Targeting Acute Myeloid Leukemia Stem Cell: Current Status and Clinical Application

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ABSTRACT

Since the discovery of leukemic stem cells by Dick and Bonnet in 1996, therapeutic targets have been searched to specifically eliminate this population and, thus, avoid recurrent episodes of relapse in these patients. To date, different molecular targets have been identified in the population of leukemic stem cells that can be pharmacologically modulated. The therapeutic targets for which a drug has been developed and the clinical trials in patients with AML are identified and correlated with the published preclinical data. The therapeutic targets and drugs described against leukemic stem cells are searched in journals indexed in MedLine. The preclinical regulatory reports are searched on the website of the FDA (US Food and Drug Administration). To identify the approved clinical trials, the registry www.clinicaltrial.gov is consulted. Therapeutic targets or drugs are grouped according to their mechanism of action and efficacy in clinical trials and a search is made to identify which preclinical data are critical for predicting positive results in patients. The analysis will allow the development of a method to identify the clinical potential of preclinical studies described with drugs against leukemic stem cells.

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AUTHORIZATION OF THE DIRECTOR OF THE TFG

I, Ruth Muñoz Risueño, hereby authorize the presentation of this manuscript as the "Treball de Fi de Grau" of the student Laia Gutierrez Tordera for the degree in Biologia Humana of Universitat Pompeu Fabra.

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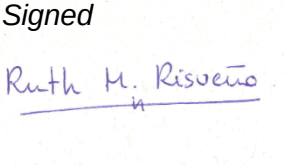


INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous clonal hematopoietic neoplasms affecting the myeloid lineage. It is the most common form of leukemia in adults and it is diagnosed at a median age of 70 years. Its incidence increases with age, with 1.3 cases per 100,000 for those under 65 years and 12.2 per 100,000 for those over 65 years[1,2]. Over the last five decades, the use of chemotherapeutic agents demonstrated to prolong survival in AML patients, with a 5-year survival rate of 40% for patients under 60 years and 10% for those above the age of 60 years[3]. However, minimal changes have been made since then and the cytarabine + anthracycline induction therapy "7+3" is still considered the first-line treatment, even when they result in poor outcomes for the majority of patients due to cytopenias and elderly age-associated complications[4]. The risk stratified classification of AML into favourable-, intermediate- and high-risk groups makes it possible to predict the prognosis of the disease based on the patient's genetic characteristics, and so to decide if there is need for immediate allogeneic hematopoietic cell transplant[5]. Moreover, more than 70% of treated patients relapse after a few months or in the first year due to resistant disease[6].

AML is composed of a set of heterogeneous cells, all of them leukemic, which can be classified as: bulk, progenitors and leukemic stem cells. This set of leukemic cells coexists with normal hematopoietic cells, both primitive and mature/differentiated. Despite LSC were originally identified as $CD34^{+}CD38^{-}$[7], today it is demonstrated that this phenotype cannot be used to identify LSC, although it is valid in most cases[8]. LSC also give rise to malignant immature myeloblasts that accumulate in more than 20% in bone marrow and also in peripheral blood[9], thus preventing normal blood cells from doing their function[10,11]. LSC are able to self-renew and differentiate into non-fully mature populations due to their stem-cell like properties, while they increase proliferation rate and block terminal differentiation due to their leukemic properties. Therefore, LSC are thought to be responsible for relapse, as they are in charge of initiation and maintenance of the disease. In consequence, self-renewal, proliferation and differentiation are an outstanding target for new drugs.

In AML, the identification of new molecular markers in LSC is making it possible to move forward to a more personalized therapy where the LSC population is removed to prevent relapse. The identification and targeting of recurrent mutations in AML is a primary aim for the development of new drugs. However, in AML there are no recurrent mutations found in high frequency, thus this strategy is only valid but really useful for few patients.

