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Perspective
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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.[1] A separate phase I/II study using ivosidenib upfront in older/less-fit patients reported CRh/CR rates of 42%/30%, respectively.[2] Similar phase I/II studies of enasidenib showed 20% CR rates in the R/R setting[3] and ORR/CR 31%/18% (overall response rate/CR) when used upfront in older patients.[4] 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.[5,6]

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 has shown meaningful improvements in ORR (68% vs 42%) and CR (50% vs 12%) rates.[7] A phase Ib study (NCT02677922) of upfront ivosidenib/azacitidine reported interim ORR/CR 78%/57%[8] and the phase III AGILE study of ivosidenib/azacitidine is enrolling (NCT03173248). *IDH*-mutated primary AML cells are also more sensitive to venetoclax,[9] and a phase Ib/II study of venetoclax/enasidenib in *IDH2*-mutated AML is currently ongoing (NCT04092179). Interim results from a phase Ib/II study of ivosidenib/venetoclax +/– azacitidine (NCT03471260) in the R/R or nonintensive 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.[10]

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HemaSphere (2021) 5:6(e589). http://dx.doi.org/10.1097/HS9.0000000000000589.
Received: 18 March 2021 / Accepted: 22 April 2021

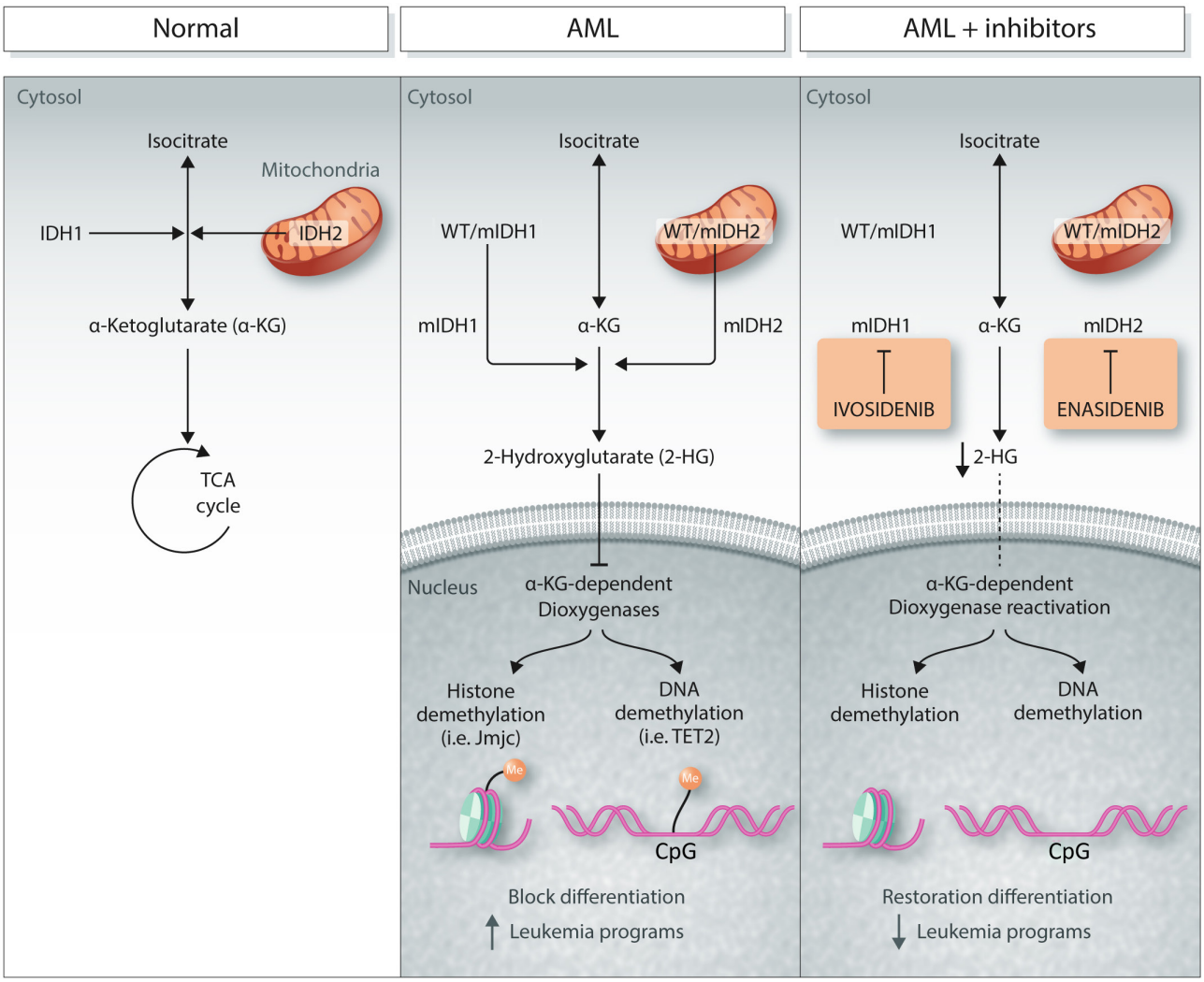

Figure 1. The role of mutant IDH proteins in AML and their therapeutic inhibition. The left "normal" panel demonstrates the standard oxidative decarboxylation of isocitrate to alpha-ketoglutarate (α-KG) in the TCA cycle by the IDH1 and 2 enzymes in the cytosol and mitochondria, respectively. In the middle panel, mutations in IDH1 and IDH2 lead to further reduction of alpha-ketoglutarate to the oncometabolite 2HG, which inhibits the activity of α-KG-dependent dioxygenase enzymes, leading to increased methylation of histones and DNA, and alterations of gene expression that block differentiation and drive leukemia. In the right panel, the activity of mutant IDH1 and 2 enzymes is inhibited by ivosidenib and enasidenib, respectively, decreasing 2-HG and restoring the function of the dioxygenase enzymes, normal differentiation, and blocking the generation of leukemia programs.

