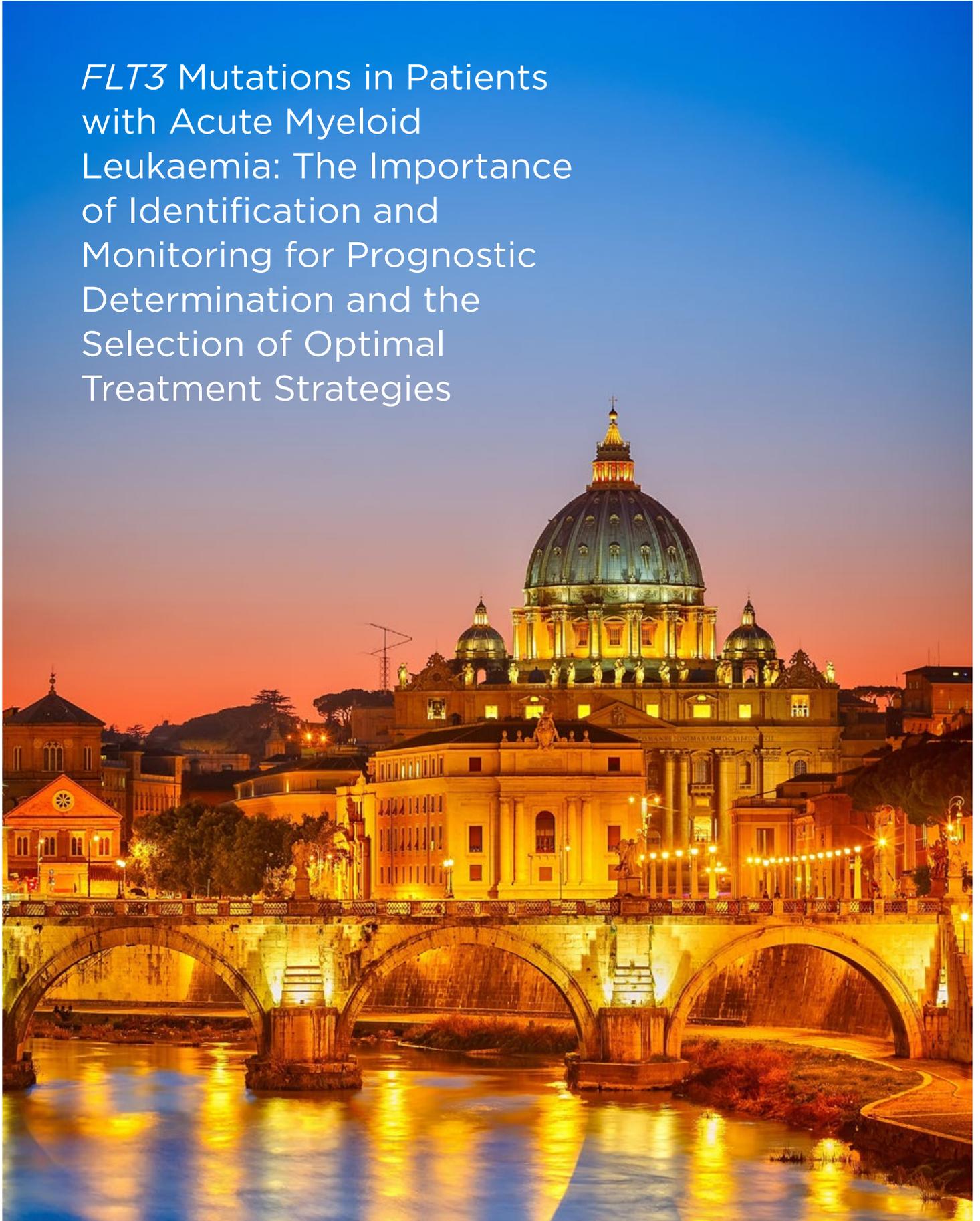


FLT3 Mutations in Patients
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***FLT3* Mutations in Patients with Acute Myeloid Leukaemia: The Importance of Identification and Monitoring for Prognostic Determination and the Selection of Optimal Treatment Strategies**

