REVIEW ARTICLE

The clinical impact of the molecular landscape of acute myeloid leukemia

Sabine Kayser[1] and Mark J. Levis[2]

[1]NCT Trial Center, National Center of Tumor Diseases, German Cancer Research Center (DKFZ), Heidelberg, Germany and [2]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University Baltimore, MD, USA

Correspondence: S. Kayser
s.kayser@dkfz-heidelberg.de

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Abstract

Research into the underlying pathogenic mechanisms of acute myeloid leukemia (AML) has led to remarkable advances in our understanding of the disease. Mutations now allow us to explore the enormous diversity among cytogenetically defined subsets of AML, particularly the large subset of cytogenetically normal AML. Despite the progress in unraveling the tumor genome, only a small number of recurrent mutations have been incorporated into risk-stratification schemes and have been proven to be clinically relevant, targetable lesions. The current World Health Organization Classification of myeloid neoplasms and leukemia includes eight AML categories defined by recurrent genetic abnormalities as well as three categories defined by gene mutations. We here discuss the utility of molecular markers in AML in prognostication and treatment decision-making. New therapies based on targetable markers include IDH inhibitors (ivosidenib, enasidenib), venetoclax-based therapy, FLT3 inhibitors (midostaurin, gilteritinib, and quizartinib), gemtuzumab ozogamicin, magrolimab and menin inhibitors.

Introduction

Acute myeloid leukemia (AML) is a genetically heterogeneous disease with identifiable somatic mutations in 97.3% of all cases.[1] Besides age and comorbidities, the prognosis for patients with AML is largely determined by the biology of the disease.[2] Targeted sequencing has identified several mutations that carry prognostic information, including mutations in *FLT3*, *NPM1*, *KIT*, *CEBPA* and *TP53*.[3] In addition, massively parallel sequencing led to the discovery of recurrent mutations in *DNMT3A* and *IDH*.[4,5] Consistently, the recurrent genetic abnormalities defining subtypes of AML are associated with distinctive clinicopathological features, impact prognosis, and are influencing treatment choices. Thus, AML with recurrent genetic abnormalities is included within the current World Health Organization (WHO) classification as a separate entity.[6] AML with *FLT3* is not included as a separate entity, because it occurs across multiple subtypes. However, the WHO classification acknowledges that *FLT3* mutations should be looked for in all AML cases.[6]

Although new molecular analysis techniques such as ultra-deep sequencing have helped to identify numerous recurrent genetic abnormalities, to date, only a limited number have been incorporated into risk-stratification schemes such as the National Comprehensive Cancer Network or European LeukemiaNet (ELN) guidelines.[7,8] Until recently most patients have been treated with similar chemotherapeutic regimens.[9] However, treatment options for AML have expanded as a result of the discovery of genetic abnormalities. Since 2017, eight new targeted drugs have been approved by the Food & Drug Administration (FDA) and six by the European Medicines Agency (EMA).[10] These new therapeutics subsume tyrosine kinase inhibitors (TKI), immune checkpoint inhibitors, monoclonal or bispecific T-cell engager antibodies, as well as metabolic and pro-apoptotic agents. Targeting *FLT3*-kinase signaling is particularly important given that approximately one third of AML patients have a *FLT3* mutation.[3] In this article, we give an overview of the clinical impact of molecular markers in AML and how they are used as improved strategies for cancer therapy.

Isocitrate dehydrogenase

Isocitrate dehydrogenase (IDH) is an enzyme that catalyzes the oxidative decarboxylation of isocitrate to α-ke-

toglutarate. Its two isoforms IDH1/IDH2 are recurrently mutated in roughly 20% of *de novo* AML.[3] Mutations in *IDH1* occur in about 8% of AML patients and are almost exclusively located at R132.[1] *IDH2* mutations can be detected in almost 12% and involve substitutions at R140 or R172.[11] *IDH* mutations are frequently associated with intermediate-risk or normal karyotype cytogenetics.[12,13] Genetically, *IDH* mutations are associated with *NPM1* mutations,[12-15] but less frequently co-occur with *TET2* or *WT1* mutations, which might be because all three classes of mutations affect DNA methylation.[16] Biologically, *IDH* mutations lead to increased levels of the oncometabolite 2-hydroxyglutarate and consecutively result in arrest of hematopoietic differentiation via inhibition of histone demethylation.[17,18]

The data regarding outcomes of patients with *IDH*-mutated AML are conflicting. Three reports from cooperative study groups showed a negative impact of cooperating *IDH1/2* mutations on relapse-free survival/relapse risk and overall survival (OS) in AML patients exhibiting the genotype mutated *NPM1* with unmutated *FLT3*-internal tandem duplication (ITD).[14,15,19] In a retrospective analysis of 319 patients with newly diagnosed, *IDH*-mutated AML (127 with *IDH1*, 135 with $IDH2^{R140}$, and 57 with $IDH2^{R172}$ mutations) treated with intensive chemotherapy in three Acute Leukemia French Association (ALFA) prospective trials the presence of *NPM1* mutations was the only variable predicting improved OS in multivariate analysis ($P<0.0001$).[20] In contrast, Patel *et al.* reported a favorable impact of the genotype mutated *NPM1* with unmutated *FLT3*-ITD only if cooperating *IDH1/2* mutations were present.[21] The prognostic significance of *IDH2* mutations in AML also seems to depend on the location of the mutation (*IDH1*: single nucleotide polymorphism *vs*. *R132;*[22] *IDH2*: R140 *vs*. 172).[23] The effects on survival are likely distinct for each of the *IDH* mutations, with the presence or absence of other mutations also affecting outcomes. Such opposing effects of genotypes on outcome highlight the statistical shortcomings of retrospective molecular studies.

