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Simple Summary: Acute myeloid leukemia (AML) is a heterogenous disease caused by a combination of molecular events in hematopoietic stem cells that drive proliferation and block differentiation. The traditional therapeutic approach of AMLs consisted in inducing the massive killing of leukemic blasts with chemotherapeutic agents. An alternative approach consisted in inducing the differentiation of leukemic cells and was successfully developed in the treatment of acute promyelocytic leukemia. For many years the extension of differentiation therapy to other AML subtypes remained challenging. However, the development of studies of molecular characterization of the genetic abnormalities of AMLs have led the identification of new therapeutic targets that can be specifically targeted with some drugs, such as inhibitors of isocitrate dehydrogenase (IDH), lysine demethylase 1 (LSD1) and Menin capable of inducing AML differentiation. This review will discuss the clinical evolution of these inhibitors, outcomes data from latest clinical studies, development of resistance mechanisms and strategies to improve outcomes and future directions.

Abstract: Acute myeloid leukemia (AML) is characterized by clonal expansion of myeloid progenitors blocked at various stages of their differentiation process and drugs that bypass this differentiation block are therapeutically efficient, as supported by retinoic acid and arsenic trioxide in acute promyelocytic leukemia. However, the successful application of differentiation therapy to APL was not translated into clinical benefit for other non-APL subtypes of AML, in which intensive chemotherapy regimens represent the standard of care. However, the development of molecular studies has led to the identification of therapeutic targets (such as mutated proteins, deregulated pathways) have led to the generation of a new category of specific pharmacologic agents. Some of these agents, such as inhibitors of mutant isocitrate dehydrogenase (IDH1 and IDH2), Lysine-specific demethylase-1 (LSD1) and Menin have shown the capacity of inducing leukemic cell differentiation and a significant therapeutic efficacy.

Keywords: Leukemia; Acute Myeloid Leukemia; Differentiation; Targeted Therapy; Genetic Abnormalities

1. Introduction

A hallmark of acute myeloid leukemia (AML) is represented by a differentiation block of myeloid progenitors/precursors that accumulate in bone marrow and in blood.

One AML subtype, acute promyelocytic leukemia (APL) displayed a unique sensitivity to retinoic acid promoting the differentiation of leukemic promyelocytes to mature neutrophils [1]. This discovery provided the rationale for initial clinical studies based on the use of all-trans retinoic acid (ATRA) in APL, which supported the capacity of this drug to promote in vivo differentiation of leukemic cells [2-4]. Subsequent studies have clarified the molecular basis of the peculiar sensitivity of APL to ATRA; in fact, APL is characterized by a 15;17 chromosome translocation with breakpoints within the retinoic acid receptor α (RARA) gene on chromosome 17 and the PML gene, which encodes a transcription factor on chromosome 15: a PML-RARA fusion protein is formed because of the translocation. The specific targeting of this protein represents the molecular basis of the peculiar

sensitivity of APL to ATRA [5]. The PML-RARA protein is responsible for all the peculiar features of APL cells, such as sensitivity to ATRA, block of cell differentiation at a promyelocyte stage and increased proliferation due to diminished apoptotic cell death [6]. This peculiar molecular vulnerability of APL was exploited and allowed the development of a curative dual differentiation therapy based on the association of ATRA and arsenic trioxide (ATO), leading to more than 90% of patients being cured from this leukemia without the use of cytotoxic chemotherapy [7].

Although differentiation therapy has completely revolutionized the therapy of APL, changing the outcome of this leukemic subtype once highly fatal to a curable disease, great challenges were observed attempting to develop differentiation-based treatments in other AML subtypes. However, some recent developments of targeted therapies have led to the identification of newer targets for differentiation therapy. Particularly, in this context, the targeting of some molecules in non-APL AMLs, such as isocitrate dehydrogenase 1 and 2 (ODH1 and IDH2), lysine-specific demethylase 1 (LSD1) and Menin was associated with induction of leukemic cell differentiation and significant therapeutic effects.

This review will analyze recent developments in the therapy of AML based on IDH, LSD1 and Menin inhibitors.

2. IDH Inhibitors

Isocitrate dehydrogenase (IDH) is an enzyme that catalyzes the oxidative decarboxylation of isocitrate, producing α-ketoglutarate and CO_2. In humans, three isoforms of IDH exist: IDH3 catalyzes the third step of the citric cycle, while converting NAD to NADH in the mitochondria; the isoforms 1 and 2 (IDH1 and IDH2) catalyze th same reaction outside of the citric acid cycle and use NADP as cofactor.

Mutations of IDH are frequently observed in AML and their prevalence increases with age. While WT-IDH1 and IDH2 catalyze the decarboxylation of isocitrate to α-ketoglutarate, mutant IDH1 and IDH2 enzymes catalyze the conversion of α-KG to 2-hydroxyglutarate (2-HG), an oncometabolite responsible for oncogenic activity of *IDH1* and *IDH2* mutations [8]. Cancer-associated IDH mutations occur at the level of distinct arginine residues in the enzyme active sites.

Inhibitors of IDH are small molecules that bind within the IDH enzymatic active site, blocking aberrant 2-HG production and inducing myeloid differentiation.

The largest study of molecular characterization of 1023 older AML patients reported a frequency of 9.7% *IDH1^{MUT}*, 18.9% *IDH2^{MUT}*, including 1% of double-mutant *IDH1/IDH2*; *IDH1^{MUT}* significantly co-occurred with *DNMT3A^{MUT}* (42.4%), while *IDH2^{MUT}* was associated with *DNMT3A^{MUT}* (35.8%), *NPM1^{MUT}* (31.1%), *SRFSF2^{MUT}* (38.3%) [9]. *IDH1^{MUT}* was less frequently associated with *TET2^{MUT}* (6.3%) and *TP53^{MUT}* (9.3%) [9]. Normal karyotype was more frequently observed in *IDH1^{MUT}* and *IDH2^{MUT}*, while complex karyotype was less frequent in *IDH1^{MUT}* and *IDH2^{MUT}* AMLs [9]. *IDH2^{MUT}* AMLs exhibited an improved survival when treated with low-intensity treatments compared to *IDH^{WT}* AMLs.

The co-mutation profile significantly differed in *IDH1^{R132C and}* *IDH1^{R132H}* AMLs in that in the former ones there is a significantly higher frequency of *SRSF2*, *ASXL1* and *RUNX1* mutations and lower frequency of *NPM1* mutations than in the latter ones [10].

The frequency of both *IDH1* and *IDH2* mutations in AML is strongly influenced by age. *IDH1+IDH2* mutations increased from 3.4%, 11.3%, 17.7% up to 21% in groups pediatric (0-12 yrs), 13-39 yrs, 40-59 yrs and >60 yrs of patients, respectively [11]. *ASXL1* and *RUNX1* mutations markedly increased in older AML patients [11].

IDH Mutations and Cell Differentiation

Experimental studies have in part elucidated the mechanisms through which *IDH* mutations exert a leukemogenic effect. Studies in mouse models have shown that *IDH* mutations alone cannot induce *in vivo* the development of a full leukemic process but need to cooperate with additional genetic lesions to initiate leukemia [12, 13]. More recently, transgenic *IDH2^{MUT}* zebrafish models have confirmed these findings and have reported in transgenic embryos co-expressing *IDH2* mutations and *FLT3^{ITD}* the development of a leukemic process recapitulating features of human *IDH^{MUT}* AML

[14]. Single-cell transcriptomic analysis showed increased myeloid skewing, differentiation blockade and leukemia-associated gene signatures [14].
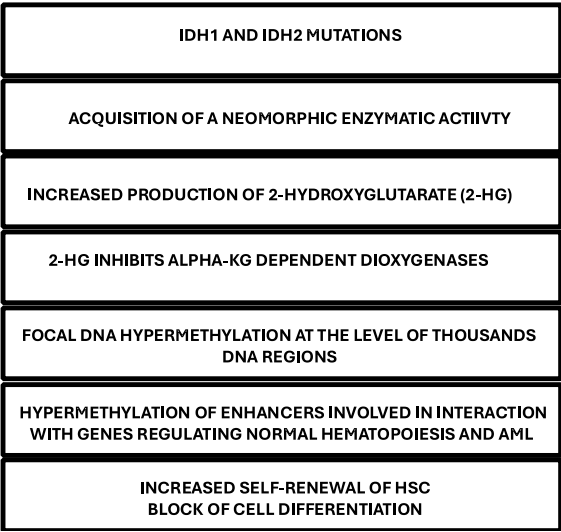

Figure 1. Various steps involved in the development of *IDH*-mutant AMLs, in relation to the pathogenetic contribution of *IDH1* and *IDH2* mutations.