In 2017, after a phase III trial[12], the FDA and the EMA approved the first tyrosine kinase inhibitor (TKI), midostaurin, in combination with chemotherapy (CT) for FLT3-mutated AML, which is found in 25% of patients[13,14]. Additionally, another FLT3 inhibitor, gilteritinib, is in clinical use[15,16]. The FDA also approved Enasidenib and Ivosidenib for relapsed or refractory isocitrate dehydrogenase (IDH)-mutated AML[17,18], which represents a total of 20% of patients with AML, 12% with IDH2 and 8% with IDH1 mutations[19]. The pro-apoptotic agents venetoclax and glasdegib were approved in combination with azacitidine, decitabine or low-dose decitabine for the treatment of newly-diagnosed AML in adults age 75 years or older, or who had comorbidities that prevented use of intensive induction CT[18,20]. Interestingly, gemtuzumab ozogamicin (GO) received FDA re-approval for CD33 positive AML after being withdrawn because of an unacceptable benefit-risk ratio[21,22]. At present, new FDA-unapproved drugs for AML are under clinical development with promising results, such as Imatinib (Gleevec), which is approved for Chronic Myeloid Leukemia (CML)[23] but still in clinical trials for AML[24]. On the contrary, compounds like arsenic trioxide were proven not useful in clinical trials even the good results in preclinical studies.

The current therapeutic approach is based on targeting leukemic cells, usually affecting both LSC and blast cells, although common unwanted side effects are also observed on normal cells due to the lack of specificity. Despite the heterogeneity among patients and clones, there are common signaling pathways and molecular components between groups of individuals that play a role in the survival of LSC. These include Wnt/β-catenin, Sonic Hedgehog, PI3K/Akt/mTor, JAK-STAT, Notch, p53, NF-κB, Hsp90, oxidative phosphorylation in the mitochondria or c-Kit receptor, among others[9,11,15,25-28]. Some of the agents targeting these molecules have shown a good benefit-risk ratio in preclinical studies, consequently moving forward to clinical trials, where part of them have proven to be successful. On the contrary, others have shown not enough benefit with overmuch toxicity, therefore the clinical trial has been paused or cancelled.

This degree thesis is not meant to be an exhaustive review of all emerging agents for the treatment of AML. Instead, main treatment strategies directed to LSC that made the step to the clinics are summarized. Here, the weak points of LSC are uncovered from the preclinical and clinical points of view in order to discuss the best mechanisms to target acute myeloid leukemia.

WORKING HYPOTHESIS

Agents targeting leukemia stem cells can be grouped together depending on their mechanism of action and efficacy and this preclinical data can be critical for predicting positive results in patients.

AIMS AND OBJECTIVES

1) To summarize the mechanisms of action and current strategies to target LSC signaling in AML.
2) Presenting the agents approved by the FDA targeting LSC in AML.
3) Identifying new therapies for AML against LSC in clinical trials and still not approved by the FDA.
4) Proposing a set of reasons to explain the results obtained in clinical trials in comparison with the results obtained in preclinical studies.

TARGETING CELL MECHANISMS TO REMOVE LSC

AML is composed of a heterogeneous leukemic population that include cells with and without stem cell properties. Standard CT has proven effective to target the highly proliferative bulk of AML, but not the LSC population. Therefore, specific approaches are needed to target the LSC compartment and block cell survival and chemoresistance, thus leading to programmed cell death or differentiation of the progenitor cells. This strategy can be achieved either in a single-drug or a combined therapy. The main strategies to target the LSC compartment are summarized in Table 1.

FDA approved drugs for LSC in AML

Bcl-2 inhibition

LSC have a less flexible metabolism than HSC and depend slightly more on OXPHOS than their normal counterparts. To target specifically the metabolism of LSC, drugs against BCL-2 anti-apoptotic protein are under development[25]. Venetoclax (VEN) is a BCL-2 inhibitor approved by the FDA for the treatment of AML[18], although it was initially approved for chronic lymphocytic leukemia (CLL)[29]. VEN inhibits BCL-2, blocks OXPHOS and reduces LSC energy, making them more susceptible to CT, while normal progenitor and blast cells survive by upregulating glycolysis[30]. On the basis of this preclinical data, a single-agent phase II clinical trial in relapsed/refractory AML (R/R AML) was launched with poor results[31]. A dose of 800 mg daily was used. The median age was 71 years (range, 19-84) and most of the patients were heavily pretreated. Single-agent VEN produced an overall response (OR) in 5 out of 32 relapsed/refractory AML patients, which was short-lived, with median time to progression of 2.5 months. Escalating dose to 1200 mg daily showed no additional activity. However, VEN was well tolerated. In view of these results, VEN was tested in a combined therapy with already approved FDA chemotherapeutic agents. The combination of VEN and hypomethylating agents (HMA) or low dose cytarabine (LDAC) has shown promise in the treatment of R/R AML and especially for newly diagnosed older patients who were not eligible to conventional CT[32], therefore both combinations have been approved by the FDA[33,34]. Approval was based on two non-randomized trials in patients with newly-diagnosed AML who were 75 years or older or had comorbidities that precluded the use of CT. On the one hand, in study M14-358 (NCT02203773), VEN was combined with azacitidine (n=67) or decitabine (n=13), achieving a complete remission (CR) of 37% and 54% respectively. Time in remission was 5.5 months and 4.7 months. On the other hand, in study M14-387

