

Impact of new prognostic markers in treatment decisions in acute myeloid leukemia

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Current Opinion in Hematology 2009, 16:98–104

Purpose of review

In recent years, new molecular markers have emerged as significant prognostic parameters and as potential targets for molecularly targeted therapy in acute myeloid leukemia (AML). However, prognostic markers cannot guide the decision for a specific treatment, as they are associated with a differential outcome regardless of the given treatment. In contrast, predictive markers indicate a treatment benefit in patients that are characterized through these markers. Thus, predictive markers can guide clinical decision-making.

Recent findings

In young adults, mutations of the nucleophosmin (gene $9NPM1^{mut}$) in the absence of concurrent *FLT3*-internal tandem duplication (ITD) (*FLT3*-ITD^{neg}) have impressive prognostic and, beyond prognostication, predictive properties. This *NPM1*^{mut}/*FLT3*-ITD^{neg} genotype predicts equivalent favorable outcome after intensive chemotherapy and allogeneic stem cell transplantation, whereas in the absence of this marker clinical outcome was significantly improved after an allogeneic transplantation. In addition, within a retrospective study performed on older adults, the same genotype predicted a significantly improved outcome if all-*trans* retinoic acid was added to intensive chemotherapy.

Summary

The discovery of new prognostic and predictive markers has increased our understanding of leukemogenesis and this may lead to improved prognostication and, more important, to novel genotype-specific treatment strategies.

Keywords

acute myeloid leukemia, all-*trans* retinoic acid, *FLT3*-internal tandem duplication, nucleophosmin, predictive markers

Curr Opin Hematol 16:98–104
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1065-6251

Introduction

Pretreatment genetic aberrations in leukemic cells are one of the most powerful prognostic parameters in acute myeloid leukemia (AML) [1–5,6*,7*]. In recent years, a number of submicroscopic gene mutations as well as deregulated gene expression have been identified, especially, in the large subgroup of patients exhibiting a normal karyotype [cytogenetically normal (CN)-AML] [6*]. The prognostic value of cytogenetic aberrations, gene mutations, and deregulated genes in AML has been evaluated almost exclusively in retrospective studies and, therefore, cautious interpretation of results and in consequence their clinical impact is necessary. Furthermore, prognostic markers are not *per se* usable for clinical decision-making in that they are associated with a differential outcome regardless of the treatment given [8,9]. In contrast, markers attributing the clinical benefit of a specific treatment to AML patients who are characterized

by the marker status are termed predictive markers [9]. From a statistical purist's point of view, only predictive markers can be used for clinicians in therapeutic decision-marking.

In this article, we first discuss some statistical aspects that have to be considered for the interpretation of results from retrospective studies and, thereafter, the discovery of novel prognostic and predictive markers in AML.

Statistical aspects

The interpretation of results from prognostic and predictive marker studies depends to a great extent on the internal and external validity of the data. The validity of results is improved by addressing potential sources of bias and using appropriated statistical methods.

Potential sources of bias

In contrast to controlled clinical trials, in which the patient population is clearly defined by inclusion and exclusion criteria, in most prognostic molecular marker studies selection bias remains an issue. The patient population in prognostic marker studies was mostly derived from a combination of several clinical trials and the main inclusion criteria for the marker studies is the availability of pretreatment peripheral blood, bone marrow samples, or both. As a consequence of this, leukemia specimens with low cell counts will be archived less frequently for further genetic analysis as compared with cell-rich samples. This fact is reflected by the observation that in AML prognostic marker studies [10^{••},11^{••}], the analyzed patient populations are frequently characterized by higher white blood cell (WBC) counts. These sources of bias can be addressed by stating the proportion of patients with available samples in relation to those without samples and by a comparison for the clinical endpoints and important covariates used in the study between the groups of patients with and without available samples. By these two simple methods, the readers are put into a position to assess whether results can be generalized to all AML patients or rather the results are valid only for the subset of patients analyzed in the particular study.

Appropriate statistical methods

For the assessment of new prognostic and predictive markers, multivariable regression analyses are a *conditio sine qua non* because they allow evaluation of the impact of new markers in relation to already known markers. However, selection of variables for the presented model impacts heavily on the obtained results and therefore the methodology of variable selection has to be taken into account for the clinical interpretation of the results. In addition, in most studies, clinical datasets are incomplete. It is important to address the issue of missing data when developing prognostic models. The procedure to exclude those individuals whose data are incomplete from the analyses is not recommended, because this practice is inefficient, leading to a reduction in statistical power and, more importantly, to biased results and massive overestimation of odds and hazard ratios [12]. Since its introduction nearly 30 years ago, multiple imputation has become an important and influential approach in the statistical analysis of incomplete data and the methodology has been recently reviewed [13[•]].