In vitro models of *IDH* mutations have shown a consistent pattern of DNA hypermethylation, similar to that observed in primary *IDH^MUT* AML cells [15]. Conditional knock-in experiments have allowed to explore the effects of DNA hypermethylation in hematopoietic progenitor cells (HPCs): using this approach, *IDH1* mutation (*IDH1^R132H*) was inserted into the endogenous murine *IDH1* locus and was expressed in all hematopoietic cells or specifically in cells of myeloid cell lineage [15]. These mutant mice showed increased numbers of HPCs and self-renewal capacity [12]. Expansion of HPC and increased self-renewal were observed also in a model of transgenic mice expressing *IDH2^R140Q* in the hematopoietic system; in addition, it was observed also a block of cell differentiation [13].

In addition to hematopoietic cells, *IDH* mutations impair histone demethylation and inhibit cell differentiation in normal astrocytes [16].

Additional studies have shown that *IDH1^R132H* mutant promotes cytokine independence and blocks differentiation in hematopoietic cells and these effects can be recapitulated by 2-HG [17].

The key role of *IDH* mutations in blocking HPC differentiation was further and strongly supported by the observation that a small molecule acting as an inhibitor of mutant *IDH2* induced cell differentiation of *IDH2^MUT* leukemic cells [18].

A recent study showed that the expression of *IDH1^R132H* and *IDH^R140Q* mutants into human CD34+ cells via lentiviral transduction markedly impaired the colony forming capacity along erythroid, monocytic and granulocytic lineages [19]. In line with this observation, CD34+ cells isolated from a *IDH2^MUT* AML patient undergoing treatment with Enasidenib showed in the time a progressive improvement in their differentiation capacity, as supported by the *in vitro* generation of colonies of mature hematopoietic cells [19].

Finally, a recent study additionally supported the role of *IDH* mutations in blocking hematopoietic differentiation. Thus, Landberg et al. have generated CD34+ cells edited by the CRISPR/Cas9 and AAV6-mediated homology-directed repair to express *IDH1^R132H* or *IDH^R140Q* mutations: mutant CD34+ cells displayed a pronounced decrease in their colony and differentiation capacity; the block of cell differentiation of *IDH1^R132H* – edited cells was rescued by Ivosidenib [20-21].

All these observations support a key role of *IDH* mutations in blocking cell differentiation of *IDH^MUT* AMLs.

It is important to underline that the effects of *IDH* mutations on cell differentiation are largely dependent on the methylation changes induced by these mutations. *IDH1* and *IDH2* mutations in AML are associated with increased DNA methylation. The effects of these DNA methylation changes are not widespread but occur at the level thousands of focal regions that are specifically

hypermethylated compared to normal CD34$^+$ cells and AMLs without *IDH* mutations [22]. The methylation profile observed at these focal regions is different compared to that observed at the level of loci commonly hypermethylated in AML [22]. The profile of hypermethylated loci is highly comparable in *IDH1* and *IDH2*-mutant AMLs, but DNA hypermethylation is more pronounced in *IDH1^{MUT}* AMLs than in *IDH2^{MUT}* AMLs [22]. AMLs with biallelic inactivating *TET2* mutations had less dramatic methylation phenotype, but many of the hypermethylated regions correspond to those observed in *IDH^{MUT}* AMLs [22]. 5 hydroxy methyl cytosine (5hmC) levels in hypermethylated DNA regions are significantly lower in *IDH^{MUT}* or *TET2^{MUT}* AMLs, thus providing evidence that both the mutations determine increased DNA methylation by blocking TET2-mediated demethylation (TET2 promotes DNA demethylation through hydroxylation of 5mC) [22]. DNA hypermethylation in *IDH^{MUT}* AML cells requires the activity of the DNMT3A metyltransferase. Importantly, *IDH^{MUT}*-specific DNA hypermethylated regions are enriched at the level of enhancers involved in the interaction with gened involved in normal hematopoiesis and AML [22].

IDH1 Inhibitors

Ivosidenib (AG-120) is a first-in-class inhibitor of *IDH1^{MUT}* that exhibits marked 2-HG lowering in *IDH1^{MUT}* AMLs and induction of cell differentiation of these leukemic blasts [23]. A pivotal study of Di Nardo and coworkers reported a rate of complete remission (CR) or complete remission with partial hematological recovery (CRi) of 30.4%, with an overall response rate (ORR) of 41.6% and a median duration of response (mDR) of 8.2 months [23]. Bone marrow studies showed that Ivosidenib induced myeloid differentiation and hematopoietic recovery without an intervening period of bone marrow aplasia, a finding consistent with a mechanism of action involving induction of myeloid cell differentiation [24]. Differentiation syndrome was observed in 11% of treated patients and all these patients showed different degrees of response to treatment [24].

17 patients enrolled in this study responded to Ivosidenib treatment and subsequently underwent allogeneic HSCT [25]. 12% of these patients had relapsed following prior transplant before starting Ivosidenib therapy, and 76% were refractory to initial therapy; most common baseline co-mutations were *NPM1* (29%), *DNMT3A* (24%) and *SRFSF2* (18%) [24]. Prior response to Ivosidenib before allo-HSCT were CR in 10/17, CRi in 4/7 and MLFS (morphologic leukemia-free state) 3/17 [25]. Post-HSCT mOS was 7.7 months, survival rates of 76.5% and 47.1% at 6 and 12 months, respectively; mRFS was 7.3 months, RFS rates of 58.8% and 47.1% at 6 and 12 months [23]. Of all patients whose best OR was CR following Ivosidenib therapy and who underwent HSCT, median OS was not reached [25].

In newly diagnosed *IDH1^{MUT}* AML patients ineligible to standard chemotherapy induction, Ivosidenib treatment achieved a CR/CRi rate of 42.4%; 18% of treated patients developed a DS [26].

The phase III trial AG120-C-009 evaluated newly diagnosed *IDH1^{MUT}* AML patients ineligible for intensive chemotherapy to receive Ivosidenib and Azacitidine (IA) or Placebo and Azacitidine (PA): event-free survival was significantly longer in IA than in PB; the median OS was 24 months with IA and 7.9 months with PA [27]. DS was observed in 14% of patients receiving IA and 8% in those treated with PA [27]. The addition of Venetoclax to the Ivosidenib and Azacitidine doublet was safe and increased the rate of response s and improved long-term survival [28].

A phase I clinical study evaluated safety and efficacy of Ivosidenib in association with induction chemotherapy in newly diagnosed *IDH1^{MUT}* AML patients, resulting in a CR+CRi rate of 72% [29]. In this clinical setting the frequency of DS was low [29]. A more careful examination of these patients at the level of BM morphology and flow cytometric phenotype at day 14 and 21 showed three different patterns of response: (i) aplasia pattern, <10% cellular, <5% blasts; (ii) >10% cellular, <5% blasts, with morphologic and flow cytometric evidence of blast cell differentiation; (iii) persistence of leukemic blasts [30]. The differentiation response was observed in 20% of patients treated with Ivosidenib and chemotherapy [30].

Fathi et al. reported the results of a multicenter phase I trial involving treatment post-HSCT with Ivosidenib for *IDH1*-mutated AML [31]. 16 patients were enrolled and 8 discontinued maintenance with Ivosidenib. Two-year PFS was 81% and two-year OS was 88% [31].

The analysis of the primary response and relapse after Ivosidenib treatment allowed to define the mechanisms of resistance and relapse in R/R $IDH1^{MUT}$ AML patients. Clinical response to Ivosidenib was not predicted by the position of $IDH1^{MUT}$ within the conal hierarchy: $IDH1^{MUT}$ was subclonal in 28% of cases and clonal in 72% of cases and there was no association between $IDH1^{MUT}$ subclonal or clonal status and response to Ivosidenib [32]. Baseline RTK mutations (*NRAS, KRAS, FLT3-ITD, FLT3-TKD*) are associated with primary treatment resistance [31]. Relapse is characterized by multiple mechanisms, including emergence of RTK pathway mutations and *IDH* mutations conferring resistance to Ivosidenib [32].

