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Differentiation therapy of myeloid leukemia: four decades of development

Vikas Madan[1] **and H. Phillip Koeffler**[1,2,3]

[1]Cancer Science Institute of Singapore, National University of Singapore, Singapore; [2]Cedars-Sinai Medical Center, Division of Hematology/Oncology, UCLA School of Medicine, Los Angeles, CA, USA and [3]Department of Hematology-Oncology, National University Cancer Institute of Singapore (NCIS), National University Hospital, Singapore.

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ABSTRACT

Acute myeloid leukemia is characterized by arrested differentiation, and agents that overcome this block are therapeutically useful, as shown by the efficacy of all-*trans* retinoic acid in acute promyelocytic leukemia. However, the early promise of differentiation therapy did not translate into clinical benefit for other subtypes of acute myeloid leukemia, in which cytotoxic chemotherapeutic regimens remained the standard of care. Recent advances, including insights from sequencing of acute myeloid leukemia genomes, have led to the development of targeted therapies, comprising agents that induce differentiation of leukemic cells in preclinical models and clinical trials, thus rejuvenating interest in differentiation therapy. These agents act on various cellular processes including dysregulated metabolic programs, signaling pathways, epigenetic machinery and the cell cycle. In particular, inhibitors of mutant *IDH1/2* and *FLT3* have shown clinical benefit, leading to approval by regulatory bodies of their use. Besides the focus on recently approved differentiation therapies, this review also provides an overview of differentiation-inducing agents being tested in clinical trials or investigated in preclinical research. Combinatorial strategies are currently being tested for several agents (inhibitors of KDM1A, DOT1L, BET proteins, histone deacetylases), which were not effective in clinical studies as single agents, despite encouraging anti-leukemic activity observed in preclinical models. Overall, recently approved drugs and new investigational agents being developed highlight the merits of differentiation therapy; and ongoing studies promise further advances in the treatment of acute myeloid leukemia in the near future.

Correspondence:

VIKAS MADAN
vikasmadan@aol.com

H. PHILLIP KOEFFLER
H.Koeffler@cshs.org

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Introduction

Acute myeloid leukemia (AML) is a heterogeneous malignancy characterized by impaired differentiation and uncontrolled clonal expansion of myeloid precursors. A varied range of genetic and epigenetic alterations disrupt normal differentiation of myeloid precursors leading to maturation arrest coupled with self-renewal capacity. Despite extensive research and remarkable advances in our understanding of the molecular mechanisms governing AML pathogenesis, chemotherapy-based treatment regimens developed in the 1970s constitute the standard of care.[1] All-*trans* retinoic acid (ATRA) for acute promyelocytic leukemia (APL) is a notable exception, which not only revolutionized the clinical outcome of APL, but also demonstrated that inducing terminal differentiation is an effective mode of treating AML.[2] This therapeutic approach of using pharmacological agents to stimulate differentiation of immature cancer cells into more mature forms is termed differentiation therapy. Apart from its overt effect on APL blasts, ATRA also differentiated some AML cell lines lacking PML-RARA and the drug showed some activity in a few clinical trials;[3-6] however, in general, ATRA has been ineffective in non-APL subtypes of AML.[7-13] Initial studies with the HL-60 cell line also suggested that many compounds could induce terminal differentiation, including cytokines, vitamin D3 analogs and ligands of PPARγ.[14] However, their therapeutic utility has been limited.[15, 16]

More recently, novel oncogenic pathways in AML have been identified, facilitat-

ing the development of targeted therapies, and heralding a new era in AML treatment. New differentiation-inducing agents that target mutant isocitrate dehydrogenase *(IDH)1*, *IDH2* or *FLT3* have gained regulatory approval. Other agents directed against distinct targets are being developed to induce differentiation of leukemic blasts. This review updates our previous description of AML differentiation therapy,[14,15] with a focus on new developments in the field, especially in the last decade.

Newly-approved targeted therapies for acute myeloid leukemia

Agents targeting two distinct oncogenic events, *IDH1/2* mutations and activating *FLT3* mutations, have been approved for AML therapy since 2017. These agents exert anti-leukemic activity by inducing differentiation of leukemic blasts and are exciting additions to differentiation therapy of AML.

Targeting mutant IDH1/2 enzymatic activity induces differentiation

IDH1 and IDH2 are $NADP^+$-dependent enzymes that normally catalyze the oxidative decarboxylation of isocitrate to produce α-ketoglutarate in the tricarboxylic acid cycle.[17] Somatic mutations in the active site of IDH1 and IDH2 are observed in about 20% cases of AML and 5% of myelodysplastic syndromes.[17] Mutations of *IDH1* and *IDH2*, which largely occur in a mutually exclusive manner, result in accumulation of the oncometabolite D-2-hydroxyglutatrate (2-HG).[18] 2-HG disrupts activity of α-ketoglutarate-dependent enzymes including members of the ten-eleven-translocation (TET) family of 5-methylcytosine hydroxylases and the jumonji-domain-containing group of histone lysine demethylases. This leads to a block of normal differentiation and promotes oncogenic transformation.[19-21]

Ivosidenib (AG-120; Tibsovo, Agios) is a first-in-class inhibitor of mutant *IDH1* developed from the prototype compound AGI-5198.[22] Ivosidenib is effective against AML cells harboring mutant *IDH1* by lowering their 2-HG levels and causing cellular differentiation.[22] A phase I dose escalation and expansion study of this drug in patients with relapsed/refractory AML harboring *IDH1* mutations resulted in about 30% of cases achieving either complete remission (CR) or CR with partial hematologic recovery (CRh), with a median overall survival (OS) of 9 months (NCT02074839) (Table 1).[23] Ivosidenib induced myeloid differentiation of AML blasts, and differentiation syndrome (DS) occurred in about 11% of patients[23] (see description of 'Differentiation syndrome' below). A subset of patients, who achieved clearance of mutant *IDH1*, showed longer CR (11.1 *vs*. 6.5 months) and OS (14.5 *vs*. 10.2 months) compared with those without clearance of the mutant *IDH1*. Notably, patients who did not respond to ivosidenib had significantly higher rates of mutations of the receptor tyrosine kinase pathway.[23] In a recent study, ivosidenib monotherapy was shown to induce durable remissions in patients with newly diagnosed *IDH1*-mutant AML ineligible for standard chemotherapy.[24] Preliminary results from an ongoing phase Ib/II trial (NCT02677922) showed that the combination of ivosidenib with 5-azacytidine was well tolerated with CR and overall response rates exceeding those achieved with 5-azacytidine alone.[25]

