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## Molecular therapy for acute myeloid leukaemia

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### Abstract

Acute myeloid leukaemia (AML) is a heterogeneous disease that is, in general, associated with a very poor prognosis. Multiple cytogenetic and molecular abnormalities that characterize different forms of AML have been used to better prognosticate patients and inform treatment decisions. Indeed, risk status in patients with this disease has classically been based on cytogenetic findings; however, additional molecular characteristics have been shown to inform risk assessment, including *FLT3*, *NPM1*, *KIT*, and *CEBPA* mutation status. Advances in sequencing technology have led to the discovery of novel somatic mutations in tissue samples from patients with AML, providing deeper insight into the mutational landscape of the disease. The majority of patients with AML (>97%) are found to have a clonal somatic abnormality on mutational profiling. Nevertheless, our understanding of the utility of mutation profiling in clinical practice remains incomplete and is continually evolving, and evidence-based approaches to application of these data are needed. In this Review, we discuss the evidence-base for integrating mutational data into treatment decisions for patients with AML, and propose novel therapeutic algorithms in the era of molecular medicine.

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Acute myeloid leukaemia (AML) is the most-common acute leukaemia in adults, and is primarily a disease of older adults (defined in this Review as those aged ≥ 60 years, unless otherwise stated), with a median age at diagnosis of 67 years<sup>1,2</sup>. The survival rates for younger adults with AML (aged <60 years) have improved, to some extent, over time, owing mostly to the development of intensive consolidation chemotherapy regimens, and improvements in supportive care and allogeneic haematopoietic-stem-cell transplantation (allo-HSCT) — the standard induction chemotherapy regimens have not changed substantially over the past 40 years<sup>3</sup>. In older patients, however, limited or no improvement

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in survival rates has been achieved, especially in patients aged >75 years, for whom no improvement in outcome has been demonstrated over the past three decades<sup>4</sup>.

The use of different therapies in the postremission treatment of AML is largely determined by prognostic risk stratification, which has classically been based on cytogenetic findings. The largest subset of patients with AML falls into the 'intermediate-risk' group, which includes those with normal-karyotype AML (NK-AML), as well as patients without either adverse or favourable cytogenetic abnormalities<sup>5,6</sup>. Considerable efforts have been made to refine risk stratification among this large and diverse subset of patients, with the emergence of multiple molecular abnormalities, including *FLT3*-ITD (*FLT3* internal tandem duplication), and mutations in *NPM1*, *KIT*, and/or *CEBPA*, which have been shown to predict outcome and have been incorporated into modern prognostic and treatment guidelines<sup>7,8</sup>, although, in addition to cytogenetic and molecular findings, clinical factors can also predict outcomes<sup>9</sup>. Advances in next-generation sequencing (NGS) technology have led to the discovery of novel somatic mutations in patients with AML, providing greater insight into the mutational landscape of the disease. The majority of patients with AML (>97%) demonstrate at least one clonal somatic abnormality on mutational profiling<sup>10–12</sup>. Through genomic analysis of samples from 200 patients with *de novo* AML, The Cancer Genome Atlas (TCGA) investigators demonstrated that the distribution of genomic aberrations includes mutations in signalling-pathway genes in 59%, DNA-methylation-related genes in 44%, chromatin-modifying genes in 30%, *NPM1* in 27%, myeloid-transcription-factor genes in 22%, transcription-factor-gene fusions in 18%, tumour-suppressor genes in 16%, spliceosome-complex genes in 14%, and cohesin-complex genes in 13% of patients<sup>12</sup> (TABLE 1), with findings from several other studies corroborating a similar distribution of mutations<sup>13–15</sup>. The use of mutation profiling in the clinic continues to evolve, and a substantial degree of variance remains among oncologists on how to apply genomic findings to clinical practice<sup>16</sup>. Consequently, evidence-based approaches are urgently required for the application of genetic data in molecular medicine for patients with AML.

In this Review, we propose that the lack of progress in improving the outcomes of patients with AML over the past four decades calls for consideration of a major paradigm shift regarding the present clinical treatment algorithms; for example, consideration of the use of upfront targeted therapy in elderly or unfit patients who are not candidates for standard intensive induction regimens, and addition of targeted agents to induction chemotherapy for younger patients, all in the setting of clinical trials (FIGS 1,2). We hypothesize that the integration of mutational profiling into the care of patients with AML will improve the outcomes associated with this characteristically devastating disorder. To accommodate the integration of mutational profiling into daily practice, more-high-throughput and more-comprehensive sequencing assays need to be used, in order to inform not only the approach to therapy for relapsed or refractory disease, but also decisions on upfront treatments.

## Genetic profiling and induction therapy

Karyotype and molecular alterations are powerful prognostic markers in AML; however, at present, these data are often unavailable at the initiation of induction therapy, particularly

outside of a clinical trial. As such, patients with AML generally receive induction therapy independent of their subsequent risk stratification; the choice of induction regimen is based on the patient's age and comorbidities, with 'fit' younger patients typically receiving standard induction therapy with cytarabine and an anthracycline<sup>7</sup>. Daunorubicin and idarubicin are the anthracyclines used most commonly in induction regimens, and neither agent is clearly superior to the other<sup>17,18</sup>. The findings of studies performed in the past decade, which are outlined in the following sections, indicate that molecular subtypes of AML correlate with improved responses to induction therapy in various subsets of patients, stressing the importance of integrating upfront comprehensive mutational profiling into initial treatment decisions.

### Candidates for intensive induction therapy

In the large, randomized, phase III Eastern Cooperative Oncology Group (ECOG) E1900 trial in which patients <60 years of age with AML were enrolled<sup>19</sup>, investigators observed an increased complete response (CR) rate and improved overall survival in patients who received induction therapy with high-dose daunorubicin (90 mg/m<sup>2</sup> daily for 3 days), compared with those given the 'standard' dose of 45 mg/m<sup>2</sup> daily for 3 days (CR rate 70.6% versus 57.3%,  $P < 0.001$ ; median overall survival 23.7 months versus 15.7 months,  $P = 0.003$ ). The improved CR rate with 90 mg/m<sup>2</sup> versus 45 mg/m<sup>2</sup> daunorubicin was replicated in a second trial conducted in Korea by the Cooperative Study Group A for Haematology (COSAH)<sup>20</sup>. By contrast, the UK National Cancer Research Institute (NCRI) AML17 trial compared 90 mg/m<sup>2</sup> daunorubicin with the more-conventionally used daunorubicin dose of 60 mg/m<sup>2</sup>, and the investigators found no differences in CR or overall survival between the treatment groups<sup>21</sup>. Notably, this trial was closed before the planned patient accrual target was met, after a signal of increased mortality within 60 days of treatment was noted in the 90 mg/m<sup>2</sup> daunorubicin arm<sup>21</sup>. In addition to the use of a higher dose of daunorubicin in the comparator arm, the AML17 trial design also incorporated a second course of induction therapy for all patients and included additional randomizations, such as the potential addition of lestaurtinib, gemtuzumab, or everolimus depending on *FLT3* status and cytogenetics<sup>21</sup>, thus limiting direct comparison of these findings with those of prior studies.

On subgroup analysis of the E1900 trial<sup>19</sup>, the benefit of high-dose daunorubicin was found to be limited to patients with favourable-risk and intermediate-risk disease, according to the definition proposed by Slovak *et al.*<sup>22</sup>, without a statistically significant benefit in patients with high-risk AML (median overall survival in the pooled favourable-risk and intermediate-risk subgroups was 20.7 months with standard-dose daunorubicin versus 34.3 months with high-dose daunorubicin;  $P = 0.004$ )<sup>19</sup>. Patel *et al.*<sup>11</sup> performed a mutational analysis of 18 genes in samples from 398 of the 657 patients enrolled in this trial to characterize whether unique mutational profiles predicted response to high-dose daunorubicin. The authors found that *DNMT3A* and *NPM1* mutations, and *MLL* translocations predicted better outcomes after receipt of high-dose daunorubicin, compared with the standard dose<sup>11</sup>. More recently, Sehgal *et al.*<sup>23</sup> confirmed the benefit of anthracycline-dose intensification for patients with AML and *DNMT3A* mutations in a retrospective cohort study. By contrast, cytogenetic findings can inform decisions on de-escalation of induction therapy, such as in the case of *PML-RARA* fusions seen in patients with acute promyelocytic leukaemia (APL), in whom

excellent outcomes have been demonstrated with the use of a chemotherapy-free regimen comprising all-*trans* retinoic acid (ATRA) and arsenic trioxide (ATO) for the treatment of low-risk disease, defined by a white-blood-cell count <10,000/ $\mu\text{L}$ <sup>24</sup>. Collectively, these findings suggest that mutational profiling might be useful in determination of the upfront induction treatment regimen in patients with AML.