The TARGET-seq⁺ method was used for single-cell genotyping to explore sequential bone marrow samples from 8 patients (6 relapsed and 2 in sustained remission), 6 treated with Ivosidenib/Venetoclax and 2 with Ivosidenib/Venetoclax/Azacitidine [32]. Relapse was associated with either genetic clonal evolution or impaired differentiation of pre-existing clones fully differentiated into mature myeloid cells prior to treatment, within HSPC/precursor cell compartments known to demonstrate LSC potential [33]. In the 2 patients remained in sustained remission, therapy eradicated all leukemic clones within 3 cycles of treatment [33].

Olutasidenib is an allosteric $IDH1^{MUT}$ inhibitor that binds at the level of hydrophobic pocket located near the IDH1 heterodimer interfaces and determines the stabilization of the mutant IDH1 molecule in an inactive conformation, thus blocking its neomorphic enzymatic activity. At structural level, Olutasidenib is a quinoline derivative [34]. Given these inhibitory properties, Olutasidenib inhibits 2-HG production and promotes granulo-monocytic differentiation in primary $IDH1^{MUT}$ AML cells and inhibits the growth of $IDH1^{MUT}$ AML xenograft models [35]. A phase I/II clinical study evaluated the safety and the efficacy of Olutasidenib in $IDH1^{MUT}$ AML and MDS patients. The phase I of the study evaluated the safety and efficacy of Olutasidenib as a single agent and in combination with azacitidine in patients with $IDH1^{MUT}$ AML and MDS; Olutasidenib monotherapy was evaluated in R/R patients and Olutasidenib as monotherapy or in combination with Azacitidine. Grade 3-4 hematologic adverse events were more common with combination therapy than with monotherapy; differentiation syndrome was observed in both monotherapy and combination treatment [36]. ORR was 41% in patients receiving monotherapy and 46% in patients treated with combination therapy; for treatment-naïve AML patients, ORR was 25% in monotherapy and 77% in combination therapy [36]. For R/R $IDH1^{MUT}$ AML patients, the median OS was 8.7 months for monotherapy and 12.1 months for combination therapy [36].

The phase II study involving monotherapy treatment enrolled 153 R/R $IDH1^{MUT}$ AML patients, with a median age fo 71 years and a median 2 prior therapy regimens; the CR+CRi was 35% and ORR was 48%; the median duration of CR+CRi was 25.9 months; the median OS was 11.6 months; response rates were similar in patients who received or not prior Venetoclax treatment [37]. OS occurred in 22% of patients and in 9% of patients was grade ≥3, with one fatal case [37]. The final analysis 5-year follow-up showed a CR+CRi rate of 35% with a median duration of CR+CRi of 25.3 months; ORR was 48%, with median duration of 15.5 months; mOS was 11.6 months and in patients R/R to prior Venetoclax mOS was 16.2 months [38]. Transfusion independence from red blood cells and platelets was achieved in 39% and 41% of patients, respectively [38]. The analysis restricted to elderly patients (≥75 years) showed that Olutasedinib was well tolerated and 31% of these patients achieved a CR+CRi, with a median time to CR+CRi of 15 months, and with a median duration of CR of 25.9 months [39].

The phase II of the combination of Olutasidenib and Azacitidine was based on a study involving 4 cohorts of patients: treatment-naïve patients with AML; R/R AML and MDS patients with no prior exposure to HMA or IDH1 inhibitors; R/R to HMA; patients with prior exposure to IDH1 inhibitors [40]. In an initial report, 72 patients with AML/MDS were reported (20 R/R without prior HMA/HD1 inhibitor therapy; 21 R/R with prior HMA therapy; 20 R/R with prior HD1 therapy; 11 treatment-naïve AML patients) [40]. In treatment-naïve AML patients, the CR+CRi rates were 45%; in R/R setting, CR+CRi rates were 47% in those without prior HMA/HD1 inhibitor therapy, 38% with prior HMA/HD1 inhibitor therapy, and 30% with prior IDH1 inhibitor therapy [40]. The duration of CR+CRi was longest among treatment-naïve patients [40].

More recently, it was presented a pooled analysis of R/R IDH1-mutant AML patients included in this trial: 43% were refractory and 57% relapsed after a prior treatment; 83% of these patients had at least 2 prior treatment regimens, including HMA (40%), IDH1 inhibitor (31%), HSCT (10%) CR+CRi was observed in 31% of patients, with a median duration of 15 months; CR was achieved in 27% of patients with a median duration of 20 months; ORR was 51%; median OS was 13 months in all patients, 24 months in overall responders, 30.6 months in patients with CR+CRi and not reached in patients with CR [41].

A multicenter, investigator-initiated phase I/II study will evaluate the safety and the efficacy of the triplet regimen based on Olutasidenib in combination with Decitabine and Venetoclax [42].

The comparison of outcomes of Olutasidenib-treated from the 2102-HEM-101 single-arm trial were compared to a real-world external central arm of Ivosidenib suggestive of favorable effectiveness of Olutasidenib for patients with IDH1-mutant AML who are R/R to a Venetoclax-based regimen [43].

A recent study reported a comparative analysis of Olutasidenib and Ivosidenib. Both these drugs have been approved by FDA for treatment of IDH1-mutant AML. In the absence of head-to-head studies, this analysis compared the outcomes observed in studies carried out in R/R AML patients: the rates of CR+CRi observed with Olutasidenib and ivosidenib were similar; however, the duration of response observed in patients treated with Olutasidenib was longer than that observed in patients treated with Ivosidenib (25.9 months vs 8.2 months, respectively). Another notable difference is that Olutasidenib is structurally smaller with a lower molecular weight than Ivosidenib thus occupying less space in the binding pocket of IDH1 dimers, making it resistant to displacement by IDH1 second-mutation [44]. In biochemical studies, Olutasidenib was found to inhibit mutant *IDH1* but not wild-type IDH1 [44].

IDH2 Inhibitors

Enasidenib (AG-221) is a clinically useful *IDH2*-mutant inhibitor developed by Agios Pharmaceuticals/Celgene, reducing 2HG levels and promoting cell differentiation [45]. A pivotal study by Stein and coworkers in relapsed or refractory *IDH2* mutant AML patients reported an overall response rate of 40.3%, with a median OS of 9.3 months (19.7 months in patients who attained a CR); responses were associated with cellular differentiation of leukemic bast and not cytotoxicity was the main driver of anti-leukemic activity of Enasidenib [46]. Enasidenib was explored also in older AML patients who are unfit for intensive chemotherapy, showing an ORR of 30.8%, with 18% of CR and with a mOS of 11.3 months [47]. The analysis of R/R AML patients treated with Enasidenib showed that: Enasidenib induced a marked decrease of 2-HG levels, preceding the clinical response; 2-HG suppression by Enasidenib did not predict response since most non-responding patients also exhibited 2-HG suppression; complete remission and normalization of stem and progenitor compartments were associated with emergence of functional neutrophils bearing *IDH2* mutations; in a subset of responding patients, *IDH2^{MUT}* burden decreased and remained undetectable during response; co-mutations in NRAS and MAPK pathway effectors are enriched in patients resistant to treatment [48].

A randomized phase 3 clinical trial explored Enasidenib vs conventional care in a population of 319 older *IDH^{MUT}* R/R AML patients who received 2 to 3 prior AML-directed therapies [48]. Enasidenib improved EFS and hematological parameters but failed to improve OS compared to standard treatment; OS could be confounded by early dropout and use of subsequent AML therapies. [49].

In a phase Ib/II clinical trial, Enasidenib was evaluated in 101 newly diagnosed *IDH2*-mutated AML patients not eligible for standard induction chemotherapy, the treatment based on Enasidenib plus Azacitidine (2/3 of patients) or Azacitidine alone (1/3 of patients) [50]. Significant differences were observed between the two groups of patients concerning ORR and CRR, but not in median OS [50]. About one-third of patients in the group Azacitidine alone received Enasidenib at progression as a post-protocol therapy and this may have confounded survival analysis [50].

Explorative correlative studies performed in patients treated with Enasidenib and Azacitidine showed that responding patients displayed a decrease of balst cell population markers and an

increase of differentiated myeloid markers, such as CD11b and these changes were paralleled by a decrease of VAF of *IDH2* mutations or *NPM1* mutations up to low/very low levels, no patients with *NRAS, KRAS* or *PTPN11* mutations achieved a CR [51].