Nevertheless, treatment with the IDH inhibitors enasidenib and ivosidenib has added to the armamentarium of targeted therapy. Currently, IDH inhibitors are approved by the FDA as treatment for relapsed/refractory AML with an *IDH* mutation as well as in newly diagnosed *IDH1*-mutated AML patients not eligible for intensive chemotherapy. In 2018, the approval of ivosidenib by the FDA was based on an open-label, single-arm phase I trial showing a complete response (CR)/complete response with incomplete count recovery (CRi) rate of 30.4% and a median OS of 8.8 months in patients with relapsed/refractory *IDH1*-mutated AML.[24] In the group of patients with newly diagnosed *IDH1*-mutated AML not eligible for intensive chemotherapy, a CR/CRi rate of 42.4% was achieved with a median OS of 12.6 months. Ivosidenib was subsequently approved by the FDA for this group of patients in 2019.[25]

In addition, enasidenib was approved by the FDA in 2017 for the treatment of relapsed/refractory AML with an *IDH2* mutation on the basis of a phase I/II trial.[26] The overall response rate (ORR) with enasidenib was 40.3% and the median OS was 9.3 months. Enasidenib also showed moderate efficacy in a phase I/II trial of 39 older patients with newly diagnosed AML, resulting in a CR/CRi rate of 21%, an ORR of 30.8%, and a median OS of 11.3 months.[27] However, primary and acquired resistance to these drugs are major clinical issues.[28] Leukemia stemness seems to be a major driver of primary resistance to IDH inhibitors, whereas the selection of mutations in *RUNX1/CEBPA* or RAS-RTK pathway genes seems to be the main driver of acquired resistance, along with *BCOR*, homologous *IDH* and *TET2* mutations, as could be shown by sequencing analysis in serial samples from 60 *IDH*-mutated AML patients treated with an IDH inhibitor.[28]

While *TET2* is directly affected by the 2-hydroxyglutarate-mediated oncometabolism, it is not yet clear how loss-of-function mutations in *BCOR*, a transcription corepressor,[29] contribute to acquired resistance to IDH inhibition.

In Europe, IDH inhibitors are currently not approved for *IDH*-mutated AML since the pharmaceutical company could not fully address the major objections raised by the Committee for Medicinal Products for Human Use to support a positive benefit/risk assessment in the proposed indication. Very recently, data from the global, randomized double-blind phase III trial (AGILE) evaluating ivosidenib + azacitidine in patients with newly diagnosed AML with an *IDH1* mutation were published.[30] Ivosidenib and azacitidine significantly improved the CR rate (47.2% *vs*. 14.9%; $P<0.0001$), event-free survival (hazard ratio [HR]=0.33, $P=0.002$) and OS (24 months *vs*. 7.9 months, $P=0.001$) as compared to placebo + azacitidine in patients with newly diagnosed *IDH1*-mutated AML ineligible for intensive induction chemotherapy. Based on these data, in March 2022 the pharmaceutical company submitted a marketing authorization application to the EMA for ivosidenib in combination with azacitidine as first-line treatment in patients with previously untreated *IDH1*-mutated AML who are not eligible for intensive chemotherapy. In addition, on May 25, 2022 the FDA approved ivosidenib in combination with azacitidine for newly diagnosed AML with an *IDH1* mutation, as detected by an FDA-approved test in adults 75 years or older, or who have comorbidities that preclude the use of intensive induction chemotherapy.

Currently, a study of ivosidenib or enasidenib in combination with induction and consolidation chemotherapy, followed by maintenance therapy in patients with newly diagnosed AML or myelodysplastic syndrome (MDS) with excess blasts 2 with an *IDH* mutation is also recruiting (HOVON150AML; NCT03839771).

A high CR rate was also achieved in the VIALE-A study in AML patients not eligible for intensive chemotherapy after treatment with hypomethylating agents (HMA) and venetoclax,[31] particularly in patients with *IDH* (CR+CRi rate: 75.4% *vs*. 10.7%; *P*<0.001) or *NPM1* mutations within a normal karyotype (CR+CRi rate: 66.7 *vs*. 23.5%; *P*=0.012; VIALE-A).[32] Regarding durable remissions with responses lasting for >12 months, *NPM1* (9/18; 50%) and *IDH2* (7/18; 39%) were among the most frequently mutated genes, with survival ongoing after 21 to 49 months follow-up.[32] Regarding *IDH1*-mutated patients, there was no difference in median OS between *IDH1*-mutated and wild-type patients (18.3 *vs*. 12.7 months; *P*=0.79).

Comparable results were achieved with low-dose cytarabine (LDAC) and venetoclax in the VIALE-C study, although the trial failed to meet its primary endpoint of improved OS with the addition of venetoclax to LDAC (7.2 months *vs*. 4.1 months; HR=0.75; 95% confidence interval [95% CI]: 0.52-1.07; *P*=0.11).[33] However, in an unplanned analysis with an additional 6 months of follow-up a significantly superior median OS of 8.4 months for venetoclax in combination with LDAC (HR=0.70; 95% CI: 0.50-0.98; *P*=0.04) as compared to 4.1 months after LDAC + placebo as well as overall response (48% *vs*. 13%; *P*<0.001) and CR rates (27% *vs*. 7%; *P*<0.001) were achieved.[33]

Based on these results, the FDA and EMA have approved venetoclax for newly diagnosed AML patients ≥75 years old or ineligible for intensive chemotherapy in combination with HMA or LDAC. Currently, these combinations of HMA or LDAC with venetoclax are standard of care in older/unfit patients with AML. There are no clear data to support the superiority of one HMA over another, although there are more data with the azacitidine combinations.

