**INTRODUCTION**

For decades, intensive induction therapy consisting of a 7-day continuous infusion of cytarabine and a 3-day course of daunorubicin ("7+3") has been the backbone of acute myeloid leukemia (AML) treatment. This regimen achieves durable response mainly in young and fit patients with favorable-risk AML. However, most patients with AML are older than 65 years with multiple comorbidities and the clinical outcomes in this population are poor, mostly due to unfavorable-risk genetics and low tolerance to intensive chemotherapy. Recently, CPX-351, a liposomal formulation with a fixed combination of cytarabine and daunorubicin, has been approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of newly diagnosed AML patients with a poor prognosis. In the randomized, phase III CITR0310-301 trial, the liposomal-encapsulated combination versus the standard combination of cytarabine and daunorubicin ("7+3" regimen) was associated with significantly prolonged median overall survival (OS) (9.6 months vs 5.9 months; HR: 0.69 [95% CI 0.52–0.90], p=0.005) in patients aged 60–75 years with newly diagnosed high-risk or secondary AML. The improved OS with CPX-351 versus "7+3" was maintained after a follow-up of 5 years in this patient population. The antitumor activity of this therapy was also demonstrated in a real-world analysis of high-risk AML patients treated with frontline CPX-351, with a median OS of 21 months and a 1-year OS rate of 64% after a median follow-up of 9.3 months. A very recent retrospective comparative analysis further demonstrated that CPX-351 versus hypomethylating agent (HMA) plus venetoclax, a B-cell lymphoma-2 (BCL-2) protein inhibitor, was associated with prolonged OS in the overall population (range, 34–93 years). However, there was no difference in outcome between the two treatment regimens in the group of patients aged 60–75 years, despite a more than doubled rate of transplant in the CPX-351 arm.

**Abstract**

Acute myeloid leukemia (AML) is the most common type of acute leukemia in adults and is associated with poor long-term survival and a high relapse rate, mainly due to relapse and resistance to available therapies. The recent advancements in the technologies for genomic profiling, particularly next-generation sequencing (NGS), have enabled the identification of recurrent and novel genetic mutations implicated in the pathogenesis of AML. This resulted in refined risk stratification and the development of more effective targeted therapies, like FMS-like tyrosine kinase 3 (FLT3) and isocitrate dehydrogenase 1/2 (IDH1/2) inhibitors. Over the last years, B-cell lymphoma-2 (BCL-2), a key regulator of the intrinsic apoptotic pathway, has emerged as a relevant target for therapy for many diseases including AML, and promising results were reported with the use of BCL-2 inhibitors. This article will present an overview of some recent breakthroughs in the field of AML, with a focus on the latest drug approvals in AML. The assessment of minimal/measurable residual disease (MRD) and its role in treatment decision-making will also be briefly discussed.

**Keywords:** acute myeloid leukemia (AML), targeted therapy, immunotherapy, epigenetics, minimal/measurable residual disease (MRD)

**Figure 1. Distribution of genetic alterations in acute myeloid leukemia.** Adapted from Döhner et al. 2017. The hypomethylating agent (HMA) azacitidine is another treatment option for elderly patients with newly diagnosed AML with >30% bone marrow blasts who are not suitable for or cannot tolerate intensive chemotherapy. The efficacy and safety of this agent was established in the randomized, open-label, phase III AZA-AML-001 trial, which showed improved median OS with azacitidine versus conventional care regimens (CCRs) in patients aged >65 years (10.4 months vs 6.5 months; HR: 0.85 [95% CI 0.69–1.03], p=0.1009), with 1-year OS rates of 46.5% and 34.2%, respectively. This OS benefit was maintained across all prespecified subgroups, including poor-risk cytogenetics. Azacitidine was generally well-tolerated in this patient population; nausea, neutropenia and thrombocytopenia were the most common treatment-emergent adverse events.