A recent study explored Enasidenib combined with Venetoclax in a population of patients with R/R AML or other myeloid malignancies, showing that this drug combination induced an ORR higher than that observed with Enasidenib alone: an ORR was significantly higher in *IDH2^{R172}* than *IDH2^{R140}*-mutant patients (83% vs 55%, respectively) [52]. In the whole treated population, ORR was 70%, 57% CRR; the median duration of response was 16.6 months, and 3 patients proceeded to HSCT following CR [52].

The combination of Enasidenib with intensive induction and consolidation chemotherapy was explored in 93 fit *IDH2^{MUT}* AML patients, showing that it was well tolerated and was associated with a CR+CRi rate of 77%, with 23% of patients achieving *IDH2* mutation clearance [29].

A recent pilot clinical trial (NCT 03728335) assessed the use of Enasidenib as a post-HSCT maintenance therapy fro 15 patients with *IDH2*-mutated AML: the patients received 24 cycles of Enasidenib therapy with leukemia-free survival rates of 100% and 93% of chromic GVHD-free survival and 87% relapse-free survival [53]. At the safety level, the treatment was well tolerated, with adverse events easily managed [53]. The results were considered highly promising and the enrollment of 20 additional patients was scheduled [53].

Concurrent RAS-signaling mutations are a great challenge in the treatment of *IDH*-mutant AML patients in that they infer resistance to IDH inhibitors. Thus, as supported by preclinical studies, it was recently proposed a phase Ib study based on the administration of Enasidenib and MEK inhibitor Cobimetinib in R/R AML patients who have co-occurring *IDH2* and *RAS* signaling mutations [54].

Interestingly, a recent study reported an ORR of 43% ih high risk *IDH2*-mutant MDS treated with Enasidenib monotherapy, with an OS of 14.9 and 25.5 months in R/R and first-line treated patients, respectively [55]. Durable responses were observed in >50% of low-risk MDS patients [55].

Differentiation Syndrome in Patients Treated with IDH Inhibitors

Treatment with IDH inhibitors was associated with the development of differentiation syndrome (DS), a potentially lethal adverse reaction triggered by agents that induce myeloid differentiation.

DS was initially described in acute promyelocytic leukemia (APL) treated with agents inducing myeloid differentiation, such as all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) [56]. DS is a relatively common and potentially severe complication observed in APL patients treated with ATRA and/or ATO; this syndrome is characterized by the association of a number of symptoms, including unexplained fever, weight gain, dyspnea with pulmonary infiltrates, pleuropericardial effusion, hypertension, and renal failure [56-57]. This syndrome may occur in a mild and in a severe form. The mechanisms responsible for the development of DS remain not fully understood but seem to be related to the induction of differentiation of leukemic blasts, resulting in the massive release of cytokines ("cytokine storm") and chemokines, and subsequent systemic inflammatory response and increased expression of adhesion molecules on the surface of differentiated leukemic cells, mediating their adhesion to vascular endothelium and then their migration into tissues [58].

The prevalence, risk factors and clinical outcomes of *IDH*-mutant AML patients treated with IDH inhibitors were not prospectively evaluated. A retrospective analysis performed by an independent differentiation review committee, composed by investigators who have participated to the development of Enasidenib showed that DS was observed in 11.7% of R/R AML patients treated with Enasidenib and that most frequent symptoms were dyspnea, fever, pulmonary infiltrates, and hypoxia; in 395 of cases DS was associated with concomitant leukocytosis [59].

The Food and Drug Administration performed a systematic analysis of DS observed in 393 AML patients treated with Ivosidenib or Enasidenib [60]. According to this analysis, DS was identified in 19% of patients; the predominant symptoms observed in these patients are pulmonary (dyspnea, pulmonary infiltrates and effusions) [60]. In these patients, the onset of symptoms occurred at a median of 19-20 days after treatment [61]. Predictors of DS were represented by higher leukemic burden in the bone marrow (≥48% of blasts) and in the blood (≥15%-25% blasts); furthermore,

concurrent mutations in *TET2* and *SRFSF2* were associated with a higher risk of developing DS, CR+CR rates were lower in patients with versus those without DS (Ivosidenib 18% vs 36%; Enasidenib 18% vs 25%) [60].

Montesinos and coworkers have performed a pooled analysis from four clinical trials involving Enasidenib in AML patients, either in monotherapy or in combination with Azacitidine or with chemotherapy [58]. Highest incidence of DS was observed in patients who received Enasidenib plus Azacitidine (17.6%) and lowest incidence in patients who received Enasidenib plus chemotherapy (2.2%) [61]. The most common symptoms were dyspnea/hypoxia (80.6%) and pulmonary infiltrate (73.1%) [61]. Baseline risk factors for developing DS were represented by higher levels of bone marrow blasts and lactate dehydrogenase [61].

Inhibitors of Lysine-Specific Demethylase 1 (LSD1 or KMD1A)

LSD1 is a member of the flavin adenine dinucleotide-dependent (FAD-dependent) amine oxidase family of demethylase, participating to the formation of various chromatin multiprotein complexes involved in gene regulation, such as rest co-repressor (CoREST) and nucleosome and remodeling and deacetylase (NuRD) [62-63]. LSD1 acts demethylating lysine 4 and 9 on histone 3 and also on non-histone substrates, such as p53 and, through these effects, it represses gene expression [62]. LSD1 is essential for the function of HSCs and its knockout results in a reduction of granulopoiesis, thrombocytopoiesis and erythropoiesis, associated with an expansion of granulo-monocytic, megakaryocytic and erythroid progenitors, thus supporting a physiological role for LSD1 in myeloid differentiation and maturation [64]. LSD1 is overexpressed in many cancer types, including AML.

A number of compounds with amine oxidase inhibitory activity (trancylcypromine, paragyline and phenelzine) and SP-2509 and GSK-LSD1 have been utilized in initial studies aiming to explore their anticancer activity. New LDS1 inhibitors suitable for clinical studies, such as ORY-1001 (Iadademstat) SP-2577 (Seclidemstat), IMG-7289 (Bodemstat) and CC-90011 (Pulodenstat) have been evaluated in clinical studies [62-63]. These compounds act as irreversible or reversible LSD1 inhbitors, with various degrees of specificity and display *in vitro* efficacy in AML with inhibition of cell proliferation and induction of cell differentiation [62-63].

Experimental studies in suitable models supported the clinical use of LSD1 inhibitors as antileukemic drugs. A pivotal study by Lynch and coworkers provided evidence that pharmacological inhibitors of LSD1 promote differentiation of myeloid leukemic cells through a mechanism independent of inhibition of LSD1 enzymatic activity (histone demethylation) but dependent of the interaction between LSD1 and transcription factor GFI1, essential to maintain the differentiation block in AML [65]. Particularly, LSD1 inhibitors disrupt the interaction of LSD1 and RCOR1 with the transcription repressor GFI1, which is bound to a set of enhancers located close to transcription factor genes involved in the regulation of myeloid cell differentiation; the inactivation of GFI1 leads to increased enhancer histone acetylation and consequent gene activation [66]. LSD1 inhibitors interfere with GFI-mediated repression of PU.1 and C/EBPα target genes and induce differentiation of AML cells [67-68]. The stabilization of the binding of LSD1 on chromatin at GFI1 binding sites requires the interaction of the HMG-box protein HMG 20B with LSD1 [69].
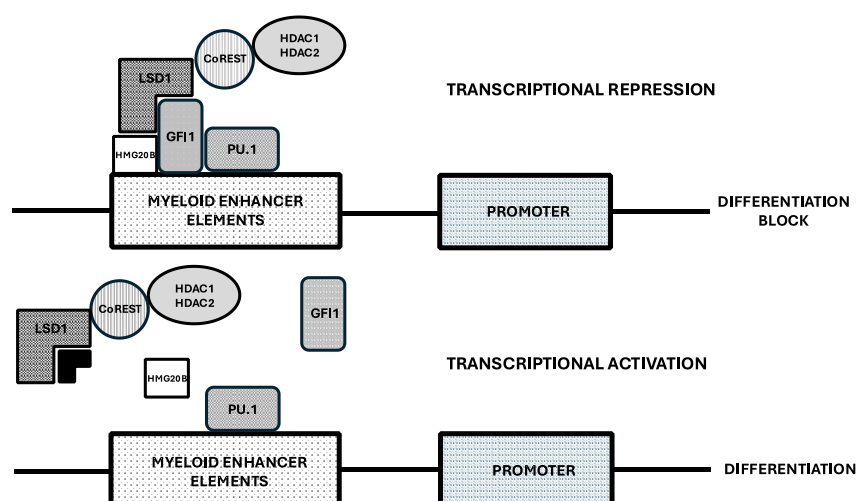
Figure 2. Molecular mechanisms underlying induction of cell differentiation by LSD1 inhibitors. LSD1 inhibition drives expression of genes involved in myeloid cell differentiation through disruption of transcriptional repression mediated by GFI1. *Top Panel*: when LSD1 interacts with the transcriptional repressor GFI1 and with HMG20B, it allows the recruitment of repressors to chromatin and catalyzes H3K4 demethylation and histone deacetylation though HDAC activity, resulting in transcriptional repression. *Bottom Panel*: LSD1 inhibitors block the interaction between LSD1 and GFi1, thus destabilizing the whole LSD1 repressor complex and leading to activation of myeloid enhancer elements with consequent transcriptional activation of master transcription factors of myeloid differentiation, such as PU.1.

