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REVIEW

The Time Has Come for Targeted Therapies for AML: Lights and Shadows

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ABSTRACT

Acute myeloid leukemia (AML) is a complex disease characterized by genetic and clinical heterogeneity and high mortality. After 40 years during which the standard of care for patients evolved very little, the therapeutic landscape has recently seen rapid changes, with the approval of eight new drugs by the Food and Drug Administration (FDA) within the last 2 years, providing new opportunities, as well as new challenges, for treating clinicians. These therapies include FLT3 inhibitors midostaurin and gilteritinib, CPX-351 (liposomal cytarabine and daunorubicin), gemtuzumab ozogamicin (GO, anti-CD33 monoclonal antibody conjugated with calicheamicin), IDH1/IDH2 inhibitors ivosidenib and enasidenib, Hedgehog inhibitor glasdegib, and BCL-2 inhibitor venetoclax. In this review, we summarize currently available data on these new drugs and discuss the rapidly evolving therapeutic armamentarium for AML, focusing on targeted therapies.

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Key Summary Points

After no substantial innovations in 40 years, the scenario of treatment in acute myeloid leukemia (AML) has recently seen a number of changes, with FDA approval of eight new non-cytostatic compounds.

The new FDA-approved targeted therapies are midostaurin, gilteritinib, glasdegib, ivosidenib, enasidenib, venetoclax, and gemtuzumab ozogamicin.

RATIFY is the first randomized trial to show that the combination of targeted therapy with standard chemotherapy significantly improves survival in AML.

Phase II trials are currently evaluating new drugs targeting EZH2, DOT1L, MLL, BET, and LSD1.

BACKGROUND

The so-called 7+3 schedule (cytarabine 100–200 mg/m^2 on days 1–7 and daunorubicin 60 mg/m^2 on days 1–3) has been the standard first-line induction therapy in adults with acute myeloid leukemia (AML) for 40 years. However, in recent years, the scenario of treatment in AML has changed substantially, with approval by the US Food and Drug Administration (FDA) of eight non-cytostatic compounds, able to interact with specific targets of different AML subtypes. Leukemogenesis is characterized by multiple somatically acquired mutations that affect genes of different functional categories. Mutations in genes encoding epigenetic modifiers, such as DNMT3A, ASXL1, TET2, IDH1, and IDH2, are commonly acquired early and can be found in the founding clone; such mutations may persist after therapy, lead to clonal expansion during hematological remission, and eventually lead to relapse. In contrast, mutations involving NPM1 or signaling molecules (e.g., FLT3, RAS) are typical secondary events that occur later during leukemogenesis; in many cases their selective inhibition may involve only a leukemia subclone, sparing other clones, and these clonal relationships need to be taken into account when designing clinical trials with molecular-targeted agents.

Immunotherapy with GO was the first targeted therapy explored and the last to be approved by the FDA, due to the non-hematological toxicity observed in patients treated with higher-dose regimens. Unfortunately, the sensitivity of calicheamicin to the drug extrusion mechanism, mediated by P-glycoprotein (PgP), strongly limited its efficacy in AML patients with unfavorable cytogenetics, and experimental studies with second-generation anti-CD33 antibodies were interrupted because of hematological toxicity.

In a seminal pivotal study [1], 1540 AML patients were studied extensively, and six patterns of co-occurrence and mutual exclusivity of genetic changes were identified:

- AML with balanced rearrangements
- AML with NPM1 mutation
- AML with mutation of genes that regulate chromatin (ASXL1, STAG2, BCOR, KMT2A PTD [partial tandem duplication], EZH2, and PHF6), RNA splicing (SRSF2, SF3B1, U2AF1, and ZRSR2), or both, or transcription (RUNX1)
- AML with IDH1 or IDH2 mutation
- AML with TP53 mutation, chromosomal aneuploidy, or both

Thanks to these achievements, AML now represents an important field for investigation of new drugs such as tyrosine kinase inhibitors (TKI), epigenetic modulators, immune checkpoint inhibitors, mitochondrial inhibitors, and molecules targeting specific oncogenic proteins and the AML microenvironment; among them, the FLT3 inhibitors midostaurin and gilteritinib, the anti-Hedgehog pathway glasdegib, the anti-IDH1 ivosidenib, the anti-IDH2 enasidenib, and the anti-BCL2 venetoclax were recently approved by the FDA for AML treatment [2].

A schematic view of the mechanism of action of recently FDA-approved AML-targeted therapies is shown in Fig. 1 (FLT3 inhibitors), Fig. 2 (glasdegib, ivosidenib, and enasidenib), and Fig. 3 (venetoclax and GO).

This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

FLT3 Inhibitors

The class III receptor tyrosine kinase (RTK) FMS-like tyrosine kinase 3 (FLT3) plays a key role in myelopoiesis. Two different types of FLT3 mutations have been detected in about 30% of AML patients: the internal tandem duplication (ITD) in the juxtamembrane region, and point mutations in the tyrosine kinase domain (TKD), mainly involving codons D835 and I836.

These mutations lead to ligand-independent activation of the receptor promoting proliferation, survival, and resistance to apoptosis of leukemic stem cells [3].

Several type I (binding the gatekeeper domain) or type II (binding the activation loop) tyrosine kinase inhibitors have been