Recently, results from a phase Ib trial evaluating venetoclax in combination with standard chemotherapy in 51 elderly AML patients (median age, 72 years; range, 63-80 years) were published.[34] During induction, a 7-day prephase/dose ramp-up (days -6 to 0) was followed by an additional 7 days of venetoclax combined with cytarabine 100 mg/m^2 intravenously on days 1-5 and idarubicin 12 mg/m^2 intravenously on days 2-3 (i.e., “5 + 2”). Consolidation chemotherapy (4 cycles) included 14 days of venetoclax (days -6 to 7) combined with cytarabine (days 1-2) and idarubicin (day 1). Maintenance venetoclax was permitted (7 cycles). The combined chemotherapy with venetoclax was safe and tolerable, leading to an ORR (CR/CRi) of 72% in fit older patients with AML. Patients with *de novo* AML benefited particularly, with an ORR of 97% as compared to 43% in patients with secondary AML. The median OS for the entire cohort was 11.2 months (95% CI: 7.3-20.1 months). Patients with *de novo* AML had a longer median OS compared to those with secondary AML (31.3 *vs*. 6.1 months; *P*=0.0001).[34] Given these promising results, venetoclax in combination with daunorubicin and cytarabine (e.g. NCT04038437; V-FAST, NCT04075747) or standard intensive chemotherapy (e.g. NCT03709758; NCT04628026) is now being studied as frontline therapy in younger and older patients with AML. Preliminary data suggest a very high ORR of 100% (n=10), with 75% (n=6/8) of the patients achieving measurable residual disease (MRD)-negative remissions assessed using multiparameter flow cytometry. No dose-limiting toxicities were reported in the 200 or 400 mg dosing cohort, whereas one dose-limiting toxicity occurred in the 600 mg dose-escalation cohort (death due to septic shock). Thus, 400 mg (the current FDA/EMA-approved venetoclax dose for AML in combination with HMA) was determined to be the maximal tolerated dose in combination with ‘7 + 3’ induction. The median time to count recovery (defined as an absolute neutrophil count ≥0.5x10^9/L and a platelet count ≥50x10^9/L) following ‘7 + 3’ + venetoclax induction was 36 days.[35] These results, if confirmed in a larger number of patients, may soon indicate the new standard of care for younger, intensively treatable patients.

Venetoclax in combination with other agents is also being evaluated in relapsed/refractory AML patients (e.g. gemtuzumab ozogamicin [GO] + venetoclax, NCT04070768; gilteritinib + venetoclax, NCT03625505; NCT04330820; oral azacitidine + venetoclax, NCT04887857). Additionally, venetoclax in combination with azacitidine is being evaluated in MRD-positive AML/MDS patients after allogeneic stem cell transplantation (allo-SCT) (NCT04809181) as well as in patients with molecular relapse/progression of *NPM1*-mutated AML (NCT04867928).

Based on the above data, patients with *IDH1* mutations should be treated with ivosidenib (+ azacitidine), whereas for patients with *IDH2*, venetoclax in combination with azacitidine currently seems the best option, at least in older patients. For younger patients, who are eligible for intensive chemotherapy, treatment with intensive chemotherapy in combination with ivosidenib (*IDH1*-mutated) or venetoclax (*IDH2*-mutated) might be an option as soon as more mature data are available.

FLT3 mutations

Approximately one third of AML patients harbor activating *FLT3* mutations, which lead to constitutive activation of a receptor tyrosine kinase.[3] Given the high incidence of these mutations, they are attractive targets for small-molecule inhibition.[36] Currently, only two FLT3 inhibitors are approved by the FDA and EMA, midostaurin and gilteritinib. Midostaurin was the first approved TKI for use in combination with standard intensive chemotherapy for adult patients without age restriction with newly diagnosed *FLT3*-mutated AML in the USA and Europe.[37,38] The approval of midostaurin was based on the positive results of the

large, international randomized phase III CALGB RATIFY trial.[39] The addition of midostaurin to intensive chemotherapy significantly improved OS in younger adults with *FLT3*-mutated AML with a median OS of 74.7 months for the midostaurin arm (range, 31.5 months - not reached) as compared to 25.6 months for the placebo arm (range, 18.6-42.9 months). Interestingly, this improvement was regardless of the *FLT3* mutational status (either ITD or tyrosine kinase domain [TKD]) or the *FLT3*-ITD allelic ratio. Furthermore, patients undergoing allo-SCT in first CR had a better outcome if they were treated with midostaurin during induction therapy (P=0.08), suggesting that the optimal treatment strategy in *FLT*3-mutated AML would be to move on to allo-SCT early in first CR.[39] Given the remarkable difference in survival after allo-SCT early in first CR in patients treated with midostaurin as compared to those treated with placebo it seems that the combination of midostaurin with intensive chemotherapy results in deeper remissions. The first evidence of this came from a small translational study evaluating the level of MRD in 17 patients treated with cytarabine and anthracycline-based intensive induction chemotherapy.[40] A TKI was given to eight (47%) of the 17 patients during induction therapy. In all cases, samples were evaluated at diagnosis and at remission by a highly sensitive combination polymerase chain reaction next-generation sequencing MRD assay for *FLT3*-ITD. In those patients who were treated with chemotherapy in combination with a TKI during induction, the average level of ITD mutation was significantly lower than that in the nine patients treated with chemotherapy alone (P=0.008).[40]

Of note, despite inclusion of maintenance therapy on the RATIFY protocol, the FDA did not approve midostaurin as maintenance therapy, whereas the EMA included maintenance in the drug's product information.[41] Lack of re-randomization prior to maintenance was cited by the FDA as a major reason; thus, the contribution of maintenance therapy to the treatment effect could not be determined.[42] Results from a *post-hoc* subset analysis of the RATIFY trial demonstrated no difference in the disease-free survival between the treatment arms during the 12 cycles of maintenance (P=0.49) and no difference in OS from the time of starting maintenance (P=0.35).[43] Nevertheless, the data suggest that midostaurin may have delayed, but not prevented relapse in some of these patients, since more relapses were observed after stopping midostaurin (17/69 [25%] *vs*. 7/51 [14%] on the placebo arm), and more of these relapses occurred within the first 6 months (14 [20%] *vs*. 2 [4%]).[43] Unfortunately, patients were not re-randomized at the start of the maintenance treatment. Moreover, the numbers were too small for any clinically meaningful comparisons. Recently published results of a phase II trial evaluating midostaurin in combination with intensive chemotherapy followed by allo-SCT and single-agent maintenance therapy for 12 months in adult (median age, 54 years; range, 18-70, 30% older than 60 years) AML patients with *FLT3*-ITD showed that midostaurin in combination with intensive chemotherapy including allo-SCT can be safely administered, also in older AML patients.[44] In contrast to the RATIFY trial, in which midostaurin maintenance therapy was only applied after high-dose cytarabine consolidation, midostaurin maintenance therapy was also administered after allo-SCT. The landmark analysis at day 100 after transplantation favored maintenance therapy after allo-SCT with better event-free survival and OS in patients starting maintenance therapy within 100 days after transplantation.[44] Further evidence came from the RADIUS trial, evaluating midostaurin maintenance after allo-SCT.[45] Inhibition of FLT3 phosphorylation to <70% of baseline (achieved by 50% of the midostaurin-treated patients) was associated with improved relapse-free survival. Thus, the addition of midostaurin maintenance therapy following allo-SCT may provide clinical benefit in those *FLT3*-ITD patients with at least 70% inhibition of phosphorylation.[45] While this provides evidence that inhibition of FLT3 as post-transplant maintenance therapy is of value, midostaurin does not seem to be the drug of choice, given that there is no way to predict who will achieve adequate *in vivo* inhibition with this compound. More importantly, these data underline the need for randomized trials to establish the concept of maintenance with targeted agents after consolidation therapy to prevent AML recurrence.

