) binds PML-RARA and converts it to an effective transcriptional activator, inducing differentiation. ATRA binding also induces proteolysis of the fusion.35,36

Prior to the discovery of ATRA and its use in the treatment of APL, APL was associated with significant early mortality and a 5-year survival rate of 35%.37-39 Treatment of patients with single-agent ATRA has been shown to result in APL remission and was one of the first successes in APL therapy.40 Although ATRA can induce APL remission, PML-RARA transcript is still detectable at the time of remission,41 and ATRA single-agent treatment generally did not lead to cure.42 More recently, it was found that arsenic binds directly to PML and triggers its SUMOylation, inducing ubiquitination and recruitment of ubiquitin ligases, leading to its degradation.43,44 The protein degradation induced by arsenic effectively targets the leukemia stem cell population and results in complete loss of the aberrant PML-RARA transcript, lending support to the utility of arsenic in APL.45,46 This combination is now the new, chemotheraphy-free, standard of care for the treatment of adults with APL.

Next-generation approaches to targeting fusions

Targeting transcription factor fusion protein-protein interactions: the example of CBFB-MYH11

Although the targeting of PML-RARA in APL is notable, transcription factors have generally been considered “undruggable.” Thus, the more systematic targeting of fusions encoding for aberrant transcription factors will require significant creativity. One strategy might disrupt the downstream protein-protein interactions of the transcription factor fusion. A recent preclinical example is the targeting of the transcription factor fusion CBFβ-MYH11 in AML.47 CBFβ is a member of the transcription factor core binding factor, where it binds to RUNX1 and enables its DNA affinity. CBFβ-MYH11 outcompetes the wild-type CBFβ for RUNX1 binding, leading to deregulation of RUNX1 transcription activity. The small molecule Al-10-49 successfully inhibited the protein-protein interactions of CBFβ-MYH11 with RUNX1 and showed efficacy against CBFβ-MYH11–driven leukemia. The molecule was selective for the fusion protein compared with wild-type CBFβ, a key selectivity for targeted therapy.

Degraders for fusion targeting

Taking advantage of the cell’s ubiquitin/proteasome system, Proteolysis-Targeting Chimeras (PROTACs) could open doors to degradation of difficult targets. These heterobifunctional molecules recruit specific protein targets to an E3 ubiquitin ligase resulting in its ubiquitination and degradation through transient binding to the target. PROTACs consist of 3 essential components, each of which require target-specific optimization. First, the warhead of a PROTAC engages the target of interest. A linker binds the warhead to the E3 ubiquitin ligase ligand. The PROTAC, with its target, forms a quaternary structure, and this structure is critical to ensure degradation.48

First insights into a “natural” PROTAC came with the understanding of the mechanism of phthalimide drugs. In 2014, Kronke et al and Lu et al independently identified cereblon
(CRBN), a component of a cullin-RING ubiquitin ligase complex, as a target of phthalimide-based drugs, such as thalidomide and lenalidomide. Furthermore, they showed that thalidomide caused CRBN-dependent proteosomal degradation of the transcription factors IKZF1 and IKZF3.49,50 This insight led to the development of bifunctional molecules that recruit specifically target protein degradation. This was demonstrated with development of dBET151 and ARV-825,52 leading to degradation of MDM2, mouse double minute 2. The von Hippel-Lindau (VHL) ubiquitin ligase has similarly been hijacked for target protein degradation, including of BRD4.53 In addition to BRD4, VHL and CRBN-mediated protein degraders have been developed for a number of targets, including FKBP12,51 BRD9,54 BCR-ABL,55 RIPK2,56 CDK9,57 FLT3,58 and BTK58 (summarized in Figure 1). Targeted protein degradation thus holds promise for targeting kinases, as well as transcription factors.

In the case of kinases, kinase degraders may be more active than small molecule inhibitors because the degraders should impair both the enzymatic and the nonenzymatic function of the protein. For example, SWI/SNF-mutant cancers depend on the catalytic and noncatalytic activity of EZH2, raising the possibility that the enzymatic inhibitors of EZH2 currently in clinical trials may not be fully effective.59 The FLOS (noncatalytic) domain of the MLL/SET methyltransferase SETD1A is critical to DNA damage response and also highlights a noncatalytic function for this protein in AML.60 In addition, kinase inhibition is subject to kinase upregulation, a potential mechanism of resistance that may be avoided with degradation of the enzyme.