Interviewees: Adriano Venditti,¹ Lars Bullinger²

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Meeting Summary

This article is based on interviews conducted in November 2020 and February 2021 with two leading experts in haematology, Prof Venditti from Italy and Prof Bullinger from Germany, who discussed the impact on management of patients with acute myeloid leukaemia (AML) and fms-related tyrosine kinase 3 gene (*FLT3*) mutations. AML is an aggressive, genetically heterogeneous, malignant clonal disorder of the haematopoietic system. Data from cytogenetic and mutational analyses categorise patients into prognostic groups. Mutations in *FLT3* are the most common genetic alteration in AML. Patients with a *FLT3* internal tandem duplication (ITD) mutation subtype have poor prognosis, especially when there is a high allelic burden. Recently, new treatment options and maintenance strategies have been investigated to improve outcomes for these patients.

ACUTE MYELOID LEUKAEMIA DIAGNOSIS AND RISK STRATIFICATION

Prof Venditti explained that AML is complex to diagnose and several tests are required. Both experts stated that initially an assessment of the bone marrow should be performed to confirm the presence of bone marrow blasts infiltration under the microscope. At this stage, Prof

Bullinger explained, the diagnosis of leukaemia can be made from the morphology. “Following this, immunophenotyping should be performed by flow cytometry to define the nature of AML based on the expression of specific antigens. This establishes whether the AML is myeloid or lymphoid in origin.” Prof Venditti highlighted that it was important to demonstrate the myeloid nature of leukaemic blasts seen

under the microscope, and that other relevant investigations include genetic and cytogenetic tests to assess the karyotypic pattern of the leukaemic blasts and whether they carry specific karyotypic alterations, which can be useful for diagnosis and prognosis. Prof Venditti went on to detail that other tests are available that can identify molecular alterations and quantitative and qualitative abnormalities such as those that are important in acute promyelocytic leukaemia, which is associated with a chromosomal translocation *t(15;17)* and results in the fusion of genes encoding a putative transcription factor (*PML*) and the retinoic acid receptor α (*RARA*).¹ The genetic evaluation is also important to detect other relevant mutations, including the nucleophosmin (*NPM1*) gene mutation, *FLT3*, or *TP53*. The investigation of mutations has become another very important analysis required by physicians.

Prof Venditti explained that physicians should wait for the results of genetic and cytogenetic testing before treatment initiation, with the exceptions of patients with acute promyelocytic leukaemia or AML with hyperleukocytosis, cases of which are considered an emergency. If the results of the mutation analysis are known, physicians can select the appropriate treatment options for their patients. Prof Venditti said that the delay in treatment initiation should not be a concern for physicians as a large, real-world cohort study has shown that time from diagnosis to treatment initiation (0–5, 6–10, 11–15, and >15 days) did not have a considerable impact on prognosis.² Thus, it was feasible to wait for genetic and other laboratory data so that clinically stable patients could be assigned to the best available therapies.

Discussing mutations associated with favourable and poor prognoses, Profs Bullinger and Venditti explained that the 2017 European LeukemiaNet (ELN) guidelines³ have three major classifications for patients with AML: favourable, intermediate, and adverse. The presence of specific mutations contributes to which group patients are categorised. Prof Venditti said that an *NPM1* mutation may be associated with favourable prognosis for patients with AML. Prof Bullinger described mutations called ‘core binding factor’ AML, such as recurrent translocations affecting *RUNX1*, gene translocation *t(8;21)*, or the inversion of the core binding effector subunit

beta *inv(16)*, as favourable. Other favourable mutations include biallelic mutations in the *CEBPA* gene, although this mutation is quite rare, occurring in fewer than 5% of AML cases.

The concomitant expression of different mutations such as *NPM1* and *FLT3* is an important factor in the management of patients with AML. The presence of *FLT3* ITD contributes to the categorisation of a patient into an intermediate or adverse group; however, if this patient also has *NPM1* and *FLT3* ITD mutation with a low allelic ratio, the combination of these two genes has been associated with a favourable prognosis. When *NPM1* is present but *FLT3* ITD has a high allelic ratio, the patient should be categorised into the intermediate prognostic group.

