Targeting Chromatin Regulation in Acute Myeloid Leukemia

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Normal hematopoiesis is sustained by multipotent hematopoietic stem cells (HSCs) that are able to both self-renew and give rise to differentiated cells throughout the lifetime of an individual. These cell fate decisions are characterized by changes in transcriptional cell states, mediated by heritable epigenetic processes, notably posttranslational modifications of nucleosome proteins and direct methylation of DNA. These changes in chromatin structure are coordinated by specific “writer” and “eraser” enzymes and specifically bound by epigenetic “readers.” Acute myeloid leukemia (AML) arises as a result of dysregulation of this ordered transcriptional progression, resulting in an aggressive disease characterized by a block in differentiation and increased proliferation. Moreover, mutations of transcriptional regulators and chromatin modifiers are recurrent in AML. Importantly, the resultant epigenetic changes are plastic, and clinical evidence suggests that targeting epigenetic alterations can reset pathological transcriptional programs with clinically relevant outcomes. In this perspective, we will outline recent progress in the development of agents that target chromatin in AML. We will focus on 3 areas: (1) targeting mutant IDH proteins; (2) therapies initially designed to target mixed-lineage leukemia (MLL)-fusions; and (3) targeting the transcriptional kinases CDK9 and CDK7.

Targeting DNA methylation in AML

DNA methylation plays a pivotal role in embryonic development, cellular differentiation, and genome stability. DNA methylation is instigated and maintained by DNMT3A/B and DNMT1, respectively, and removed by TET family enzymes and is generally associated with transcriptional repression. However, while DNMT3A and TET2 are commonly mutated in AML, they are not yet therapeutically targetable. However, indirect changes in DNA methylation can occur as a consequence of gain-of-function mutations in the isocitrate dehydrogenase 1 and 2 (IDH) enzymes. These neomorphic proteins generate the “oncometabolite” 2-hydroxyglutarate (2-HG), which interferes with dioxygenase enzymes, including TET, Jumonji-C histone lysine demethylases, and prolyl hydroxylase enzymes, resulting in increased DNA and histone methylation and aberrant transcription.

IDH mutations are present in 10%–20% of AML (Figure 1). As gain-of-function mutations, IDH1/2 mutations are amenable to small molecule inhibition, dramatically decreasing levels of 2-HG and inducing differentiation in leukemic blasts. Clinical-grade inhibitors of both IDH1 (ivosidenib; Tibsovo) and IDH2 (enasidenib; Idhifa) are now US Food and Drug Administration approved. Early phase trials demonstrated good tolerability, although a specific side effect was the IDH inhibitor–associated differentiation syndrome, managed with corticosteroids and drug interruption. A phase I study of ivosidenib in relapsed/refractory (R/R) AML reported 30%/21% CRh/CR complete hematologic response/complete response) rates with a median duration of 8 months. A separate phase I/II study using ivosidenib upfront in older/less-fit patients reported CRh/CR rates of 42%/30%, respectively. Similar phase I/II studies of enasidenib showed 20% CR rates in the R/R setting and ORR/CR 31%/18% (overall response rate/CR) when used upfront in older patients. The efficacy results from a phase III study comparing enasidenib to conventional care after failure of two to three lines of previous therapy have yet to be published, but early reports suggest a failure to meet the primary endpoint of overall survival (OS) benefit (IDHENTIFY NCT02577406). Notably, as with other “epigenetic” therapies, responses can take several months, highlighting the need to judge responses differently to conventional cytotoxic agents.

Interest has therefore shifted to generating novel combination therapies, the most advanced of which takes advantage of preclinical synergism between IDH inhibition and azacitidine. Interim results from a phase II study (NCT02677922) comparing upfront enasidenib +/- azacitidine have shown meaningful improvements in ORR (68% vs 42%) and CR (50% vs 12%) rates. A phase Ib study (NCT02677922) of upfront ivosidenib/azacitidine reported interim ORR/CR 78%/57% and the phase III AGILE study of ivosidenib/azacitidine is enrolling (NCT03173248). IDH-mutated primary AML cells are also more sensitive to venetoclax, and a phase I/II study of venetoclax/enasidenib in IDH2-mutated AML is currently ongoing (NCT04092179). Interim results from a phase I/II study of ivosidenib/venetoclax +/- azacitidine (NCT03471260) in the R/R or non-intensive settings has demonstrated the tolerability of the triple-combination demonstrating overall rates of CR/Cri of 78% (with 50% minimal residual disease negative), with a median time to best response of 2 months.
Targeting MLL-rearranged AML