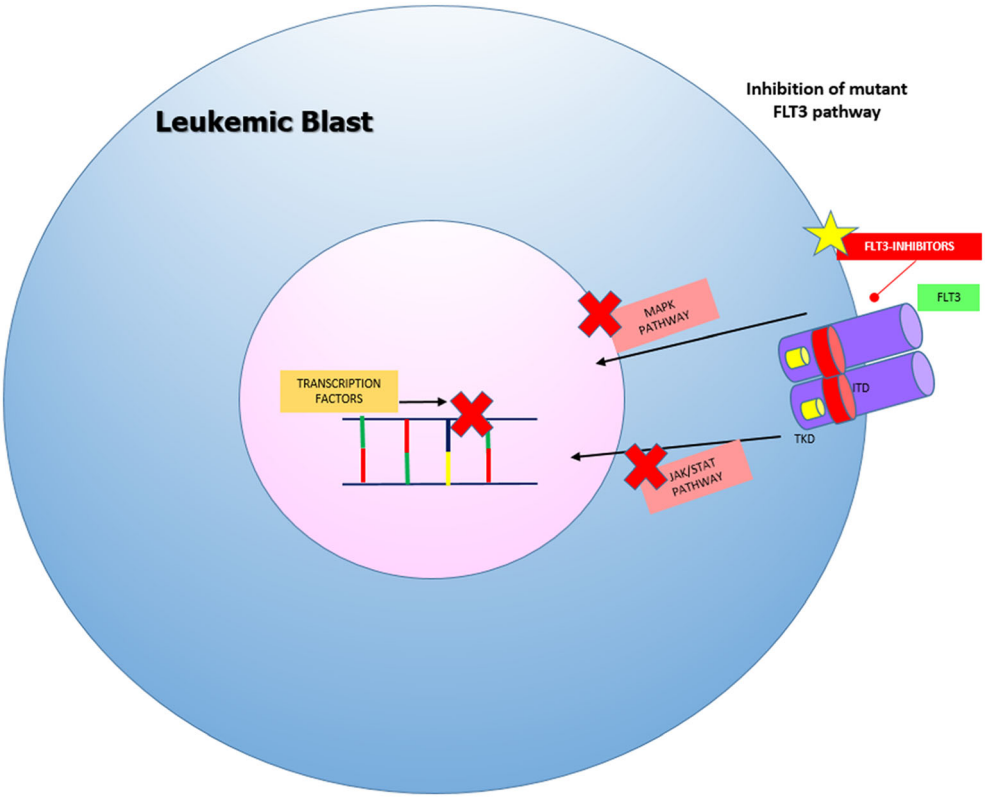

Fig. 1 Mechanism of action of FLT3 inhibitors

investigated, with variable pharmacokinetics, selectivity for FLT3, and in vitro efficacy [4].

First-generation FLT3 inhibitors (midostaurin, lestaurtinib, sorafenib) are multi-targeted kinase inhibitors, showing potent in vitro inhibition of mutant FLT3. Clinically, they have shown insufficient activity as single agents [5] but interesting synergy with chemotherapy [6, 7].

Second-generation FLT3 inhibitors, such as quizartinib, crenolanib, and gilteritinib, are more selective and more potent than midostaurin and are currently in clinical development.

Results in Upfront Therapy

Midostaurin is a multi-kinase inhibitor, initially developed as a protein kinase C inhibitor and later as an inhibitor of VEGF/angiogenesis [8] and FLT3 [9, 10].

The CALGB 10603/RATIFY trial, a multicenter phase III study, enrolled 717 adult patients (18–59 years of age) with newly diagnosed AML and FLT3 ITD or TKD mutation [11]; patients were randomized to receive standard induction chemotherapy ± midostaurin 50 mg orally b.i.d., given on days 8–22, and four courses of high-dose cytarabine (HDAC) ± midostaurin as consolidation. The trial also included a 12-month maintenance phase of midostaurin/placebo.

Patients were allowed to receive post-consolidation with allogeneic hematopoietic stem cell transplantation (HSCT), but in this case maintenance with midostaurin was not permitted.

The rate of complete remission (CR) was similar between the two arms (58.9% vs. 53.5%; $p = 0.15$), but the overall survival (OS), the study primary endpoint, was 74.7 months (median) in the midostaurin group, significantly better than 25.6 months in the placebo group (HR = 0.78, $p = 0.009$). Also, both event-free survival (EFS) and disease-free survival (DFS)

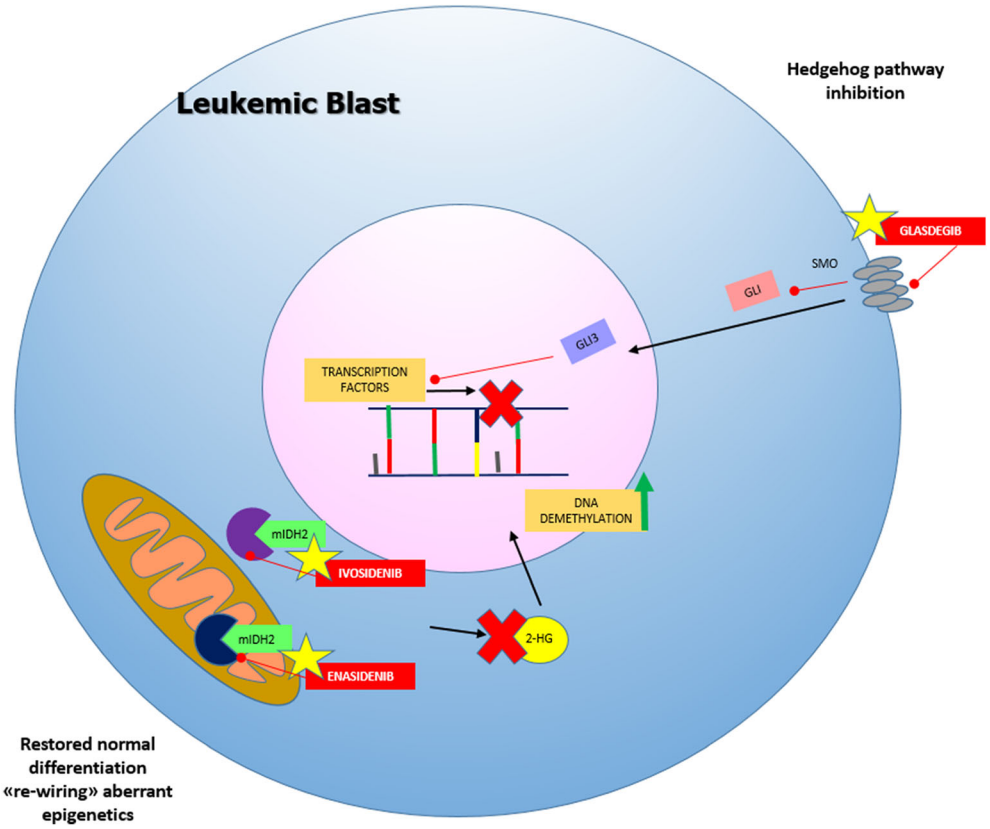

Fig. 2 Mechanism of action of glasdegib, ivosidenib, and enasidenib

were significantly better in the midostaurin arm (8.2 vs. 3.0 months, $p = 0.002$; 26.7 vs. 15.5 months, $p = 0.01$, respectively), with a 21.6% lower risk of relapse (HR = 0.78, $p = 0.002$).