A randomized study conducted by the European Organisation for Research and Treatment of Cancer (EORTC) and the Gruppo Italiano Malattie Ematologiche dell' Adulto (GIMEMA) Leukaemia Groups compared the efficacy of remission-induction therapy with daunorubicin, etoposide, and either standard-dose cytarabine (100 mg/m<sup>2</sup> per day by continuous infusion for 10 days) or high-dose cytarabine (3,000 mg/m<sup>2</sup> every 12 h by 3-hour infusion on days 1, 3, 5, and 7)<sup>25</sup>. In a planned subgroup analysis of this trial<sup>25</sup>, the investigators found that patients of all ages with *FLT3*-ITD mutations ( $n = 263$ ) benefited from high-dose cytarabine, compared with the standard-dose regimen; however, the benefit was statistically significant in patients aged <46 years (hazard ratio (HR) 0.70,  $P = 0.02$ ), but not in patients aged 46–60 years (HR 0.80,  $P = 0.14$ )<sup>25</sup>. Additional molecular abnormalities were not examined in this study, limiting the application of the findings to broader mutational profiling results. Nevertheless, the results of this trial suggest that certain genetically-defined subsets of patients with AML might benefit from dose-intensification, and that it might be possible to tailor chemotherapy regimens based on genetic profiles.

The randomized AML HD98B trial<sup>26</sup>, performed by the German–Austrian AML Study Group (AMLSG), demonstrated an improvement in the outcome of older patients (aged 61 years) with non-APL AML who received ATRA during and after intensive induction chemotherapy, as compared with those who received intensive induction chemotherapy alone. A subsequent correlative study demonstrated that *NPM1* mutations in the absence of *FLT3*-ITD mutation are a predictive marker of response to ATRA<sup>27</sup>. This correlative study was limited, however, by the relatively small numbers of patients in each subgroup ( $n = 60$  with *NPM1* mutation and  $n = 51$  with *FLT3*-ITD mutation), owing to incomplete profiling of the study cohort. Similar results were observed in the larger prospective AMLSG 07–04 trial<sup>28</sup>, in which younger adults were randomly assigned to receive intensive induction chemotherapy with and without ATRA. This trial included 289 patients with *NPM1* mutations<sup>28</sup>. The CR rate was significantly increased by addition of ATRA to the treatment regimen in only the patients with *NPM1*-mutated AML (OR 2.20;  $P = 0.05$ ), although the overall survival of all patients treated with ATRA ( $n = 549$ ) was significantly improved ( $P = 0.02$ ), compared with that of the patients who received chemotherapy only ( $n = 562$ )<sup>28</sup>. In a UK Medical Research Council (MRC) trial, however, Burnett and co-investigators<sup>29</sup> found no difference in outcomes with the addition of ATRA to induction chemotherapy with daunorubicin, standard-dose or high-dose cytarabine, and thioguanine in any of the molecular subgroups examined (*NPM1*, *FLT3*-ITD, and *CEPBA* mutated). Cross-trial comparisons limit the conclusions that can be drawn regarding whether ATRA improves outcomes among certain molecular subgroups of patients with AML, as the individual induction regimens used, and the dose and schedule of ATRA administration differed in these studies. For example, lower cumulative ATRA doses were used in the AMLSG trials<sup>26,28</sup> compared with the MRC trial<sup>29</sup>, in which ATRA was administered at the full 45 mg/m<sup>2</sup> dose on days 1–60. Taken together, the role of ATRA in any specific subset of

patients with non-APL AML remains unclear. Preclinical data have demonstrated that ATRA and ATO induce proteasome-mediated degradation of mutant NPM1 protein, and apoptosis of *NPM1*-mutant AML cell-lines and primary AML-cell samples from patients with such mutations<sup>30,31</sup>, suggesting another potential therapeutic approach that merits exploration.

On the basis of data showing valproic acid (VPA) acts as a potent histone deacetylase (HDAC) inhibitor, Tassara and colleagues<sup>32</sup> examined the therapeutic potential of the addition of VPA to standard induction therapy plus ATRA in patients with AML. This trial was stopped early owing to a lack of efficacy of the investigational VPA arm, the emergence of a trend towards a lower CR rate (40% versus 52%;  $P = 0.14$ ) and higher early mortality (26% versus 14%;  $P = 0.06$ ) in the VPA group<sup>32</sup>. Nevertheless, in an explorative subset analysis, it was noted that patients carrying *NPM1* mutations might be more sensitive to the addition of VPA, based on an improvement in 5-year overall survival on univariate analysis (52% in patients who received VPA versus 37% in patients treated with standard therapy;  $P = 0.03$ )<sup>32</sup>. The absolute number of patients with *NPM1* mutations enrolled in this trial was, however, low ( $n = 40$ ), and as such these data are hypothesis generating only and cannot be used to guide clinical practice.

### Lower-intensity therapy

Molecular data can also influence therapeutic decisions among patients who are not candidates for standard induction regimens. Data from TCGA Research Network indicates that 44% of patients with *de novo* AML carry mutations in genes encoding proteins that regulate DNA modifications, which have been demonstrated to have therapeutic implications<sup>12</sup>. In a small cohort of elderly patients with AML (median age of 74 years; range 32–85 years, with only one patient <60 years of age), Metzeler *et al.*<sup>33</sup> reported a significantly greater CR rate to the hypomethylating agent (HMA) decitabine in patients with *DNMT3A* mutations ( $n = 8$ ) versus those without such mutations ( $n = 38$ ): 75% versus 34% ( $P = 0.05$ ). The mechanistic link between mutations in *DNMT3A* — which reduce DNA methyltransferase activity — and increased response to HMAs has not, however, been elucidated. In addition, Itzykson and colleagues<sup>34</sup> have reported an increased response rate to HMA therapy with azacitidine in 13 patients with *TET2*-mutant myelodysplastic syndrome (MDS) or low-blast-count AML, compared with that observed in 73 patients without *TET2* mutations (82% versus 45%;  $P = 0.007$ ). Furthermore, in a small retrospective analysis of 42 patients with AML who received either decitabine or azacitidine, those with *IDH* mutations ( $n = 7$ ) had a higher response rate compared with those without mutations in the *IDH1/2* genes (71% versus 23%;  $P = 0.01$ )<sup>35</sup>. Finally, preclinical evidence has demonstrated that *WT1* mutations lead to a loss of TET2 function, suggesting that *WT1*-mutated AML might also be sensitive to HMA therapy<sup>36,37</sup>. These findings indicate that unified epigenetic pathway regulation by mutations in *TET2*, *IDH1/2*, *WT1* and/or *DNMT3A* might confer increased sensitivity to HMAs; although promising, validation of the findings in prospective trials is needed.

In contrast to these data, Quintás-Cardama *et al.*<sup>38</sup> noted a similar CR rate in patients with and those without *FLT3*-ITD mutations when treated with HMAs (51% versus 45%;  $P = 0.74$ ); however, they demonstrated an inferior CR rate in patients with *NPM1* mutations who

received HMAs as opposed to intensive chemotherapy (0% versus 70%;  $P=0.02$ ), although only three patients with *NPM1*-mutations received HMAs<sup>38</sup>. By contrast, in a randomized, phase II trial that examined responses to low-dose cytarabine with and without the polo-like kinase 1 (PLK1) inhibitor volasertib (a mitotic regulator that has been shown to inhibit the proliferation of leukaemia cells in preclinical studies<sup>39</sup>), the response rate among *NPM1*-mutant patients was 50% (7 of 14 patients)<sup>40</sup>. Low absolute numbers of *NPM1*-mutant patients in both studies prevent firm conclusions, although chemotherapy, in suitable candidates, seems to lead to more favourable responses than lower-intensity therapy with HMAs and volasertib in such patients.