In addition to age and the presence of comorbidities, the genetic background of the patient should be considered when assessing disease prognosis and planning the treatment. Recurrent mutations, identified mainly by next-generation sequencing (NGS), play an important role in AML prognosis and the response to the therapy (Figure 1). Their identification led to the development of genetic classification systems, which underlie the variability of this heterogeneous disease and refine risk stratification. In 2010, the European LeukemiaNet (ELN) defined the first genetic-based stratification system for AML, which was recently revised, following the better understanding of the impact of recurrent mutations on outcomes after intensive chemotherapy. The most important changes in the updated guidelines include the refined definition of the three prognostic (genetic-risk) subgroups (favorable, intermediate and adverse) based on leukemia cell cytogenetics and mutations. Another novelty is the quantification of the FLT3-internal tandem duplication (ITD) allelic burden defined by the ratio of mutated and normal alleles. In addition, a specific AML subtype should be defined by genetic aberrations at the time of diagnosis, as well as the presence of particular gene mutations during or after treatment. For example, the detection of a residual mutation in NPM1 transcripts during complete remission in patients with NPM1-associated AML indicates an increased probability of relapse. This topic is briefly discussed in the section Monitoring disease progression with MRD.
Mutations in FLT3, which are present in about 30% of patients with AML, result in constitutive activation of the receptor and its downstream pathways.23 FLT3 mutations are specific to the AML phenotype and may be detected in almost all AML subtypes, whereas they are rarely present in other myeloid neoplasms.24 The first-in-class FLT3 inhibitor midostaurin was approved in 2017 by the European Medicines Agency for the treatment of patients with newly diagnosed FLT3-mutated AML in combination with standard chemotherapy for induction and consolidation and as monotherapy for maintenance treatment.25 The approval was based on the OS benefit with midostaurin plus chemotherapy versus placebo in patients with AML and an FLT3 mutation, as demonstrated in the RATIFY trial (Figure 2).26 Following the FDA and EMA authorizations, the Swedishmed approved also gilteritinib,27 a more potent and specific FLT3 inhibitor, as a single agent for the treatment of patients who have relapsed or refractory (R/R) AML with FLT3 mutations. Efficacy was demonstrated in the international, controlled phase III ADMIRAL trial.28 In this study, 371 adult patients with R/R FLT3-mutated AML were randomized to receive either gilteritinib (n=247) or salvage chemotherapy (n=124). In the final analysis, gilteritinib was associated with significantly prolonged OS versus chemotherapy (9.3 months vs 5.6 months; HR: 0.64 [95% CI: 0.49–0.83]; p=0.001). Furthermore, the proportion of patients who achieved complete remission (CR) with full or partial hematologic recovery was 34.9% in the gilteritinib group and 15.3% in the chemotherapy group, with a CR rate of 21.1% and 10.5%, respectively. The follow-up analysis at a median follow-up of 37.1 months showed consistent superior median OS (Figure 3), with a 1-year OS rate of 36.6% with gilteritinib and 19.2% with chemotherapy and a 2-year OS rate of 20.6% and 14.2%, respectively.29 These results were corroborated in the phase III, open-label, multicenter COMMODORE trial on Asian patients with R/R FLT3-mutated AML after first-line therapy, which demonstrated that gilteritinib versus salvage chemotherapy significantly prolonged OS (median, 9.0 months vs 4.7 months; HR: 0.54 [95% CI: 0.37–0.79]; p=0.00126) and event-free survival (EFS) (median, 2.8 months vs 0.6 months; HR: 0.55 [95% CI: 0.39–0.76]; p=0.00004) in this patient population.30 The ongoing head-to-head HOVON 156 trial further aimed to compare the efficacy of gilteritinib and midostaurin when combined with intensive chemotherapy in fit patients with newly diagnosed AML.31

Gilteritinib was also investigated in combination with azacitidine in newly diagnosed patients with FLT3-mutated AML ineligible for intensive induction chemotherapy.32 Although the trial did not meet the primary endpoint of OS and the key secondary endpoint of EFS, the composite CR (CRc) rates were significantly higher with gilteritinib plus azacitidine versus azacitidine alone (58.1% vs 26.5%; p<0.001).33 These results underline that achieving higher CR rates does not necessarily translate into better OS in elderly AML patients.