Biologically, maintenance with a TKI to inhibit FLT3 signaling seems to be reasonable, particularly in light of the positive results from the SORMAIN,[46] RADIUS[45] and ADMIRAL[47] trials.

Gilteritinib, a novel, highly selective, potent oral FLT3 inhibitor with activity against ITD and TKD mutations, is the only FDA- and EMA-approved TKI for the treatment of relapsed/refractory *FLT3*-mutated AML in the USA and Europe.[48] The ideal dose of gilteritinib was identified from the multicenter, open-label phase I/II Chrysalis trial evaluating 252 *FLT3*-mutated patients. Gilteritinib resulted in prolonged responses in patients with heavily pre-treated, relapsed or refractory AML.[49] The ORR was 40%, with 8% achieving CR, 4% CRi, 18% CR with incomplete hematologic recovery (CRh), and 10% partial remission. The median OS was 25 weeks (95% CI: 20-30 weeks) and median duration of response 17 weeks (95% CI: 14-29 weeks).[49] In addition, gilteritinib was evaluated within a randomized, open-label, multicenter phase III trial (ADMIRAL trial) of relapsed/refractory *FLT3*-mutated patients, who were randomized 2:1 to receive gilteritinib or salvage chemotherapy.[50] Salvage chemotherapy options were LDAC, azacitidine, mitoxantrone/etoposide/cytarabine, or fludarabine/cytarabine/idarubicin and granulocyte colony-stimulating factor (FLAG-IDA). Randomization was strat-

ified by response to first-line AML therapy and pre-specified chemotherapy (intensive *vs*. low-intensity). The CR + CRh rate was 21%; the median time to response was 3.6 months (range, 0.9-9.6 months) and the median duration of response was 4.6 months.[50] The median OS was significantly longer after gilteritinib than in the salvage chemotherapy arm (9.3 months *vs*. 5.6 months), and 37.1% compared to 16.7% of the patients were alive at 12 months.[50] Furthermore, the OS benefit was observed in patients preselected for both high-intensity (HR=0.66, 95% CI: 0.47-0.93) and low-intensity chemotherapy (HR=0.56, 95% CI: 0.38-0.84).[50] Overall, the results support the use of gilteritinib in patients with relapsed/refractory AML. Based on these results, gilteritinib is now approved by the FDA and EMA for the treatment of relapsed/refractory patients with *FLT3*-mutated AML.

Recently, results from the phase III randomized trial comparing gilteritinib in combination with azacitidine *versus* azacitidine monotherapy for adult patients with newly diagnosed *FLT3*-mutated AML ineligible for standard chemotherapy were presented (LACEWING trial, NCT02752035).[51] While gilteritinib + azacitidine led to significantly higher composite CR rates, the combined therapy failed to improve OS compared to azacitidine alone. Thus, an independent data monitoring committee recommended terminating the study for futility, since concluding results are unlikely to show a statistically significant increase in OS. As a consequence, the pharmaceutical company has stopped enrollment.

In addition, results from a phase I/II study evaluating gilteritinib combined with the "7+3" regimen and consolidation treatment were presented at the annual meeting of the European Hematology Association.[52] In this dose-escalation study, 68 *FLT3*-mutated AML patients received gilteritinib (40, 80, 120, or 200 mg/day) in one of two schedules, in combination with the "7+3" regimen (schedule 1: days 4-17; schedule 2: days 8-21). The maximum tolerated dose of gilteritinib was determined to be 120 mg, which was evaluated in a dose expansion cohort. Remarkably, the composite CR rate of *FLT3*-mutated patients receiving gilteritinib on schedule 1 (n=22) was 100% and on schedule 2 (n=11) 81.8% with a median disease-free survival of 297 days.[52] Very promising results were also achieved in a phase I/II trial in 26 patients with either relapsed/refractory *FLT3*-mutated AML or high-risk MDS/chronic myelomonocytic leukemia (n=15) or patients with newly diagnosed *FLT3*-mutated AML (n=11) unsuitable for intensive chemotherapy.[53] All patients received azacitidine 75 mg/m^2 subcutaneously/intravenously on days 1-7, venetoclax for up to 28 days, and gilteritinib on days 1-28. The gilteritinib dose ranged from 80 mg to 120 mg daily during the phase I dose escalation (3+3 design). However, the triple therapy was so myelosuppressive that a bone marrow evaluation was performed on day 14 and if aplasia was noted, venetoclax was stopped. Encouraging results were achieved with an ORR (CR+ CRi + morphologic leukemia-free state) of 67% in relapsed/refractory patients and 100% in newly diagnosed patients (CR/CRi, n=9, 82%).[53]

Currently, several trials of gilteritinib are underway, including a trial of gilteritinib *versus* placebo as maintenance therapy after consolidation (NCT02927262) or after allo-SCT in patients with *FLT3*-ITD mutations (NCT02997202). There are ongoing trials combining gilteritinib with atezolizumab (NCT03730012) and venetoclax (NCT03625505) in patients with relapsed/refractory AML as well as a randomized phase II trial of gilteritinib *versus* midostaurin in combination with induction and consolidation chemotherapy (NCT03836209).