### Addition of targeted agents

A series of small-molecule FLT3 tyrosine kinase inhibitors (TKIs) have been developed and tested in patients with AML, including midostaurin, lestaurtinib, lefitinib, sorafenib, quizartinib, and crenolanib. The preclinical and clinical experience with FLT3 inhibition has been the subject of numerous dedicated review articles<sup>41–44</sup>, and a comprehensive analysis of these agents is outside the scope of this Review. These agents were among the first targeted agents to be added to standard induction chemotherapy for patients with AML, and current evidence for this approach will be briefly discussed in the following paragraph.

Sorafenib inhibits a broad spectrum of kinases, including FLT3, PDGFR, VEGFR, KIT, and the RAF proteins, although an active metabolite, sorafenib *N*-oxide, is a more potent inhibitor of FLT3 than the parent compound<sup>45</sup>. Serve *et al.*<sup>46</sup> reported the results of a randomized, placebo-controlled trial investigating sorafenib in combination with intensive induction chemotherapy, using the 7 + 3 regimen comprising cytarabine and daunorubicin dosed at 60 mg/m<sup>2</sup>, in older patients with AML, and found that treatment in the sorafenib arm did not improve either event-free or overall survival. Moreover, use of sorafenib was associated with greater treatment-related mortality and lower CR rates, compared with the intensive chemotherapy regimen only<sup>46</sup>. This study was not restricted to patients carrying *FLT3*-ITD mutations; however, on subgroup analysis, the patients with *FLT3*-ITD-mutated AML also had poorer event-free and overall survival with sorafenib versus placebo treatment<sup>46</sup>. In the randomized, placebo-controlled SORAML trial in patients of 60 years of age with AML<sup>47</sup>, investigators evaluated the efficacy of addition of sorafenib to standard induction chemotherapy, followed by high-dose cytarabine consolidation therapy; patient enrolment was not restricted by genotype, and the findings of the study demonstrated an improvement in 3-year relapse-free survival (RFS) in the patients who received sorafenib versus the placebo group (56% versus 38%;  $P=0.017$ )<sup>47</sup>. This benefit did not translate into an improvement in overall survival (63% with sorafenib versus 56% with placebo;  $P=0.382$ ), and a higher incidence of some toxicities (fever, bleeding, and hand-foot syndrome) was observed in the sorafenib arm, calling into question the overall clinical benefit of this approach<sup>47</sup>. In a phase I/II study<sup>48</sup>, sorafenib was combined with low-dose cytarabine therapy for elderly patients (median age of 77 years (range 63–83 years)) who were ineligible for intensive induction therapy, independent of tumour genotype, and the results demonstrated a favourable safety profile; however, efficacy was limited, with an overall response rate (ORR) of 10%<sup>48</sup>. Finally, sorafenib has been combined with azacitidine in a phase II trial restricted to patients with *FLT3*-ITD mutations<sup>49</sup>, in which investigators

demonstrated a 46% ORR rate and a 27% CR rate. The favourable results of this trial, although not directly comparable to the permissive trials (that is, those that did not select patients based on genotype), nonetheless are suggestive of the importance of genotype-selected approaches in this patient population. The lack of specificity of sorafenib, coupled with a limited dataset in *FLT3*-ITD-positive patients, limits the ability to use these data to assess the importance of *FLT3*-ITD as a therapeutic target in newly diagnosed, and in relapsed or refractory, AML. Additional *FLT3* inhibitors have been combined with standard intensive induction therapy, or used as monotherapies in patients deemed unfit for intensive induction, including midostaurin (in the phase Ib setting<sup>50</sup>; a phase III trial is currently ongoing<sup>51</sup>) and lestaurtinib<sup>52,53</sup>. In the phase III setting, lestaurtinib treatment did not result in an improved CR rate compared with standard first-line chemotherapy<sup>52</sup>. Studies with newer agents, including crenolanib<sup>54</sup>, AC220 (REF. 55), and ASP2215 (REF. 56) are all ongoing.

### Future directions

As discussed, the presence of somatic mutations can clearly influence the response of patients with newly diagnosed AML to induction therapy, highlighting the underlying heterogeneity of this disease. The turnaround time required for high-throughput, comprehensive DNA sequencing, in addition to standard cytogenetic testing, to deliver clinically actionable data is the major barrier to clinical implementation of mutational data at time of diagnosis. The addition of targeted agents to induction therapy in a small set of AML trials demonstrates that rapid testing for alterations in a single gene is possible, but needs to be coordinated across cooperative sites in order to allow for adequate accrual of patients with rare mutations. Thus, future directions for research should include the continued development of faster DNA-sequencing platforms to allow for a reasonable turnaround time from sample receipt to reporting of results, enabling integration of these data to inform decisions on induction therapy<sup>57–60</sup>. To obtain statistically meaningful results from clinical trials, multicentre studies with upfront application of mutational data, acquired through a central lab or via a platform that can be performed at the different centres, will be required to investigate which molecularly targeted therapies are most effective in newly diagnosed patients with AML. A reasonable approach to the evaluation of novel agents would be to first examine their use as single agents in older patients who are not candidates for standard induction regimens, and subsequently apply those agents that show promise to the treatment of younger patients, in combination with standard induction therapy (FIGS 1,2).

### Application to postremission therapy

Mutational profiling has been demonstrated to facilitate decision-making regarding postremission therapy, such as the decision to proceed with allo-HSCT in patients with high-risk AML versus proceeding with chemotherapy-based consolidation alone in lower-risk groups; the data supporting this approach are reviewed in the following sections. As further mutational data emerge, determining whether unique mutational patterns can predict outcome after allo-HSCT will be important, as such patterns might inform the development of investigational agents in patients whose genomic profile is predictive of an adverse outcome with allo-HSCT.

## Postremission allografting

Studies assessing the effect of allo-HSCT on outcome are inherently limited by selection bias, in that often patients who do not undergo transplantation are ‘sicker’ than those who do<sup>61,62</sup>. Prospective studies that compare patients with available donors to ‘no-donor’ subsets limit this bias, but have other important limitations, including exclusion of patients without siblings and patients who never underwent HLA-typing<sup>63</sup>. Overall, the results of analyses have demonstrated a benefit of allo-HSCT for patients with intermediate-risk and high-risk cytogenetics<sup>64–67</sup>. Another approach to establish the benefit of allo-HSCT is the use of matched-pairs analyses, which incorporate patients with both sibling and unrelated donors to limit selection bias<sup>68</sup>. Many ‘donor versus no-donor’ studies have, however, been conducted using traditional cytogenetic-risk categories, without incorporating comprehensive mutational data.

**The influence of NPM1 and FLT-ITD mutations**—Schlenk *et al.*<sup>69</sup> evaluated the prognostic and therapeutic relevance of *NPM1*, *FLT3*, *CEBPA*, *MLL*, and *NRAS* mutations in 872 adults <60 years of age with NK-AML who were enrolled in one of four multicentre prospective AMLSG trials; 663 patients received postremission therapy, with 150 undergoing allo-HSCT from an HLA-matched related donor. Among the patients without a donor, who received either chemotherapy or autologous HSCT (auto-HSCT), no statistically significant differences were observed in patient outcome according to mutational status of the five genes analysed<sup>69</sup>. The authors performed Cox regression analyses of the RFS data to explore the role of allo-HSCT according to genotype; they concluded that patients with *FLT3*-ITD mutations, and patients with wild-type *NPM1* and *CEBPA* without the *FLT3*-ITD allele benefited from transplantation, whereas other subgroups either did not benefit or the numbers of patients were too small to conduct meaningful statistical analyses<sup>69</sup>. In 2015, Röllig *et al.*<sup>70</sup> reported the findings of another donor versus no-donor analysis regarding the role of allo-HSCT in patients with *NPM1*-mutant AML, which indicated that allo-HSCT led to improved RFS in this group; however, no improvement in overall survival was demonstrated, probably owing to the excellent response to salvage therapy in this patient subset<sup>70</sup>.