(NCT02287233), VEN was combined with LDAC (n=61) and achieved a CR of 21%, with a time in remission of 6 months. Toxicity was well tolerated (>30% patients), the more frequent adverse events (AE) were nausea, diarrhea, constipation, fatigue, hypokalemia, decreased appetite and febrile neutropenia. Currently, confirmatory phase III trials are ongoing to evaluate VEN in combination with azacitidine (NCT02993523) or LDAC (NCT03069352)[35].

Hedgehog pathway inhibitors

Self-renewal and cell cycle progression are regulated by the Hedgehog pathway (Hh), among others. Previous studies have established a role for aberrant activation of Hh signaling in solid tumors and hematologic malignancies such as chronic myeloid leukemia (CML)[36,37]. In LSC, the overexpressed Smoothened (SMO), a G-protein coupled receptor, activates the glioma-associated oncoprotein transcription factor (GLI), leading to cell dormancy and increased self-renewal. Targeting of SMO with the antagonist glasdegib (PF-04449913) has shown promising effects on eradicating dormant LSC, as it downregulates self-renewal and promotes cell cycle progression, where they are more easily target by chemotherapeutic agents or tyrosine kinases inhibitors, while normal progenitor cells do not lose their capacity for self-renewal[38]. In 2018, the FDA approved glasdegib in combination with LDAC for the treatment of newly-diagnosed AML in adult patients who are ≥75 years old or who have comorbidities that preclude use of intensive induction CT[20]. Approval was based on a multicenter phase Ib/II study that included 115 patients who received glasdegib plus LDAC or LDAC alone (NCT01546038). Median survival was 8.3 months for the glasdegib + LDAC condition and 4.3 months for LDAC alone. The most common AE were anemia, fatigue, hemorrhage, febrile neutropenia or nausea, among others. The recommended glasdegib dose is 100 mg orally, once daily[39]. Another phase II study (NCT01546038) evaluated glasdegib plus cytarabine or daunorubicin in 69 patients with untreated AML or high-risk myelodysplastic syndromes (MDS), age of 64 (27-75) years. In 46.4% of the cases, patients achieved CR[40], and in patients ≥55 years CR was 40%. Median OS was 14.9 months with 12-month survival probability 66.6%. Again, the combination was well tolerated. Thus, a randomized phase III trial of glasdegib with CT (7+3) is ongoing (NCT03416179).

Tyrosine Kinase inhibitors

FMS-like tyrosine kinase receptor 3 (FLT3), a cytokine receptor mainly expressed in hematopoietic cells[41], is associated with cell proliferation and survival[42], as well as the

most frequently mutated gene in AML, with FLT internal tandem duplications (FLT3-ITDs) and point mutations in tyrosine kinase domain (TKD) that results in constitutive tyrosine kinase signaling[43]. FLT-ITD is highly frequent and related with poor prognosis, thus specific FLT3 inhibitors have been developed. First generation FLT3 inhibitors like midostaurin and sorafenib were not designed to specifically target FLT3 and also target c-KIT, PDGFR and VEGFR, thereby leading to more off-target effects[44]. Midostaurin is a multi-kinase inhibitor showing limited efficacy as a single-agent but improves in combination with standard intensive CT. In a phase Ib study of post-remission newly diagnosed young (≤60 years) AML patients, midostaurin 50 mg twice-daily dose in combination with 7+3 high-dose cytarabine (HDAC) achieved CR rates of 92% (FLT3-mutants) and 74% (FLT3-wild-type)[45]. In an international, randomized placebo-controlled phase III trial, midostaurin plus with 7+3 in patients with FLT3 mutation, demonstrated a median OS of 74.7 months for the combination versus only 26 months for the placebo. Midostaurin is approved by the FDA only in combination with CT.