Mutations in the nucleophosmin gene

Nucleophosmin (NPM1) is a highly conserved phosphoprotein that physiologically resides in nucleoli and shuttles between nucleus and cytoplasm. It is involved in several cellular processes such as ribosome biogenesis, response to stress stimuli, maintenance of genomic

stability, regulation of activity and stability of tumor-suppressor genes such as *p53* and *ARF*, and transcriptional regulation [14]. Falini *et al.* [15] first discovered the abnormal cytoplasmic localization of NPM1 that is caused by mutations in exon 12 of the gene. Subsequent studies revealed that cytoplasmic accumulation of NPM1 mutants results from two major alterations acting in concert, loss of tryptophan residues normally required for NPM1 binding to the nucleoli and generation of an additional export signal motif at the C-terminus [16[•]]. In addition, NPM1 leukemic mutants were shown to recruit wild-type NPM1 from nucleoli to nucleoplasm and cytoplasm through dimerization [16[•]]. Mutations of the *NPM1* gene are the most frequent genetic aberration in adult AML detectable in 24–35% of all cases [15,17–19] and in 43–62% of cases exhibiting a normal karyotype [10^{••},15,17–21,22[•],23[•]]. In childhood AML, the incidence of *NPM1* mutations is much lower at 8%, and *NPM1* mutations are age dependent in that they are found in older children (median 10.9 years) and not seen in pediatric patients below the age of 3 years [24[•]]. In adult AML, the incidence of *NPM1* mutation increases with age [25[•]] until the age of 60 years and, thereafter, seems to slightly decrease [22[•]]. Clinically, *NPM1* mutations are associated with specific features, including predominance of female sex, higher bone marrow blast percentages, lactate dehydrogenase levels, WBC and platelet counts, and high CD33 but low or absent CD34 antigen expression. Of note, *NPM1* mutations are significantly associated with cytogenetics in that more than 85% occur in CN-AML patients [10^{••},15,17–21,22[•]–24[•]]. The differences in clinical characteristics at diagnosis between *NPM1*^{mut} and *NPM1*^{wt} AML are not only related to the *NPM1* mutational status but also to the interaction with cooperating gene mutations such as *FLT3*-internal tandem duplication (ITD) [20].

Mutations in the FMS-like tyrosine kinase 3 gene

FMS-like tyrosine kinase 3 (*FLT3*) is a member of the class III receptor tyrosine kinase family that is normally expressed on the surface of hematopoietic progenitor cells. *FLT3* and its ligand play an important role in proliferation, survival, and differentiation of multipotent stem cells. The gene that encodes *FLT3* is localized on chromosome 13q12, containing 24 exons. In AML patients, somatic mutations that result in the constitutive activation of *FLT3* have been identified in two functional domains of the receptor, the juxtamembrane domain and the activation loop of the tyrosine kinase domain (TKD) [26,27]. The juxtamembrane domain that has been shown to be crucial for kinase autoinhibition is disrupted by ITDs in 28–34% of CN-AML cases, whereas juxtamembrane domain point mutations occur less frequently [27–29]. *FLT3*-ITDs are located in exons

14 and 15 and vary in insertion site and length of the duplicated segment (from three to 400 nucleotides). Mutations occurring in the activation loop in the carboxy-terminal lobe of the TKD are usually point mutations, small insertions, or deletions mainly involving codons 835 (D835) and 836 (I836) in 11–14% of CN-AML patients [28,29,30]. However, additional point mutations or insertions affecting other codons in the TKD have been reported in single AML cases. In-vitro studies and results from global gene expression profiling revealed that there are not only similarities but also important differences in signal transduction properties between *FLT3*-ITDs and TKD mutations that may explain differences in clinical phenotypes [31]. AML patients harboring a *FLT3*-ITD are characterized by certain pretreatment features such as increased WBC count, higher percentages of blood and bone marrow blasts, and a more frequent diagnosis of *de novo* rather than secondary AML [28].