DOT1L-inhibition in MLL-mutated AML

The MLL genes encode for a family of histone methyltransferases that are essential for embryonic and adult hematopoiesis. The MLL1 (KMT2A) gene is recurrently mutated in AML, either as a result of a partial tandem duplication (PTD) or as part of a rearrangement, leading to the formation of fusion chimeric proteins with up to 70 different partners. Although all MLL-rearranged (MLL-r) chimeras lose their C-terminus methyltransferase activity, the majority fuse with translocation partners that are members of multi-subunit protein complexes involved in chromatin remodeling/transcriptional elongation, particularly the super elongation complex or disruptor of telomeric silencing 1-like (DOT1L) containing complex.

DOT1L is the only known histone H3 lysine 79 (H3K79) methyltransferase in mammals, where it plays an important role in the regulation of cell proliferation, DNA repair, and active transcription. Whereas DOT1L is essential for embryonic erythropoiesis and development, its role in adult hematopoiesis appears non-essential, suggesting a therapeutic window. DOT1L inhibition reduces H3K79 modification and the expression of critical MLL-r target genes, including the HOXA cluster and MEIS1, correlating with reduced proliferation and survival (Figure 2). Intriguingly, DOT1L inhibitors may also be effective in other AML genotypes driven by NUP98-NSD1, MLL PTD, IDH1/2, NPM1c, and DNMT3A. Mechanistically, DOT1L inhibition in NPM1c and DNMT3A-mutated AML appears to involve downregulation of HOXA genes and MEIS1.

Pinometostat (EPZ-5676) is the most clinically-advanced DOT1L inhibitor. A pediatric phase I study in R/R MLL-r leukemias (NCT02141828) reported good tolerability, albeit that no objective responses were observed in this difficult group of patients. The adult phase I dose escalation study (NCT01684150) confirmed pinometostat as well tolerated, with some CRs observed, despite its pharmacokinetic limitations (continuous infusion up to 28 d). A phase Ib/II study of pinometostat with azacitidine in adult MLL-r AML has now completed enrollment (NCT03701295) and a phase Ib/II study of pinometostat alongside intensive chemotherapy upfront in MLL-r adult AML is ongoing (NCT03724084). New generations of orally available inhibitors with improved pharmacokinetics are also under development.
being developed, which will improve clinical utility and may increase responses to DOT1L-inhibition.13,14

**Targeting menin**

Menin, another preclinically validated MLL-r target, binds to the N-terminus of wild-type MLL and MLL-fusion proteins, and is required for aberrant gene expression. Preliminary data from the ongoing trial of KO-539 (KOMET-001 NCT04067336), which acts by disrupting the menin-MLL interaction (Figure 2), has shown evidence of remarkable efficacy even at low doses, with some patients achieving CR and reports of tumor lysis.15 This was associated with reduction in the expression of the key MLL targets HOXA/MEIS1, a fact that may account for responses in non-MLL-r rearranged AML, including NPM1c, IDH, EZH2, DNMT3A, and EZH2-mutant genotypes. A phase I/II study of another MLL-menin inhibitor, SNDX-5613, in MLL-r and NPM1c AML is ongoing (AUGMENT-101 NCT04065399).

Further novel menin inhibitors are in development16,17 with the orally available VTP-50469 showing remarkable preclinical efficacy in a mouse model of Npm1c/Dnmt3a-mutant preleukemia and the NPM1c-mutant OCI-AML3 human leukemia cell line. Mechanistically, this activity was associated with down-regulation of MEIS1 and PBX3, although interestingly without disruption of HOXA gene expression.18

**Histone demethylases and LSD1**

Histone demethylation is also a potential target in MLL-r and other AML subtypes. The prototypic LSD1 an exemplar of the lysine specific demethylases (LSD)—one of the 2 main classes of histone demethylase, demethylates H3K4me1/2 and H3K9me1/2 histone marks—acting as both a transcriptional repressor or activator in a context-specific manner. LSD1 is part of the MLL supercomplex associated with sites of active transcription and LSD1 inhibition modulates H3K4me2 levels at genes specifically bound by the MLL-r protein. Furthermore, LSD inhibition increases leukemic stem cell sensitivity to all-trans-retinoic acid (ATRA)-mediated differentiation across AML genotypes, irrespective of PML-RARA status. LSD1 inhibitors demonstrate some toxicity towards normal hematopoiesis, particularly erythropoiesis, although this is reversible on drug discontinuation, suggesting a possible therapeutic window.