Several first- and second-generation FLT3 inhibitors are currently being investigated in phase II and phase III clinical trials, mostly in combination with chemotherapy.28 These include quizartinib, which provided a survival benefit and manageable toxicity profile versus chemotherapy in patients with R/R FLT3-ITD-positive AML.34 Furthermore, crenolanib, a potent type I pan-FLT3 inhibitor effective against both ITD and resistance-conferring tyrosine kinase domain (TKD) mutations, demonstrated clinical benefit in R/R AML patients both as monotherapy and in combination with the 7/8 s regimen.35 In the phase II SORMAIN study, treatment with sorafenib, another multikinase inhibitor, led to a reduced risk of relapse and death after allogeneic hematopoietic cell transplantation (HCT) for FLT3-ITD-positive AML.36 Other emerging combinations also demonstrated anti-leukemic activity, including sorafenib plus azacitidine in patients with untreated or relapsed AML and FLT3-ITD.37,38 as well as quizartinib plus azacitidine/low-dose cytarabine (LDAC) in patients with FLT3-ITD-mutated myeloid leukemias, including AML.39 Quizartinib combined with decitabine and venetoxan was also shown to be active in heavily pretreated and prior FLT3 inhibitor-exposed patients with FLT3-ITD-mutated AML in an ongoing, single-arm, open-label, phase II trial.40 Among those with R/R AML (n=23), the CR rate was 78% and the median OS was 7.6 months. However, the additional benefit compared with decitabine and venetoxan remains to be clarified. Data also suggested that RAS/RAF/MEK and FLT3 F691L mutations were associated with resistance to the treatment. There were no major safety signals and no grade ≥2 QTc prolongation was reported.

**IDH inhibition**

Mutations in the IDH1 and IDH2 genes are present in 8–15% of patients with AML and are associated with normal cytogenetic status.28,29 Authorized in 2017, enasidenib was the first IDH2 inhibitor that was approved for the treatment of adult patients with R/R AML harboring an IDH2 mutation,30 based on the results from the open-label, single-arm, phase II AG221-C-001 trial (Figure 4).31,32 The phase III randomized, open-label IDHentyrule trial further compared enasidenib monotherapy with CC1RIs including intermediate-dose cytarabine (IDAC), LDAC, azacitidine or best supportive care (BSC) in older patients with late-stage IDH2-mutated R/R AML previously treated with multiple AML therapies.33 The study did not meet the primary point, as no statistically significant improvement in OS was shown among patients treated with enasidenib versus BSC. At the 2021 ASH Annual Meeting & Exposition, results were reported from post hoc analyses of this study that included patients preselected to lower-risk azacitidine, LDAC or BSC only.34 The overall response rate (ORR) was substantially increased with enasidenib versus CC1R (41% vs 11%), with CR rates of 26% and 3%, respectively, and rates of hematologic improvement of 41% and 13%, respectively (p=0.001 for all comparisons). Enasidenib versus CC1R was also associated with prolonged OS (HR: 0.74 [95% CI: 0.56–0.97]; p=0.029) and EFS (HR: 0.68 [95% CI: 0.50–0.92]; p=0.011).

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In 2019, the FDA approved ivosidenib, an IDH1 antagonist, as a single agent for the first-line treatment of adult patients with IDH1-mutant AML who are ≥75 years old or who have comorbidities that preclude intensive induction chemotherapy. This was due to data from the open-label, single-arm, phase I/II AG120-C-001 trial, which showed that ivosidenib monotherapy induced durable remissions, favorable OS outcomes (Figure 5) and transfusion independence in patients with newly diagnosed AML, with tolerable safety profiles. The ongoing SAKK/HOVON 150 trial further aimed to assess whether ivosidenib or enasidenib can improve treatment outcomes in patients with newly diagnosed AML or myelodysplastic syndrome (MDS)-ER2 and an IDH1 or IDH2 mutation.

Many phase I and II trials are currently investigating different combination therapies encompassing IDH1/2 inhibitors, with encouraging results. These include enasidenib plus azacitidine in IDH2-mutated newly diagnosed AML14 and ivosidenib in combination with azacitidine for newly diagnosed AML15 and ivosidenib plus venetoclax with or without azacitidine in IDH1-mutated myeloid malignancies. The efficacy and safety of ivosidenib plus azacitidine in patients with newly diagnosed AML with an IDH1 mutation was assessed in AGILE, a randomized, double-blind, phase III study on 146 patients who were randomized 1:1 to receive either ivosidenib plus azacitidine or placebo plus azacitidine. Results showed that ivosidenib-containing regimen significantly improved clinical outcomes compared with placebo-containing regimen, including EFS (HR: 0.53 [95% CI: 0.31–0.91]; p=0.01), OS (median: 19.0 months vs 9.9 months; HR: 0.44 [95% CI: 0.27–0.73]; p=0.0005) and ORR (62.5% vs 18.9%; p<0.0001), with CR rates of 47.2% and 14.9% (p<0.0001), respectively, and CR plus partial hematologic recovery (CRh) rates of 52.8% and 17.6% (p=0.001), respectively. However, it remains to be clarified whether combinations with IDH1/2 inhibitors are superior to those with venetoclax.