High CR rates have also been observed with standard induction therapy with the "7+3" regimen combined with crenolanib (72%)[54] and quizartinib (84%),[55] surpassing the 59% CR rate observed within the RATIFY trial.[39] In addition, quizartinib (40 mg/day on days 8-21) *versus* placebo in combination with standard induction and post-remission therapy (including allo-SCT) followed by up to 3 years of maintenance therapy with quizartinib (30-60 mg/day) or placebo was evaluated in 539 adult patients with *FLT3*-ITD AML within the global, randomized, double-blind, placebo-controlled phase III QuANTUM-First trial (NCT02668653).[56] Therapy with quizartinib resulted in a significantly longer median OS of 31.9 months as compared to 15.1 months with placebo. The safety of quizartinib was shown to be manageable and consistent with the known safety profile. Thus, the pharmaceutical company will share the data with regulatory authorities for possible global approval. Currently, quizartinib is approved in Japan as treatment for adult patients with relapsed/refractory *FLT3*-ITD mutated AML (approval granted in October 2019). While these results are premature, the high CR rates suggest that the benefit of selective FLT3 inhibitors is not just in depth of response, as with midostaurin, but that more patients may respond overall.

Nevertheless, although AML patients may respond to FLT3 inhibitors, the duration of the response is still mostly short due to primary and acquired resistance. The most common mechanism of resistance is due to acquired *FLT3*-TKD mutations, such as F691L and D835.[57] These mutations hinder TKI binding and lead to an active kinase conformation.[58,59] This mechanism of resistance was reported for type II inhibitors, including quizartinib. In contrast, gilteritinib and crenolanib showed clinical activity against *FLT3*-TKD D835 mutations. Nevertheless, the data suggest that this is mainly the case if the *FLT3*-TKD mutation coexists with *FLT3*-ITD, but not as much if *FLT3*-TKD D835 is the only mutation.[60] Moreover, they had limited activity against F691L mutations.[61] Further mechanisms of resistance are related to the acquisition of multiple RAS/MAPK pathway gene mutations at relapse, frequently in alter-

native clones, suggesting a high level of pathway reactivation.[62]

Recently, the selective and irreversible FLT3 inhibitor, FF-10101, was found to have significant activity against *FLT3*-ITD and -TKD mutations, including F691L and D835, both *in vitro* and *in vivo*.[63,64] Thus, the inhibitor was evaluated in a phase I dose escalation study in 52 patients with refractory/relapsed AML.[65] Continuous treatment with FF-10101 at a dose of 10-225 mg four times a day or 50-100 mg twice daily in pretreated patients (median number of prior therapies, n=3) resulted in a composite CR rate of 13% and a partial response rate of 8%, including those with activating *FLT3*-TKD mutations resistant to gilteritinib and other FLT3 kinase inhibitors.[65] Doses of 50-75 mg twice daily were well tolerated and resulted in sustained FLT3 inhibition. The trial is currently active, but not recruiting patients (NCT03194685).

We suggest treatment with gilteritinib for relapsed/refractory *FLT3*-mutated AML patients, particularly in those patients not eligible for intensive therapy strategies. In younger relapsed/refractory patients, gilteritinib could be used as bridge to transplant. Based on the QuANTUM-First data, quizartinib in combination with standard chemotherapy might soon be available as intensive first-line treatment in younger patients.

Whether patients with *NPM1*-mutated or core binding factor-rearranged (both CD33-positive), newly diagnosed AML and a *FLT3* mutation might benefit from combined therapy (midostaurin + GO + standard "7+3" chemotherapy) is currently being evaluated in a phase I/II trial (MOSAIC trial, NCT04385290).

Acute myeloid leukemia with mutated *NPM1*

NPM1 mutations are one of the most frequent molecular abnormalities in AML, particularly in patients with a normal karyotype.[1] *NPM1* mutations result in cytoplasmic accumulation of the protein, although it is presently still unclear how they contribute to leukemic transformation.[66] The *NPM1* mutations subtypes A, B, and D comprise 90% of all variants. These three mutation subtypes have been shown to be reliable markers for MRD detection with high sensitivity.[67,68] To date, however, more than 50 different *NPM1* mutations have been reported.[69] The same assay can be adapted for cases with rare *NPM1* mutation variants by replacing mutation-specific primers, but case-specific quantitative, reverse transcriptase polymerase chain reactions (RT-qPCR) need to be carefully established to avoid non-specific background amplification from the wild-type *NPM1* allele.[70]

In *NPM1*-mutated AML, concurrent mutations typically occur in *FLT3*, *DNMT3A*, *IDH1/2* or *TET2*.[1] Thus, the prognostic impact of *NPM1* mutations should be interpreted in the context of a *FLT3*-ITD, which occurs in roughly 45% of normal karyotype AML.[1,71-74] Particularly in *NPM1*-mutated patients with a concurrent high *FLT3*-ITD allelic ratio (≥0.5)[8,75-77] the favorable prognostic effect of *NPM1* is mitigated or even abolished as compared to that in patients with a low allelic ratio.[76,77] In comparison, patients with mutated *NPM1* without *FLT3*-ITD or *FLT3*-ITD with a low allelic ratio (<0.5) have a somewhat better outcome.[8,77] These data have recently been confirmed in a large cohort of intensively treated adult AML patients.[78] Moreover, *IDH1/2* mutations may also exert a negative prognostic impact on relapse-free survival and OS in patients with mutated *NPM1* without *FLT3*-ITD.[14,15] In a retrospective analysis of 319 patients with newly diagnosed AML and an *IDH* mutation (127 with *IDH1*, 135 with $IDH2^{R140}$, and 57 with $IDH2^{R172}$ mutations) treated with intensive chemotherapy in three Acute Leukemia French Association prospective trials the presence of *NPM1* mutations was the only variable predicting improved OS in multivariate analysis (P<0.0001).[20] In contrast, Patel *et al.* reported on a favorable impact of mutated *NPM1* without *FLT3*-ITD only if cooperating *IDH1/2* mutations were present.[21] Such opposing effects of genotypes on outcome highlight the statistical shortcomings of retrospective molecular studies.