Prof Bullinger stated that in the adverse group, mutations are more complex, with recent additions, but generally include *RUNX1*, *ASXL1*, or *TP53*. The adverse group contains cases with complex karyotypic changes, i.e., three or more chromosomal abnormalities or with a monosomal karyotype (two or more monosomies). Prof Bullinger explained recent additions to this group include the loss of chromosome 5 or 7, rare translocations such as *t(6;9)* or *t(9;22)*, mutations involving the histone-lysine N-methyltransferase 2A (*KMT2A*) locus, or inversion *inv(3)* involving the *MECOM1* locus. Overall, the characterisation of mutations is very important to define prognosis, and is also useful for management decision-making, including assessing the need for allogeneic haematopoietic stem cell transplantation (HSCT).

Both professors concurred that the mutation pattern evolves over time. Under the selective pressure of treatment, molecular aberrations change and on relapse a mutation associated with an adverse prognosis or outcome may be detected. For example, a *FLT3* ITD mutation can have erratic behaviour and be absent at diagnosis but found on relapse or refractory disease (R/R). Therefore, both professors recommended that at the time of relapse there is a need to retest the mutation profile with cytogenetics.

Mutations can be point mutations or ITD that activate the gene, but they can also be a therapeutic target. “The *FLT3* ITD mutation can be targeted by using *FLT3* inhibitors, which is ground-breaking for patients with AML,”

said Prof Venditti. He explained that this has paved the way for a new field of research and investigation. Prof Bullinger highlighted that *FLT3* inhibitors can be combined with intensive 7+3 chemotherapy; the approved agent in Europe to be used with chemotherapy is midostaurin. In the R/R setting, gilteritinib targeting *FLT3* mutation is also approved. Prof Venditti explained that other mutations such as mutations in the genes encoding isocitrate dehydrogenase 1 and 2 can be targeted with ivosidenib and enasidenib. These therapies are approved by the U.S. Food and Drug Administration (FDA) for the treatment of R/R AML. In addition, ivosidenib is approved for frontline treatment of patients not eligible for intensive chemotherapy.

ROLE OF MEASURABLE RESIDUAL DISEASE IN PROGNOSTIC ASSESSMENT AND TREATMENT DECISIONS

In the treatment of patients with AML, it is important to completely eradicate the leukaemic cells to reduce the risk of relapse; both professors explained that “measurable residual disease (MRD) is the measurable leukaemic burden.” Prof Bullinger considered that the measurement of MRD depends on the sensitivity of the assay, with flow cytometry being a less sensitive assay than reverse transcriptase PCR. MRD is an important prognostic tool. Prof Bullinger explained that patients who are MRD-positive at any timepoint during treatment have a less favourable prognosis compared with those who are MRD-negative. Prof Venditti cited a meta-analysis and systematic literature review that evaluated 11,151 patients with AML and found that the estimated 5-year disease-free survival was 65% for those without MRD at the time of the first response, compared with 25% for those with MRD at the same analysis point.⁴ The overall survival was also higher for patients without MRD (68%) than those with MRD (34%).⁴

In addition, Prof Bullinger explained that MRD is becoming increasingly important in the management of patients with AML due to the availability of targeted therapies. He said that “the impact of the selective pressure exerted by treatments can be monitored and therapeutic decisions can be modified accordingly.” MRD can be further utilised to assess the need for allogeneic HSCT in patients who have received

7+3 chemotherapy and have experienced a response but not received maintenance therapy. If these patients become MRD-positive, they can be monitored and appropriate salvage therapy or HSCT can be planned. Furthermore, MRD is a useful marker to predict whether a patient is likely to have a favourable or poor outcome following allogeneic HSCT.

THERAPY FOR *FLT3*-POSITIVE PATIENTS

Prof Venditti and Prof Bullinger stated that the *FLT3* ITD mutation is a common mutation in patients with AML, occurring in approximately 20–30% of patients. Prof Bullinger explained that 5–10% of patients have a point kinase mutation within the gene that activates gene function to confer a proliferative advantage for leukaemic cells. The point mutation on its own is not associated with negative prognostic impact, but if the allelic burden is high without the favourable *NPM1* mutation then this is an adverse risk factor. Prof Venditti commented that this is why allogeneic HSCT has been an effective therapy for patients with this mutation.