The LSD1/CoREST/HDCA2 complex is recruited by the SNAG domain of GFI1 [66]. In addition to GFI1, GFI1B is another repressor containing a SNAG domain and mediating the recruitment of LSD1/CoREST/HDCA2 complexes [70]. These molecular complexes involving LSD1 act as epigenetic regulators of hematopoietic differentiation [70]. GFI1 plays an important role in the control of granulo-monocytic differentiation, while GFI1B plays a role in erythroid and megakaryocytic differentiation [71].

The N-terminal intrinsically disordered region (IDR) is required for the regulation of LSD1-transcription factors interactions, controlling enhancer activation that is necessary for AML cell differentiation [72].

PRY-1001 is a potent and selective LSD1 inhibitor that induces H3K4me2 accumulation on LSD1 target genes, differentiation of leukemic cells and inhibition of leukemic cells and inhibition of leukemic stem cell activity in AML models [73]. ORY-1001 synergizes with other anti-leukemic drugs currently used in the treatment of AML and in a patient-derived xenograft model of T-ALL significantly reduces leukemia development and improves survival [73]. These observations have supported the clinical evaluation of ORY-1001 (Iadademstat) in AML patients.

A phase I clinical study evaluated Iadademstat in relapsed or refractory AML patients: the dose-escalation section of the study carried out in 27 patients showed the recommended dose of Iadademstat for an extension cohort was 140 μg/m² administered as single agent from day 1 to 5 of 28-day cycles; in the extension cohort 14 additional patients were treated, including 5 patients with MLL/KMT2A-rearranged AMLs [74]. Reduction of blood and bone marrow percentages of blasts were observed, with one patient achieving CR; inhibition of blast cell differentiation was frequently observed, particularly in patients with MLL translocations: cell differentiation was observed in 80% of these patients and was particularly pronounced in two patients, with one of these patients developing a rapid and fulminant differentiation syndrome [74].Preclinical studies have shown a synergism between Azacitidine and Iadademstat, thus supporting the clinical evaluation of this drug combination. Thus, the phase II ALICE clinical study evaluated 36 newly diagnosed AML patients with intermediate or advanced-risk disease, with a median follow-up of 22 months; dose-response studies showed that optimal dose of Iadademstat in combination with Azacitidine was 90 μg/m² [75].

In 27 patients, the ORR was 22727 with 14/27 CR+CRi (11/27 MRD⁻) [75]. 3 patients displayed DS and one patient had fatal grade 5 intracranial hemorrhage.

Iadademstat is also being evaluated in combination with Venetoclax and Azacitidine in newly diagnosed AML patients in an investigator-initiated phase I clinical trial at Oregon Health & Science University Knight Cancer Institute (NCT 06357182) and in company-sponsored phase Ib clinical trial in combination with Gilterinib in patients with R/R AML patients harboring *FLT3* mutations (NC 05546580).

Particularly, concerning the association of Iadademstat with Gilertinib, he FRIDA study aims to establish he safety, tolerability and the recommended phase II dose in R/R *FLT3*-mutant AML patients [76]. This trial was supported by preclinical studies showing a strong synergy of Iadademstat with FLT3 inhibitors and particularly with Gilterinib in *FLT3*-mutant AML cells [76]. The FRIDA sudy was based on classical design for phase I/II studies, with an escalation phase (from 75 to 150 μg/orally of Iadademstat) and an expansion phase at the selected safe and pharmacologically active dose [76]. Preliminary results on the FRIDA trial were presented at the EHA 2024 Meeting on the first 13 patients: the combination of Iadademstat and Gliteritinib appeared to be safe and well tolerated, with no dose limiting toxicities at initial dose (75 μg) and DL1 (100 μg) of Iadademstat; encouraging antileukemic activity was shown, with 5 out of 13 patients (38%) achieving CR+CRi and 9 out of 13 patients (69%) achieving bone marrow blast clearance in the first cycle of treatment [77].

Two other LSD1 inhibitors, SP-2577 (Seclidemstat) and CC-90011 (Purodemstat) are under evaluation in patients with hematological malignancies but their evaluation in AML patients is at the moment very limited.

Menin Inhibitors in the Treatment of AML

Menin protein is encoded by the *MEN 1* gene, whose germline mutations are the causation of sporadic or autosomal dominant hereditary cancer syndromes affecting the endocrine system; menin plays a key role as epigenetic regulator of gene expression for its role as scaffold protein able to interact with various partners; thus, Menin acts as an adaptor protein between H3K4 (histone 3 protein with the lysine amino acid al position 4) methyltransferase KMT2A (also known as MLL) and LEDGF (lens epithelium derived growth factor). Menin interacts with both the wild-type and rearranged *KMT2A*, regardless of the fusion partner. Menin was found to be crucial for KMT2A activity and the maintenance of *HOXA* expression but not essential for normal hematopoiesis. The normal regulation of the KMT2A activity is required for the maintenance of expression of HOX family cluster genes in tissues.

Upregulated HOXA/MEIS1 expression is observed in AMLs characterized by the presence of *NPM1* mutations or by rearrangements of the *MLL* gene [78-80]. In *NPM1*-mutant AMLs (20-30% of total AMLs) the nucleolar protein is mutated and is usually delocalized in the cytoplasm; however, a part of the mutated NPM1 protein resides in the nucleus where it interacts and is co-localized with KMT2A at the level of the *HOXA* locus [78-80]. The mutant NPM1c protein amplifies the function of KMT2A [79] and inhibits also the activity of histone deacetylases [80] to maintain active transcription of *HOXA/B* cluster genes and *MEIS1*. Mutant *NPM1* upregulates the expression of *HOXA/B* gene clusters also through another mechanism dependent upon the interaction between mutant NPM1c protein and FOXM1 protein with its consequent inactivation and delocalization in the cytoplasm: the transcriptional inactivation of FOXM1 by cytoplasmic sequestration induces de-repression of *HOX A/B* cluster genes [81].

KMT2A-rearranged AMLs represent 5-10% of adult AMLs, but are much more frequent in younger patients, while they are rare in older patients; in these leukemias the rearrangement of *KMT2A* gene upregulates HOX-MEIS1 expression [82]. KMT2A protein directly binds to and uses its SET domain H3 (Lys4) methyltransferase to regulate *HOX* gene promoters. A third AML subset associated with increased HOX A/B expression is represented by partial tandem duplication (PTD) of *KMT2A* gene, occurring in 3-11% of adult *de novo* AML and associated with adverse outcome. At the level of gene expression, *KMT2A-PTD* are characterized by a peculiar profile, showing increased expression of several *HOX* genes, including *HOX-B5, HOX-B7, HOX-B8* and *HOX-B9*. The comparative analysis of the profile of *HOX-A/B* genes deregulated in these AMLs overexpressing

HOX genes showed a different profile in *KMT2A-PTD* and *KMT2A*-rearranged AMLs and a similar profile in *KMT2A-PTD* and *NPM1*-mutant AMLs [83]. The analysis of the whole gene expression profile allowed to define three gene signatures along the HOX-targeted gene axis (HOX primitive, HOX transient and HOX committed profiles); *KMT2A*-rearranged AM Ls were mostly classified as HOX-committed, while *KMT2A-PTD* as HOX-primitive/transient and *NPM1*-mutant AMLs were distributed along the three HOX profiles [83].