In a study of 245 adult patients with *NPM1*-mutated AML, relevant MRD checkpoints could be defined.[79] Achievement of RT-qPCR negativity after two courses of induction therapy identified patients with a low cumulative incidence of relapse (6.5% after 4 years) as compared to that of RT-qPCR-positive patients (53% after 4 years; P<0.001), translating into significant differences in OS (90% *vs*. 51%, respectively; P=0.001). After completion of therapy, the cumulative incidence of relapse was 15.7% in MRD-negative patients as compared to 66.5% in MRD-positive patients (P<0.001).[79] Another study indicated that a *NPM1* mutation cut-off level of 0.01 after induction therapy, as measured by RT-qPCR (with a sensitivity of 10^{-6}), was associated with a cumulative incidence of relapse after 2 years of 77.8% for patients with ratios above the cut-off as compared to 26.4% for those with ratios below the cut-off. In addition, *NPM1* MRD positivity by RT-qPCR before allo-SCT is independently associated with a significantly increased risk of relapse and inferior survival.[81,82] Assuming that a further reduction of MRD levels optimizes outcome after allo-SCT, this relationship would justify risk-stratified treatment allocation, including the use of additional pre-transplant chemotherapy. However, as MRD might simply reflect reduced sensitivity of leukemia cells to chemotherapy, the presence of MRD might only mark those patients who are unlikely to be cured with subsequent similar-type therapies, even if disease levels are brought temporarily below the level of detection. Therefore, a further approach could be pre-emptive immune or antibody therapy

(NCT02789254) in MRD-positive patients, such as pembrolizumab and azacitidine (PEMAZA trial, NCT03769532) or venetoclax and azacitidine (VIALE-M trial, NCT04102020).

Increasing levels of *NPM1* MRD were also predictive of an impending relapse after chemotherapy (MRD increase >1% *NPM1*^{mut}/*ABL1*) or allo-SCT (MRD increase >10% *NPM1*^{mut}/*ABL1*).[83] Importantly, MRD status has been found to be a better predictor of relapse risk than *FLT3*-ITD in *NPM1*-mutated AML.[84]

In the randomized French ALFA-0701 trial showing the superiority of intensive chemotherapy in combination with GO over intensive chemotherapy alone *NPM1*-MRD was predictive for response to therapy since more MRD-negative results were obtained in patients treated in the GO arm than in those treated in the control arm after induction therapy (39% *vs*. 7%; *P*=0.006) as well as at the end of treatment (91% *vs*. 61%; *P*=0.028).[85] In addition, positive *NPM1*-MRD (defined as >0.1% in the bone marrow) after induction and at the end of treatment also predicted a higher risk of relapse, but did not influence OS.[85]

Patients with *NPM1* or *IDH2* mutations respond very well to venetoclax + HMA treatment with 2-year OS rates of 71.8% and 79.5%, respectively.[32] Similar results were documented within the phase III trial VIALE-A of venetoclax + HMA in newly diagnosed AML.[31] Thus, elderly patients with *NPM1* mutations or patients not eligible for intensive treatment should be treated with venetoclax in combination with azacitidine. *NPM1*-mutated AML also appears to be responsive to menin inhibitors (see below).[86]

CD33-positive acute myeloid leukemia

CD33 is highly expressed on cells of myeloid lineage, thus making it an attractive therapeutic target.[87] GO is a humanized anti-CD33 monoclonal antibody linked to the cytotoxic agent calicheamicin.[88] GO initially received accelerated approval from the FDA in 2000 for the treatment of patients with CD33-positive AML in first relapse who were ≥60 years and not suitable for intensive chemotherapy.[89,90] However, GO was voluntarily withdrawn from the market by the pharmaceutical company in 2010 when a phase III trial comparing standard induction chemotherapy