The standard of care for newly-diagnosed patients who are *FLT3*-positive (*FLT3*+) is 7+3 chemotherapy plus midostaurin given as induction and consolidation therapy.⁵ The schedule for the induction phase of the treatment for AML is cytarabine (200 mg/m², Days 1–7) and daunorubicin (60 mg/m², Days 1–3) for up to four cycles in one load.⁵ The addition of midostaurin (50 mg every 12 hours) with high-dose cytarabine every 12 hours on Days 1, 3, and 5 has been shown in the RATIFY trial to prolong overall and event-free survival in patients with AML and a *FLT3* mutation.⁶ Therefore, midostaurin should now be included in the treatment approach for patients who are *FLT3*+. Moreover, a post hoc analysis of patients with the *NPM1* mutation but with a decreased allelic ratio of *FLT3* mutation found that allogeneic HSCT was not required if midostaurin was used as part of the treatment strategy.⁷

Prof Bullinger explained that the standard of care also depends on age and fitness: “So, a young and fit patient would receive 7+3 and midostaurin and if they experienced R/R, they may qualify for monotherapy with gilteritinib.”

Prof Venditti highlighted that “in addition to these therapies, allogeneic HSCT is a formidable tool to improve prognosis.” He further stated that the use of midostaurin and allogeneic HSCT are not mutually exclusive; midostaurin, which is only approved in Europe for maintenance therapy, could be utilised for maintenance therapy following transplantation. Prof Venditti confirmed the role of MRD to help determine the success of therapy: if MRD remains positive, this is a sign of poor prognosis, and if this persists following both induction and consolidation chemotherapy, the patient should be a candidate for allogeneic HSCT. Furthermore, Profs Venditti and Bullinger mentioned that allogeneic HSCT following 7+3 with midostaurin was an important option for patients classified to the adverse group by the guidelines.³

Available FLT3 inhibitors include midostaurin and gilteritinib. Prof Bullinger noted that there are another two, quizartinib and crenolanib, that are not yet licensed but are being investigated in clinical trials.

Prof Venditti explained that the mechanism of action of FLT3 inhibitors is based on the ligation with the FLT3 receptor and the ability of these agents to inhibit the downstream metabolic pathway, which induces an arrest of differentiation of the apoptotic proliferative alterations that are caused by a mutation in *FLT3* in the leukaemic cells. Prof Bullinger went further to detail the mechanism of action of midostaurin: a broad, multiple-kinase FLT3, which inhibits both the tyrosine kinase domain mutation and the *FLT3* ITD mutation. Other agents include sorafenib, which is not approved for patients with *FLT3* ITD but is used by many haematologists for paediatric patients, off-label following HSCT for MRD-positive patients, or for those with R/R AML. Prof Bullinger went on to detail that gilteritinib is approved in Europe for patients with R/R AML and that quizartinib is approved in Japan. He stated that there are many investigational agents in clinical trials, such as crenolanib.

MAINTENANCE THERAPY: A NEW CONCEPT IN ACUTE MYELOID LEUKAEMIA TREATMENT

Prof Venditti stated that maintenance therapy is a burning issue. Prof Bullinger also explained

that maintenance therapy is a new concept as there are now agents for this approach. “We know that some patients can’t consolidate as aggressively as we would like, and these patients are unable to receive HSCT as they are too old or have complications. These patients may have persisting leukaemic cells that will ultimately result in relapse, so there is a large unmet clinical need for these individuals. In addition, there are patients who receive consolidation therapy and are candidates for HSCT but then something goes wrong, such as a persisting fungal infection, and then they cannot have HSCT, but we are able to give these patients maintenance FLT3 inhibitor therapy.”