Extensive analyses regarding the effect of *FLT3*-ITD mutation status on outcomes of allo-HSCT have been performed, both in regard to the benefit of allo-HSCT and to the risk of relapse post-transplantation in this high-risk patient population. Firstly, in a prospective study in patients with intermediate-risk AML ( $n = 555$ ; 175 with *FLT3*-ITD mutations) by Bornhauser *et al.*<sup>71</sup>, patients with *FLT3*-ITD mutations had a significantly higher risk of relapse compared with those without *FLT3*-ITD mutations after consolidation chemotherapy (94% versus 59%, HR 4.0;  $P < 0.001$ ) or allo-HSCT (35% versus 19%, HR 2.7;  $P = 0.03$ ), but not auto-HSCT; however, the absolute rates of relapse for all patients undergoing allo-HSCT were lower than those for other treatments, and overall survival was significantly worse for the *FLT3*-ITD-mutant patients after consolidation with chemotherapy only (21% versus 46% in those without *FLT3*-ITD mutations, HR 2.2;  $P = 0.001$ )<sup>71</sup>. The difference in the cumulative incidence of relapse (CIR) in recipients of allo-HSCT compared with patients not undergoing allo-HSCT has been confirmed in a smaller retrospective analysis of 66 patients, 24 of whom had *FLT3*-ITD mutations<sup>72</sup>. Results have also indicated that the benefit



of allo-HSCT might be restricted to patients with a high *FLT3*-ITD allelic ratio 0.51 ( $P=0.02$  for RFS and  $P=0.03$  for overall survival)<sup>73</sup>. In a retrospective analysis of 206 patients who underwent allo-HSCT, 42% of whom had *FLT3*-ITD mutations, Brunet *et al.*<sup>74</sup> found that *FLT3*-ITD-mutant patients had a higher incidence of relapse and worse leukaemia-free survival compared with patients without *FLT3*-ITD mutations. In a study of data from 511 patients in the Center for International Blood and Marrow Transplant Research (CIBMTR) database, 31% of whom were *FLT3*-ITD positive, Deol and colleagues<sup>75</sup> found that the presence of a *FLT3*-ITD mutation increased the CIR after allo-HSCT, but did not have an effect on overall survival. Given the high risk of post-transplantation relapse in patients with *FLT3*-ITD-mutant AML, several investigators have examined incorporating *FLT3*-inhibitors as maintenance therapy after allo-HSCT. For example, phase I trials that examine the use of sorafenib<sup>76</sup> and quizartinib<sup>77</sup> in this context were reported at the 2014 American Society of Haematology (ASH) meeting, and the organizers of a midostaurin trial are currently recruiting patients<sup>78</sup>. Conclusions regarding these trials are restricted to the safety of the agents, and subsequent trials are needed to determine treatment efficacy.

**The influence of *MLL* rearrangements**—Wang *et al.*<sup>79</sup> have prospectively evaluated the effect of *MLL* rearrangements on the outcomes of 56 consecutive patients with acute leukaemias who underwent allo-HSCT; during the study period, an additional 29 patients with *MLL*-rearranged leukaemias did not undergo allo-HSCT for various reasons, including disease relapse while awaiting transplant, disease resistance, patient preference, donor availability, and death during induction therapy<sup>79</sup>. In total, 26 of the 56 patients who underwent allo-HSCT were adults with AML<sup>79</sup>. Of the patients who underwent allo-HSCT, 12 relapsed (11 died from relapsed disease and one was subsequently lost to follow up), seven died from other causes, and 37 were alive without disease recurrence (median follow-up duration of 742 days)<sup>79</sup>. Of note, a lower relapse rate was noted among the patients transplanted in first CR (CR1) compared with patients transplanted beyond CR1 (17.9% versus 48.1%;  $P=0.03$ )<sup>79</sup>. By contrast, patients who did not undergo transplantation had a median overall survival of 145 days<sup>79</sup>. These findings, although preliminary and from a heterogeneous population of adult and paediatric patients with AML or ALL, suggest that allo-HSCT might be a viable option for patients with *MLL*-rearranged leukaemias. A particularly high-risk subset of *MLL*-rearranged AMLs has been identified by Gröschel *et al.*<sup>80</sup>, who demonstrated that patients with *EVII* overexpression had inferior overall survival versus those with other *MLL*-rearranged AMLs; however, the outcomes of this patient group improved with the use of consolidation therapy with allo-HSCT, compared with other consolidation approaches<sup>80</sup>.

**The influence of *RUNX1* mutations**—The effect of *RUNX1* mutations on AML-treatment outcomes has been examined in 945 patients treated in two consecutive AMLSG multicentre trials (AML HD98A and AMLSG 07–04); 53 patients in these trials had a total of 59 different *RUNX1* mutations<sup>81</sup>. Of the 53 patients with *RUNX1* mutations, 32 attained a CR after induction chemotherapy (60%)<sup>81</sup>. In an exploratory analysis, the investigators examined the role of allo-HSCT, which was performed in 14 of the 32 patients with *RUNX1*-mutations who achieved a CR. In the patients undergoing allo-HSCT, 4-year RFS was 52%, compared with 0% among patients who received postremission therapy with high-

dose cytarabine or auto-HSCT ( $P < 0.0001$ )<sup>81</sup>. Moreover, Chou *et al.*<sup>82</sup> have reported improved overall survival of patients with *RUNX1* mutations, compared with those without such mutations, after allo-HSCT (HR 0.33;  $P = 0.04$ ), and poor outcomes in patients with *RUNX1* mutations, compared with patients who were *RUNX1* wild type, among those who did not receive allo-HSCT (HR 1.74,  $P = 0.04$ ). Caution is required not to overinterpret these findings, however, given that the nontransplanted patients might have been sicker than the patients who underwent allo-HSCT.

**The influence of TP53 mutations**—Patients with complex-karyotype AMLs (CK-AML) have been demonstrated to have dismal outcomes with chemotherapy alone, and the current recommendation is to consider these patients for allo-HSCT in CR1 (REF. 7). Rucker *et al.*<sup>83</sup> examined a group of 234 patients with CK-AML to assess the frequency of *TP53* alterations and the effect of these genetic aberrations on clinical outcomes. In total, 70% of patients had *TP53* alterations (60% of whom had *TP53* mutations and 40% had *TP53* losses)<sup>83</sup>. Patients with *TP53* alterations had substantially lower CR rates after induction chemotherapy and inferior overall survival; this effect persisted after multivariable analysis<sup>83</sup>. 30 patients in this series underwent allo-HSCT for consolidation therapy, 15 of whom had *TP53* alterations; 14 of the 15 patients with *TP53* mutations relapsed and died, compared with 9 of the 15 patients who were *TP53* wild type ( $P = 0.04$ )<sup>83</sup>. The absolute number of patients who underwent allo-HSCT in this study was low; however, these findings suggest extremely poor post-transplant outcomes in patients with *TP53* alterations, and investigational therapies should be considered in lieu of, or in addition to, allografting.