The better outcome was not related to the FLT3 mutational site (ITD vs. TKD) or the FLT3-ITD/wild-type (WT) ratio; 28.1% of AML patients treated with midostaurin underwent HSCT during their first CR, versus 22.7% in the placebo arm ($p = 0.10$). Patients in the midostaurin arm receiving transplants demonstrated a trend toward improved 4-year OS in comparison with those receiving placebo (63.7% vs. 55.7%; $p = 0.08$); the benefit of midostaurin was observed only in patients who received HSCT during the first remission and not at later time points.

Most of the adverse events were similar between the two arms, and no significant treatment-related adverse events (TRAE) of grade ≥ 3 were reported in the midostaurin arm, with only a slightly increased incidence of rash, nausea, and anemia.

RATIFY was the first randomized trial to show that the combination of a targeted therapy with standard chemotherapy significantly improved survival in AML.

Based on this pivotal trial, on April 28, 2017, the FDA approved midostaurin in combination with intensive induction and consolidation therapy for patients with FLT3-mutant, newly diagnosed AML, while in Europe it was approved for induction, consolidation, and maintenance (Table 1).

Sorafenib is an oral multikinase inhibitor of RAF-1, VEGF, c-KIT, PDGFR, ERK, and FLT3. Currently, sorafenib is approved for hepatocellular carcinoma and renal cell carcinoma, but also has a potent anti-leukemic effect on FLT3-mutated AML. In a previous study, sorafenib in combination with intensive chemotherapy failed to increase OS [12], but in a subsequent phase III trial, sorafenib prolonged OS and

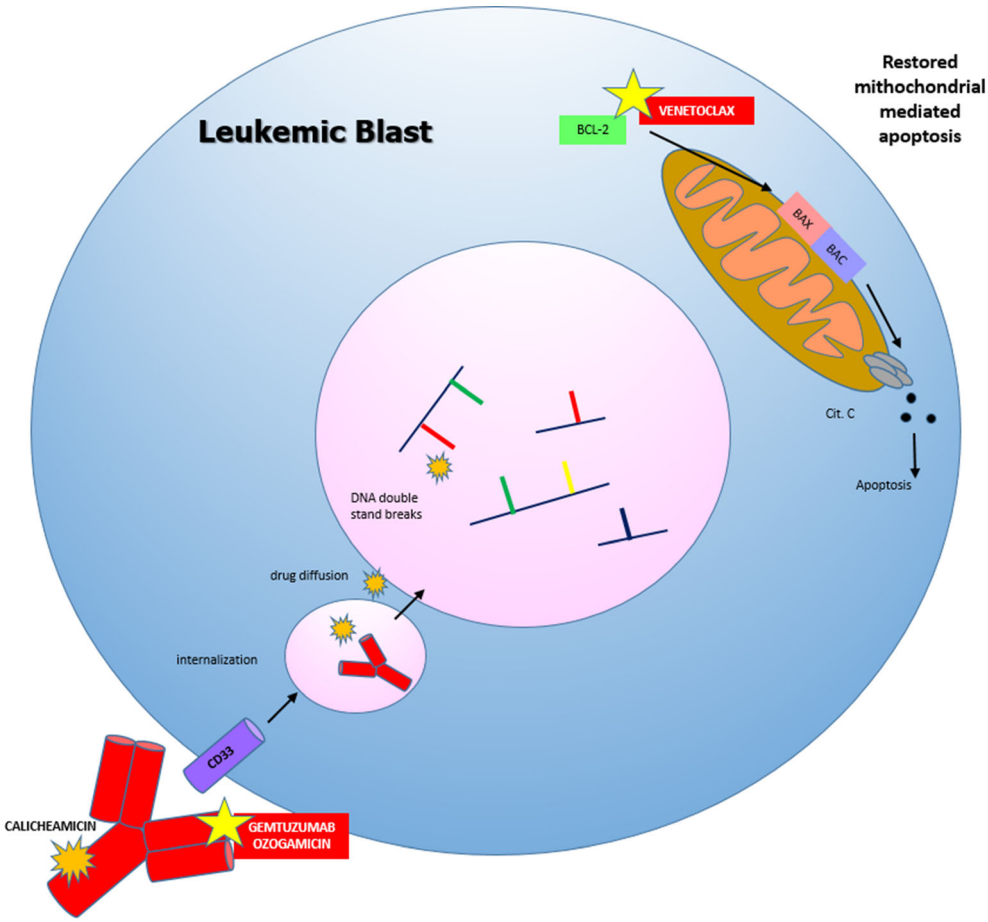

Fig. 3 Mechanism of action of GO and venetoclax

relapse-free survival (RFS) when administered as maintenance after HSCT [13].

Quizartinib is a selective second-generation inhibitor of FLT3-WT and FLT3-ITD, without activity on FLT3-TKD. A phase III trial in which it is being administered with standard induction chemotherapy in younger adults with newly diagnosed FLT3-ITD-mutated AML is still ongoing (NCT02668653).

Crenolanib is a type-1 FLT3 inhibitor active against both FLT3-ITD- and FLT3-TKD-mutant AML, originally developed as a selective inhibitor of the platelet-derived growth factor receptors (PDGFR). It is also a potent inhibitor of mutated FLT3, particularly the secondary mutation D835 [14], which is one of the mechanisms of resistance to FLT3 inhibitors [15]. The addition of crenolanib (100 mg, three times/day) to standard 7+3 induction chemotherapy resulted in CR/incomplete count recovery (CRi) rates of 24/25 (96%) among patients with FLT3-mutant AML, and was able to overcome the poor prognostic impact of co-occurring driver mutations such as FLT3-ITD, NPM1, and DNMT3A [16, 17].

Gilteritinib, a pyrazinecarboxamide derivative also known as ASP-2215, is a selective and potent inhibitor of FLT3 [18]; when administered at doses $\geq$ 80 mg/day in combination with induction and consolidation chemotherapy, gilteritinib achieved CR/CRi rates of 89% in a phase I study [19].