In 2018, the Food and Drug Administration (FDA) approved the use of ivosidenib for patients with *IDH1*-mutant relapsed/refractory AML; and subsequently the FDA approval was granted for newly diagnosed *IDH1*-mutant patients who are ≥75 years old or who have comorbidities precluding the use of intensive induction chemotherapy. Other inhibitors of mutant *IDH1*, which were effective in preclinical models include AG-881 (Agios Pharmaceuticals), IDH-305 (Novartis Pharmaceuticals), FT2102 (Forma Therapeutics) and BAY1436032 (Bayer); however, only FT2102 is being developed further for hematologic cancers (Table 1).[26]

The first selective inhibitor of mutant *IDH2* was AGI-6780, which potently inhibited its aberrant enzyme activity in AML cells and induced differentiation of these cells *in vitro*.[27] Subsequently, a clinically useful inhibitor, enasidenib (AG-221; IDHIFA), was developed by Agios Pharmaceuticals/Celgene Corporation. This drug reduced 2-HG levels and induced differentiation of *IDH2*-mutant AML blasts *ex vivo* and in xenograft mouse models, conferring these mice with a survival advantage.[28] In a phase I/II study of relapsed/refractory AML with *IDH2* mutation (either R140 or R172), the drug was well-tolerated and induced hematologic responses associated with maturation in 40% of patients, half of whom achieved CR (NCT01915498).[29] The median OS among patients with relapsed/refractory AML was 9.3 months, while it was 19.7 months in those who attained CR with enasidenib.[29] Moreover, older patients who were not candidates for standard cytotoxic therapy had a 30% response rate to enasidenib, including an 18% CR rate.[30] The differentiated neutrophils retained the *IDH2* mutation, supporting differentiation as the mechanism of action of enasidenib.[31,32]

While enasidenib potently suppressed 2-HG in most patients, levels of 2-HG were not predictive of clinical response. Lack of clinical response despite suppression of 2-HG levels suggests the need for additional biomarkers of drug response. An absence of response to enasidenib could also occur because of other genetic or epigenetic abnormalities in the leukemic clones. For example, co-occurring mutations of genes in the RAS pathway are significantly associated with the lack of response to enasidenib.[32] In 2017, enasidenib received FDA approval for use in adult relapsed/refractory AML patients with *IDH2* mutations. Ongoing clinical studies are evaluating the efficacy of combining mutant *IDH1/2* inhibitors with either 5-azacytidine or other therapeutic agents (Table 1). Interestingly, an interim analysis of an ongoing study (NCT02677922) showed significantly higher response and CR rates with a combination of enasidenib and 5-azacytidine than with 5-azacytidine monotherapy in patients with newly diagnosed AML, who were not candidates for intensive chemotherapy.[33] Encouraging response rates noted in a preliminary analysis of an ongoing trial also support the feasibility of combining either ivosidenib or enasidenib with standard AML induction therapy in frontline treatment of the newly diagnosed patients harboring mutations of *IDH1* and *IDH2*, respectively.[34]

Despite the clinical benefit of selective inhibitors of mutant *IDH1/2*, acquired resistance to this targeted therapy poses new challenges in treating AML. For instance, *IDH2*-mutant patients developed resistance to enasidenib by acquisition of a second mutation at residues located at the interface where enasidenib binds to the IDH2 dimer.[35] Resistance to enasidenib can also occur through acquisition

of additional oncogenic mutations in other genes.[36] Similarly, secondary resistance to ivosidenib was associated with acquisition of either *IDH1*-second site mutations or mutations of *IDH2* or RTK pathway genes.[37]

Differentiation syndrome following mutant IDH1/2 therapy

DS, originally described as retinoic acid syndrome, is a common side effect observed in APL patients treated with ATRA or arsenic trioxide. It is a serious complication that develops within 1-3 weeks of initiation of differentiation therapy in APL.[38] The pathogenesis of DS involves release of pro-inflammatory cytokines from differentiating blast cells, leading to a systemic inflammatory state. This, combined with increased vascular permeability, leads to a myriad of clinical symptoms that include unexplained fevers, weight gain, hypotension, rash, hypoxia, renal failure, dyspnea with pulmonary infiltrates and pleuropericardial effu-

Table 1. Clinical trials of IDH1 and IDH2 inhibitors.

Target	Inhibitor	Clinical trial identifier	Phase	Recruitment status	Combination	Study population
Mutant IDH1	Ivosidenib (AG-120)	NCT03245424	Approved for marketing	-	-	IDH1-mutated relapsed/refractory AML
		NCT04044209	Phase II	Temporarily suspended	Nivolumab	IDH1-mutated relapsed/refractory AML and high-risk MDS
		NCT04493164	Phase II	Not yet recruiting	CPX-351	IDH1-mutated AML and high-risk MDS
		NCT03471260	Phase Ib/II	Recruiting	Venetoclax + 5-Aza	Advanced hematologic malignancies with an IDH1 mutation
		NCT04250051	Phase I	Not yet recruiting	FLAG chemotherapy	IDH1-mutated relapsed/refractory AML
		NCT02632708	Phase I	Active, not recruiting	Standard chemotherapy	IDH1-mutated newly diagnosed AML
		NCT02677922	Phase Ib/II	Active, not recruiting	5-Aza	IDH1-mutated newly diagnosed AML
		NCT02074839	Phase I	Recruiting	-	Advanced hematologic malignancies with an IDH1 mutation
		NCT03173248	Phase III	Recruiting	5-Aza	IDH1-mutated newly diagnosed AML
		NCT03839771	Phase III	Recruiting	Standard chemotherapy	IDH1-mutated newly diagnosed AML
	Olutasidenib (FT 2102)	NCT02719574	Phase I/II	Recruiting	5-Aza or cytarabine	IDH1-mutated relapsed/refractory AML and MDS
		NCT04013880	Phase Ib/II	Withdrawn (loss of funding)	ASTX727	IDH1-mutated relapsed/refractory AML and MDS
Mutant IDH2	Enasidenib (AG-221)	NCT01915498	Phase I/II	Active, not recruiting	-	Advanced hematologic malignancies with an IDH2 mutation
		NCT04092179	Phase Ib/II	Not yet recruiting	Venetoclax	IDH2-mutated relapsed/refractory AML
		NCT02632708	Phase I	Active, not recruiting	Standard chemotherapy	IDH2-mutated newly diagnosed AML
		NCT02677922	Phase Ib/II	Active, not recruiting	5-Aza	IDH2-mutated newly diagnosed AML
		NCT03683433	Phase II	Recruiting	5-Aza	IDH2-mutated relapsed/refractory AML
		NCT03825796	Phase II	Recruiting	CPX-351	IDH2-mutated relapsed AML
		NCT02577406	Phase III	Active, not recruiting	-	Older subjects (≥60 years old) with IDH2-mutated relapsed/refractory AML (compared to conventional therapy)
		NCT03881735	Phase II	Recruiting	-	IDH2-mutated relapsed/refractory AML
		NCT03839771	Phase III	Recruiting	Standard chemotherapy	IDH2-mutated newly diagnosed AML
		NCT03515512	Phase I	Recruiting	-	Maintenance therapy for IDH2-mutant AML or CMML following allogeneic stem cell transplantation
		NCT03728335	Phase I	Recruiting	-	Maintenance therapy for IDH2-mutant AML following allogeneic stem cell transplantation
	Vorasidenib (AG-881)	NCT02492737	Phase I	Completed	-	Advanced hematologic malignancies with either IDH1 or IDH2 mutation