In order to prevent off-target effects, a new generation of more selective tyrosine kinase inhibitors was developed. Again, gilteritinib, quizartinib and crenolanib demonstrated better activity when combined with CT rather than as single agents[46]. In 2018, the FDA approved gilteritinib for treatment of adult patients who have R/R AML with FLT3 mutation as detected by an FDA-approved test[16]. Approval was based on a phase III study (NCT02421939) assessing oral gilteritinib 120 mg daily versus CT, which included 138 adults with R/R AML having FLT3-ITD or FLT3 mutations. After a median follow-up of 4.6 months (2.8-15.6), 29/138 patients (21%) achieved CR or CR with partial hematologic recovery (CRh). The most common AE were myalgia/arthralgia, transaminase increase, fatigue, fever, diarrhea and dyspnea, among others[47]. Recently, more phase III trials have begun (NCT02421939, NCT02997202, NCT02752035, NCT02298166). In AML, the development of agents targeting FLT3 mutated subtypes has led to a big improvement in the treatment of AML, starting with first generation agents and moving to an improved new generation of drugs.

IDH inhibitors

The isocitrate dehydrogenases IDH1 and IDH2 enzymes are responsible for the catalysis of isocitrate to α-ketoglutarat (α-KG). Mutations in these proteins encode for neomorphic enzymes that catalyze the conversion of α-KG to the oncometabolite R-2-hydroxyglutarate, which perturbs histone methylation and the DNA of HSC[48]. These mutations are found in 20% of AML patients[49]. Inhibitors of mutant IDH enzymes reduce R-2-hydroxyglutarate and histone hypermethylation and induce myeloid differentiation

into neutrophils[50]. IDH2 inhibitor enasidenib and IDH1 inhibitor ivosidenib were approved in 2017 and 2018 by the FDA for patients with IDH2- and IDH1-mutated R/R AML, respectively[17,18]. Enasidenib approval was based on the study AG221-C-001 (NCT01915498), a single-arm phase I/II clinical trial that included 199 adults with R/R AML who had an IDH2 mutation[51]. At a 100 mg orally daily dose, 23% experienced CR or CRh lasting a median of 8.2 months. The median duration of response was 5.8 months in all responders and 8.8 months in those who achieved CR. The median OS was 9.3 months. More than 20% of patients experienced nausea, vomiting, diarrhea, elevated bilirubin and decreased appetite. Differentiation syndrome occurred in 14% of patients, although it was effectively managed. Currently, enasidenib is under further evaluation in randomized phase III trials (NCT03839771, NCT02577406) [52]. IDH1-inhibitor ivosidenib was also approved in a single-arm phase I study (AG120-C-001, NCT02074839) that included 174 adult patients with IDH-1 mutated R/R AML[32]. At a dose of 500 mg daily, the CR+CRh rate was 32.8%, the median time-to-response was 2 months and the median response duration was 8.2 months. The CR and CRh rates were 24.7% and 8.0%, respectively. The most common any-grade AEs were fatigue, leukocytosis, diarrhea, nausea, febrile neutropenia and pneumonia. Differentiation syndrome and QT prolongation also occurred in 11% and 23% of patients. Ivosidenib is currently tested in combination therapy in randomized phase III trials (NCT03173248, NCT03839771) [52].

Other cell-surface antigens inhibition

Monoclonal antibodies (mAb) have emerged as effective targeted therapies for human AML stem cells given their high specificity and low toxicity. The strategy involves identifying cell surface antigens preferentially expressed on AML LSC compared with normal HSC[53]. An increasing number of antibodies have been identified in recent years, including gemtuzumab ozogamicin (GO), CD123, CD44, CLL-1, CD96, CD47, CD32 and CD25. At present, only GO received FDA approval[22], although CD123, CD44, CLL-1 and CD25 are under clinical trials (NCT03631576, NCT00060372, NCT03222674, NCT03912064, NCT02678338). Antibody engineering also offers a potential path to improve the efficacy and reduce the immunogenicity of normal therapeutic mAb, such as AMG330, a T-cell construct to attack CD33 that is now in phase I (NCT02520427) [54]. CD33 is differentially expressed in LSC and normal HSC, therefore the humanized CD33 antibody lintuzumab was developed in an effort to target specifically LSC. Two randomized trials reported controversial results for lintuzumab (HuM-195[55], SGN-33[56]), as CD33 is internalized in hours when engaged with bivalent antibodies[57]. To date, most efforts are focused on GO, a humanized anti-CD33 antibody conjugated with an