PROTAC optimization will be target dependent and critical to the success of these compounds. For example, in the targeting of BCR-ABL, dasatinib, but not imatinib or ponatinib, proved to be a potent warhead to induce target degradation.55 Similarly, engagement of CRBN as the E3 ubiquitin ligase was more successful than engagement of VHL for BCR-ABL1 degradation.55 Target-specific optimization of all 3 components will be critical for successful degrader design. Nevertheless, protein degradation holds significant promise for inhibiting previously undruggable targets, such as transcription factors, and may be particularly efficacious in disrupting large transcription factor complexes formed as a result of leukemia-associated fusions.

### Targeting the complexes associated with transcription factor fusions

In addition to the gain of novel functions resulting from chromosomal translocations, fusions can lead to leukemia transformation via formation of unique protein complexes. In particular, newly formed translocationary complexes, such as those formed in KMT2A-rearranged (KMT2A-r) leukemias, may alter target gene transcription. Understanding of these complexes, their localization, structure, and function, will lead to insights into how they can be disrupted to reverse the leukemia phenotype. New technologies, such as cryoelectron microscopy61 and CRISPR/Cas9 domain scanning62,63 will help to define these complexes, their functional domains, and their interactions with other proteins and putative inhibitors, informing future drug discovery efforts.

### KMT2A-rearranged leukemia as a paradigm

The fusion of KMT2A to >80 described partners forms a chimeric oncogene. This fusion preserves the amino terminal of KMT2A fused with sequences derived typically from the AF4, AF9, AF10, and ENL genes. These binding partners interact with DOT1L, a histone methyltransferase that targets lysine 79 of histone H3 for mono-, di-, or trimethylation, to promote transcriptional elongation.64 Thus, the KMT2A-fusion protein recruits DOT1L to its target genes, leading to hypermethylation at H3K79 and aberrant gene expression. This translocation causes upregulation of HOX gene expression, enhancing proliferation and blocking differentiation, leading to acute leukemia. The activity of the KMT2A fusion is also dependent on its direct interaction with the tumor suppressor Menin.56-67 Suppression of Menin and DOT1L can individually reverse the leukemogenic potential of KMT2A translocations. Thus, pharmacological inhibition of both DOT1L and Menin has been of high interest for therapeutic intervention for the treatment of KMT2A-r leukemias, both ALL and AML.68,69
EPZ-5676 was developed by Epizyme, Inc as the first clinical inhibitor of DOT1L. Incubation of KMT2A-r cell lines with EPZ-5676 led to a decrease in H3K79 methylation, without an effect on other histones. Gene expression analysis showed inhibition of KMT2A-r target gene expression, and KMT2A-r cell lines were uniquely sensitive to DOT1L inhibition compared with KMT2A-wild-type cell lines. In rat models of KMT2A-r leukemia, EPZ-5676 led to target inhibition, demonstrated by a decrease in H3K79 methylation and in HOXA9 and MEIS1 gene expression, and a decrease in tumor volume. Despite promising preclinical data, there was limited activity of EPZ-5676 in phase 1 clinical trials. The phase 1 study of EPZ-5676 in adult patients evaluated 51 patients, with 9 patients showing leukocytosis or other evidence of differentiation with DOT1L therapy. Responses included complete response (2) and resolution of leukemia cutis (3). In pediatrics, an open-label dose escalation study in patients with relapsed/refractory KMT2A-r leukemia was completed in 2016. This study enrolled 18 patients and had an acceptable safety profile. H3K79me2 ChIP-sequencing on leukemic blasts demonstrated that EPZ-5676 reduced methylation at KMT2A-r target genes of ≥80% at all tested time points and doses, consistent with DOT1L inhibition. Transient reductions in peripheral or bone marrow blasts were detected in ~40% of patients, although without objective responses. Further studies to identify effective drug combination with DOT1L inhibitors and resistance mechanisms will be needed to address the full utility of this compound class. In an initial study using a cell line–based evaluation of EPZ-5676, treatment-resistant cell lines were developed and demonstrated to have increased expression of a drug efflux transporter ABCB1 as one mechanism of acquired resistance. Moreover, one liability of EPZ-5676 has been the need for continuous infusion of the drug. Improvements in the pharmacokinetics of DOT1L inhibitors will also be important for advancement of these molecules in the clinic.

Numerous studies have also demonstrated the importance of Menin interaction with the KMT2A fusion protein, prompting the development of selective inhibitors. KMT2A-r cell lines were selectively more sensitive to the Menin inhibitors, MI-463 and MI-503, compared with KMT2A-wild-type cell lines. Cell-line treatment with Menin inhibitors induced leukemia cell differentiation and decreased KMT2A target gene expression. A decrease in tumor burden and prolonged survival was observed with in vivo treatment of KMT2A-r leukemia models. Menin inhibitors are expected to enter clinical trials in the near term, and the combined inhibition of Menin and DOT1L may be particularly effective.