**The influence of CEBPA mutation**—Schlenk *et al.*<sup>84</sup> examined the clinical outcomes of patients with *CEBPA* mutations, who were treated using various consolidation approaches, among a large cohort of patients with AML ( $n = 2,983$ ) who were enrolled in four Dutch–Belgian Haemato-Oncology Cooperative Group and Swiss Group for Clinical Cancer Research (HOVON/SAKK) trials and three AMLSG trials. 124 of the patients had AML with biallelic *CEBPA* mutations and achieved CR1 (REF. 84). In CR1, 32 of these patients underwent allo-HSCT, with 29 having matched related donors and three with matched unrelated donors; 20 underwent auto-HSCT, and 72 received chemotherapy<sup>84</sup>. The authors of this analysis reported that the patients who underwent allo-HSCT or auto-HSCT had improved RFS compared with those who received chemotherapy, but did not have better overall survival<sup>84</sup>. Furthermore, in the patients who relapsed ( $n = 45$ ; one after allo-HSCT, five after auto-HSCT, and 39 after chemotherapy), re-induction chemotherapy followed by allo-HSCT was associated with favourable outcomes: 83% (35 of 42) of patients undergoing reinduction therapy achieved a second CR<sup>84</sup>.

### Nontransplant postremission therapies

In a study of 185 patients who achieved a CR in the Cancer and Leukaemia Group B (CALGB) study 8525, in which investigators randomly assigned patients to postremission treatment with one of three cytarabine doses, Neubauer and colleagues<sup>85</sup> examined the influence of *RAS* mutations on clinical outcomes — as *in vitro* data provided evidence of cytarabine sensitivity in *RAS*-mutant cells<sup>86</sup>. *RAS* mutations were present in 34 of the 185 patients<sup>85</sup>. The authors found that patients with *RAS* mutations who received high-dose

cytarabine consolidation (3 g/m<sup>2</sup> every 12 h on days 1, 3, and 5; or 400 mg/m<sup>2</sup> per day for 5 days) had the lowest 10-year CIR (45% compared with 68% for patients with wild-type *RAS*)<sup>85</sup>. In patients with wild-type *RAS*, those who received high-dose cytarabine had a lower relapse risk than those who received low-dose cytarabine (HR 0.67; *P* = 0.04); this reduction in relapse risk with high-dose cytarabine was magnified in patients with *RAS* mutations (HR 0.28; *P* = 0.002), after adjusting for confounding variables such as presence of core-binding factor (CBF) cytogenetics<sup>85</sup>. Findings of *in vitro* studies have suggested that the presence of *RAS* mutations leads to altered cellular responses, ranging from cytostatic to cytotoxic, in the presence of cytarabine, potentially explaining these differences<sup>86,87</sup>.

### Future directions

Data are limited regarding the relationships between other mutations, including those in *ASXL1*, *DNMT3A*, *TET2*, *IDH1/2*, *WT1*, *EZH2*, and *PHF6*, and postremission outcomes of patients with AML. Furthermore, the existing data are often from small cohorts of patients, thus limiting the power of statistical analysis, especially with regard to infrequent mutations, such as those in *EZH2*, *WT1*, and *PHF6*. A meta-analysis of published trials with available biospecimens and/or comprehensive mutational profiling results would increase the number of patients with data available for meaningful statistical analysis of the effects of different mutations, or combinations of mutations. Alternatively, prospective comprehensive sequencing of well-annotated, homogeneously treated patient cohorts would assist in understanding the clinical implications of integrated mutational profiling in AML. Questions for future research include the role of mutation-directed inhibitors for maintenance therapy in the post-transplantation setting, or for maintenance therapy in patients harbouring targetable mutations in genes, such as *IDH1/2* and *FLT3*, who achieve CR, but are not candidates for transplantation. In nontransplant candidates with mutations in genes affecting DNA methylation, such as *TET2*, *DNMT3A*, *IDH1/2*, and *WT1*, the role of postinduction therapy with HMAs merits further investigation.

### Novel therapies for patients with AML

Over the past decade, novel molecular findings obtained using NGS technology have resulted in a rapid expansion of the armamentarium of targeted agents, which is expected to continue over time. At present, new therapies are typically offered to patients in the relapsed/refractory disease setting, although, as we have proposed, these agents should be strongly considered (in the setting of a clinical trial) for the treatment of newly diagnosed elderly or unfit patients who are not candidates for intensive induction therapy (FIGS 1,2). Some of the novel molecular medicine approaches that are under investigation in patients with AML are discussed in the following sections.

#### IDH1/IDH2

Preclinical data demonstrated the efficacy of *IDH2* inhibition in models of AML<sup>88,89</sup>, leading to the development of a multicentre, open-label, phase I dose-escalation study examining AG-221, a first-in-class, potent, reversible, selective inhibitor of the mutant form of IDH2 (REF. 90). Data from this phase I trial were first presented at the ASH meeting 2014 (REF. 91), and were updated at the European Haematology Association (EHA)

meeting in 2015 (REF. 92). Eligibility criteria included the presence of an *IDH2* mutation in patients with advanced-stage haematological malignancies, with 75% of patients having relapsed/refractory AML<sup>91</sup>. The data from this trial indicate that AG-221 is well tolerated, and the maximum-tolerated dose has not yet been reached<sup>91,92</sup>. Of the 11 deaths reported to date, most were disease-related, with only two deaths being reported as ‘possibly’ related to effects of the study drug<sup>91,92</sup>.

The updated findings presented at the EHA meeting revealed that among 45 efficacy-evaluable patients, objective responses were observed in 25 patients (ORR of 56%): six CRs, four bone-marrow CRs, five CRs with incomplete count recovery (CRi), and 10 partial responses (PRs)<sup>92</sup>. Five patients who achieved a CR proceeded to allo-HSCT<sup>91,92</sup>. Ultimately, the final, mature results from this trial must be awaited before we can draw any firm conclusions and decide whether these findings warrant any further investigation in the phase II setting. Additional considerations for the future include whether *IDH2* inhibition can be moved into the front-line setting, alone and/or in combination with conventional induction therapy, and whether *IDH2* blockade should be continued post-transplantation as maintenance therapy.

Early results from a phase I trial of the *IDH1* inhibitor AG-120 were also presented at the EHA meeting in 2015 (REF. 93), and demonstrated that seven of 14 efficacy-evaluable patients had objective responses (four CR, two bone-marrow CRs, and one PR). Development of a phase I trial to evaluate AG-881, a dual *IDH1*–*IDH2* inhibitor, is currently underway<sup>94</sup>.

### Kinase inhibitors

**FLT3 inhibitors**—Numerous *FLT3* inhibitors have been developed for the treatment of *FLT3*-mutant AML; typically, these agents have been first used in the relapsed or refractory disease setting, with some advancing to phase III trials, as discussed previously. Use of the early *FLT3* inhibitors as single agents generally failed to produce robust or sustainable responses in phase I/II trials in this setting<sup>44</sup>. Newer-generation *FLT3* inhibitors have been demonstrated to have higher potency and selectivity for *FLT3* (REF. 42). In a phase I clinical trial<sup>95</sup>, the second-generation *FLT3* inhibitor quizartinib was assessed as a single agent, and responses were reported in patients with *FLT3*-mutant, *FLT3*-indeterminate, and *FLT3*-wild-type disease (response rates of 53%, 41%, and 14%, respectively). Quizartinib progressed to phase II clinical trials, with the results showing a high degree of activity as a single agent in both patients with mutant and wild-type *FLT3* (composite CR (CRc) and PR rates of 68% and 47%, respectively)<sup>96</sup>. Crenolanib is a newer *FLT3* inhibitor, which has demonstrated activity against both *FLT3*-ITD and *FLT3*-TKD mutations, with high selectivity for *FLT3* relative to the closely-related protein *KIT*<sup>97,98</sup>. In the phase II setting<sup>99</sup>, crenolanib was associated with an ORR of 47%, with greater responses rate and longer overall survival seen in the *FLT3*-inhibitor-naïve arm, compared with patients who had previously received a *FLT3* inhibitor, suggesting that on-target *FLT3* inhibition is responsible for the efficacy of this agent.