Results in Relapsed/Refractory (R/R) AML

Gilteritinib and quizartinib have demonstrated a survival benefit compared with chemotherapy in prospective randomized trials in R/R patients: the ADMIRAL phase III trial (NCT02421939)

Table 1 Completed clinical trials with midostaurin

Study	Phase	Population	Association	Results (efficacy)	Results (safety)	Reference	Status
Midostaurin							
RATIFY	III	de novo AML (aged 18-60 years)	+ IC vs. IC alone	> OS (74.7 vs. 25.6 m) 22% reduced risk of death; 4-year OS 51% vs. 44%. > EFS (8.2 vs. 3.0 m). > DFS (26.7 vs. 15.5 m). No differences in CR rate	No differences vs. placebo except for anemia, rash, nausea	[11]	Completed

Mechanism: inhibition of mutant and wild-type FMS-like tyrosine kinase 3 (FLT3)
Status: approved by FDA, in combination with intensive induction and consolidation therapy for patients with FLT3-mutant newly diagnosed AML (April 28, 2017)
IC intensive chemotherapy, *OS* overall survival, *EFS* event free survival, *DFS* disease free survival, *CR* complete response

randomized 138 adults with R/R AML with FLT3 ITD, D835, or I836 mutations to oral gilteritinib 120 mg daily versus investigators' choice of low-dose cytarabine (LDAC), azacitidine, or second-line therapy [mitoxantrone, etoposide, and cytarabine (MEC), or fludarabine, cytarabine, granulocyte colony-stimulating factor, and idarubicin (FLAG-IDA)]. The median OS in the gilteritinib arm was 9.3 months, compared with 5.6 months in patients who received standard chemotherapy (SC) [hazard ratio = 0.637 (95% CI 0.490, 0.830), $p = 0.0007$)]; 1-year OS was 37% in the gilteritinib arm versus 17% in the SC arm. The CR/CRh = CR with incomplete hematologic recovery: All CR criteria except for residual neutropenia (< 1.0 x 10e3/L) or thrombocytopenia (100 x 10e9/L) rates for gilteritinib and SC were 34% and 15.3%, respectively ($p = 0.0001$); CR rates were 21.1% and 10.5% (two-sided $p = 0.0106$). Median EFS was 2.8 months and 0.7 months in the gilteritinib and SC arms, respectively (HR 0.793, $p = 0.0830$) [20]. Based on the interim data [21], in November 2018 the FDA approved gilteritinib for the secondary treatment of AML in adults with a FLT3 mutation.

Quizartinib achieved positive results in the phase III QuANTUM-R trial, which randomized (2:1) 367 patients with FLT3-ITD allelic burden ≥ 3% to either single-agent quizartinib ($n = 245$; 60 mg, with a 30-mg lead-in of 15 days) or investigators' choice chemotherapy (total = 122). Median OS improved from 4.7 to 6.2 months (HR 0.76; 95% CI 0.58–0.98; $p = 0.0177$) in favor of quizartinib. A higher response rate (composite CR 48% vs. 27%) was reported in the quizartinib arm, lasting a median of 12.1 months. The subsequent SCT rate was higher for patients receiving quizartinib (32 vs. 12%). Significant non-hematological grade ≥ 3 TRAEs were limited to QTc prolongation (10%) and reversible gastrointestinal symptoms [22]. However, the internal FDA analysis could not confirm a significant EFS benefit with quizartinib versus chemotherapy: median EFS was 6.0 weeks in the quizartinib arm (95% CI 0.1–8.3) versus 3.7 in the control arm (95% CI 0.4–6.0), respectively (HR 0.9; 95% CI 0.71–1.16; $p = 0.114$). Moreover, the FDA focused on cardiac toxicity as a key concern with quizartinib. Additional safety issues identified in the FDA analysis included potentially fatal differentiation syndrome, acute febrile neutrophilic dermatosis, and prolonged cytopenia [23].

A phase II study in relapsed/refractory FLT3-mutated AML showed that crenolanib at 200 mg three times/day continuously (28-day cycles) achieved 23% CR with CRi in naive patients, and 5% CR in patients previously treated with other FLT3 inhibitors [24].

IDH1 AND IDH2 INHIBITORS

The isocitrate dehydrogenase (IDH) family is involved in the cellular energy pathway, by

catalyzing the oxidative decarboxylation of isocitrate to α-ketoglutarate. While IDH1 is localized in peroxisomes in the cytosol, IDH2 resides in mitochondria [25, 26].

IDH mutations occur in approximately 20% of AML patients [IDH1 (8%) and IDH2 (12%)], and are more common in the elderly (25–28%). They are usually associated with intermediate-risk cytogenetic, *FLT3*, and NPM1 mutations [27–29]; Bose et al. [30].

Somatic mutations in catalytically active arginine residues decrease their enzymatic activity and also confer a gain in functional activity, leading to the production of the oncometabolite 2-hydroxyglutarate (2-HG) instead of alpha-ketoglutarate (α-KG).

2-HG competitively inhibits the function of αKG-dependent oxygenases involved in DNA or histone demethylation, resulting in global DNA hypermethylation of regulatory genes and arrested myeloid differentiation. The mutation also increases reactive oxygen species (ROS) output and cell-cycle transition through the activation of MAPK (mitogen-activated protein kinase) signaling and the repression of cyclin-dependent kinase inhibitors Cdkn2a and Cdkn2b, resulting in metabolic changes leading to upregulation of NF-Kb and BCL-2 proteins [31–36].

Ivosidenib and enasidenib are two orally available selective inhibitors of mutant IDH1 and IDH2, and have been shown to decrease cellular 2-HG production by more than 90%, thus reducing histone and DNA hypermethylation and inducing myeloid differentiation [37, 38].

Results in R/R AML

Enasidenib, formerly known as AG-221, binds to mutated IDH2, reducing its affinity to NADPH, leading to impaired catalytic activity. It was administered at 100 mg/day in a phase I/II trial [39] in 239 patients (median age 67 years, range 19–100) with mutant IDH2 and advanced myeloid malignancies. The maximum tolerated dose was not reached. The same dose (FDA-approved) was designated for the expansion phase (n = 126) based on favorable pharmacokinetic and pharmacodynamic profiles.