Combinations with BCL-2 inhibitors
Targeted therapies may also be directed towards relevant pathways independent of mutational status. Swissmedic’s authorization of venetoclax16 in combination with an HMA (azacitidine or decitabine) or LDAC for the treatment of previously untreated AML in patients ineligible for induction chemotherapy, was based on the CR data of two open-label, non-randomized trials (Study M14-35817 and Study M14-38718) in this patient population. Efficacy was further confirmed in two randomized, double-blind, placebo-controlled trials. In VIALE-A, 431 patients underwent 2:1 randomization to receive venetoclax plus azacitidine (n=286) or placebo plus azacitidine (n=145). At a median follow-up of 20.5 months, the median OS was 14.7 months in the venetoclax-containing arm and 9.6 months in the placebo-containing arm (HR: 0.66 [95% CI: 0.52–0.85]; p=0.001) (Figure 6). Venetoclax-treated patients also achieved improved CR rate (37% vs 18% with placebo). The efficacy of venetoclax plus azacitidine versus azacitidine alone was evaluated in a subgroup of untreated AML patients with poor-risk cytogenetics, without or without TP53 mutations. Analysis of pooled data from VIALE-A and a phase Ib trial of venetoclax plus azacitidine/detriment presented at the 2021 ASH Annual Meeting & Exhibition indicated improved CR plus CR with incomplete blood count recovery (CRi) in patients with wild-type versus mutated TP53 (70.0% vs 40.4%). Venetoclax plus azacitidine versus azacitidine alone was further associated with increased remission rates in patients with wild-type and mutated TP53, while this treatment regimen prolonged duration of response and OS only in patients with wild-type TP53.

In VIALE-C, patients were randomized to receive venetoclax plus LDAC (n=143) or placebo plus LDAC (n=68). At a median follow-up of 12.0 months, CR+CRi rates were 48% with venetoclax plus LDAC and 13% with LDAC alone with CR achieved in 27% and 7% of patients, respectively. At the primary analysis, the study did not meet its primary endpoint of OS. However, after an additional 6-months of follow-up, the median OS was 8.4 months in the venetoclax plus LDAC arm versus 4.1 months in the LDAC alone arm, corresponding to a 70% reduction in the risk of death with venetoclax (HR:0.30 [95% CI: 0.20–0.49]; p<0.001).

When combined with gilteritinib, venetoclax induced a high ORR in patients with FLT3-mutated AML, particularly in patients with previous exposure to FLT3 tyrosine kinase inhibitor (TKI), in a multicenter, open-label, phase Ib clinical trial. In the final report, the modified CRi rate, defined as CR plus CR with incomplete platelet recovery (CRp) plus CRi plus morphologic leukemia-free state (MLFS), was 79% in patients with FLT3-ITD-mutated AML, 78% in FLT3-mutated patients with prior TKI exposure and 75% in all FLT3-mutated patients. At a median follow-up of 15.1 months, the median OS in patients with FLT3-ITD-positive AML was 10 months. Of note, gilteritinib plus venetoclax achieved molecular clearance of FLT3 allotypic burden <10−1 in 60% of patients with FLT3-ITD who attained modified CRi. Similarly, the treatment with venetoclax in combination with ivosidenib resulted in high ORR, with a CR rate of 44% and CR+CRi rate of 78% in patients with IDH1-mutated myeloid malignancies in a phase Ib/II study.