**KIT inhibitors**—A phase I/II study was performed to examine the safety and efficacy of imatinib (an inhibitor of KIT, as well as BCR–ABL1-kinase fusion proteins) combined with mitoxantrone, etoposide, and cytarabine in patients with KIT-positive relapsed/refractory AML<sup>100</sup>. Of 21 patients treated with imatinib at a dose of 400 mg per day, 62% achieved a CR<sup>100</sup>. Investigators determined that the patients who responded to therapy had a higher degree of phospho-AKT inhibition compared with nonresponders, indicating that agents that more-effectively inhibit AKT might have greater clinical utility<sup>100</sup>. Furthermore, imatinib has been examined as a single agent at higher doses, but with no responses observed<sup>101</sup>. In the phase I setting in a population of patients with relapsed/refractory AML, imatinib in combination with cytarabine and daunorubicin was associated with a frequency of CR or CR with incomplete platelet recovery (CRp) of 57%<sup>102</sup>, and in combination with low-dose cytarabine, an objective haematological response rate of 11% was reported in older patients who were not candidates for intensive induction therapy<sup>103</sup>. In all of these studies, KIT-positive patients were defined as those with AML blasts showing positivity for CD117 (KIT receptor), most commonly by flow cytometry, without evaluation of *KIT*-mutation status. Prolonged therapy with KIT TKIs can lead to a resistant phenotype via acquired secondary *KIT* mutations<sup>104</sup>. In preclinical models of gastrointestinal stromal tumours, which also harbour *KIT* mutations, the heat-shock-protein inhibitor AUY922 demonstrated growth inhibition in both imatinib-sensitive and imatinib-resistant cell-lines, implicating a resistance pathway that demands further investigation<sup>105</sup>. Owing to the high incidence of CD117 expression and *KIT* mutations in CBF AMLs, dasatinib has been examined as a maintenance therapy in CR1 for patients with high-risk disease, with a low 2-year disease-free survival rate of 25.7% reported<sup>106</sup>. Of the four patients with CBF AML harbouring *KIT* mutations at initial diagnosis who received dasatinib maintenance therapy, 75% no longer had *KIT* mutations detected at the time of relapse, suggesting that clonal evolution contributed to relapse<sup>106</sup>.

**JAK2 inhibitors**—*JAK2* mutations are rarely found in patients with AML, and are observed most often in the setting of an antecedent myeloproliferative neoplasm (MPN)<sup>107</sup>. Nevertheless, JAK2 inhibition with ruxolitinib has been tested in the phase II setting in patients with refractory leukaemias, including post-MPN AML — 12 of the 38 patients treated harboured the *JAK2*<sup>V617F</sup> mutation<sup>108</sup>. Three of the 18 patients with post-MPN AML had a response to therapy in this study, with two achieving a CR and one a CRi<sup>108</sup>; of the three patients who achieved a CR, two harboured *JAK2*<sup>V617F</sup> mutations<sup>108</sup>. Despite these promising initial findings, a subsequent study was terminated early owing to a lack of satisfactory clinical benefit, with only one CRp observed among 13 evaluable patients with AML — the patient with the CRp was negative for the *JAK2*<sup>V617F</sup> mutation<sup>109</sup>. JAK2 inhibitors are currently under continued development in combination with HMAs for the treatment of post-MPN AML<sup>110</sup>.

### Targeting the RAS pathway

**Farnesyltransferase inhibition**—RAS activity is dependent on post-translational farnesylation; therefore, farnesyltransferase inhibitors have been tested in clinical trials involving patients with AML<sup>111</sup>. In a trial examining the farnesyltransferase inhibitor tipifarnib, however, no correlation between treatment response and *RAS*-mutation status or

inhibition of protein farnesylation was found<sup>112</sup>, thus calling the precise mechanism of action of this agent into question. Overall, responses to farnesyltransferase inhibitors have been disappointing; for example, in a trial in which investigators examined 348 elderly patients with AML (aged > 70 years) who received tipifarnib, the highest ORR, 20%, was observed in patients treated at a dose of 300 mg twice daily<sup>113</sup>.

**MEK–AKT-pathway inhibition**—Given the disappointing results obtained with farnesyltransferase inhibition, further avenues of targeting the RAS-signalling pathway have been explored, predominantly inhibition of downstream mediators, such as MEK and AKT — with a preclinical rationale for dual-pathway inhibition<sup>114</sup>. The efficacy of combination therapy targeting MEK and AKT has been established in patients with *BRAF*-mutant melanoma, and is currently being investigated in clinical trials in patients with AML and *RAS* mutations<sup>115,116</sup>.

### Chromatin modulators

**DOT1L inhibition in MLL-rearranged AML**—DOT1L is a histone methyltransferase that is required for the development and maintenance of *MLL*-rearranged leukaemias, and preclinical data have supported the potential clinical utility of DOT1L inhibition in this setting<sup>117,118</sup>. Subsequently, a phase I clinical trial was initiated to examine the safety of EPZ-5676, a small-molecule inhibitor of DOT1L, with preliminary results reported at the ASH meeting, 2014 (REF. 119). At the time of reporting, 37 patients had been enrolled in the study, 31 of whom had AML, with 36 patients evaluable for safety outcomes (having received at least one dose) and 28 evaluable for antileukaemic activity (having completed one or more post-baseline bone-marrow biopsy)<sup>119</sup>. Median time on therapy was 29 days, and EPZ-5676 was generally found to be well tolerated<sup>119</sup>. Best responses included one morphological CR, one cytogenetic CR, and two patients had resolution of leukaemia cutis; six patients had a treatment-related increase in neutrophils and/or monocytes<sup>119</sup>. These data support ongoing clinical investigation of EPZ-5676 and further exploration of DOT1L as a therapeutic target in patients with AML.

**EZH2 inhibitors**—EZH2 is a member of the polycomb group complex, which has histone methyltransferase activity. EZH2 is critical for haematopoietic-stem-cell development, influencing the balance between cell self-renewal and differentiation<sup>120</sup>. EZH2 inhibitors are currently under clinical development, with most clinical trials of such agents enrolling patients with diffuse large-B-cell lymphoma<sup>121</sup>, although inactivating mutations in *EZH2* are also associated with AML<sup>122</sup>. Preclinical data suggest that the treatment of *WT1*-mutant AML cells with EZH2 inhibitors promotes myeloid differentiation, highlighting another potential application of this class of drugs<sup>123</sup>. The observation that loss-of-function *EZH2* mutations are observed in myeloid malignancies, however, suggests a complex role of EZH2 in AML, and indicates the need for carefully designed preclinical studies and judicious patient selection when evaluating the use of EZH2 inhibitors in AML.

**BET inhibitors**—Inhibitors of the bromodomain and extraterminal (BET) family proteins act by targeting the epigenetic regulators that maintain aberrant chromatin states commonly associated with AML<sup>124</sup>. The BET-bromodomain-containing protein 4 (BRD4) was

identified using an advanced RNA-interference screening method as a critical factor for maintenance of the AML-cell phenotype<sup>125</sup>, and murine models have supported the clinical use of BET inhibition across multiple cytogenetic and molecular subtypes of this disease<sup>126</sup>. Data from a phase I trial of a BET inhibitor, OTX015, were recently reported at the ASH meeting 2014 (REF. 127), and demonstrated single-agent antileukaemic efficacy at a wide range of doses (evidence of activity reported in five of 28 patients (18%)), with a reasonable toxicity profile. These data necessitate further investigation of OTX015 in the phase II setting.

**HDAC inhibitors**—Histone deacetylases (HDACs) are a class of enzymes that influence gene expression by altering the acetylation status of nucleosomal histones and other, nonhistone, proteins in chromatin<sup>128</sup>. HDAC inhibitors promote cell-cycle arrest, growth inhibition, and apoptosis in multiple cell types, including leukaemia cell-lines<sup>129</sup>. In a phase II study<sup>130</sup>, the HDAC inhibitor vorinostat had minimal single-agent activity in patients with untreated or relapsed AML, leading to the initiation of combination studies this agent. In particular, the use of vorinostat in combination with gemtuzumab ozogamicin in patients >60 years of age was associated with CR and CRp rates of 19% and 3%, respectively, with better responses observed in patients with normal or favourable AML karyotypes, compared with patients of the same age and performance status with other cytogenetic profiles (CR plus CRp rate of 46% versus 0%)<sup>131</sup>. Further complicating investigation of this combination, gemtuzumab ozogamicin was voluntarily withdrawn by Pfizer in 2010, at the request of the FDA, owing to concerns regarding an initial lack of clinical benefit and liver toxicity<sup>132</sup>. In 2014, however, the results of a meta-analysis of randomized studies demonstrated that addition of gemtuzumab ozogamicin to induction chemotherapy was associated with a survival benefit; thus, this drug could conceivably return to the market in the future<sup>133,134</sup>.