antimitotic drug (Mitomycin or Calicheamicin)[58,59]. In 2017, GO was approved for the treatment of newly-diagnosed CD33-positive AML in adult and pediatric patients ages 2 years and older. It may be used in combination with daunorubicin and cytarabine or as a stand-alone treatment. Approval of GO in combination with CT for adults was based on ALFA-0701 (NCT00927498), a phase III study of 271 patients with *de novo* AML aged 50 to 70 years old. Induction therapy consisted of daunorubicin (60 mg/m^2) and cytarabine (200 mg/m^2) with or without GO (3 mg/m^2). The median event-free survival (EFS) was 17.3 months for patients receiving GO versus 9.5 months for those receiving CT alone. The most common AE were hemorrhage, infection, fever, nausea, vomiting, hepatotoxicity, infusion-related reactions and hemorrhage. Curiously, GO had already received FDA approval in 2000 on the basis of phase I and II clinical trials[60,61]. However, a phase III study showed limited efficacy with high hepatotoxicity (NCT00085709)[62,63], and GO was withdrawn from the US Market in 2010. Seven years later, it has been reapproved after several considerations.

Delivery mechanisms: CPX-351

Not only single or combined drugs are being developed. Recently, studies have also focused on the mechanism to delivery drugs to their site of action. In 2017, the FDA approved CPX-351, a liposome-encapsulated combination of daunorubicin and cytarabine for the treatment of adults with newly-diagnosed therapy-related AML or AML with myelodysplasia-related changes[64]. Each vial contains 44 mg daunorubicin and 100 mg cytarabine encapsulated together in liposomes, so the volume required for each dose and patient is calculated using the body surface area. Approval was based on the phase III study CLTR0310-301 (NCT01696084), where CPX-351 was compared to standard combination of daunorubicin and cytarabine (7+3). 309 patients aged 60-75 years old were enrolled in the study. The median overall survival was 9.6 months compared with 5.9 months for the 7+3 control. AE were mainly hemorrhage, febrile neutropenia, nausea and diarrhea[65].

Drugs (still) not approved by the FDA

Tyrosine-kinase inhibitors

Many agents are being developed in the field of AML. Some of them have been discarded for further modification, while others are currently under confirmatory clinical trials. It is the case of imatinib and dasatinib, which are not approved for AML but for CML[66,67]. Imatinib inhibits the protooncogen tyrosine kinase ABL. In a phase II study for R/R AML

(n=38), imatinib in combination with CT had an ORR of 37% with a median overall survival of 11.1 months. Among responders, 8/14 patients proceeded to allogeneic hematopoietic cell transplant. Moreover, the regimen was well tolerated[68]. These encouraging results lead to a similar compound, dasatinib, which is also administrated in combination with CT. Dasatinib prevents human AML stem/progenitor cell growth in vitro through inhibition of c-Kit along with the SRC family kinases (SFK), which are overexpressed in LSC, and results in enhanced p53 activity[69,70]. In the AMLSG 11-08 phase Ib/IIa study (NCT00850382) and another phase II study (n. EudraCT: 2006-006555-12)[71,72], dasatinib showed an acceptable toxicity profile. Currently, a phase III trial with dasatinib in adults with CBF-AML is ongoing (NCT02013648)[69]. In phase I/II there is also volasertib, an inhibitor of Plk1 and cell-cycle progression[73], which at the moment shows promising results.

mTORC1-S6K1 inhibitors

Dysregulation of the mechanistic target of rapamycin complex 1 (mTORC1)-p70 ribosomal protein kinase 1 (S6K1) signaling pathway is common among AML patients. The mTORC1-S6K1 pathway regulates proliferation, metabolism and autophagy, both in LSC and normal HSC. In 1999, rapamycin, an allosteric inhibitor of mTORC1, was approved for immunosuppression in organ transplant[74], and semi-synthetic analogues of this drug followed for AML therapy. Rapalogues such as temsirolimuns, everolimus and rifaforolimus are first-generation mTORC1 inhibitors, while ATP analogues are second-generation mTORC1 inhibitors. Both first- and second-generation inhibitors demonstrated biological activity in single-drug preclinical studies[75-78]. However, at long-term malignant cells develop drug resistance[79]. Thus, a phase II study using RapaLink-1, a bivalent mTORC-1 inhibitor, was performed. RapaLink-1 consists of rapamycin and MLN0128, an ATP analogue, together in a single drug. It inhibits phosphorylation of S6K1 and Akt, therefore inhibiting mTORC1 and mTORC2[80]. Nonetheless, combined inhibition of mTORC1-S6K1 pathway by RapaLink-1 or single-drug agent along with another target such as CT has proven to be the most successful strategy[81].