However, Prof Venditti stated that maintenance is still an area of uncertainty. For example, the RATIFY trial assessed the use of maintenance therapy in 174 patients who did not receive allogeneic HSCT and found no difference between the placebo arm and the midostaurin maintenance arm (12 4-week; 336 days) in disease-free survival duration (hazard ratio: 0.83; $p=0.49$).⁸ However, the RATIFY trial was not sufficiently powered or designed to evaluate the benefit of each treatment stage individually;⁸ Prof Venditti explained, therefore, that maintenance therapy should be explored further in clinical trials. He mentioned a study that showed promising evidence of the effectiveness of maintenance therapy with sorafenib compared with placebo in 83 adult patients with *FLT3* ITD-positive AML, who were in complete haematological remission after allogeneic HSCT.⁹ The study found that the 24-month relapse-free survival rate was higher in patients in the sorafenib arm compared with the placebo arm (hazard ratio: 0.256; 95% confidence interval: 0.10–0.65; log-rank $p=0.002$).⁹

MAINTENANCE THERAPY IN *FLT3*-POSITIVE PATIENTS

Prof Bullinger said that all patients were suitable for maintenance therapy if they were *FLT3* ITD-positive, but many haematology centres may select HSCT for these patients if the patients did not have the favourable *NPM1* mutation. However, the 2017 ELN guidelines,³ which both professors cited as the major guidelines for AML, indicate that the allelic ratio of the *FLT3* mutation impacts selection, and that if patients

had a low allelic ratio with the *NPM1* mutation then maintenance therapy would be an optimal strategy. There is also a cohort of patients who are classified as intermediate prognosis and who do not want to undergo HSCT; these patients should also receive maintenance therapy. Prof Venditti explained that, additionally, MRD was a driver for the selection of patients suitable for maintenance therapy. Prof Venditti agreed with Prof Bullinger that the patients in the adverse category should receive HSCT, since their baseline cytogenetic characteristics mean that if they do not receive an HSCT, prognosis is very unfavourable. For patients in the intermediate category or favourable category, MRD could also be a driver in the context of dynamic risk evaluation and treatment decision-making. Prof Bullinger concurred that maintenance therapy is more effective in patients without MRD at therapy initiation but noted that it can be used successfully in those patients who are MRD-positive, stating that “MRD monitoring will assist decision-making in maintenance therapy.”

Discussing safety and efficacy profiles of maintenance therapy options, both professors considered the tolerability of therapies used for maintenance in patients with *FLT3* mutations to be favourable, but noted that some patients experience cardiac adverse events. Prof Bullinger added that these events could be managed. They both agreed that the benefit of tyrosine kinase inhibitors is not yet fully demonstrated, with Prof Bullinger specifying that some of the studies are not sufficiently powered to demonstrate outcome benefits and that the efficacy depends on the type of kinase inhibitor. Overall, Prof Bullinger stated, in patients who are unable to receive consolidation therapy, maintenance buys time, delays relapse, and patients experience some good quality of life. Prof Venditti mentioned an ongoing large international trial¹⁰ that aims to assess gilteritinib

versus midostaurin in patients with *FLT3*-mutated AML. Prof Venditti thought that this trial would answer many questions and demonstrate the differences in efficacy and/or the benefit of maintenance therapy.¹⁰

When asked what data are anticipated from ongoing trials and what impact these data could have for patients with *FLT3+* AML, Prof Venditti said he was anticipating that data from studies evaluating the use of crenolanib in patients with newly diagnosed AML¹¹ and R/R AML¹² would impact clinical practice. The ongoing QuANTUM-F trial may have such effects, and Prof Venditti is looking forward to these results. Prof Bullinger was hopeful that the use of sorafenib maintenance may change the landscape. He explained that a preclinical assessment in mice found that sorafenib blocked the immune escape mechanism so that lymphocytes can recognise leukaemia cells again.¹³

Both professors were optimistic that in the future the agents approved in the R/R setting would demonstrate a benefit in the first-line setting. Prof Bullinger highlighted that the German-Austrian AML Study Group (AMLSG) is performing a Phase III trial evaluating 7+3 chemotherapy with gilteritinib compared with 7+3 chemotherapy with midostaurin.¹⁴ Prof Bullinger’s hypothesis was that the use of potent *FLT3* inhibitors at an earlier timepoint may improve outcomes for patients.¹⁴

Throughout the interview, both professors highlighted that the use of MRD is very important in assessing the prognosis and management approach of patients with AML, in addition to the determination of mutations that categorise patients into groups so that physicians can identify those patients who should receive HSCT and those who are candidates for maintenance therapy.

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