A trial in which investigators are combining vorinostat with azacitidine for the treatment of patients with newly diagnosed AML who are not eligible for intensive induction is currently ongoing<sup>135</sup>. Panobinostat, a pan-HDAC inhibitor, has also been combined with azacitidine in a phase Ib/II study involving patients with AML and high-risk MDS<sup>136</sup>, the results of which demonstrated a 31% ORR (CR, CRi, and PR) in the subgroup of patients with AML. The combination of the HDAC inhibitor pracinostat with azacitidine is also under investigation in an ongoing trial<sup>137</sup>. Of note, data from a preclinical model of AML indicate a synergism between the HDAC inhibitor pracinostat and the JAK2 inhibitor pacritinib; further studies are needed to determine if this approach has any clinical utility<sup>138</sup>.

## Future directions

At present, targeted agents are typically used in the setting of relapsed and/or refractory AML, at a time when patients are generally sicker and when the disease is more resistant to therapy. To facilitate the future development of targeted therapies, we propose a paradigm shift in the general approach to therapy for newly diagnosed AML, in which mutational profiling should be performed upfront in all patients. If a targetable mutation is identified in older patients who are not candidates for intensive induction, they should be considered for frontline treatment with the relevant targeted agent in the context of a clinical trial — or an alternate investigational agent if no molecular targets are identified (FIG. 1). For younger

patients, the addition of a targeted agent to standard induction therapy should be considered if an appropriate trial is available (FIG. 2). At first relapse, targeted agents should be considered, again, in the setting of a clinical trial.

To achieve these goals, the current mechanism for mutational profiling needs to be adjusted in order to enable quicker access to mutational data and to prevent unnecessary delays in administering upfront therapy. Admittedly, concerns exist regarding the timing of therapy relative to the window when informative mutational profiling is available. In a study of 599 patients with newly diagnosed AML, Bertoli *et al.*<sup>139</sup> determined that time from diagnosis to treatment (TDT) had no effect on survival, CR, or early death rates. By contrast, Sekeres *et al.*<sup>140</sup> found that a TDT >5 days negatively affected the CR rate and overall survival of patients aged <60 years, but not older patients. Both of these studies are limited by their nonrandomized nature, as the TDT among patients with more-favourable disease could conceivably have been longer than in patients who seemed to have more-aggressive disease — who might have been treated quicker. A randomized trial is unlikely to be performed to address this question, however, and the findings of both studies indicate that reasonable delays in the initiation of induction therapy might be safe in older patients with AML.

On the basis of the seminal work regarding clonal evolution in AML<sup>141–144</sup>, additional concerns regarding the use of targeted therapies are bound to arise over time: in a clonally heterogeneous disorder, does the mutant-allele frequency influence responsiveness to targeted agents? Are patients with more clonally heterogeneous AML less responsive to a single targeted agent than those with more-homogenous disease? Are combinations of targeted agents safe and effective? The design of clinical trials involving targeted agents should include correlative studies with well-organized biospecimen collection to help answer these questions.

Given the rarity of certain mutations in AML, accrual of patients for trials is often difficult. To examine the role of targeted agents in the treatment of patients with rare mutations, performing multicentre and/or cooperative-group trials could help to increase accrual by providing geographically diverse access to patients, enabling sufficient statistical power for meaningful comparisons. Finally, standardized, high-throughput, and rapid DNA-sequencing techniques are imperative for the clinical application of genomic data to patient care.

## Conclusions

We are currently at a crossroads where our knowledge of AML biology is rapidly expanding, and we must endeavour to apply this knowledge to the clinical context as soon as possible in order to improve the outcomes of our patients. To enhance the clinical care of patients with AML, especially older patients for whom clinical outcomes have improved little over the past several decades, we advocate for a paradigm shift in the way that novel agents are introduced into the clinic. Instead of delaying introduction of novel agents to the setting of relapsed/refractory disease, we propose consideration of frontline treatment with targeted agents either alone or in combination with chemotherapy, in the context of multicentre and/or cooperative-group clinical trials, when available.



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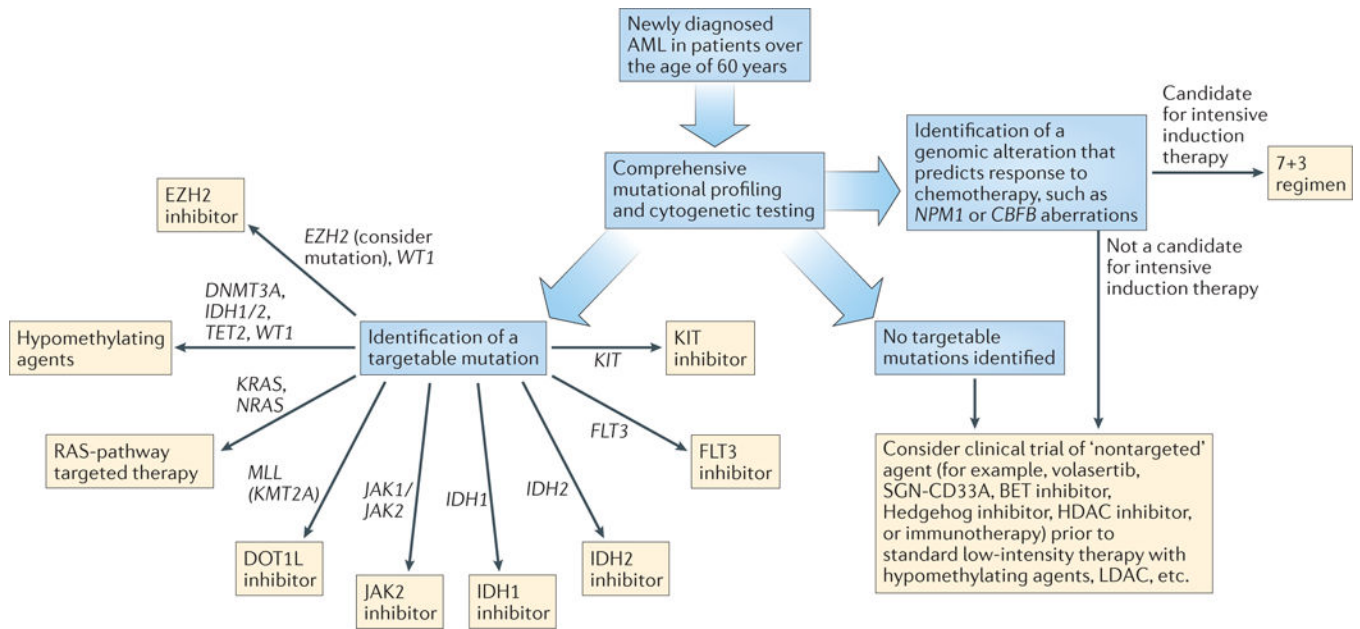
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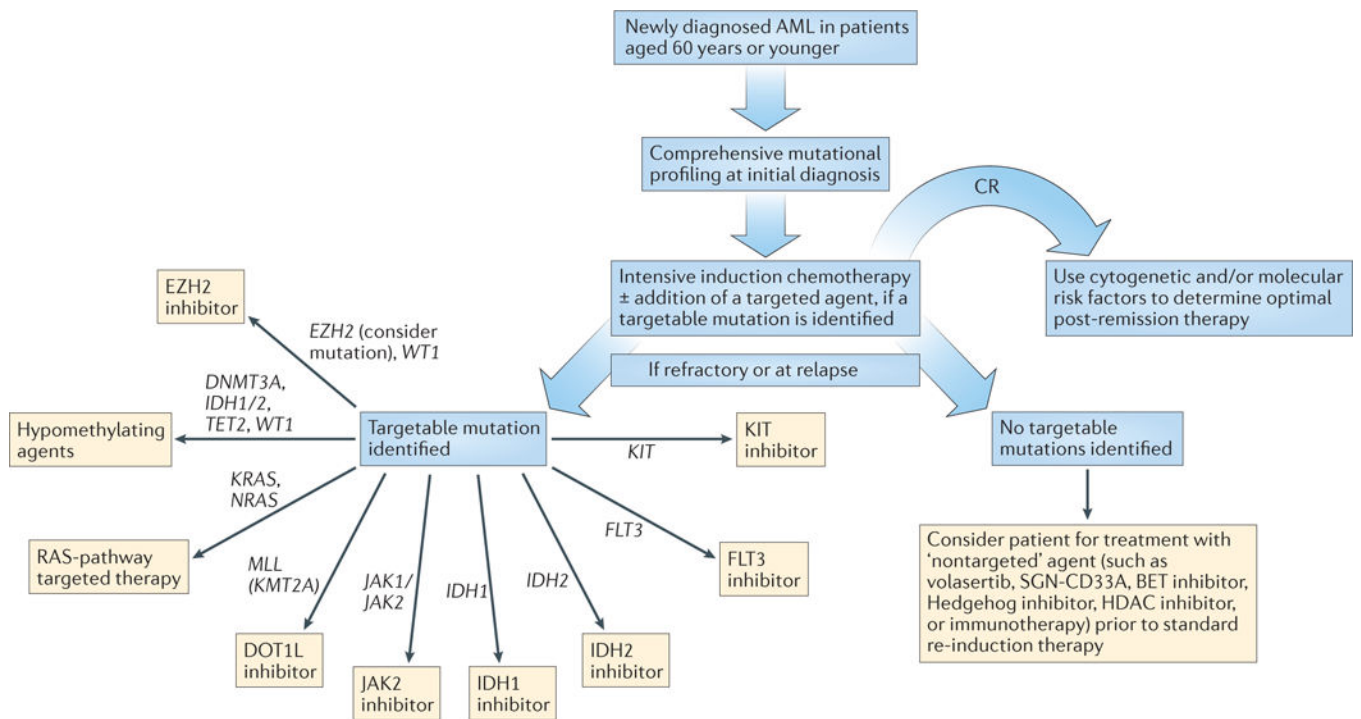
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### Key points