Two LSD1 inhibitors, GSK2879552 (NCT02177812) and ORY-1001 (EudraCT 2013-002447-29), are in AML clinical trials. The phase I trial of Iadademstat (ORY-1001) reported low toxicity, with evidence of hematological responses, especially in MLL-r cases.19 A phase II trial of Iadademstat + azacitidine is ongoing (EudraCT No.: 2018-000482-36) with encouraging preliminary results (ORR 73%, time to response of 36 d, longest-CR 405 d) (Salamero et al, EHA Annual Congress 2020, Abstract EP580).20 A further study investigating the ability of the LSD1 inhibitor tranylcypromine to sensitize AML to ATRA is also recruiting (NCT02717884).

**Targeting oncogenic transcription by CDK7/9 inhibition**

Small molecule inhibitors of the cyclin-dependent kinases CDK7/9 have shown activity in AML. CDK9 is a key member...
of the P-TEFb complex that regulates transcriptional elongation, whilst CDK7 activates RNA polymerase II (RNA Pol II) by CDK7-dependent phosphorylation (Figure 2). Targeting these proteins is thought to work through reducing oncogenic over-expression of critical leukemia-regulators such as MYC.

The CDK9 inhibitor dinaciclib inhibits MLL target genes, demonstrating efficacy in preclinical models of MLL-r AML. Voruciclib overcomes MCL1-mediated venetoclax resistance in a preclinical model of AML and is undergoing phase I study (NCT03547115). Alvocidib has proven tolerable and shown encouraging responses in combination with intensive chemotherapy in a phase I study (NCT03298984). A randomized phase II study of alvocidib, cytarabine, and mitoxantrone versus cytarabine and daunorubicin (7+3) in newly diagnosed high-risk AML reported higher rates of CR (70% vs 47%), but no improvement in OS.

Conclusions

Hematological malignancies are characterized by mutation or dysregulation of epigenetic regulators. This has led to the development of targeted therapies aimed at eradicating malignant cells through the restoration of normal epigenetic and transcriptional states. Epigenetic regulators represent attractive therapeutic targets as they often have enzymatic activities or binding domains amenable to small molecule inhibition, and the states that they govern are reversible. However, despite good preclinical evidence of efficacy and safety, only a few of these therapies have reached clinical development with encouraging results. It is therefore important to address the potential pitfalls that currently prevent us from taking full advantage of these rationally-designed therapies.

One obvious problem is that AML is a highly heterogeneous disease and that epigenetic modifiers can act as both tumor suppressors and oncogenes in different cellular contexts. Furthermore, individual patients harbor a complex clonal architecture that often evolves and can be selected for by treatment during the course of the disease, allowing significant opportunity for subclonal escape or acquired resistance. Further understanding mechanisms of resistance will aid rational design of combination therapies.

In this context, clinical trials must be tailored to appropriately measure clinical benefits and harms. With a large number of clinical-grade agents now available, it will be important to predict or otherwise identify genetic subgroups that respond to specific inhibitor classes, particularly those harboring truncal mutations, such as MLL-r or IDH-mutated AML. Early phase clinical trials inevitably test these therapies as single agents in highly pretreated populations, thereby decreasing the likelihood to observe significant benefit. Thus, promising agents might be discarded because of a lack of single-agent efficacy. Moreover, the standard response criteria used for cytotoxics are not conducive to measure the likely slower response of an epigenetic inhibitor. In addition, as with cytotoxics, single agents are unlikely to eradicate such a complex disease, necessitating the rational development of combination therapies. In so doing, it will be important to anticipate and monitor for compound toxicities, and be mindful to either avoid the danger of inadvertently activating oncogenic programs or adversely affect beneficial immune-mediated tumor responses.

We therefore favor expediting their use in rationally designed combinations with standard or other well-understood targeted therapies, or testing them in previously untreated patients, perhaps those not suitable for standard therapies. Given their relative lack of cytotoxicity, trial design should anticipate prolonged treatment to demonstrate efficacy, consider their use as maintenance therapy, and potentially develop novel outcome measures using rationally designed biomarkers of response.

In conclusion, facilitated by the remarkable advances in our knowledge of the role of dysregulated epigenetics in AML, translation of therapeutically targeting the epigenome in AML patients is ongoing. However, achieving their full clinical potential will require an even deeper understanding of the role of epigenetic dysregulation in malignant transformation, coupled with rationally designed clinical trials.

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