**Targeting other rare leukemia fusion oncoprotein interactors**

As in the case of KMT2A translocations, in the case of fusions involving the nucleoporin NUP98, the amino terminus of NUP98 translates to at least 31 different partners, with NUP98 being the most frequent partner. NUP98 translocations have been described in patients with AML, myelodysplastic syndrome, and T-cell acute lymphoblastic leukemia. These translocations have been associated with a poor prognosis in children and adults with AML. The NUP98 amino terminus contains the Gle2-binding sequence, which is responsible for the binding of Ribonucleic Acid Export 1. Although NUP98 primarily localizes in the nuclear membrane as part of the nuclear pore complex, the NUP98 fusions localize inside the nucleus. The NUP98 fusions have been shown to inhibit CRM1 (chromosome region maintenance 1; exportin 1)–mediated nuclear export of known substrates, leading to accumulation of CRM1-regulated transcription factors and altered transcription. More recently, NUP98 fusions have been shown to interact with KMT2A (MLL1) and the nonspecific lethal complex. There is significant overlap in the target genes for NUP98-HOX and KMT2A-r, suggesting that the HOX/Meis1 transcriptional program is also important in NUP98 translation-driven leukemias. In support of an interaction between NUP98-HOXA9 and wild-type KMT2A, the deletion of Kmt2a (Mll1) in a NUP98-HOXA9–driven mouse model attenuated leukemia development. Thus, inhibition of the protein complex formed by translocations of NUP98 with other transcription factors may be a viable option for targeting this high-risk leukemia subgroup.

**Targeting cooperating kinases in transcription factor–fusion leukemias**

In addition to targeting the fusion directly or to targeting its interaction partners, another therapeutic opportunity is to target cooperating kinases in the leukemia. For example, 11% to 30% of RUNX1-RUNX1T1–positive AML express an activating c-KIT mutation, and these events are targetable with drugs, such as imatinib, dasatinib, midostaurin, crenolanib, or newer KIT inhibitors. Interestingly, a pediatric patient with AML characterized by a DEK-NUP214 fusion had an extraordinary response to treatment with selinexor, a CRM1 inhibitor. In light of this extraordinary responder, and given the interaction of both DEK-NUP214 and NUP98-HOXA9 with CRM1, it will be interesting to see whether these and other nucleoporin fusions impart sensitivity to CRM1 inhibitors.
drive its expression. SIK3 maintains MEF2C function in AML, and inhibition of SIK3 has been demonstrated to be therapeutically in KMT2A-r leukemia in vitro.

Resistance to single-agent targeted therapies is a common problem. For example, mutations in BCR-ABL1 have rendered CML cells resistant to imatinib and dasatinib, a problem which has been overcome with the development of ponatinib.12,95 Similar findings in other drug-target contexts are incentivizing the scientific community to be forward-thinking, to anticipate resistance mechanisms, and to develop second-generation inhibitors and novel drug combinations before the first-generation drugs have completed testing. With this in mind, Loxo Oncology developed Loxo-195, based on the predicted resistance mechanisms to larotrectinib.96 The use of targeted therapy for fusion-driven leukemias is predicted to select for resistant cells over time. Understanding the mechanisms of resistance will be crucial to the successful implementation of these new targeted therapies.

Conclusions

Since the discovery of imatinib for BCR-ABL1-positive CML, leukemia-selective, targeted therapy has been the holy grail of leukemia treatment. Efforts to genomically characterize leukemia have resulted in the identification of new targets, although many have been difficult to “drug.” New chemistry approaches and technologies such as CRISPR/Cas9 and cryoelectron microscopy offer the promise of targeted therapies even for these most challenging oncogenic drivers, including transcription factor fusions. In anticipation of these advances, it will be important to develop sensitive clinical assays to comprehensively identify fusions and to develop pharmacodynamic assays to ensure target engagement and proper trial interpretation. Finally, highly effective drug combinations will ultimately be needed as successful leukemia therapy has nearly always required a multi-drug approach.

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ORCID profiles: Y.P., 0000-0002-5336-0216; K.S., 0000-0003-0218-7895.

Correspondence: Kimberly Stegmaier, Broad Institute of Massachusetts Institute of Technology and Harvard University, 450 Brookline Avenue, Boston, MA 02215; e-mail: kimberly_stegmaier@dfci.harvard.edu.

Footnote

aberrant H3K79 methylation by DOT1L. 