AML: acute myeloid leukemia; 5-Aza: 5-azacytidine; ASTX727: decitabine + cedazuridine (E7727; cytidine deaminase inhibitor; Astex Pharmaceuticals); CMML: chronic myelomonocytic leukemia; CPX-351: liposomal cytarabine and daunorubicin; FLAG: fludarabine, cytarabine, plus granulocyte colony-stimulating factor; MDS: myelodysplastic syndrome.

sion.[38] Although potentially life-threatening, clinical symptoms of DS are routinely managed using corticosteroids, while discontinuation of differentiation therapy might be required in cases with severe DS.[38,39]

DS, similar to that observed in APL following ATRA/arsenic trioxide therapy, has also been reported in patients treated with inhibitors of mutant *IDH1/2*. The most common clinical features of IDH-DS were dyspnea and pulmonary infiltrates or pleuropericardial effusion, while hypotension was the least common symptom.[40] IDH-DS was effectively managed by dose interruption and treatment with glucocorticoids and hydroxyurea.[23,29,41] In contrast to a rapid occurrence of DS in APL (median of 12 days),[38,42,43] the onset of DS was delayed in patients treated with IDH inhibitors.[23,40,41] A recent comprehensive analysis concluded that the incidence of DS in IDH inhibitor-treated patients (19%)[40] is similar to the rate observed for ATRA-treated APL (~25%).[39,42,43] This necessitates routine monitoring for DS in patients on IDH inhibitor therapy.

Further mechanistic studies are necessary to establish how inhibition of mutant *IDH1* and *IDH2* relieves the block in myeloid differentiation. Inhibitors of mutant *IDH1/2* potently suppress 2-HG, and this is likely to restore the activity of α-ketoglutarate-dependent enzymes that regulate DNA and histone methylation. Therefore, studies need to focus on investigating the function of TET enzymes and jumonji histone lysine demethylases, and whether restoration of their function correlates with clinical outcome in subjects treated with inhibitors of mutant *IDH1/2*. A better understanding is also required of why the clinical response to IDH inhibitors is variable despite robust inhibition of 2-HG levels. In addition, better predictive biomarkers are needed to enhance the therapeutic utility of IDH inhibitors.

FLT3 inhibitors promote differentiation of acute myeloid leukemia blasts

FMS-like tyrosine kinase 3 (*FLT3*) is one of the most frequently mutated genes in AML (~30%), with two distinct alterations, either an internal tandem duplication (*FLT3*-ITD) in the juxtamembrane domain or point mutations in the tyrosine kinase domain (TKD). These activating mutations lead to ligand-independent, constitutive FLT3 signaling which promotes proliferation of leukemic cells. Activation of FLT3 also suppresses myeloid differentiation by inhibiting the function of CEBPA *via* ERK1/2-mediated phosphorylation, while pharmacological inhibition of FLT3 causes granulocytic differentiation of AML cells.[44]

Early studies with FLT3 inhibitors demonstrated clinical manifestation of differentiation with evidence of neutrophilic dermatoses.[45-47] Since then, several inhibitors of FLT3 have been investigated in clinical trials and two agents, gilteritinib and midostaurin, have received regulatory approval. In clinical studies, the differentiation-inducing effect of FLT3 inhibition is clearly evident with selective FLT3 inhibitors, gilteritinib and quizartinib.

Gilteritinib (Xospata, Astellas Pharma) is a type I FLT3 inhibitor active against both ITD and TKD mutations. In a murine xenograft model, gilteritinib induced regression of tumors expressing mutant *FLT3*.[48] In an initial clinical study, 49% of *FLT3*-mutant AML patients responded to gilteritinib. Of note, 37% of the patients who had received prior treatment with another FLT3 inhibitor responded to gilteritinib [NCT02014558 (CHRYSALIS study)] (Table 2).[49] Significantly, gilteritinib stimulated differentiation towards granulocytic and monocytic lineages in about half of the *FLT3*-mutant patients, who exhibited significant reduction in marrow blast percentage, even though the mutation burden of *FLT3* was unchanged.[50] In this subgroup of patients, DS was reported in two patients, in whom it was resolved with glucocorticoid therapy.[50] Intriguingly, the authors noted no apparent signs of myeloid differentiation in the other half of patients treated with gilteritinib although reductions in AML blasts and bone marrow cellularity were observed. This alternate pattern of response to gilteritinib was associated with a reduction in allele frequency of mutant *FLT3* and indicated that mechanisms other than induction of myeloid differentiation, might contribute to the anti-leukemic effect of gilteritinib. Promising data emerging from an interim analysis of a phase III clinical study (NCT02421939 [ADMIRAL study]) comparing gilteritinib monotherapy to salvage chemotherapy in *FLT3*-mutant relapsed/refractory AML patients led to the FDA (November 2018) and European Medicines Agency (EMA) (September 2019) approving single-agent gilteritinib therapy in adult patients with *FLT3*-mutated relapsed/refractory AML. This trial demonstrated significantly longer OS (9.3 months *vs*. 5.6 months) and higher response rates (CR/CRh rates of 34% *vs*. 15%) in gilteritinib-treated patients than in those treated with chemotherapy.[51] The efficacy of gilteritinib is also being explored in a variety of other clinical settings, including in combination with either 5-azacytidine or other chemotherapeutic agents, as well as for maintenance therapy in patients with *FLT3*-mutated AML (Table 2).

Quizartinib is a type II inhibitor that binds adjacent to the ATP-binding domain when the FLT3 protein is in its inactive conformation and, therefore, lacks activity against TKD-mutant *FLT3*.[52] It is a highly potent and selective inhibitor of FLT3 and showed comparatively greater efficacy as a single agent than chemotherapy in a larger phase III trial (QuANTUM-R study; NCT02039726). In this study of relapsed/refractory AML with FLT3-ITD, quizartinib monotherapy prolonged OS compared with salvage chemotherapy.[53] Quizartinib caused terminal myeloid differentiation of leukemic blasts with a surge in the number of FLT3-ITD-retaining neutrophils, and development of clinical DS.[54] Superior response rates were obtained in relapsed/refractory AML harboring *FLT3*-ITD when quizartinib was combined with either 5-azacytidine or low-dose cytarabine.[55] A larger, placebo-controlled trial (QuANTUM-First; NCT02668653) combining quizartinib with standard induction and consolidation chemotherapy in newly-diagnosed FLT3-ITD-positive AML patients is ongoing (Table 2).