Heat shock protein 90 inhibitors

Another field that is being explored are the heat shook protein 90 (Hsp90) inhibitors[82]. These 90-kDa proteins regulate the stability of many “client” proteins, such as the AML-overexpressed FLT3, c-Kit, AKT, and others[83]. The Hsp90-inhibitor 17-AAG agent, tanamycin, prevents stabilization and activation of client proteins, which are ubiquitinated and degraded. However, in a phase I study of 17-AAG with bortezomib[84], the

combination led to toxicity without measurable response in patients with R/R AML, emphasizing the need for modified next generation Hsp90 inhibitors.

Wnt/β-catenin inhibitors

LSC are highly dependent on the Wnt/β-catenin pathway. Thus, compounds like the synthetic antifungal ciclopirox olamine, also used for topical dermatological treatment, is used to attenuate this pathway, as it binds iron needed for the proper function of this pathway[85]. In a phase I study it demonstrated biological activity (NCT00990587), but if sustained plasma levels are required for optimal tumor effect, oral administration of ciclopirox may not be feasible due to gastrointestinal toxicity[86].

NF-κB inhibition and ROS generation

Interestingly, traditional medicine uses some compounds that are attractive for AML. The main problem with these drugs is their limited biological activity together with high toxicity. It is the case of parthenolide, a derivate from *Tanacetum parthenium* (feverfew) that induces apoptosis through inhibition of NF-Kβ, activation of p53 and increased ROS[87]. In a phase I dose escalation trial it demonstrated poor pharmacological activity due to few solubility[88], thus dimethylamino-parthenolide was developed with a 70% bioavailability and at present is being evaluated in a phase II clinical trial[89,90]. Another agent which neither succeeded in the clinical trials was arsenic trioxide (ATO) [91]. ATO is highly effective in acute promyelocytic leukemia (APL), but it has no activity in R/R AML[92]. Despite ATO induces apoptosis in AML cell lines by depleting glutathione and generating ROS in vitro, it has limited clinical meaningful antileukemia activity in patients, even in combination with ascorbic acid[93].

Table 1. Selected trials for FDA-approved drugs against LSC for AML.

Target	Agent	Investigation	Phase	Identifier	Remarkable improvements	Current status
	Venetoclax	Combined with azacitidine or decitabine in R/R and newly diagnosed older AML	2	NCT02203773	CR[1] 37% (azacitidine) and 54% (decitabine) Time in remission of 5.5 and 4.7 months	Phase 3 (NCT02993523)
		Combined with LDAC in R/R and newly diagnosed older AML	2	NCT02287233	CR 21% Time in remission of 6 months	Phase 3 (NCT03069352)
Smoothened (SMO)	Glasdegib	+/- LDAC in newly diagnosed older AML	1b/2	NCT01546038	CR 17% Median survival of 8.3 months	Phase 3 (NCT03416179)
		Combined with cytarabine or daunorubicin in untreated AML (or MDS)	2	NCT01546038	CR 46.4% OS of 14.9 months 12-month survival probability of 66.6%	
Multi-kinase inhibitor (FLT3 receptor)	Midostaurin	Combined with HDAC (7+3) in post-remission newly diagnosed young AML	1b	NIHMS584703	CR 92% in FLT3 mutants	Phase 3 (NCT00651261)
	Gilteritinib	Adult R/R AML with FLT3 mutation	1/2	NCT02014558	CR 8%	Phase 3 (NCT02421939, NCT02997202)
IDH2	Enasidenib	IDH2-mutated R/R AML	1/2	NCT01915498	CR 19.3% Median response duration of 5.8%	Phase 3 (NCT03839771, NCT02577406)
IDH1	Ivosidenib	IDH-1 mutated R/R AML	1	NCT02074839	CR 21.6% Median response duration of 8.2%	Phase 3 (NCT03173248, NCT03839771)
CD33	Gemtuzumab Ozogamicin	Newly-diagnosed CD33-positive adults and >2 years pediatric AML	3	NCT00927498	CR + CRh[2] 40.6%	Phase 1 (NCT03848754)
Delivery mechanism	CPX-351	Newly-diagnosed AML	2	NCT00788892	CR + CRi[3] 66.7%	Phase 1b (NCT03904251), Phase 3 (NCT01696084)

[1]Complete Remission; [2]Complete Remission with partial hematological response; [3]Complete Remission with incomplete hematologic recovery.