Among patients with R/R AML (n = 176), the ORR was 40.3%, while complete clinical remission (cCR) and CR rates were 26% and 19%, respectively. The median time to first response was 1.9 months (range 0.5–9.4 months) and median time to CR was 3.7 months (range 0.7–11.2). The median response duration was 5.6 months (range 3.8–9.7). Among those patients achieving CR, the median response duration was 8.8 months (range 5.3–not reached).

Over a median follow-up of 7.7 months, the median OS was 9.3 months (95% CI 8.2–10.9), with an estimated 1-year survival of 39%.

In patients achieving CR, the median OS was 19.7 months (95% CI 11.6–not reached).

Moreover, 10% of patients proceeded to transplantation, suggesting that enasidenib could be a bridge to curative treatment. Approximately 35–43% of patients became transfusion-independent, including those with non-CR/CRi responses.

Enasidenib showed an acceptable tolerability profile; nevertheless, the overall TRAE incidence was 82%, although most of these were mild: 46% nausea (5% grades 3/4), 45% hyperbilirubinemia (18% grades 3/4), and 40% fatigue (8% grades 3/4) or diarrhea (4% grades 3/4). Thrombocytopenia was seen in 27% of patients (23% grades 3/4), anemia in 27% (19% grades 3/4), and IDH differentiation syndrome (IDH-DS) in 10% (6% grades 3/4). Median time to onset of IDH-DS was 48 days (range 10–340), significantly longer than that of DS induced by all-*trans* retinoic acid, which usually occurs within 1–2 weeks. IDH-DS was managed with temporary drug interruption, dexamethasone 10 mg orally every 12 h for 3 days or until improvement, and hydroxyurea 2–4 g/day. Permanent drug discontinuation was not required in any patients.

Several mechanisms of resistance leading to late relapse have already been proposed, including acquisition of IDH1-mutated subclones or additional non-catalytic second-site mutations of IDH2 [40, 41].

Ivosidenib, formerly known as AG-120, a selective inhibitor of mutant IDH1, was explored in a phase I trial and in an expanded study including 258 patients with IDH1-mutated hematologic malignancies [42]; when

administered at 50 mg/day in 125 R/R AML patients, ivosidenib achieved ORR, cCR, and CR rates of 41%, 30%, and 22%, respectively. Median time to cCR was 2.7 months and median duration of response was 6.5 months (8.2 months for patients with CR/CRi). During a median follow-up of 14.8 months, the median OS was 8.8 months, and in patients achieving cCR, the 18-month OS was 50%. IDH1 mutational clearance was observed in 21% of patients with CR or CRi.

Ivosidenib was well tolerated, with QTc prolongation (7% grade $\geq$ 3) and IDH-DS (4.7% grade $\geq$ 3) the main toxicities, and no dose-limiting toxicity. Similar to enasidenib, patients with a high co-mutational burden were less likely to respond to ivosidenib; however, in contrast to enasidenib, RAS mutations did not affect the clinical response to ivosidenib.

Based on these non-randomized studies, both enasidenib (August 2017) and ivosidenib (July 2018) were approved by the FDA as a single agent for relapsed AML with IDH2 and IDH1 mutations, respectively.

Results in Untreated AML

In the frontline AML setting, monotherapy with enasidenib and ivosidenib achieved CR/CRi rates of 21–43% [43–45] and 41% [46], respectively. IDH inhibitors have also been tested in combination with intensive chemotherapy (7+3 schedule) for induction, achieving an ORR of 93% and 73% in the ivosidenib and enasidenib arms, respectively, with mutational clearance of 41% and 30%, respectively [44].

A summary of clinical trials with ivosidenib and enasidenib is shown in Tables 2 and 3.

Table 2 Completed AML clinical trials with ivosidenib

Study	Phase	Population	Association	Results (efficacy)	Results (safety)	Reference	Status
Ivosidenib							
NCT03245424	II	R/R AML	–	ORR 41%, cCr 30%, CR 22%, OS 8.8 months	QTc pr. 7% G > 3 IDH-DS 4.7% G > 3	[93]	Completed

Mechanism: inhibition of IDH1-mutant enzyme
Status: approved by FDA as a single agent in R/R AML patients with proven IDH1 mutation (July 20, 2018)
CR complete response, *OS* overall survival

Table 3 Completed AML clinical trials with enasidenib

Study	Phase	Population	Association	Results (efficacy)	Results (safety)	Reference	Status
Enasidenib							
NCT01915498	I-II	R/R AML	–	ORR 40.3% cCR 26%, CR 19%, OS 9.3 months	Hyperbilirubinemia G3/4 18% IDH-DS G3/4 6% Thrombocytopenia G3/4 23% Anemia G3/4 19%	[39]	Active

Mechanism: inhibition of IDH2-mutant enzyme
Status: approved by FDA as a single agent in R/R AML patients with proven IDH2 mutation (August 1, 2017)
CR complete response, *OS* overall survival

GEMTUZUMAB OZOGAMICIN AND ANTI-CD33 ANTIBODIES

In recent years, a number of antigen-specific immunotherapies, including antibodies against both leukemic myeloid antigens (CD33, CD123) and more recently against some leukemia stem cell markers (CD123, CD25, CD44, CD96, CD47, CD32), have been tested in preclinical studies [4, 47].

Gemtuzumab ozogamicin is a recombinant humanized anti-CD33 antibody coupled with the cytotoxic drug calicheamicin, delivered to CD33-expressing leukemic cells after internalization and intracellular release.

GO was approved in 2000 for relapsed non-chemo-eligible CD33+ AML, based on non-randomized phase 2 trials in which it was administered at a dose of 9 mg/m^2 every 2 weeks, yielding a 26% CR rate [48, 49].

Unfortunately, the phase III SWOG (Southwest Oncology Group) study, S0106, comparing GO at 6 mg/m^2 coupled with conventional induction therapy versus conventional induction therapy alone in newly diagnosed AML patients (< 60 years of age), failed to demonstrate any advantage in either ORR or OS, and showed significantly higher mortality in the GO arm (5.5% vs. 1.4%) [50]. Of note, an increase in both hematological and liver toxicity, including high rates of veno-occlusive disease (VOD), especially after HSCT, have been reported. The lack of clinical benefits, as well as the emerging safety concerns raised by the interim analysis of the SWOG S0106 study, led to an early termination of this trial and the withdrawal of GO from the market in 2010.