Although gilteritinib and quizartinib act as differentiating agents, differentiation is not universally observed in all patients treated with FLT3 inhibitors, suggesting alternative mechanisms of clearing AML blasts. Midostaurin (Rydapt, Novartis) is a first-generation FLT3 inhibitor that received FDA and EMA approval in 2017 for use in adults with *FLT3*-mutated newly diagnosed AML when combined with intensive chemotherapy; the EMA also approved midostaurin for single-agent maintenance therapy. As a single-agent, midostaurin causes a reduction in leukemic blasts in *FLT3*-mutant relapsed/refractory AML patients, without achieving CR.[56] Combining midostaurin with chemotherapy significantly improved its efficacy, with a 92% CR rate being observed in FLT3-mutated patients (Table 2).[57] Moreover, in a large, phase III, double-

Table 2. An overview of acute myeloid leukemia clinical trials of FLT3 inhibitors.

Inhibitor	Clinical trial identifier	Phase	Recruitment status	Combination	Study population
Gilteritinib	NCT02014558 (CHRYSALIS)	Phase I/II	Completed	-	Relapsed/refractory AML
	NCT03730012	Phase I/II	Recruiting	Atezolizumab	FLT3-mutated relapsed/refractory AML
	NCT04293562	Phase III	Recruiting	CPX-351	Newly diagnosed AML with or without FLT3 mutations (standard chemotherapy compared to therapy with CPX-351 and/or gilteritinib)
	NCT04336982	Phase I/II	Not yet recruiting	CC-90009	FLT3-ITD positive relapsed/refractory AML
	NCT04240002	Phase I/II	Recruiting	FLAG chemotherapy	Children, adolescents and young adults with FLT3-mutated relapsed/refractory AML
	NCT02927262	Phase II	Active, not recruiting	-	Maintenance therapy following induction/ consolidation therapy for FLT3-ITD positive AML in CR
	NCT02421939 (ADMIRAL)	Phase III	Active, not recruiting	-	FLT3-mutated relapsed/refractory AML (gilteritinib compared to salvage chemotherapy)
	NCT03625505	Phase Ib	Recruiting	Venetoclax	Relapsed/refractory AML
	NCT02997202	Phase III	Recruiting	-	Maintenance therapy following allogeneic transplant for FLT3-ITD positive AML in CR
	NCT03182244	Phase III	Recruiting	-	FLT3-mutated relapsed/refractory AML (gilteritinib compared to salvage chemotherapy)
	NCT02752035	Phase II/III	Recruiting	Single agent or combined with 5-Aza	FLT3-mutated newly diagnosed AML not eligible for intensive induction chemotherapy
	NCT03836209	Phase II	Recruiting	Standard chemotherapy	FLT3-mutated newly diagnosed AML
	NCT02310321	Phase I/II	Active, not recruiting	Standard chemotherapy	FLT3-mutated newly diagnosed AML
	NCT02236013	Phase I	Recruiting	Standard chemotherapy	Newly diagnosed AML
	NCT04027309	Phase III	Not yet recruiting	Standard chemotherapy	FLT3-mutated newly diagnosed AML (also for one-year maintenance therapy)
	NCT04140487	Phase I/II	Not yet recruiting	5-Aza and venetoclax	FLT3-mutated relapsed/refractory AML and high risk MDS
Quizartinib	NCT03135054	Phase II	Recruiting	Omacetaxine mepesuccinate	Newly diagnosed or relapsed/refractory AML carrying FLT3-ITD
	NCT02039726 (QuANTUM-R)	Phase III	Active, not recruiting	-	Monotherapy vs salvage chemotherapy in FLT3-ITD positive relapsed/refractory AML
	NCT04107727	Phase II	Recruiting	Sandard chemotherapy with or without quizartinib	Newly diagnosed AML wild-type for FLT3
	NCT03989713	Phase II	Not yet recruiting	high-dose cytarabine, mitoxantrone	Relapsed/refractory AML with FLT3-ITD
	NCT03793478	Phase I/II	Recruiting	Re-Induction Chemotherapy	Pediatric relapsed/refractory AML with FLT3-ITD (also for maintenance therapy)
	NCT03552029	Phase I	Recruiting	Milademetan	FLT3-ITD positive relapsed/refractory AML FLT3-ITD positive newly diagnosed AML patients unfit for intensive chemotherapy
	NCT04112589	Phase I/II	Recruiting	FLAG-IDA chemotherapy	Relapsed/refractory AML
	NCT02668653 (QuANTUM-First)	Phase III	Active, not recruiting	Standard chemotherapy	Newly diagnosed AML with FLT3-ITD (also for continuation therapy)
	NCT03735875	Phase Ib/II	Recruiting	Venetoclax	FLT3-mutated relapsed/refractory AML
	NCT04128748	Phase I/II	Not yet recruiting	CPX-351	AML and high risk MDS
	NCT04209725	Phase II	Recruiting	CPX-351	FLT3-ITD positive relapsed/refractory AML
	NCT03661307	Phase I/II	Recruiting	Decitabine and venetoclax	Newly diagnosed or relapsed AML and high risk MDS
	NCT04047641	Phase II	Recruiting	Cladribine, idarubicin and cytarabine	Newly diagnosed or relapsed/refractory AML and high risk MDS
	NCT01892371	Phase I/II	Active, not recruiting	5-Aza or cytarabine	Relapsed/refractory AML and MDS