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.[11] 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).[12] 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

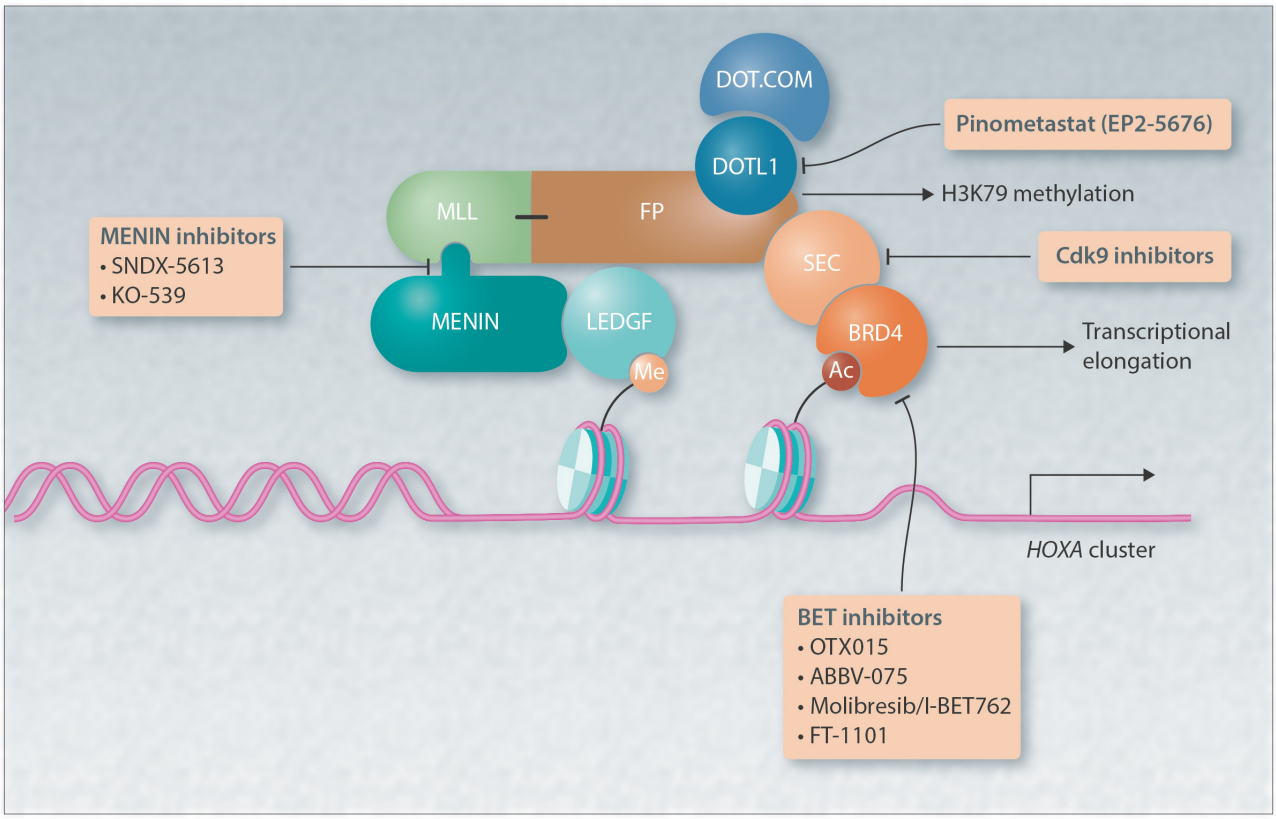

Figure 2. Graphic of a prototypic MLL-fusion protein (FP). Shown are the targets of and inhibitors that block the activating program downstream of MLL-FP; menin inhibitors, such as SNDX-5613 and KO-539, that block the interaction of the N-terminus of the MLL-FP with menin to tether the protein at its target loci; inhibitors of the DOT1L H3K79 methyltransferase protein, such as pinometastat, that block H3K79methylation and transcriptional elongation; BET inhibitors, such as OTX015, ABBV-075, molibresib/I-BET762 and FT-1101, that block the interaction of BRD4 with acetylated histones and with it malignant transcription; and the CDK7 and 9 inhibitors that block phosphorylation of RNA polymerase II (RNA Pol II) and malignant transcription.

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*-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 development[16,17] 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 overexpression of critical leukemia-regulators such as *MYC*.

The CDK9 inhibitor dinaciclib inhibits MLL target genes, demonstrating efficacy in preclinical models of *MLL*-r AML.[21] 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).[22] 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.[23]

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,[24–26] 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.

Acknowledgments

We would like to thank all the members of the Huntly laboratory and our funders including Cancer Research UK (C18680/A25508), the European Research Council (647685), MRC (MR-R009708-1), Blood Cancer UK, the Kay Kendall Leukaemia Fund (KKL1243), the Wellcome Trust (205254/Z/16/Z), the Cambridge NIHR Biomedical Research Centre. We apologize to those authors whose work we were unable to cite due to space constraints.

Disclosures

The authors have no conflicts of interest to disclose.

Sources of funding

SER is supported by a Clinician Scientist Fellowship from Cancer Research UK (C67279/A27957). Research in the Wellcome—MRC Cambridge Stem Cell Institute is funded by a grant from the Wellcome Trust (203151/Z/16/Z). Research in the Cancer Research UK Cambridge Centre is supported by a Cancer Research UK Major Centre Award (C9685/A25117).

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