A recent study provided an analysis of pediatric AMLs subdivided according to *HOX* family gene expression. No expression or very low expression of *HOXA* and *HOXB* genes was observed in normal bone marrow cells, while 25% of pediatric AML had significant upregulation of *HOX A/B* gene expression. The cluster of AMLs with highest *HOX A* expression contained 88% of *KMT2A*-rearranged AMLs and these patients displayed a significantly worse outcome compared to those with low/moderate *HOX* expression; patients without *KMT2A* rearrangements that clustered within the group at highest *HOX A* expression had a dismal outcome [84]. Patients with high *HOXB* expression mainly pertain to patients with *FLT3-ITD* or *NUP98-NSD1* gene alterations [84]. AMLs with *HOX-A* expression display a clear co-expression of *MEIS-1* [84].
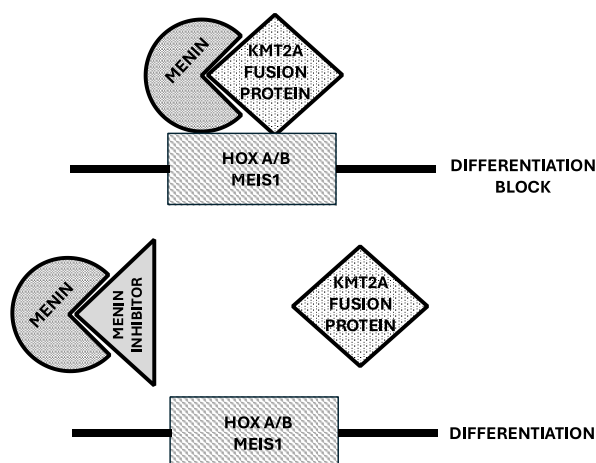

Figure 3. Role of Menin in the regulation of HOX a/B gene expression and cell differentiation in KMT2A-rearranged AMLs. *Top Panel:* KMT2A fusion protein binding to its cofactor Menin acts as a stimulator of HOX A7B gene expression, with consequent effects resulting in a block of cell differentiation. *Bottom Panel:* Menin inhibitors bind to Menin and impair its interaction with HOX A/B genes, inhibit HOX A/B gene expression, with consequent induction of cell differentiation.

Efficient drug discovery techniques have led to the development of potent and selective Menin inhibitors that block the Menin-KMT2A interaction through a hydrophobic pocket on surface of Menin protein. Preclinical studies have supported the efficacy of Menin inhibitors in *MLL*-rearranged and *NPM1*-mutated models of leukemia. Thus, Krivstov et al showed that the highly selective Menin inhibitor VTP50469 displaced Menin from protein complexes and inhibited chromatin occupancy by *MLL* of selected genes in leukemic cells bearing *MLL* rearrangements resulting in induction of cell differentiation and apoptosis [81]. Patient-derived xenograft models derived from *MLL*-rearranged AML cells showed dramatic reduction of leukemia when treated with VTP50469 [85]. Klossowski et al. confirmed that the menin-specific inhibitor MI-3454 inhibited cell proliferation and induced differentiation in primary patient samples with *MLL* translocations or *NPM1* mutations and induced remission or regression of leukemia in mouse models of *MLL*-rearranged or *NPM1*-mutated leukemia [85]. Menin inhibitors silence both a canonical *HOX* and *MEIS-1*-dependent oncogenic gene expression program and a noncanonical transcriptional program involving tumor suppressor genes and both these events are required to achieve a good therapeutic response [86].

In vitro studies in *NPM1*-mutant or *MLL*-rearranged leukemic cell lines or primary AML cells using the Menin inhibitor DS-1594b showed that the primary effect was induction of cell

differentiation and not of apoptosis; when the Menin inhibitor was associated to the BCL-2 inhibitor Venetoclax the apoptotic effect was predominant [87].

Table 1. *A*-rerrangend and *NPM1*-mutant AML patients treated with Menin inhibitors in monotherapy.

Compound	Revumenib	Bleximenib	Enzomenib	Ziftomenib
Trial	AUGMENT-101 Phase I/II	CAMELOT-1 Phase I/II	DSP-5336-101 Phase I/II	KOMET-001 Phase I/II
Number of patients	161	21	40	58
ORR	KMT2Ar 64% NPM1m 47%	KMT2Ar 30% NPM1m 50%	KMT2Ar 59% NPM1m 54%	KMT2Ar 17% NPM1m 42%
CR+CRi	KMT2Ar 23% NPM1m 23%	KMT2Ar 33% NPM1m 33%	KMT2Ar 30% NPM1m 47%	KMT2Ar 17% NPM1m 35%
MRD negativity (in CR+CRi)	KMT2Ar 58% NPM1m 64%	NR	NR	KMT2Ar 100% NPM1m 63%
HSCT among responders	KMT2Ar 36% NPM1m 17%	NR	NR	KMT2Ar 33% NPM1m 33%
Differentiation Syndrome (%)	22%	19%	11%	11%

Revumenib

Revumenib ia a potent oral small molecule inhibitor of the Menin-KMT2A interaction. This compound was evaluated in monotherapy in R/R AML patients with *KMT2A* rearrangements or *NPM1* mutations. The AUGMENT-101 trial (NCT 04065399) is a phase I clinical stud evaluating the safety and the efficacy of Revumenib in patients with R/R heavily pretreated *NPM1*-mutant or *KMT2A*-rearranged AMLs [88]. Since Revumenib is a substrate of CP3A4, the study was subdivided into two cohorts: a cohort A of patients receiving a CP3A4 inhibitor and a cohort B of patients not receiving a CP3A4 inhibitor [85]. A first evaluation of this study was based on 60 patients (46 with *KMT2A*-rearranged AMLs and 14 with *NPM1*-mutant AMLs); the ORR was 59% in *KMT2A*-rearranged AMLs and 36% in *NPM1*-mutant AMLs; the rate of CR+CRi was 30%; with a median follow-up of 11.9 months in patients who achieved morphologic CR+CRi the median duration of response was 9.1 months; mOS was 14.3 months; 56% of the responder patients achieved MRD negativity and 38% underwent allogeneic hematopoietic stem cell transplantation [88]. Interestingly, in many patients with *KMT2A* rearrangements achieving morphological remission after one cycle of treatment, there was continued evidence of *KMT2A* fusions, a phenomenon seemingly related to the induction of leukemic blast differentiation elicited by Revumenib. Differentiation syndrome was observed in 16% of patients, with all cases classified as grade 2 [88]. Additional 8 AML patients *NPM1-WT* and *KMT2A-WT* did not respond to Revumenib [88]. Analysis of the pharmacodynamic effects of Revumenib assessed through transcriptional analysis of RNA expression of bone marrow cells showed that Menin inhibition resulted in downregulation of HOXA9 and MEIS1, associated with an increase in the expression of genes related to differentiation, such as CD11b and CD14 [88]. Single cell studies carried out on 4 patients treated with Revumenib showed a differentiation continuum that starts with immature AML blasts (CD34+/c-kit+), progresses through intermediate blast cells (CD68+, CD11b-, CD14-) and ends with more differentiated monocytic cells (CD68+, CD11b+, CD14+); intermediate AML blasts and monocytic cells were enriched in post-treatment samples [89].

A second study reported the results of phase II carried out in 94 R/R *KMT2A*-rearranged acute leukemia (78 with AML and 14 with ALL); 13% of patients discontinued treatment for adverse events; DS was observed in 26% of patients (14.9% had grade 3 and 1% grade4) [90]. CR+CRi rate was 22.8% with an ORR of 63.2%; the median duration of response was 6.4 months; mOS was 8.0 months; among responding patients, 38.9% received allo-HSCT [90]. Transcriptional changes in bone marrow cells showed a decrease of MEIS1 and HOXA expression and an increase of differentiation-related genes CD11b and CD14 [90]. An updated evaluation of *KMT2A*-rearranged AML patients enrolled in AUGMENT-001 trial extended to a total of 116 patients showed 23% of patients achieving CR+CRi,

with an ORR of 64% and with 5.8% of MRD negativity among patients achieving CR+CRi; 34% of patients who achieved ORR proceeded to HSCT [91].

Recently, the Syndex Pharmaceuticals announced the results of the phase II of the AUGMENT-001 study evaluating the efficacy of Revumenib in 64 R/R heavily pretreayted (75% relapsing after Ventoclax therapy) *NPM1*-mutated AML patients; 23% of patients achieved a CR+CRi; ORR was 47%; MRD negativity was 64% among patients who achieved CR+CRi; 17% of the responding patients undergo HSCT [92].