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Inhibitor	Clinical trial identifier	Phase	Recruitment status	Combination	Study population
	NCT02984995	Phase II	Completed	-	FLT3-ITD positive relapsed/refractory AML
	NCT01565668	Phase II	Completed	-	FLT3-ITD positive relapsed/refractory AML
Midostaurin	NCT00651261 (RATIFY)	Phase III	Active, not recruiting	Standard chemotherapy	FLT3-mutated newly diagnosed AML (< 60 years old)
	NCT04385290	Phase I/II	Not yet recruiting	Gemtuzumab Ozogamicin	FLT3-mutated newly diagnosed AML
	NCT04496999	Phase I	Not yet recruiting	HDM201	Relapsed/refractory AML with FLT3 mutation and wildtype TP53
	NCT03512197	Phase III	Recruiting	Standard chemotherapy	Newly diagnosed AML negative for FLT3 mutation
	NCT04097470	Phase II	Not yet recruiting	Decitabine	AML patients ineligible for intensive chemotherapy
	NCT03280030	Phase II	Active, not recruiting	Standard chemotherapy	FLT3-mutated newly diagnosed AML
	NCT03591510	Phase II	Suspended	Standard chemotherapy	Pediatric patients with FLT3-mutated newly diagnosed AML
	NCT03258931	Phase III	Recruiting	Standard chemotherapy	FLT3-mutated newly diagnosed AML
	NCT03900949	Phase I	Recruiting	Gemtuzumab ozogamicin plus chemotherapy	FLT3-mutated newly diagnosed AML
	NCT03379727	Phase IIIb	Active, not recruiting	standard chemotherapy	FLT3-mutated newly diagnosed AML
	NCT03836209	Phase II	Recruiting	Standard chemotherapy	FLT3-mutated newly diagnosed AML
	NCT01477606	Phase II	Completed	Standard chemotherapy	Newly diagnosed AML carrying FLT3-ITD (also for maintenance therapy)
	NCT03951961 (MAURITIUS)	Phase II	Not yet recruiting	-	MRD-positive FLT3-mutant AML after allogeneic stem cell transplantation
	NCT04027309	Phase III	Recruiting	Standard chemotherapy	FLT3-mutated newly diagnosed AML (also for maintenance therapy)
	NCT03686345	Phase II	Recruiting	Standard chemotherapy	CBF-AML (also for 1-year maintenance therapy)
	NCT01202877	Phase II	Completed	5-Aza	Relapsed/refractory AML and MDS
Crenolanib	NCT03258931	Phase III	Recruiting	Standard chemotherapy	FLT3-mutated newly diagnosed AML
	NCT02283177	Phase II	Completed	Standard chemotherapy	FLT3-mutated newly diagnosed AML
	NCT03250338	Phase III	Recruiting	Standard chemotherapy with or without crenolanib	FLT3-mutated relapsed/refractory AML subjects ≤ 75 years of age
	NCT02400255	Phase II	Recruiting	-	Maintenance therapy following allogeneic transplant for FLT3-mutant AML
	NCT01657682	Phase II	Completed	-	FLT3-mutated relapsed/refractory AML
	NCT02400281	Phase I/II	Completed	Standard salvage chemotherapy or 5-Aza	FLT3-mutated relapsed/refractory AML

AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; 5-Aza: 5-azacytidine; CBF: core binding factor; CPX-351: liposomal cytarabine and daunorubicin; FLAG-IDA: fludarabine, cytarabine, idarubicin and glycosylated granulocyte colony-stimulating factor (G-CSF); MRD: minimal residual disease, FLAG: fludarabine, cytarabine plus G-CSF.

blind, placebo-controlled trial in newly diagnosed *FLT3*-mutated AML, addition of midostaurin to chemotherapy resulted in significant improvements in OS and event-free survival.[58] However, none of these studies demonstrated that midostaurin exerted its anti-leukemic activity via differentiation of AML blasts. Midostaurin is a broad-range multikinase inhibitor, which targets KIT, PDGFRα/β, VEGFR2, and members of the serine/threonine kinase PKC family, apart from inhibiting FLT3. Thus, it is possible that its clinical efficacy results from blocking additional pro-survival and proliferation signals. Indeed, midostaurin also caused clinical responses in FLT3-negative AML and further trials are carefully investigating its clinical activity in *FLT3* wild-type AML (Table 2). Crenolanib is another selective FLT3 inhibitor, which produced high response rates in newly-diagnosed and relapsed *FLT3*-mutant AML patients, as well as those refractory to other tyrosine kinase inhibitors.[52,59] It is being investigated in several late-stage clinical studies, primarily in combination with chemotherapy (Table 2). Several preclinical studies have demonstrated that FLT3 inhibitors broadly induce apoptosis of AML cells.[60,61] Hence, FLT3 inhibitors possibly function through multiple pathways, among which induction of apoptotic cell death and terminal differentiation of leukemic blasts are prominent.

Secondary resistance frequently develops in AML patients treated with FLT3 inhibitors, and complex patterns of clonal selection and evolution have been report-

ed.[52,59,62-64] Acquired resistance to type II FLT3 inhibitors often involves gain of secondary mutations either in the TKD or the gatekeeper residues of the *FLT3* gene,[65] and type I inhibitors, such as gilteritinib and crenolanib, which have activity against both ITD and TKD mutations are effective in these patients. Overall, rational combination therapies with agents targeting known resistance pathways are needed to eradicate leukemic clones.

Other differentiation-inducing agents evaluated in clinical trials

The approval of new targeted drugs bodes well for AML differentiation therapy, but improving therapeutic options for all subgroups remains a major challenge. Epigenetic enzymes are attractive targets for AML therapy and inhibitors of histone deacetylases (HDAC), KDM1A, DOT1L and BET proteins have been extensively studied in AML. We discuss below several of these drugs, which stimulated differentiation of leukemic blasts in preclinical models, but their single-agent activity in clinical trials has been disappointing.

Inhibition of KDM1A (LSD1) promotes myeloid differentiation

KDM1A is a demethylase enzyme with specificity for monomethyl- and dimethyl-histone H3 lysine-4 (H3K4) and lysine-9 (H3K9). It is highly expressed in AML cells and is required to maintain the oncogenic program downstream of MLL-AF9.[66] Pharmacological inhibition of this enzyme potentiates ATRA-induced differentiation of AML cells and diminishes their engraftment in immune-deficient mice.[67] Combined treatment with ATRA and tranylcypromine (an inhibitor of KDM1A) caused a gene-specific increase in H3K4me2 levels and upregulation of myeloid-differentiation associated gene expression.[67] MLL-AF9-expressing cells treated with KDM1A inhibitors also exhibit gain in chromatin accessibility at sites bound by PU.1 and CEBPA; and depletion of either of these transcription factors confers resistance of MLL-AF9-expressing cells to KDM1A inhibition.[68] Preclinical results have also demonstrated sensitivity of other AML subtypes (e.g., AML with RUNX1-RUNX1T1) to KDM1A inhibitors.[69] The efficacy of tranylcypromine combined with ATRA is being evaluated in clinical trials (Table 3).

ORY-1001 (Iadademstat), a more potent and selective derivative of tranylcypromine, effectively reduced growth of AML cell lines, induced expression of differentiation markers and increased survival of murine xenograft models.[70] The drug is synergistic with ATRA as well as with other epigenetic inhibitors targeting DOT1L, DNMT1 or HDAC.[70] Because of favorable pharmacological characteristics and tolerability in a phase I/IIa study in relapsed/refractory AML,[71] ORY-1001 is now being investigated in combination with 5-azacytidine in elderly AML individuals who are not eligible for intensive chemotherapy (Table 3). Other KDM1A inhibitors are currently being investigated as either monotherapy or in combination with ATRA in early phase clinical trials (Table 3).