Over the next 8 years, several clinical trials, including ALFA-0701, AML-19, and Mylo-France-1, exploring different schedules of these drugs in order to reduce the toxicity, contributed to the favorable reassessment of GO (Table 4).

In the GIMEMA randomized trial, GO monotherapy was tested in patients aged > 60 years who were ineligible for induction chemotherapy. The study population was randomized to GO (either 3 mg/m^2 on days 1, 4, and 7 or 6 mg/m^2 on day 1 and 3 mg/m^2 on day 8) versus best supportive care. An ORR (CR + CRi) of 27% was achieved in the GO arm, with median OS of 4.9 vs. 3.6 months in favor of the GO arm [51]. This study showed that even lower doses of GO could achieve meaningful responses with high saturation of the CD33 sites. Moreover, the rapid re-expression of CD33 molecules on the cell surface after a first exposure to the drug suggested that the administration of fractionated doses could be beneficial [52].

Furthermore, the AML 17 trial confirmed that single doses higher than 3 mg/m^2 should not be employed because of increased incidence of VOD and early mortality [53].

In the ALFA-0701 phase III study, patients aged 50–70 with de novo AML were randomized to receive standard induction with or without GO, administered in three fractionated doses of 3 mg/m^2 on days 1, 4, and 7. Patients who achieved a CR/CRi underwent two consolidation cycles with intermediate-dose cytarabine with or without GO, based on the initial randomization. The CR rate did not differ between the two arms (81% GO arm vs. 75% control arm; OR 1.46, 95% CI 0.20–2.59; $p = 0.25$).

Nevertheless, GO was associated with significantly longer EFS (median 15.6 vs. 9.7 months; 2-year EFS 40.8% vs. 17.1%) and RFS (median 28.1 vs. 11.4 months; 2-year RFS: 50.3% vs. 22.7%) and better OS (median 34 vs. 19 months; 2-year OS 53.2% vs. 41.9%) [54]. A post hoc analysis showed that EFS was improved only in patients with high levels of AML blast with CD33+ expression > 70% (49% in GO arm vs. 17% in control arm; HR, 0.56; 95% CI 0.37–0.85; $p = 0.0051$) [55].

A higher incidence of grade ≥ 3 hemorrhage (22.9% vs. 9.5%) and a longer time to platelet recovery were observed with GO, but the incidence of VOD (two cases out of 139 patients in the GO group) was low. A subsequent study suggests that delaying HSCT 90 days after GO administration may reduce the risk of this serious complication [56].

Finally, a meta-analysis of five randomized studies including 3325 patients with AML showed that combining GO with intensive chemotherapy in the treatment-naive AML patients was associated with significantly better

Table 4 Clinical AML studies with GO after its withdrawal from the market (2010)

Study	Phase	Population	Association	Results (efficacy)	Results (safety)	References	Status
Gemtuzumab ozogamicin							
ALFA-0701	III	De novo AML (age 50–70 years) GO 3 mg/m^2 D1–D4–D7	+ IC vs. IC alone	No difference in CR (81% vs. 75%); > EFS (15.6 vs. 9.7 months) > RFS (28.1 vs. 11.4 months), > OS (34 vs. 19 months)	G ≥ 3 hemorrhage (22.9% vs. 9.5%); longer time to platelet recovery. SOS: 2/139 cases	[54]	Completed
MRC AML15	III	De novo AML (age 18–60 years) GO 3 mg/m^2 D1	+ IC vs. IC alone (different regimens)	No differences in CR, OS; > OS in favorable cytogenetic risk	No > hematological or extra-hematological toxicities	[61]	Completed
SWOG S0106 (NCT00085709)	III	De novo AML (age 18–60 years) GO 6 mg/m^2 D4	+ IC vs. IC alone	No differences in CR rate (69 vs. 70%), 5-year OS (46 vs. 50%), 5-year RFS (43 vs. 42%)	> Fatal toxicity (5.5 vs. 1.4%)	[50]	Completed
NCRI AML16	III	De novo AML (unfit/ > 60 years of age) GO 5 mg/m^2 D1	+ LDAC vs. LDAC	> ORR (30 vs. 17%); no improved OS	No > hematological or extra-hematological toxicities	[62]	Completed
GOELAMS AML 2006 IR	III	De novo AML (age 18–60 years) GO 6 mg/m^2 D4	+ IC vs. IC	> EFS in GO arm (53.7 vs. 27%) in patients not undergoing allo-SCT No differences in CR rate (91 vs. 86.5%), early death (10 vs. 4.5%), OS (53 vs. 46%)	> G3/4 hepatic toxicities in the GO arm (23 vs. 13%). No differences in TRM	[60]	Completed
AML-19 (NCT00091234)	III	De novo AML (unfit/ > 60 years of age) GO 6 mg/m^2 D1 and 3 mg/m^2 D8	vs. BSC	ORR 27%; mOS 4.9 vs. 3.5 m	No > hematological or extra-hematological toxicities	[51]	Completed

Mechanism: recombinant humanized anti-CD33 antibody, which delivers the linked cytotoxic drug calicheamicin to CD33-expressing leukemic cells
Status: approved by FDA for treatment of newly diagnosed and R/R CD33-positive AML, alone or in combination with daunorubicin and cytarabine (September 1, 2017)
CR complete response, *EFS* event free survival, *IC* intensive chemotherapy, *OS* overall survival

OS, due to a reduced risk of relapse. The CR rates were similar with and without GO, but an improved 5-year OS was observed in patients treated with GO [34.6% vs. 30.7%; HR 0.90 (95% CI 0.82-0.98), $p = 0.01$]. A survival benefit was observed in patients with core binding factor (CBF) AML [6-year OS 75.5% vs. 545.8%; HR 0.47 (0.31–0.73), $p = 0.0006$] and in those with normal cytogenetics, while no benefit was seen in patients with adverse cytogenetics [57]. Finally, the results of a pediatric study (AAML0531), suggesting that CD33-splicing single-nucleotide polymorphism (SNP) affects the response to GO, were not confirmed in the UK MRC/NCRI AML15 trials in young AML patients [58, 59].