ALLG AMLM26 INTERCEPT multiarm study was design to obtain proof-of-concept for novel theraoies targeting MRD or early relapse in AML [93]. In the context of this study, preliminary analysis on 9 *NPM1*-mutant AML patients with MRD-positivity showed a a *NPM1* mutant ≥ 1 $\log_{10}$ MRD reduction in 62.5 of patients, including 37.5 of patients achieving MRD negativity [93].

A phase I/II study evaluated combination of Revumenib with decitabine/cedazuridine and Venetoclax in R/R AML patients (*NPM1*-mutant, *KMT2A*-rearranged and *NUP98*-rearranged), reporting an ORR of 88%, with a CR+CRi of 58% and with a MRD negativity by flow cytometry of 93% among patients with CR+CRi [94].

In pediatric patients, KMT2A rearrangements are frequently associated with RAS pathway mutations (51% of cases) and with a worse prognosis [95]. A recent preclinical study showed that the combination of a Menin inhibitor (VTP-50469, an analog of Revumenib) and Selutenib (a MEK 1-2 inhibitor) exerted a synergistic antitumor effect *in vitro* and in PDX models of *KMT2A*-rearranged AML cells bearing RAS pathway mutations [95].

About 40% of patients treated with Revumenib developed *MEN1* mutations and some acquired resistance to Menin inhibitors without *MEN1* mutations. Particularly, Perner et al. identified Menin gene mutations that were not present at diagnosis and developed on Revumenib treatment; clonal expansion of these mutations was observed in about 39% of evaluable patients who have performed at least two cycles of treatment [96]. These mutations were found at residues M327, G331 or T349 which do not impact the interaction between KMT2A and Menin or its oncogenic properties but decrease binding affinity of Revumenib to KMT2A and mediate therapeutic resistance [96-97]. A recent study characterized in detail the consequences of Menin mutations in the interaction with Menin inihbitors [93]. The crystal structure of Menin mutants T349M, M327I, G331R and G331D and the N-terminal of MLL1 showed that: drug-resistant mutations in Mnwin occur at a site located in proximity of the MLL1 binding site, but do not affect t MLL1 binding to Menin; all these point mutations in Menin generate a steric collision with Mwnin inhibitors; the G331D mutant show a particularly slow dissociation of MLL1 from Menin and thus seems to be particularly difficult to be inhibited with small molecule inhibitory drugs [98].

The identification of *MEN1* mutations in AML patients undergoing treatment with Revumenib is important because may offer opportunities for patients with some *MEN1* mutations to derive benefit from treatment with some of new Menin inhibitors.

Bleximenib

JNJ-75276617 (Bleximenib) is a novel potent inhibitor of the protein-protein interaction between Menin and KMT2A; in *KMT2A*-rearranged and *NPM1*-mutant leukemia cells, this compound inhibited the interaction of the Menin-KMT2A complex with chromatin at the level of target genes resulting in reduced expression of several target genes such as MEIS1 and FLT3 and exerted a potent antiproliferative activity [94]. Bleximenib displayed a synergistic antileukemic activity with Gilteritinib, Venetoclax and Azacitidine [93]. Interestingly, Bleximenib displayed a strong antiproliferative activity in leukemic cells bearing mutations (*MEN1^{M371}* or *MEN1^{T349M}*) observed in patients refractory to Revumenib [99]. In these mutant AMLs, Bleximenib was still able to displace KMT2A and prevent its interaction with Menin despite the presence of *MEN1* mutations that block the acivity of other Menin inhibitors.

A first-in-human phase I clinical study evaluated the safety and preliminary the efficacy of Bleximenib in 58 R/R acute leukemia patients (56 AML and 2 ALL) showing an acceptable safety profile and preliminary evidence of antileukemic efficacy and biologic activity [100].

A phase I clinical study (NCT 04811560) carried out in 121 R/R acute leukemias (mostly AML, 108) patients identified the optimal dose of Bleximenib (100 mg) in monotherapy to be used in phase II studies; at this dose, the ORR was 50% and the CR+CRi was 40% [101]. The enrolled patients had either *KMT2A* rearrangements or *NPM1* mutations. Another recent phase I clinical study explored Bleximenib in association with standard chemotherapy in a group of 22 newly diagnosed AML patients (11 *KMT2A*-rearranged and 11 *NPM1*-mutated) showing an acceptable safety profile with no DS or dose-limiting toxicities [102]. The ORR was 93% (83% in *KMT2A*-rearranged AML and 100% in *NPM1*-mutated AML); 6 patients proceeded to HSCT [102]. A phase Ib (NCT 05453903) clinical study explored the safety and the efficacy of Bleximenib in combination with Venetoclax and Azacitidine in R/R AML patients with *KMT2A* rearrangements or *NPM1* mutations [98]. In the safety dataset, 45 patients received the triplet combination treatment, with a median of 2 prior lines of treatment, including prior Venetoclax treatment in 56% of cases and allo-HSCT in 27% of cases [102]. The safety profile was acceptable and no patient developed DS or tumor lysis syndrome [103]. In the efficacy dataset, ORR was was 86%, CR+CRi was 48%; for patients with prior Venetoclax exposure, ORR was 82% and CR+CRi was 36% [103]. 9 responder patients discontinued treatment and proceeded to allo-HSCT [103].

Hogeling et al. recently reported a large *in vitro* careening of primary AML cells incubated in the presence of Bleximenib [104]. This study showed that AML cells bearing *NPM1* mutations or *KMT2A* rearrangements are sensitive to the antiproliferative and differentiation-inducing effects of Bleximenib [104]. Concerning *NPM1*-mutant AMLs, those bearing both *NPM1* and *DNMT3A* mutations are those markedly sensitive to the induction of differentiation [104]. In addition to these AML subtypes, also AML characterized b a granulo-monocyte progenitor (GMP)-like phenotype display sensitivity to Bleximenib: these cases include leukemias bearing CEBPA mutations [104]. Gene expression studies showed that AMLs responding to Bleximenib display some epigenetic alterations inducing a striking upregulation of HLA class I and class II expression through a mechanism independent of *MEIS 1* loss [99]. These epigenetic changes resulted in an enhanced sensitivity of leukemic cells to T cell-mediated cytotoxicity in allogeneic and autologous settings [104].

In line with this observation, previous studies have shown that: CEBPA is an essential collaborator in HOXA9/MEIS 1-mediated leukemogenesis [105]; CEBPA and KMT2A are co-localized on chromatin and CEBPA-mutated HPCs are hypersensitive to pharmacologic targeting of KMT2A complex using Menin inhibitors [106].

DSPP-5336 (Enzomenib)

Enzomenib is a potent, orally bioavailable inhibitor of menin-MLL interaction exhibiting antiproliferative activity *in vitro* in cell lines, primary leukemic blasts and patient-derived xenograft mouse models of *KMT2A*-rearranged or *NPM1*-mutant AMLs [102]. Enzomenib directly binds to Menin (Kd 6.0 nM) and inhibits Menin-KMT2A interaction (IC_{50} 1.4 nM) [107].

A phase I/II clinical study evaluated 81 patients in Arm A (without CYYP3A4 inhibitor) or in Arm B (with CYP3A4 inhibitor) for the safety and efficacy of a treatment based on Enzamenib monotherapy [108]. In the phase I portion of the stud it was evaluated that the active doses of Enzomenib correspond to ≥140 mg [109]. 35 patients were treated at the active Enzomenib dose: 22 *KMT2A*-rearranged (20 AML and 2 ALL) with an ORR of 59% and a CR+CRi of 22.7%; 13 *NPM1*-mutant AML patients with an ORR of 53.8% and CR+CRi of 23% [109]. Pharmacodynamic changes supported induction of cell differentiation with decrease in stemness markers (HOXA9, MEIS1) and increase in differentiation markers (CD11b) [104]. DS was observed in 11% of treated patients.