Mutations in the CCAAT enhancer-binding protein alpha gene

The transcription factor CCAAT enhancer-binding protein alpha (CEBPA) is a key molecule in the mediation of lineage specification and differentiation of multipotent myeloid progenitors into mature neutrophils [32]. *CEBPA* mutations were first discovered in 2001 and the majority of the mutated patients have normal cytogenetics. There are two major types of *CEBPA* mutations; nonsense mutations affecting the N-terminal region of the molecule preventing expression of the full-length CEBPA protein, thereby upregulating the formation of a truncated isoform with dominant negative properties, and in-frame mutations in the C-terminal basic region-leucine zipper domain resulting in CEBPA protein with decreased DNA binding or dimerization activity. N and C-terminal mutations often occur simultaneously, either affecting the same (monoallelic) or different (biallelic) alleles. CN-AML patients carrying a *CEBPA* mutation are characterized by distinct clinical features such as higher peripheral blood blast counts, lower platelet counts, less lymphadenopathy, or extramedullary leukemia and *CEBPA* mutations are less frequently associated with *FLT3*-ITD or TKD mutations [33].

Prognostic and predictive value of the nucleophosmin, FMS-like tyrosine kinase 3, CCAAT enhancer-binding protein alpha genotypes

NPM1^{mut} has consistently been reported as a favorable prognostic marker for achievement of a complete remission after intensive induction therapy, either as a single marker [15,21,22,23] or in combination with the *FLT3*-ITD in that a favorable response was only seen in patients

with the combined genotype *NPM1*^{mut}/*FLT3*-ITD^{neg} [10,19,20]. Actually, no data are available attributing the favorable impact of *NPM1*^{mut} to induction success to specific chemotherapeutic agents or strategies.

NPM1^{mut} has also been reported as a favorable prognostic marker for relapse-free survival (RFS) and overall survival (OS). In most reports, this favorable impact on survival endpoints was evident in the genotype *NPM1*^{mut}/*FLT3*-ITD^{neg}, whereas the unfavorable prognosis of patients with the genotype *NPM1*^{mut}/*FLT3*-ITD^{pos} was mainly determined by the negative impact of *FLT3*-ITD [10,19–21,22,23]. The favorable outcome of patients with the genotype *NPM1*^{mut}/*FLT3*-ITD^{neg} was achieved not only after intensive consolidation chemotherapy but also after autologous or allogeneic blood stem cell transplantation (SCT). However, there is some controversy about the value of allogeneic SCT in first complete remission in patients with the favorable genotype *NPM1*^{mut}/*FLT3*-ITD^{neg}. In a large individual patient data meta-analysis [10] focused on patients with CN-AML, the favorable genotype *NPM1*^{mut}/*FLT3*-ITD^{neg} could be established as a predictive marker for RFS in that patients exhibiting this genotype did not benefit from an allogeneic SCT in first complete remission. In contrast, in the subgroup of patients defined either by *FLT3*-ITD^{pos} as a single marker or by the genotype *NPM1*^{WT}/*FLT3*-ITD^{neg}/*CEBPA*^{WT}, an allogeneic SCT led to a 40% reduction in the risk of relapse or death. Of note, in this meta-analysis of four prospective clinical trials, allogeneic SCT was restricted to matched family donors and the allocation to an allogeneic SCT was strictly based on a so-called genetic randomization [34]. The benefit in RFS did not translate into a significantly better OS, which was mainly due to the excellent outcome of relapsed patients after a matched unrelated donor SCT. These results strongly argue for an allogeneic SCT from a matched related and probably also unrelated donor in first complete remission in AML with these high-risk genotypes [10]. Very similar data were reported by Bornhäuser *et al.* [35], who showed a lower relapse rate in *FLT3*-ITD^{pos} patients after SCT; however, in this study, uncontrolled selection bias relativizes the results in that allocation to the treatment strategies was performed in a prioritized rather than a randomized manner with first priority for allogeneic SCT, second priority for autologous SCT, and finally, if the other strategies had not been feasible, chemotherapy. In contrast, Gale *et al.* [36] found no beneficial effect of an allogeneic SCT in AML defined by the single marker *FLT3*-ITD^{pos}. However, their data were hampered by a low adherence to the protocol with only 63% of the patients receiving the allocated allogeneic SCT, a high treatment-related mortality of 30% after allogeneic SCT, and an enormous potential selection bias due to the fact that only about one-third of the total clinical trial

population (MRC AML-10 and AML-12 trials) has been analyzed.