DISCUSSION

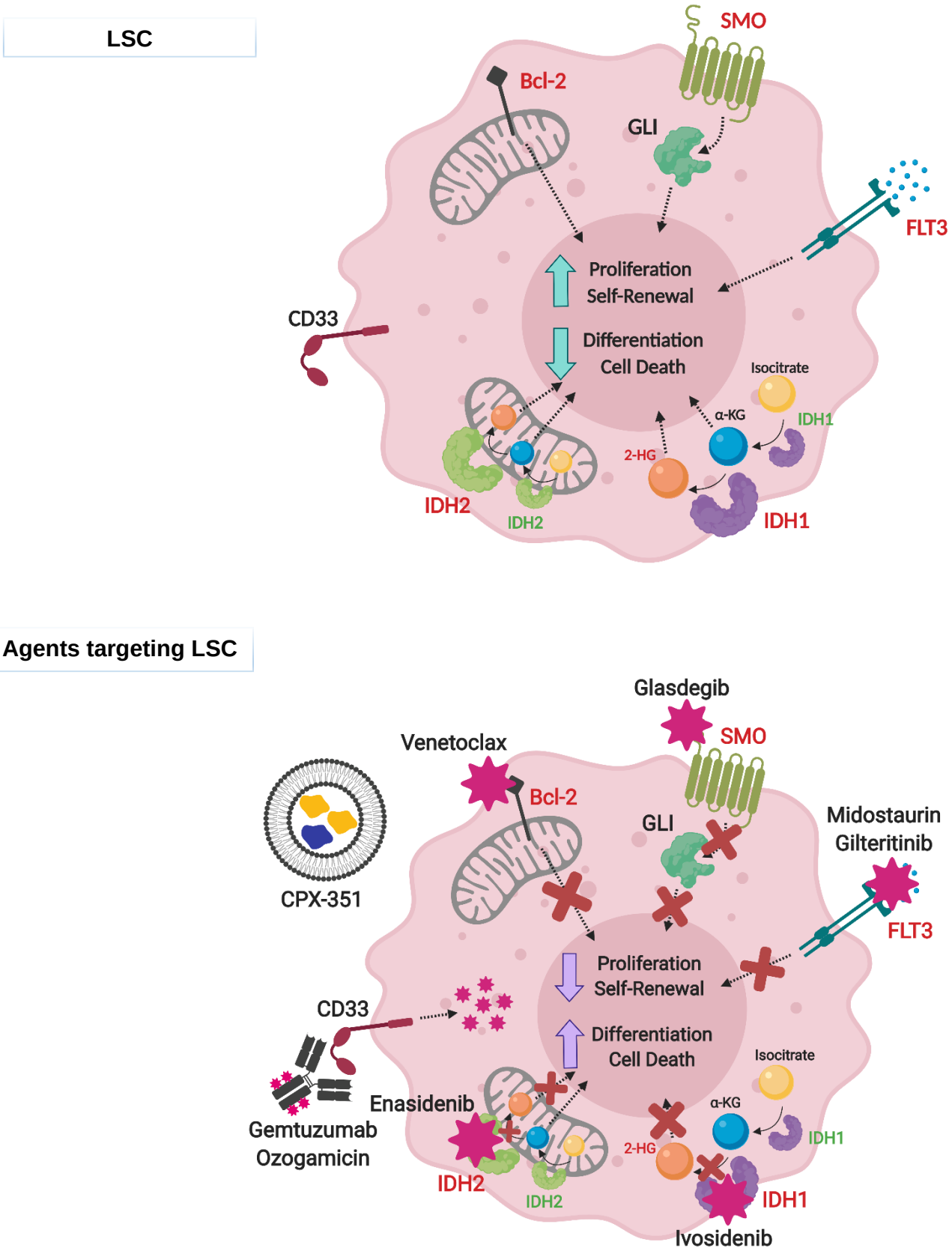
Acute Myeloid Leukemia is a heterogeneous clonal hematopoietic malignancy affecting cells from the myeloid lineage. In the last decades, standard CT agents have been considered the first-line treatment for patients with AML. However, most of these patients relapse several months or years after treatment. Thus, targeting of LSCs with few collateral damage to normal tissues represents a major approach for the treatment of AML. Notably, the LSC compartment shows some distinct features compared with more differentiated blast cells or normal stem cells that likely contribute to standard CT resistance, which can effectively kill leukemic blasts in majority of patients (Figure 1). Moreover, older AML patients tend to be more resistant to CT and they may present comorbidities. Therefore, recent studies have focused on targeting leukemia cells, both as regards in blast and LSC populations, so to induce cell death or cell differentiation (Figure 1). In leukemia cells, survival is mediated by deregulated pathways within the cell that increase self-renewal, proliferation and block differentiation. These pathways involve Bcl-2 and OXPHOS metabolism, Hedgehog, Wnt/β-catenin, tyrosine kinases such as FLT3 and PI3 kinase, STAT, Notch, isocitrate dehydrogenases IDH1 and IDH2, mTORC1-S6K1, Hsp90, NF-κB and other surface antigens. Similarly, re-activation of p53-mediated apoptosis pathways induce programmed cell death in the LSC population. Selective inhibitors of these pathways are used in order to eliminate the origin of the tumor and the differentiated cells, hence reducing the probability of relapse. Outstandingly, in clinical trials practically all AML selective inhibitors have demonstrated better efficacy in combination with standard CT. Among the list of strategies to target LSC summarized above (Table 1), at the moment only nine drugs or combinations have been approved by the FDA. For a few years, many clinical trials involving AML have begun, some of them currently in phase III with promising expectations. These agents show good bioavailability, clinical efficacy and tolerable AE similar to those observed in standard CT. Moreover, AML drug combinations extent the disease-free survival and overall survival in comparison with CT. For example, glasdegib plus LDAC increased median survival more than four months compared to LDAC alone. In addition, screening of patients' genotype has shown useful to predict the patient outcome and choose the best treatment. It is the case of ivosidenib and evosidenib, aimed at IDH mutations, or midostaurin and gilteritinib, directed at FLT3-ITD and FLT3-mutations. Interestingly, the main reasons for discarding a new drug is the low ratio between clinical activity and toxicity, which leads to abandonment of the research on that drug or further modifications for the preparation of more efficacious analogues. For example, plant-derived agents were typically administered as a mixture of different compounds, which results in high cytotoxic and low efficacy, but purification and formulation into manageable forms is not always possible. Other reasons for discarding a drug are low solubility, such as the case of parthenolide that was modified to dimethylamino-parthenolide, or rapid metabolism. To improve bioavailability, delivery techniques such as CPX-351 or nanoparticles are studied. Gold nanoparticles containing antisense