DOT1L inhibition in *MLL*-rearranged acute myeloid leukemia

Rearrangements of the *MLL* gene occur in 5-10% of AML cases and are associated with an adverse prognosis. DOT1L, a H3K79 histone methyltransferase, is recruited by MLL-fusion proteins resulting in increased H3K79 methylation at target gene loci.[72,73] This mis-targeting of DOT1L activity is essential for the transforming ability of MLL fusion proteins, and enhances expression of HOXA9 and MEIS1.[74-76] Depletion of DOT1L in MLL-AF9 leukemic cells induces differentiation and apoptosis.[75]

EPZ004777 was the first selective inhibitor of DOT1L, which inhibited H3K79 methylation and reversed the gene expression signature of MLL fusion leukemias, including downregulation of *HOXA* cluster genes.[74,75,77] This drug stimulated myeloid differentiation in murine and human cells harboring MLL fusions.[76-78] Pinometostat (EPZ-5676) is closely related to EPZ004777 but is more potent and has better pharmacokinetic properties.[79] However, in a phase I trial of pinometostat in advanced stage *MLL*-rearranged leukemia, only two of 51 patients achieved a CR, while seven non-responding patients also exhibited morphological changes consistent with myeloid differentiation (NCT01684150).[80] Despite apparent biological activity (inhibition of H3K79 methylation) in patients, pinometostat as a stand-alone therapy was not sufficient to produce a clinical benefit. This lack of therapeutic activity may be attributed to heterogeneity of MLL fusion proteins, which might result in differential sensitivity to DOT1L inhibitors.

Given their insufficient clinical efficacy as single agents, combinatorial strategies incorporating DOT1L inhibitors are being tested. Preclinical studies have shown synergistic anti-leukemic effects of combining DOT1L inhibition with either cytotoxic chemotherapy, hypomethylating agents, or inhibition of KDM1A or menin (a MLL cofactor),[81-84] suggesting that dual targeting of the MLL-fusion complex may prove superior to DOT1L inhibitor monotherapy. Phase Ib/II trials of pinometostat plus either standard chemotherapy or 5-azacytidine are ongoing in AML with *MLL* gene rearrangements (Table 3).

Myeloid differentiation induced by inhibition of BET proteins

Bromodomain and extra terminal (BET) family of proteins bind to acetyl-modified lysine residues of histone tails and activate transcription. A screen of RNA interference in a mouse AML model expressing the *MLL-AF9* fusion and oncogenic *NRAS* identified BRD4, a BET family member, as indispensable for disease progression.[85] Genetic and pharmacological inactivation (JQ1 and I-BET151) of BRD4 led to downregulation of MYC, BCL2 and CDK6 in AML blasts, inhibited growth of MLL-rearranged AML cell lines and induced differentiation of primary leukemic samples and murine hematopoietic cells transformed with MLL fusions.[85-87] Another BET bromodomain inhibitor, AZD5153, which ligates two bromodomains of BRD4 simultaneously, has prominent anti-proliferative activity against AML cell lines *in vitro* and in xenograft models.[88] In other preclinical studies, BRD4 inhibitors exhibited anti-leukemic effects in AML driven by mutations of either *IDH2*, *NPM1* or *FLT3*-ITD.[89-91] Several BET inhibitors are currently under clinical investigation (Table 3); however, emerging results from these early phase trials are disappointing. In a phase I trial of OTX015 (MK-8628), this BET inhibitor elicited a clinical response in only five of 36 AML patients, with the response lasting 2–5 months.[92] The clinical response to mivebresib (ABBV-075) was similarly inadequate and frequently associated with adverse effects including cytopenia.[93] A number of trials of

BET inhibitors have been terminated because of a combination of issues including low response rates, dose-limiting toxicities and inter-patient variability in pharmacokinetic properties (Table 3).

Improved drug design and combination strategies might establish the therapeutic utility of BET inhibitors. For instance, hetero-bifunctional compounds, BET-PROTAC (proteolysis-targeting chimera, ARV-825 and ARV-771), in

Table 3. Clinical trials on drugs targeting epigenetic regulators.

Target	Inhibitor	Clinical trial identifier	Phase	Recruitment status	Combination	Study population
KDM1A	ORY-1001 (Iadademstat)	EudraCT 2013-002447-29	Phase I	Completed	-	Relapsed/refractory AML
		EudraCT 2018-000482-36	Phase I	Ongoing	5-Aza	Older AML patients ineligible for standard treatment
	Tranylcypromine	NCT02717884	Phase I/II	Recruiting	ATRA and AraC	Non-APL AML or MDS who failed azanucleoside treatment
		NCT02273102	Phase I	Completed	ATRA	Adult AML and MDS
		NCT02261779	Phase I/II	Unknown	ATRA	Relapsed/refractory AML or patients not eligible for intensive treatment
	IMG-7289	NCT02842827	Phase I	Completed	ATRA	AML and MDS
	INCB059872	NCT02712905	Phase I/II	Terminated (business decision)	5-Aza ATRA	Newly diagnosed AML Relapsed/refractory AML
	GSK2879552	NCT02177812	Phase I	Terminated (unfavorable risk-benefit ratio)	ATRA	Relapsed/refractory AML
DOT1L	Pinometostat (EPZ-5676)	NCT01684150	Phase I	Completed	-	Relapsed/refractory AML with MLL-rearrangements or PTD
		NCT02141828	Phase I	Completed	-	Pediatric relapsed/refractory AML with MLL-rearrangements
		NCT03724084	Phase Ib/II	Recruiting	Standard chemotherapy	Newly diagnosed AML with MLL-rearrangements
		NCT03701295	Phase Ib/II	Recruiting	5-Aza	Relapsed, refractory or newly diagnosed AML with MLL-rearrangements
BET proteins	OTX015 (MK-8628)	NCT02303782	Phase Ib/II	Withdrawn	5-Aza	Newly diagnosed AML not eligible for intensive treatment
		NCT01713582	Phase I	Completed	-	*De novo* and secondary AML
		NCT02698189	Phase Ib	Active, not recruiting	-	*De novo* and secondary AML
	Molibresib (I-BET 762, GSK525762)	NCT01943851	Phase I/II	Completed	-	Relapsed/refractory AML
	RO6870810 (TEN-010)	NCT02308761	Phase I	Completed	-	Relapsed/refractory AML
	FT-1101	NCT02543879	Phase I/Ib	Completed	5-Aza	Relapsed/refractory hematologic malignancies
	INCB057643	NCT02711137	Phase I/II	Terminated (safety issues)	-	Advanced solid tumors and hematologic malignancies
	INCB054329	NCT02431260	Phase I/II	Terminated (PK variability)	-	Advanced malignancies including AML
	Mivebresib (ABBV-075)	NCT02391480	Phase I	Completed	-	Relapsed/refractory AML
	PLX51107	NCT02683395	Phase 1b/IIa	Terminated (business decision)	-	Advanced hematologic malignancies
		NCT04022785	Phase I	Recruiting	5-Aza	AML and MDS
Aberrant DNA demethylation	Vitamin C	NCT02877277		Completed	DNMTi	AML and MDS
		NCT03397173	Phase II	Recruiting	5-Aza	AML and MDS with TET2 mutations
		NCT03999723	Phase II	Recruiting	5-Aza	MDS, CMML and AML

AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; APL: acute promyelocytic leukemia; ATRA: all-trans retinoic acid; 5-Aza: 5-azacytidine; AraC: cytarabine; CMML: chronic myelomonocytic leukemia; DNMTi: DNA methyltransferase inhibitor; PTD: partial tandem duplication.

which a BET inhibitor (OTX015/JQ1) is linked to a ligand for an E3 ubiquitin ligase (either cereblon or von Hippel–Lindau),[94,95] have exhibited high potency in attenuating MYC and inducing apoptosis of AML cells.[96] Moreover, concomitant inhibition of both BRD4 and DOT1L had significant synergistic activity against *MLL*-rearranged leukemia cell lines, primary human AML cells and murine leukemia models.[97] Clearly, additional studies are needed to establish the clinical efficacy of drug combinations involving BET inhibitors.

Histone deacetylase inhibitors

Recruitment of HDAC is a major pathogenic event in AML subtypes driven by PML-RARA, inv(16) (CBFA-MYH11) and RUNX1-RUNX1T1 chimeric proteins, indicating that inhibiting the activity of HDAC might be therapeutic. In preclinical studies, a combination of a HDAC inhibitor (trichostatin A) with ATRA relieved the differentiation block in ATRA-resistant APL cells. Panobinostat, another HDAC inhibitor, caused proteosomal degradation of the RUNX1-RUNX1T1 oncoprotein, triggering myeloid differentiation of leukemic cells in a murine model of human t(8;21) AML.[98] Although several classes of HDAC inhibitors showed promise in preclinical models, their clinical efficacy as monotherapy was negligible in early phase clinical trials.[99-102] Combinations of HDAC inhibitors with hypomethylating agents, chemotherapeutic agents or immunotherapy and targeted therapies are currently in different stages of clinical testing. Despite evidence of synergism between HDAC inhibitors and hypomethylating agents in preclinical and early clinical studies, large multi-arm clinical studies showed increased toxicity and decreased OS in patients treated with a HDAC inhibitor plus 5-azacytidine compared with 5-azacytidine alone.[103-105] Collectively, the data suggest that HDAC inhibitors have limited clinical benefit, illustrating the potential problem of off-target effects arising from a lack of specificity of this approach.

Differentiation therapies in early clinical development

Differentiation of acute myeloid leukemia blasts using Aurora kinase inhibitors

Aurora kinases are serine/threonine kinases that regulate chromosomal alignment and segregation during mitosis and meiosis. Expression of Aurora kinase A and B is higher in leukemic blasts than in normal $CD34^+$ cells.[106] Inhibition of Aurora kinase A in acute megakaryocytic leukemia (AMKL) increases polyploidization of blasts and induces their differentiation.[107] Furthermore, these inhibitors significantly prolonged survival of mice in an AMKL model, as well as mice engrafted with primary human AMKL cells.[107,108] Alisertib, an Aurora kinase A inhibitor, is currently under clinical evaluation for use in the treatment of AMKL (NCT02530619). Several trials of either specific or dual inhibitors of Aurora kinase A and B showed moderate efficacy for treating other types of AML. In a phase I study, a combination of alisertib and conventional induction therapy (cytarabine and idarubicin) resulted in an 86% remission rate in newly diagnosed AML patients (NCT01779843),[109] suggesting enhanced activity of this drug combination. Thus, Aurora kinase inhibitors may hold promise in AML therapy.

Restoration of TET activity with vitamin C promotes differentiation

TET proteins are dioxygenase enzymes that catalyze oxidation of 5-methylcytosine to 5-hydroxymethylcytosine, leading to DNA demethylation. The *TET2* gene is frequently mutated in AML and other hematological malignancies, as well as in clonal hematopoiesis of indeterminate potential.[110] Stimulation of the remaining wild-type allele of *TET2* is a plausible therapeutic approach.

In a murine model of reversible silencing of *TET2*, re-expression of *Tet2* in hematopoietic cells primed the cells towards myeloid differentiation and promoted cell death.[111] Vitamin C is a co-factor that activates TET proteins; and its depletion impairs the function of TET2 and accelerates tumorigenesis in a murine model of AML.[112] Importantly, vitamin C treatment mimics pharmacological restoration of TET2 activity as evidenced by increased 5-hydroxymethylcytosine levels in AML cells and Tet2-deficient mice.[111] In AML with biallelic *TET2* mutations or copy number neutral loss of heterozygosity of the *TET2* locus, vitamin C might still enhance 5-hydroxymethylcytosine levels by potentiating TET3 activity, which can potentially compensate sufficiently for lack of TET2.[111] Serum ascorbic acid (vitamin C) levels are significantly reduced in patients with diverse hematologic malignancies,[113,114] and high doses of oral vitamin C might improve their outcome. The clinical efficacy of vitamin C combined with 5-azacytidine in patients with AML or myelodysplastic syndrome with or without *TET2* mutations is currently being investigated (Table 3). Conceivably, dietary supplements of vitamin C may also help to augment TET2 activity and impede clonal hematopoietic progression in individuals with clonal hematopoiesis of indeterminate potential, especially those harboring mutant *TET2*.

Inhibition of DHODH relieves the block in myeloid differentiation in acute myeloid leukemia

HOXA9 is suppressed during myeloid differentiation, but its upregulation occurs in a large proportion of AML driven by different oncogenic events. In a murine model of inducible *Hoxa9* expression, inhibition of dihydroorotate

Table 4. Clinical trials of DHODH inhibitors in acute myeloid leukemia.