KO-539 (Ziftomenib)

Ziftomenib is an oral menin inhibitor targeting the Menin-KMT2A protein-protein interaction; Ziftomenib is metabolized to two metabolites with comparable activity to Ziftomenib itself. Preclinical studies have supported the antileukemic activity of Ziftomenib in models of *NPM1*-mutant and *KMT2A*-rearranged AMLs. Particularly, Ziftomenib induced Menin protein degradation through the ubiquitin-proteasome pathway, reduces MEIS1, FLT3, CDK6 and BCL2 protein levels, in

association with induction of differentiation and induction of cell apoptosis of leukemic cells harboring *MLL*-rearrangements or *NPM1* mutations [110]. Furthermore, co-treatment with Ziftomenib with BCL-2,CDK6 or BET inhibitors induced synergistic lethality in *MLL*-rearranged or *NPM1*-mutant leukemic cells [110]. These findings were confirmed and extended in a second study showing that Ziftomenib had a marked anti-proliferative activity in combination with drugs from various classes, including those targeting chromatin regulation and DNA damage as well as apoptosis and cell cycle block [111]. Particularly pronounced was the synergistic interaction between Ziftomenib and Venetoclax resulting in a pronounced anti-proliferative activity in *MLL*-rearranged and *NPM1*-mutant leukemic cells [111].

The KOMET-001 multicentre, open-label, multicohort, phase I/II clinical trial evaluated Ziftomenib in adult R/R AML patients; the study was subdivided into two phases: a phase Ia (dose-response) in which the patients received Ziftomenib (from 50 to 1000 mg) orally once daily in 28 day-cycles; a phase Ib in which patients with *KMT2A* rearrangements or *NPM1* mutations were randomly assigned to two parallel dose cohorts (200 and 600 mg Ziftomenib) [112]. In phase Ib no responses were observed in patients treated at 200 mg of Ziftomenib; at the recommended dose for phase II (600 mg) the rate of CR+CRi was 12.5% (2/16) in *KMT2A*-rearranged and 35% (7/20) in *NPM1*-mutant AML patients; the ORR in *KMT2A*-rearranged was 17% and 45% in *NPM1*-mutant AMLs; the median OS was 6.0 and 5.6 months, respectively in *KMT2A*-rearranged and *NPM1*-mutant AMLs [112].

The KOMET-007 phase I clinical study evaluated the safety and the efficacy of Ziftomenib combined with standard chemotherapy in 34 newly diagnosed AML patients with *KMT2A* rearrangements or *NPM1* mutations; the patients were treated either with 200 or 400 mg of Ziftomenib [113]. For *NPM1*-mutant AML patients, the CR+CRi rate was 100% at 200 mg and 86% at 400 mg, with MRD negativity among responders of 100% and 80%, respectively; for *KMT2A*-rearranged patients, CR+CRi rates were 90% at 200 mg and 63% at 400 mg, with MRD negativity among responders of 83% and 100%, respectively [113].

The ongoing KOMET-008 trial is an open-label, dose-escalation and expansion study aiming to determine the safety, tolerability, and preliminary efficacy of Ziftomenib in association with standard of care regimens for treatment of either R/R *NPM1*-mutant AML (arm A) or *KMT2A*-rearranged AML (arm B). The arm A is subdivided into 3 cohorts: cohort A-1 (Ziftomeniob+FLAG-IDA), cohort A-2 (Ziftomenib plus LDAC) and cohort A-3 (Ziftomenib plus Gilteritinib in *NPM1/FLT3* double mutant AML). The arm B is subdivided in two cohorts: cohort B-1 (Ziftomenib plus FLAG-IDA), cohort B-2 (Ziftomenib plus LDAC) [114].

BMF-219 (Icovamenib)

BMF-219 is the only covalent Menin inhibitor in clinical development under evaluation in multiple hematologic malignancies, solid tumors and diabetes mellitus, Preclinical studies have shown that BMF-219 show sustained and marked inhibition of Menin-dependent oncogenic signaling.

The phase I COVALENT-101 phase I dose-escalation and dose-expansion study evaluated BMF-219 in R/R acute leukemia (cohort 1), DLBCL (cohort 2), multiple myeloma (Cohort 3) and CLL (cohort 4) [115] A recent report analyzed the results observed in 26 R/R AL patients (24 AML and 2 ALL) enrolled in two parallel arms, with or without a CYPP314 inhibitor [115]. BMF-219 was usually well tolerated, with no dose-limiting toxicities; DS was observed in 13% of cases [115]. 5 patients were evaluable for response and 2 achieved a CR (1 CR and 1CRi) [115].

Recurrent chromosomal rearrangements involving the Nucleoporin 98 (NUP98) gene, detected in 5-10% of pediatric AML cases and in 2-4% of adult AMLs, are classified as high-risk AMLs [116]. In NUP98 rearrangements the NUP98 gene is fused with various partners, the most frequent being NSD1 and KDM5A [116]. NUP98 fusions elicit leukemogenesis through interaction with histone modifying complexes: particularly, NUP98 fusion proteins interact with KMT2A complexes and are co-localized with KMT2A at the level of *HOX A/B* genes [117]. *NUP98*-rearranged AMLs are Menin-dependent as supported by the observation that the Menin inhibitor VTP50469 induced antiproliferative effects and prolongation of survival in mouse AML models driven NUP98 fusion proteins [118].

Another study showed that the Menin inhibitor Revumenib inhibited proliferation and survival of primary NUP08 fusion protein-positive AML cells and inhibited numerous NUP fusion protein target genes, such as MEIS1 and CDK6 [114].

In order to exert their leukemogenetic activity NUP98 fusion proteins and KMT2A-Menin antagonize the non-canonical polycomb repressive complex 1.1 (PRC1.1) [115].

Interestingly, Carraway et al. reported the case of a patient with NUP98-fusion relapsed after prior treatments, including allo-HSCT; the patient was treated with BMF-219 and achieved a CR after few cycles of treatment; unfortunately, after 5 months of treatment, the patient relapsed with *NUP98-NSD1*-positive AML [115].

The findings of this case report suggest that Menin inhibitor monotherapy is not sufficient to obtain a complete eradication of leukemic clones in *NUP98* fusion-positive AML patients and that the cooperation between Menin inhibition and kinase inhibitors targeting either CDK6 or nFLT3 strongly cooperate in *NUP98* rearranged primary AML cells and in PDX models [116].

3. Conclusions

In AML, different genetic events determine a block in the differentiation of leukemic cells at various stages of the differentiation/maturation process. Targeted therapy developed in the late twentieth century have introduced a new therapeutic approach based on the targeting of a specific abnormality present in a subset of AML cells. One of the aims of target therapy consists in forcing leukemic cells to differentiate. The prototype of successful differentiation therapy was represented by the treatment of APL using differentiation-inducing agents ATRA and ATO that have completely revolutionized the therapy of this AML subtype, transforming the outcome of this leukemic subtype from the deadliest to the most curable.

However, ATRA and ATO resulted to be not effective in AML outside APL, thus providing evidence that differentiation block observed in various AML subtypes does not have a uniform underlying mechanism causing differentiation impairment. Thus, it emerged the idea that the strategy to induce differentiation in other AML subtypes could be induced through the targeting of specific alterations during the leukemic process. This view was strongly supported by recent studies showing that IDH inhibitors, LSD1 inhibitors and Menin inhibitors induce the differentiation of some AML subtypes. Although these agents have shown a marked capacity to induce leukemic cell differentiation associated with antileukemic effects, the therapeutic success was clearly lower than that observed using ATRA+ATO in APL. This lower efficacy of differentiation therapy based on IDH, LSD1 and Menin inhibitors compared to ATRA+ATO in APL is seemingly related to a higher genetic complexity and heterogeneity of *IDH*-mutant AMLs, *KMT2A*-rearranged and *NPM1*-mutant AMLs compared to APL cells in which all the pathogenic events are driven by PML-RARA fusion protein.

However, in spite these difficulties, these agents represent an important therapeutic tool for improving the outcomes of *IDH*-mutant, *IDH*-mutant and *KMT2A*-rearranged AML patients and possibly also of other AML subtypes. Optimal responses induced by IDH, LSD1 and Menin inhibitors may be significantly improved by strategic partnering with other therapies: ongoing and future clinical studies will clarify the impact of these association therapies, appropriately extended to AML patients at various disease stages. In this context, promising is the introduction of some of these agents into first-line treatment of elderly non-fit patients using triplet drug combinations.

Future studies will be required to evaluate the impact of these agent on overall survival in the context of various therapeutic settings involving R/R and first-line patients. Future studies will assess also the potential therapeutic impact of these drugs as maintenance therapy or treatment for eradication of MRD.

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