Based on this background, including other favorable experiences [60–62], GO, at a fractionated dosing schedule, was re-approved by the FDA on September 1, 2017, for treatment of newly diagnosed and R/R CD33-positive AML in combination with daunorubicin and cytarabine or as a stand-alone treatment.

THE HEDGEHOG INHIBITOR GLASDEGIB

The Hedgehog (Hh) signaling pathway is vital for embryogenesis and fetal development. Aberrant signaling in this pathway affects the proliferation of leukemic stem cells, and its upregulation has been suggested as an important mechanism of chemoresistance in AML cell lines [63–66].

The Hh pathway is tightly regulated by two transmembrane proteins, patched (PTCH), which is a negative regulator, and smoothened (SMO), a positive regulator [67, 68].

Glasdegib is an oral agent that inhibits the Hh pathway by interacting with smoothened protein [69]. In vitro and in vivo studies with this agent showed that it inhibited the growth of AML cell lines and human leukemia stem cells [70].

In two phase I trials in adult patients with myeloid malignancies, glasdegib was well tolerated and was associated with an ORR of up to 49% [71, 72], with muscle spasms, dysgeusia, and alopecia being the most common TRAEs. The dose of 100 mg daily was recommended for phase II studies.

A separate phase II study, designed to evaluate the combination of glasdegib given at 100 mg daily for 28 days with standard 7+3 induction in patients over 55 years of age with de novo AML, demonstrated a CR rate of 46.4% [73], with median OS in this older population of 14.9 months.

In the randomized phase II BRIGHT AML 1003 study, 115 patients with newly diagnosed AML who were either $\geq$ 75 years of age or unfit for intensive chemotherapy were randomized to LDAC 20 mg subcutaneously b.i.d. on days 1–10 plus glasdegib 100 mg daily ($n = 77$) versus LDAC alone ($n = 38$) [74]. Despite the low CR rate (18.2% in patients receiving glasdegib, compared with 2.3% for LDAC alone), median OS was significantly better: 4.9 vs. 8.8 months (HR 0.51, 95% CI 0.39–0.67) (Table 5). Glasdegib was well tolerated: the most common AEs occurring at higher rates in the glasdegib/LDAC arm were cytopenia and gastrointestinal symptoms (mostly grade 1–2); cytopenia was not associated with an increased incidence of sepsis or bleeding as compared with LDAC.

Based on these results, in November 2018, glasdegib was approved by the FDA for use in combination with LDAC for AML patients either aged $\geq$ 75 years or with comorbidities precluding intensive induction chemotherapy.

VENETOCLAX AND OTHER PRO-APOPTOTIC AGENTS

B-cell leukemia/lymphoma-2 (BCL2) is an anti-apoptotic protein that promotes leukemic cell survival through regulation of the mitochondrial apoptotic pathway. An increased level of BCL-2 expression relative to the pro-apoptotic BAX protein is associated with poor outcome in patients receiving intensive chemotherapy for AML [76].

Sensitizer BCL-2 homology 3 (BH3) proteins are antagonists of these antiapoptotic proteins and activate downstream BAX and BAK, which induce the release of mitochondrial intermembrane molecules such as cytochrome c, resulting

Table 5 AML clinical trials with glasdegib

Study	Phase	Population	Association	Results (efficacy)	Results (safety)	References	Status
Glasdegib							
NCT01546038	II	De novo AML, MDS	DCA, LDAC, IC	CR/CRi: 31%	G3–5 AE: 87%(+ LDAC); 85.7% (+ DCA); 86.4% (+ IC)	[75]	Completed
NCT01546038	II-R	De novo AML, elderly/unfit	Glasdegib + LDAC vs. LDAC	CR 18.2% vs. 2.3%; mOS 8.8 m vs. 4.9 m	28.6% G5 AE; 64.3% G3/4 AE (glasdegib + LDAC)	[74]	Completed
NCT01546038	II	De novo AML	IC	CR 46.4%; mOS 14.9 m	7.5% G5 AE; 91.3% G3/4 AE	[73]	Completed

Mechanism: inhibition of SMO protein of Hedgehog pathway

Status: approved by FDA in combination with low-dose cytarabine for AML patients either aged $\geq$ 75 years or with comorbidities precluding intensive induction chemotherapy (November 21, 2018)

IC intensive chemotherapy, *CR* complete response

in activation of the caspase cascade and, finally, apoptosis [77].

Venetoclax is an oral, selective BH3-mimetic that binds to and inhibits the BH3 domain of BCL2 proteins and thereby dislodges proapoptotic factors, such as BIM, from their BCL2 binding site, reactivating the mitochondrial apoptotic pathway [78]. It was previously approved by the FDA (April 2016) for the treatment of chronic lymphocytic leukemia [79].

In a phase II study in high-risk R/R AML patients (n = 32), treatment with venetoclax at 800 mg/day as a single agent achieved an ORR of 19%; interestingly, patients with IDH mutations showed a higher ORR (33%), as IDH-mutant AML cells depend on BCL-2 for survival. Eighty-one percent of the patients experienced grade 3/4 adverse events, including febrile neutropenia (31%) and hypokalemia (22%) [80].

Preclinical studies have shown that resistance to venetoclax on a molecular basis is mediated by the antiapoptotic proteins Bcl-xL and MCL1, which can be overcome by combination therapy with hypomethylating agents (HMAs) [81, 82], anthracyclines (idarubicin and daunorubicin) [83], and the MDM2 antagonist idasanutlin [84–87].

Nevertheless, these preliminary results, considering the potent proapoptotic activity of this drug in the presence of cytotoxic stress, led to a phase Ib-II trial in which venetoclax at 400 mg/day was combined with HMA (azacitidine or decitabine based on institutional preference) as upfront treatment in 145 AML patients $\geq$ 65 years of age who were unfit for intensive chemotherapy; this treatment was well tolerated, yielding an impressive 61% CRi rate [88].