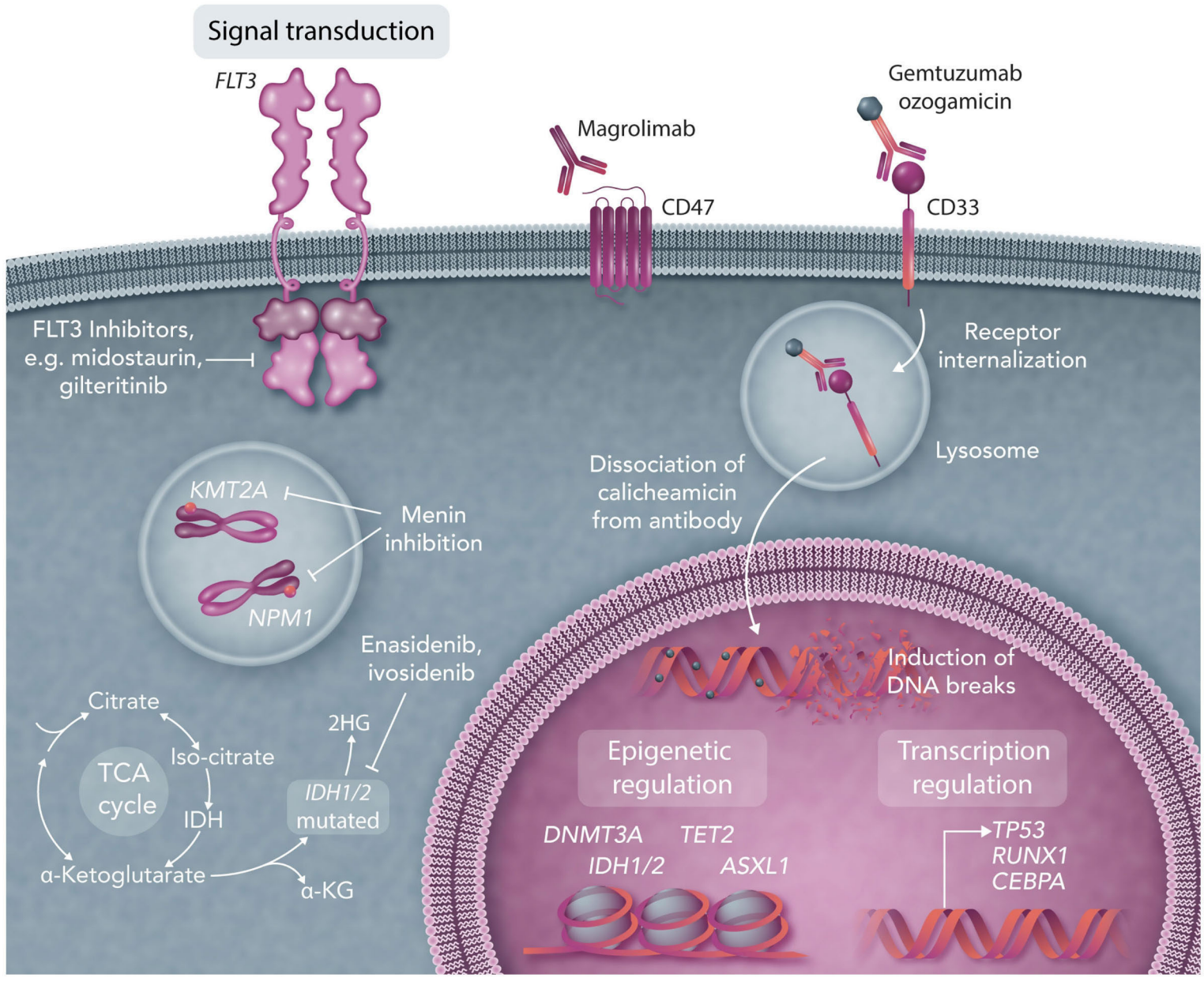

Figure 1. Genes recurrently mutated in acute myeloid leukemia as well as mechanism of action of targeted therapies. TCA: tricarboxylic acid cycle; IDH: isocitrate dehydrogenase; αKG: alpha-ketoglutarate; 2HG: 2-hydroxyglutarate.

("7+3") with or without GO in patients younger than 60 years showed an increased early mortality rate (6% *vs*. 1%).[91] Nevertheless, the early mortality rate in the standard arm was unexpectedly low. Consecutively, three other randomized trials showed improved OS rates with the addition of GO in patients with favourable- and intermediate-risk cytogenetics without increased induction mortality.[92-94] A reduced risk of relapse (*P*=0.0001) and improved survival (*P*=0.01) without increased rates of induction mortality in patients with favorable- and intermediate-risk cytogenetics were reported in a meta-analysis of five trials including 3,325 AML patients randomized to receive GO along with intensive induction chemotherapy.[95] Thus, GO was reapproved by the FDA in 2017 and by the EMA in 2018 for the treatment of adult patients (EMA: aged 15 years and older) with newly diagnosed CD33-positive AML. In addition, GO is licenced in the USA as monotherapy for the treatment of patients aged 2 years and older with relapsed or refractory CD33-positive AML as well as in patients with newly-diagnosed AML.

Thus, we suggest the use of GO in combination with intensive chemotherapy in patients with CD33-positive, favorable-risk AML (according to risk-stratification schemes such as the National Comprehensive Cancer Network[7] or ELN[8] guidelines).

Acute myeloid leukemia with *TP53* mutations

The tumor protein p53 (TP53) encodes a transcription factor that is involved in cell cycle arrest and apoptosis.[96] *TP53* mutations occur in roughly 12% of AML patients,[97] predominantly in therapy-related or secondary AML as well as in elderly patients.[98] Moreover, *TP53* alterations are found in roughly 70% of AML patients with a complex karyotype.[99] *TP53* mutations predict for very low CR rates (less than 30%) and were shown to be an independent poor prognostic factor among the subgroup of AML with complex karyotype.[99] Interestingly, TP53 could be identified in hematopoietic stem and progenitor cells in chemotherapy-naïve controls and in therapy-related or secondary AML patients years prior to development of overt disease, suggesting that hematopoietic stem and progenitor cells carrying TP53 may be chemotherapy-resistant and expand after treatment.[100] Individuals with clonal hematopoiesis with indeterminate potential have a 13-fold increased risk of developing a hematologic malignancy, and this risk may be increased in the context of cytotoxic therapy, at least if a *TP53* mutation is present.[100]

Recently published data suggest that treatment with decitabine at a dose of 20 mg/m² per day for 10 consecutive days in monthly cycles may improve the dismal outcome of AML with *TP53* alterations.[101] Although responses were not durable, they resulted in OS rates that were similar to those of AML patients with an intermediate-risk cytogenetic profile and who also received serial 10-day courses of decitabine.[101]

Moreover, in 55 patients with *TP53*-mutated MDS or AML, APR-246 (eprenetapopt), a novel, first-in-class, small molecule, in combination with azacitidine led to an ORR of 73% with a CR rate of 50% in MDS patients (n=20/40) and of 64% and 36% in AML patients (n=4/11). The median survival was 10.8 months with a significant improvement in responding *versus* non-responding patients by landmark analysis (14.6 *vs*. 7.5 months; *P*=0.0005).[102] Overall, 35% (n=19/55) of the patients underwent allo-SCT with a median OS of 14.7 months. APR-246 was also evaluated in a phase II trial in 52 *TP53*-mutated patients (34 with MDS, 18 with AML [including 7 with >30% blast cells]). The ORR among the patients with MDS was 62%, including a CR rate of 47%, with a median response duration of 10.4 months. The ORR among those with AML was 33%, including a CR rate of 17% (27% and 0% CR in the AML patients with less than and more than 30% bone marrow blast cells, respectively). The main treatment-related adverse events were febrile neutropenia (36%) and neurological events (40%), the latter correlating with a lower glomerular filtration rate at treatment onset (*P*<0.01) and higher age (*P*=0.05), and resolving with temporary drug interruption without recurrence after adequate APR-246 dose reduction. With a median follow-up of 9.7 months, the median OS was 12.1 months in MDS, and 13.9 and 3.0 months in AML with less than and more than 30% marrow blasts, respectively.[103] Recently, a randomized phase III trial of APR-246 in combination with azacitidine *versus* azacitidine alone in *TP53*-mutated MDS completed accrual (NCT03745716); the final results are pending. Additionally, novel doublet and triplet therapy with venetoclax and azacitidine in combination with APR-246 (NCT04214860, completed, final results are pending) or as post-transplant maintenance are being investigated (NCT03931291, completed, final results are pending).