Inhibitor	Clinical trial identifier	Phase	Recruitment status	Combination	Study population
ASLAN003	NCT03451084	Phase IIa	Recruiting	5-Aza (≥60-years old)	AML patients ineligible for standard treatment and relapsed/refractory AML
BAY2402234	NCT03404726	Phase I	Recruiting	-	AML and MDS
PTC299	NCT03761069	Phase Ib	Recruiting	-	Relapsed/refractory AML
Brequinar	NCT03760666	Phase Ib/IIa	Recruiting	-	Relapsed/refractory AML

AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; 5-Aza: 5-azacytidine; DHODH: dihydroorotate dehydrogenase.

dehydrogenase (DHODH), a key enzyme in uridine synthesis that catalyzes conversion of dihydroorotate to orotate, induced terminal differentiation of leukemic cells.[115] In an independent CRISPR/Cas9 screen of AML cell lines, loss of DHODH also decreased growth of AML cells.[116] This suggests that targeting the *de novo* pyrimidine synthesis pathway may induce AML differentiation.

DHODH is neither mutated nor overexpressed in cancers; but cancer cells that are metabolically reprogrammed may have an increased dependency on *de novo* pyrimidine production,[117] thus creating a potential therapeutic window. Earlier attempts to target DHODH with inhibitors (e.g., teriflunomide, brequinar, and leflunomide) in several cancers were unsuccessful because of either low potency or off-target effects.[118] Subsequently, more potent and selective DHODH inhibitors (ASLAN003, BAY2402234 and PTC299) (Table 4) have been developed, which show antileukemic activity in various preclinical models;[117,119-121] and are in early phase clinical trials.

The mechanism of action of DHODH inhibitors is unclear; but these drugs appear to have potential therapeutic value for the treatment of AML. However, by analogy with ATRA and the differentiating agents discussed above, it is unlikely that monotherapy will achieve durable responses.

Potential new targets for acute myeloid leukemia differentiation therapy

Recent studies have brought to light previously uncharacterized modulators of myeloid differentiation which can be targeted to overcome the differentiation block in AML. Pharmacological agents targeting these candidate modulators need to be developed to explore their clinical utility.

The CAF1 complex as a novel target for differentiation therapy of acute myeloid leukemia

CHAF1B is a subunit of the chromatin assembly factor 1 complex (CAF1) that facilitates assembly of H3-H4 tetramers at replication forks during the S phase. High *CHAF1B* levels are observed in leukemia and are associated with a poor prognosis.[122] Overexpression of CHAF1B enhances the leukemic potential of the MLL-AF9 oncoprotein, and its inhibition promotes differentiation of leukemic cells.[122] CHAF1B protein occupies discrete genomic loci and interferes with the CEBPA-mediated differentiation of leukemic cells;[122] and is a potential therapeutic target.

Inhibiting transcriptional repressors to re-activate the myeloid differentiation program

A common feature of AML driven by different oncogenic events is increased recruitment of transcriptional co-repressors, which affects transcription of differentiation-promoting genes. Two co-repressors (SMARCA5 and CHD4) are enriched in the PU.1/RUNX1/CEBPA master transcription factor hub specifically in AML cells.[123] An inhibitor of SMARCA5/CHD4 suppresses growth of AML cell lines *via* their terminal differentiation while not affecting growth of normal hematopoietic progenitors.[123]

Cell cycle regulators as therapeutic targets in acute myeloid leukemia

CDK6 is required for the growth and maintenance of an immature state of MLL-rearranged AML cells, and its suppression (by PD-0332991) induced myeloid differentiation in human and murine AML cells expressing the MLL fusion.[124] Another cell cycle regulator, CDK1 phosphorylates CEBPA, thereby inhibiting its differentiation-inducing activity. In preclinical studies, pharmacological and genetic inhibition of CDK1 relieved the differentiation block in AML cells harboring mutations of *FLT3*.[125] Inhibition of CDC25 using IRC-083864 can also overcome the differentiation block in *FLT3*-ITD AML cells..[126] Overall, cell cycle regulators may be potential therapeutic targets in AML.

Combination therapies using differentiation-inducing agents

Combinatorial therapeutic approaches may be needed to increase efficacy and reduce development of resistance in AML. In APL, ATRA is effective in achieving remission by inducing terminal differentiation, but does not eradicate the leukemic clone; however, high cure rates are attained when ATRA is combined with arsenic or anthracycline. It is postulated that ATRA differentiation therapy in APL is unable to eliminate the leukemic stem cell, and this may also be relevant to differentiation therapy of AML more widely. In fact, a recent study revealed that mature APL cells become leukemogenic upon termination of ATRA treatment, suggesting that withdrawal of differentiation therapy can lead to reacquisition of clonogenic and leukemogenic properties through de-differentiation.[127] Hence, combination regimens involving newly approved differentiation therapies (mutant *IDH1/2* and *FLT3* inhibitors) should be optimized for efficient clearing of the leukemic clones. Similarly, rational combination strategies might enhance the clinical utility of epigenetic inhibitors that have been largely ineffective as single agents.

Concluding remarks

Since 2017, four drugs (inhibitors of mutant *IDH1/2* and *FLT3*) that induce differentiation of AML cells have been approved by the FDA. These novel therapeutic agents mark significant strides forward in differentiation therapy of AML. However, for other differentiation-inducing agents (e.g., inhibitors of HDAC, BET proteins, DOT1L), which showed promise in preclinical studies, early reports from clinical trials have not been encouraging. This highlights the limitations of preclinical efficacy models usually performed in immunocompromised mice lacking the immune component, as well as species-specific differences in drug pharmacokinetics and metabolism. Animal models are also limited in their ability to adequately replicate the molecular heterogeneity of AML. In addition, there is a pressing need to identify molecular subgroups which might be responsive to the new agents, as illustrated by the success of the RATIFY trial in assessing the benefit of midostaurin in *FLT3*-mutant AML. Another concern is that the new drugs may have been tested on limited cohorts of patients. For example, DOT1L inhibitors have been clinically evaluated in AML patients with rearranged *MLL*, but their differentiation-promoting activity has also been demonstrated in *DNMT3A*-mutant AML cells in preclinical studies,[128,129] suggesting that investigation of DOT1L inhibitors could be expanded to other cohorts of patients. In addition, initial trials usually involve patients with relapsed/refractory disease which can

be biologically and clinically distinct from newly diagnosed AML. Overall, this illustrates that numerous difficulties need to be overcome for translation of preclinical candidates into clinically useful differentiation therapy. APL is arguably the least molecularly complex type of AML, which may be relevant to the early success of differentiation therapy in APL.

In summary, new insights into disease pathogenesis acquired from mutational and gene expression analyses have directed AML differentiation therapy to several novel targets. For many targets, pre-clinical potential has not translated into clinical success; nonetheless, additional promising targets (DHODH, Aurora kinases) have emerged and are being actively explored. Overall, we are in an exciting era of new therapeutic targets and drugs not imagined 10 years ago.

Disclosures

No Conflicts of interest to disclose

Contributions

VM performed literature search and wrote the review, HPK conceived and wrote the manuscript.

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