- More than 97% of patients with acute myeloid leukaemia (AML) demonstrate at least one clonal somatic abnormality on comprehensive mutational profiling, which is increasingly being performed in the clinic
- Unique mutational profiles can be used to predict a response to both standard and investigational therapies in patients with newly diagnosed or relapsed and/or refractory AML
- Molecular abnormalities associated with AML are also predictors of outcome following allogeneic haematopoietic-stem-cell transplantation
- Comprehensive mutational profiling should be performed in all newly diagnosed patients with AML using standardized high-throughput assays
- Comprehensive mutational profiling will enable consideration of novel targeted agents in the upfront setting, either alone or in combination with chemotherapy, and we hypothesize that this approach will improve outcomes of patients with this disease



**Figure 1. Proposed treatment algorithm for patients aged >60 years with newly diagnosed AML**  
 All novel agents, both targeted and ‘non-targeted’, should be administered in the setting of a clinical trial. HDAC, histone deacetylase; LDAC, low-dose cytarabine.



**Figure 2. Proposed treatment algorithm for patients aged 60 years with newly diagnosed AML**  
 All novel agents, both targeted and ‘non-targeted’, should be administered in the setting of a clinical trial. CR, complete response; HDAC, histone deacetylase.

Table 1

Frequency of mutations in relevant AML-associated genes\*

Gene	Overall frequency	Frequency in patients aged <60 years	Frequency in patients aged ≥ 60 years
<i>FLT3</i>	19–28% ( <i>FLT3</i> -ITD) <sup>145</sup> and 5–10% ( <i>FLT3</i> -TKD) <sup>12,146</sup>	30% ( <i>FLT3</i> -ITD) and 7% ( <i>FLT3</i> -TKD) <sup>10</sup> NK-AML only: 35% ( <i>FLT3</i> -ITD) and 8% ( <i>FLT3</i> -TKD) <sup>147</sup>	17–21% ( <i>FLT3</i> -ITD) <sup>27,148,‡</sup> and 5% ( <i>FLT3</i> -TKD) <sup>27</sup> NK-AML only: 23% ( <i>FLT3</i> -ITD) and 5% ( <i>FLT3</i> -TKD) <sup>147</sup>
<i>NPM1</i>	27–35% <sup>12,13</sup>	29% <sup>10</sup> NK-AML only: 57% <sup>147</sup>	24–34% <sup>27,148,‡</sup> NK-AML only: 42% <sup>147</sup>
<i>DNMT3A</i>	26% <sup>12</sup>	18–23% <sup>10,149–151</sup>	NA
<i>NRAS</i>	8–9% <sup>12,152,153</sup>	10% <sup>10</sup>	NA
<i>ASXL1</i>	17–19% <sup>154,155,§</sup>	3–6% <sup>10,156,  </sup> NK-AML only: 3% <sup>157</sup>	NA NK-AML only: 16% <sup>157</sup>
<i>CEBPA</i> (biallelic)	4–6% <sup>12,153,158,159</sup>	8–9% <sup>10,159</sup> NK-AML only: 10% <sup>147</sup>	NA NK-AML only: 9–10% <sup>27,¶,147</sup>
<i>TET2</i>	8–27% <sup>12,160,161</sup>	8% <sup>10</sup>	NA
<i>WT1</i>	6–7% <sup>12,162</sup>	8–11% <sup>10,163</sup>	NA
<i>IDH2</i>	8–9% <sup>12,164</sup>	8–9% <sup>10,165</sup>	NA
<i>IDH1</i>	9% <sup>12,152,164</sup>	7–8% <sup>10,165</sup>	NA
<i>KIT</i>	2–4% <sup>12,153</sup>	6% <sup>10</sup>	NA
<i>RUNX1</i>	5–10% <sup>12,153</sup>	5% <sup>10</sup> NK-AML only: 8% <sup>166</sup>	NA NK-AML only: 16% <sup>166</sup>
<i>MLL</i> -PTD	5% <sup>145</sup>	5% <sup>10</sup> NK-AML only: 4% <sup>147</sup>	4% <sup>27</sup> NK-AML only: 11% <sup>147</sup>
<i>NRAS</i>	8–9% <sup>12,152,153</sup>	10% <sup>10</sup>	NA
<i>PHF6</i>	3% <sup>167</sup>	3% <sup>10</sup>	NA
<i>KRAS</i>	2–4% <sup>12,153</sup>	2% <sup>10</sup>	NA
<i>TP53</i>	2–8% <sup>12,153,168</sup>	2% <sup>10</sup>	NA
<i>EZH2</i>	2% <sup>169</sup>	0% <sup>10</sup>	NA
<i>JAK2</i>	1–3% <sup>153,170</sup>	NA	NA

AML, acute myeloid leukaemia; *FLT3*-ITD, *FLT3* internal tandem duplication mutation; *FLT3*-TKD, *FLT3* tyrosine-kinase-domain mutation; *MLL*-PTD, *MLL* (*KMT2A*) partial tandem duplication; NA, not available; NK-AML, normal-karyotype acute myeloid leukaemia.

\* Inclusive of all karyotypes, except when noted; discrepancies between the mutational frequencies in younger (age <60 years) and elderly (age ≥ 60 years) patients with NK-AML have been reported, when available.

‡ Ostronoff *et al.*<sup>148</sup> used the age of >65 years as the cut-point definition for 'elderly' patients.

§ The study by Schnittger *et al.*<sup>155</sup> included only patients with intermediate-risk AML.

|| Paschka *et al.*<sup>156</sup> defined younger adult patients as those aged 18–61 years.

¶ Schlenk *et al.*<sup>27</sup> analyzed *CEBPA* mutations in elderly patients with normal karyotypes only.