oligonucleotides and anti-CD33/CD34 aptamers inhibit oncogenes expression[95]. Unfortunately, resistance mechanisms like clonal evolution are also arising in LSC, as well as increase in the uptake of iron used for the Wnt/β-catenin pathway, or second-site mutations in already mutated proteins, such as IDH2[96,97]. Altogether, AML selective inhibitors of above-mentioned survival pathways in LSC have demonstrated more durable remissions along with traditional regimens. Indeed, targeting LSC is fundamental for eradication of AML and without doubt many agents currently in preclinical studies will proceed to clinical trials.

Figure 1. Model for selective targeting of LSCs.

Upper panel: AML stem cell under baseline conditions. Differential signaling pathways ensure cell survival.

Lower panel: agents targeting the main survival signaling pathways inhibit tumor progression.



CONCLUSIONS

Acute Myeloid Leukemia represents a challenge not only at the moment of diagnosis, but also in future relapses. In a few years, the standard chemotherapy treatment will represent a poor solution to manage the disease, as it is inadequate to prevent relapse with origin in leukemia stem cells. For two decades, consistent improvements in the knowledge of leukemia stem cells and their molecular signaling pathways have offered new mechanisms to target AML, especially in combination with chemotherapeutic agents. Taking into account that more than five drugs or combinations for AML have been approved in the past three years with great improvements in complete remission and overall survival, we will definitely witness the approval of more refined AML therapies in the near future.

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