Recent data from two phase I trials suggest a high response rate after combination therapy with venetoclax and decitabine, azacitidine[104] or low-dose cytarabine[105] in newly diagnosed elderly (≥60 years) AML patients not eligible for intensive chemotherapy, a group in whom a high incidence of *TP53* mutations would be suspected. Responses were also achieved in newly diagnosed patients with *TP53* mutations after treatment with venetoclax/azacitidine within the VIALE-A trial, although these were mostly short-lived and not durable.[31]

Although these data seem to be promising, durable responses are seldom observed. Thus, new treatment approaches are urgently needed for these very high-risk patients.

CD47-positive acute myeloid leukemia

CD47 is a "do not eat me" signal, strongly expressed in solid tumors and myeloid malignancies, which enables cells carrying this protein to evade macrophages.[106,107] In preclinical models of AML and MDS, it could be demonstrated that blocking CD47 enhances antitumor response[108,109] and that anti-CD47 antibodies stimulate antibody-dependent cellular phagocytosis, promoting priming and memory responses of CD8 T cells.[110]

Magrolimab targets CD47 on tumor cells, including macrophage phagocytosis.[10] Magrolimab is currently being evaluated in several early clinical trials in AML (e.g. NCT04435691). Recently, a phase I trial of magrolimab with azacitidine documented an ORR of 91% in patients with MDS and a CR rate of 42%.[111] In addition, high response rates were observed in *TP53*-mutated MDS patients (NCT03248479). Of note, AML patients with a *TP53* mutation (n=12) showed a CR/CRi rate of 75%. With a median follow-up of 8.8 months, the median duration of response or OS was not met.[112] Overall, the therapy was well tolerated, and no treatment-related febrile neutropenia occurred. Common treatment-related adverse events were anemia (44%), fatigue (18%), infusion reactions (18%), neutropenia (8%) and thrombocytopenia (5%). In addition, no patient discontinued treatment due to an adverse event. The mean decline in hemoglobin levels with the first dose of magrolimab + azacitidine was 0.4 g/dL. Fifty-eight percent of the patients no longer required red blood cell transfusions.[112] Encouraging results were also reported in a single-arm phase Ib trial of magrolimab + azacitidine at the European Hematology Association meeting 2022 in 72 *TP53*-mutated AML patients, unsuitable for intensive chemotherapy.[113] Magrolimab + azacitidine produced durable responses with an ORR of 48.6% (CR, 33.3%; CRi/CRh, 8.3%; morphological leukemia-free state, 1.4%; partial response, 5.6%) and an encouraging OS of 10.8 months (95% CI: 6.8-12.8 months). The combined therapy had an acceptable safety profile, although there were some safety concerns due to anemia (overall 29.2%; grade 3, 26.4%; grade 4, 2.8%). The most common grade ≥3 adverse events were febrile neutropenia (37.5%), thrombocytopenia (29.2%), pneumonia (26.4%), and neutropenia (20.8%). Grade 3 hemolysis was reported in one patient (1.4%); no grade 4 hemolysis was reported. Adverse events led to discontinuation of magrolimab in 30.6% of the patients and of azacitidine in 29.2% of the patients. Patients who proceeded to allo-SCT had an encouraging 1-year OS of 63%.[113] These observations are being further evaluated in a randomized, open-label phase III trial for adult AML patients with *TP53* mutations (ENHANCE-2, NCT04778397) as well as a randomized phase III study of triple combination therapy with venetoclax/azacitidine + magrolimab *versus* venetoclax/azacitidine + placebo (NCT05079230).

Lysine-specific methyltransferase 2A (*KMT2A*)

Mutations in the *KMT2A* gene (formerly known as Mixed Lineage Leukemia, *MLL*) at 11q23 occur in roughly 10% of acute leukemias.[114] *KMT2A* encodes a histone methyltransferase, which regulates homeobox genes affecting hematopoiesis.[115]

Menin is essential for the proliferation and survival of *KMT2A*-rearranged and *NPM1*-mutated AML.[116,117] *NPM1* mutations have also been associated with the upregulation of *HOXA* genes, similar to gene expression patterns observed in patients with *KMT2A* rearrangements.[118,119] These findings have led to the hypothesis that AML patients with *NPM1* mutations might also benefit from menin inhibition. Menin inhibitors are a novel class of agents targeting the underlying biology of *NPM1*- and *KMT2A*-mutated AML. In preclinical models, small-molecule inhibitors of the menin-KMT2A protein-protein interaction induce differentiation, downregulate critical gene expression programs, and confer a survival advantage in patient-derived xenograft models of *NPM1*- and *KMT2A*-mutated AML.[120-122] Four different menin-MLL1 inhibitors (SNDX-5613, NCT05326516; JNJ-75276617, NCT04811560; KO-539, NCT04067336; and DSP-5336, NCT04988555) are currently in early-phase clinical trials. Preliminary results in relapsed/refractory AML with *NPM1* and *KMT2A* mutations have shown promising clinical activity.[123] In particular, the ORR after single-agent therapy with SNDX-5613 in heavily pretreated patients with relapsed/refractory *NPM1*- or *KMT2A*-mutated AML was 55% with a composite CR MRD-negative rate of 31% (n=16/51).[86] In addition, responses were durable, lasting more than 6 months in six of the 12 patients who achieved a composite CR.[86] Finally, the highly selective, irreversible menin-inhibitor BMF-219 is currently being evaluated in a multicenter phase I trial in relapsed/refractory patients with acute leukemia, diffuse large B-cell lymphoma or multiple myeloma (NCT05153330).

Conclusions

Progress in unraveling the molecular pathogenesis of AML and the identification of the genetic determinants of response to treatment have been impressive and translation of these findings into clinical decision-making has been increasing in recent years. The availability of a molecular profile enables targeted-based treatment (Figure 1). The interplay between various molecular abnormalities suggests that the combined inhibition of several signaling pathways is required to achieve maximum clinical benefit. Thus, evaluation of the genetic profile at diagnosis, but also at relapse is mandatory in all AML patients. Open questions remain about whether patients should be

treated with intensive or non-intensive approaches and how best to incorporate maintenance therapy, monoclonal antibodies as well as immunotherapy.

Disclosures

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Contributions

SK and MJL wrote the manuscript.

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