Adapted from Roboz et al. 2020.11

Adapted from DiNardo et al. 2020.12
Inhibition of Hedgehog signaling pathway

Both the FDA and EMA also approved glagidase, a small molecule HMG-CoA reductase inhibitor signaling pathway, in combination with low-dose cytarabine for the treatment of patients aged at least 75 years or those ineligible for induction chemotherapy, but based on OS benefit reported in the randomized, phase II H BRIGHT AML-103 trial (median, 8.3 months with glagidase plus LDAC vs 4.4 months with LDAC alone; HR: 0.46 [95% CI: 0.30–0.71]; p=0.0002). At data cutoff, the ORR, defined as CR+CRi+MLFs, was 26.9% in the glagidase plus LDAC arm compared with 18% in the LDAC alone arm, including CR rates of 17.9% versus 2.6%, respectively, and CRi rates of 6.4% versus 2.6%, respectively.

IMMUNOTHERAPY FOR AML

In AML, immunotherapy includes antibody-drug conjugates (ADCs), mono- and bispecific antibodies, dual affinity re-targeting (DART) molecules and cell-based approaches, including chimeric antigen receptor (CAR) T-cell therapy. In December 2019, the ADC gemtuzumab ozogamicin, an antibody against CD33, a myeloid differentiation molecule inhibitor of the Hedgehog signaling pathway, in combination with either conventional chemotherapy, stem cell transplantation and participation in clinical trials.

Recent data also showed that the oral formulation of azacitidine, known as CC-486, is effective and well-tolerated as maintenance therapy in haematopoietic stem cell transplantation (HSCT)-ineligible patients with AML in first remission following induction chemotherapy, in an phase III, double-blind, placebo-controlled QUAZAR AML-001 trial, patients receiving CC-486 versus placebo experienced significantly prolonged overall and relapse-free survival. At a follow-up of 51.7 months, the median OS was maintained, with 5-year OS rates of 26% with azacitidine and 19.2% with placebo (HR: 0.69 [95% CI: 0.56–0.86]; p=0.0008). Results from QUAZAR AML-001 led to the authorization of azacitidine as induction therapy for newly diagnosed AML and high-risk MDS who are unfit for intensive induction chemotherapy. The high prognostic impact of MRD assessed by flow cytometry has been indicated in the HOVON/SAKK AML 42A Study. A meta-analysis showed that low MRD negativity is associated with superior disease-free survival and overall survival. A study conducted by Jongen-Lavresnic et al. (2018) further demonstrated that the detection of molecular MRD during CR had significant independent prognostic value regarding relapse and survival rates. Finally, a meta-analysis evaluating the association between MRD status and survival in hematologic malignancies showed that low MRD negativity is associated with superior disease-free survival and OS in patients with AML, with the value of MRD negativity being consistent across age groups, AML subtypes, time of MRD assessment, specimen source and MRD detection methods.

Although intensive induction chemotherapy can provide complete remission in most patients with newly diagnosed AML, relapse rates remain above 50%, mainly due to the emergence of resistant clones. Thus, MRD monitoring during the treatment course is important for prognosis assessment and subsequent management, such as intensive chemotherapy, stem cell transplantation and participation in clinical trials. In MRD-negative patients with favorable-risk or intermediate-risk AML, stem cell transplantation is a potential curative option. However, in MRD-positive patients, stem cell transplantation is considered a second-line therapeutic option.
fusion transcripts, with increased levels after consolidation therapy and during remission being predictive of relapse risk. Based on these data, the ELN Working Party consensus document recommends quantification of NPM1 mutations, as well as PML-RARA, RUNX1-RUNX1T1 and CBFB-MYH11 fusion transcripts in AML patients at diagnosis, at least after 2 cycles of induction/consolidation therapy and every 2 months (for 24 months) after the end of consolidation. Ongoing molecular MRD monitoring beyond 24 months of follow-up should be based on individual clinical features.

CONCLUSIONS

In the past few years, the introduction of more effective and less toxic targeted therapies has improved the understanding of disease biology, refined diagnostic criteria and new advanced tools for disease monitoring have improved response rates and survival of patients with acute myeloid leukemia (AML). The personalized approach, which provides the opportunity to deliver treatments tailored to each patient, has achieved clinical benefit in a proportion of patients with AML, especially of those who do not respond to intensive chemotherapy regimens or cannot tolerate them. AML, however, remains incurable in most cases and aggressive stem cell transplantation (SCT) is still an important treatment option. To fill this medical need, many investigational agents and immuno-therapies, are currently being investigated, including mono- and bispecific antibodies, cell-based therapies and immune checkpoint inhibitors.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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