The phase II data showed a CR + CRi rate of 73%, with median OS of 17.5 months; 46% of the total population was alive at 2 years [89]. Venetoclax showed a good safety profile, with the main TRAEs being neutropenia and nausea.

Given these results, the combination of venetoclax with HMAs is a candidate as the new standard of care for elderly AML patients unfit for standard chemotherapy.

Table 6 AML clinical trials with venetoclax

Study	Phase	Population	Association	Results (efficacy)	Results (safety)	References	Status
Venetoclax							
NCT02287233	I-II	De novo AML, unfit	LDAC	CR/CRi 62%, OS 11.4 months	30-day mortality 36%	[90]	Active
NCT01994837	II	R/R, de novo AML, unfit	–	CR/CRi 19%, CR 6%	TRAE G3/4 81% Febrile neutropenia 31% Hypokalemia 22%	[80]	Completed
NCT02203773	I-II	De novo AML, elderly/ unfit	DCA, AZA	CR/CRi 73%, CR 61% OS 17.5 months	G3–5 TRAE 64–59%	[42]	Active, not recruiting

Mechanism: pro-apoptotic by inhibiting the BH3 domain of BCL2 proteins
Status: approved by FDA in combination with azacitidine, decitabine, or LDAC in de novo unfit/elderly (November 21, 2018)
OS overall survival, *CR* complete response

A phase I/II trial with LDAC plus venetoclax [90] showed similar results in 71 untreated, unfit AML patients. Based on these response rates, far exceeding the historical outcome observed with azacitidine alone (composite CR: 28%, median OS: 10.4 months) [91], venetoclax, in combination with azacitidine, decitabine, or LDAC, was granted breakthrough designation by the FDA on November 21, 2018, for previously untreated patients with AML who were older than 75 years or unfit for intensive chemotherapy.

Phase 3 randomized placebo-controlled registration studies in unfit elderly AML patients are currently ongoing, in order to confirm the benefit of venetoclax plus azacitidine versus azacitidine alone (NCT02993523) or venetoclax plus LDAC versus LDAC alone (NCT03069352).

Given these encouraging data, a number of studies have been proposed combining venetoclax with other agents, including 7+3 induction (NCT03709758), multi-CDK inhibitor dinaciclib (NCT03484520), gilteritinib (NCT03625505), 10-day schedule decitabine (NCT03404193), and the Mcl-1 inhibitor S64315 (NCT03672695).

In the R/R AML setting, HMAs + venetoclax achieved an ORR of up to 76% in small retrospective series, including cases with previous HMA exposure [92]. In a retrospective study in heavily pretreated patients, venetoclax plus either HMAs or LDAC yielded a response rate of 21%, with median OS of 3.0 months [93].

A list of completed clinical trials with venetoclax in AML is shown in Table 6.

CONCLUSIONS AND PERSPECTIVES

AML is a biologically and clinically heterogeneous disease. Although advances in supportive care and prognostic risk stratification have optimized the performance of standard established therapies, overall long-term survival

remains poor. Elderly patients, who represent the majority of AML cases, are more likely to have more aggressive disease, often with adverse cytogenetic features. At the same time, the risk of treatment-related mortality and several toxicities often precludes this population from receiving intensive chemotherapy or stem cell transplantation. Novel targeted therapies combine effective anti-leukemic activity with reduced toxicity, but the multiple biological pathways involved in leukemogenesis hamper the development of a single "magic bullet" against this disease. In particular, the eradication of leukemic stem cells remains the main issue in the development of effective new drugs. Leukemic stem cells (LSC) are quiescent and therefore could be more sensitive to drugs affecting different targets such as mitochondrial activity, heat shock protein 90 (Hsp 90) and inflammasome, NF-Kb and proteasome activity, and epigenetic modulators, probably with selective susceptibility in comparison with normal stem cells [94]. It has been demonstrated that chemotherapy-resistant AML LSCs reside in the endosteal region of the bone marrow and adipose tissue, where microenvironmental factors alter the intracellular metabolism, leading to chemotherapy resistance [95, 96]. Modulation of niche interactions may increase the chemosensitivity of LSCs. Finally, there is also evidence that a proinflammatory state can influence LSC growth and survival [97].

Inhibition of proinflammatory factors may both reduce LSC activity and facilitate an environment more favorable for normal stem cells. This is particularly important in the context of post-chemotherapy treatment, where the need for suppression of residual disease and promotion of normal cell regeneration is perhaps most acute.

The future scenario of new experimental drugs is very intriguing and includes the epigenetic regulators targeting DNMT3A, IDH1/2, TET2, and ASXL1, interacting with enzymes involved in writing (EZH2, DOT1L, MLL), reading (BET, bromodomains), or erasing (LSD1) histone marks involved in activating or repressing gene expression and the disruptor of telomeric silencing 1-like (DOT1L) inhibiting MLL fusion genes [98].

Furthermore, in addition to mutated enzymes and upregulated pathways, the identification of unique cell surface markers can provide a therapeutic target for recombinant monoclonal antibodies or chimeric antigen receptors (CAR) [99].

The immunosuppression related to the disease represents another important mechanism of minimal residual disease and immune escape. Immune checkpoint inhibitors have been widely studied in solid tumors, and their migration to hematological malignancies has become more prominent since the success in Hodgkin's lymphoma. So far, clinical studies on immune checkpoint inhibitors (nivolumab, pembrolizumab, and ipilimumab) have provided limited results in AML [100–103]. However, immune checkpoint inhibitors given in association with HMAs could represent an interesting strategy to enhance the activity of immunotherapies.

The last—but not least—new drug approved in this setting is CPX-351, a dual-drug liposomal encapsulation of cytarabine and daunorubicin with a synergistic 5:1 molar ratio of cytarabine and daunorubicin within the liposome. This formulation was aimed at increasing the release of drugs specifically in bone marrow, reducing extra-hematological toxicity and bypassing P-glycoprotein-based efflux pumps, which are important mediators of chemotherapy resistance [104].

Studies with CAR T-cell therapy are ongoing, with promising preliminary results [105, 106], but an optimal response requires a specific and stable myeloid leukemic antigen that has yet to be identified. Lastly, the development of well-tolerated oral therapies will improve both the feasibility of treatment and the quality of life in this very difficult-to-treat setting.

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