Approximately 11–15% of *NPM1* mutations are detected in combination with various recurring cytogenetic abnormalities, raising the question as to whether *NPM1* mutant AML defines a distinct biological and clinical entity [18,19,25,37]. This question might gain further importance when a targeted therapy becomes available for *NPM1*^{mut} AML. In a more recent study [38], *NPM1* was shown to act as a corepressor in retinoic acid-associated transcriptional regulation in a manner such that during retinoic acid-induced cellular differentiation, activating protein transcription factor 2 (AP2) recruits *NPM1* to the promoter of certain retinoic acid-responsive genes. The German–Austrian AML Study Group (AMLSG) reported on a beneficial effect of all-*trans* retinoic acid (ATRA) given as adjunct to conventional chemotherapy on complete remission rate, event-free survival, and OS in elderly patients with nonacute promyelocytic (non-APL) AML [39]. Interestingly, in retrospective analyses, it could be shown that the beneficial effect of ATRA in this trial was restricted to patients whose leukemic cells exhibited the genotype *NPM1*^{mut}/*FLT3*-ITD^{neg} [22]. So the genotype *NPM1*^{mut}/*FLT3*-ITD^{neg} appears as a predictive marker for the beneficial effect of ATRA in non-APL AML. Although this analysis is retrospective in nature, the fact that the AMLHD98B trial was a randomized trial reduces selection bias. In addition, AMLSG is currently validating these findings in a separate patient population within the ongoing prospective AMLSG 07-04 trial (clinicaltrials.gov, NCT00151242), which randomizes for ATRA in younger adults.

The high CD33 expression in *NPM1*^{mut} AML specifically points to gemtuzumab ozogamicin as a targeted therapy, especially on the basis of new data showing a positive correlation between expression level and response to gemtuzumab ozogamicin [40]. Actually, no data evaluating the specific impact of gemtuzumab ozogamicin in this molecularly defined subgroup of AML patients are available.

FLT3-ITD has been reported consistently as an unfavorable prognostic marker for RFS and OS [23,27–29]. Whether other molecular markers, in particular *NPM1*^{mut}, add to prognostication in *FLT3*-ITD^{pos} AML is unclear. Gale *et al.* [23] claimed a more favorable prognosis for patients with the genotype *NPM1*^{mut}/*FLT3*-ITD^{pos} compared with those with the genotype *NPM1*^{WT}/*FLT3*-ITD^{pos}; however, these findings could not be confirmed by several other studies [10,19]. More recent data provide evidence that outcome is also related to the level of the mutant allele, and not just its mere presence [23,29,41]. However, if *NPM1* mutation status was added to the prognostic model in these studies, the

mutant wild-type ratio of *FLT3*-ITD was no longer considered as prognostic [19,23]. At present, mutant wild-type ratio (high versus low) is used within the upfront randomized multicenter phase III trial [Cancer and Leukemia Group B (CALGB) 10603; clinicaltrials.gov, NCT00651261] for stratified randomization of midostaurin (PKC412) in young adult AML patients. This study is based on the favorable phase I/II studies in *FLT3*-mutated AML suggesting a clinical efficacy of *FLT3* inhibitors especially in combination with standard chemotherapy [42,43].

In contrast to *FLT3*-ITD mutations, the prognostic significance of *FLT3*-TKD^{mut} is still controversial. A previous meta-analysis [44] on 1160 cases including *FLT3*-TKD^{mut} cases ($n=84$) showed a negative prognostic impact of TKD mutations. However, no subset analysis for CN-AML patients was performed. In contrast, a study [30] performed by the British MRC group on 1107 young adults showed a positive impact of *FLT3*-TKD^{mut} on RFS and OS, especially if patients had a high allelic wild-type/mutant ratio. Of note, in the study from the MRC, there was a large potential selection bias starting from 3803 patients who were treated in the MRC trials AML-10 and AML-12 and only 1107 patients who had been finally analyzed (29%). In addition, other gene mutations, in particular *NPM1* mutations, have not been taken into account for multivariate analysis. In a study by Bacher *et al.* [45] and Schlenk *et al.* [10], the interaction of *FLT3*-TKD^{mut} with *NPM1*^{mut} has been addressed showing a favorable prognosis for the genotype *NPM1*^{mut}/*FLT3*-TKD^{mut} in the absence of an *FLT3*-ITD. This is in contrast to a study [31] from CALGB revealing a negative prognostic impact of *FLT3*-TKD^{mut} irrespective of the *NPM1* status. However, in the large meta-analysis [10] of CN-AML, *FLT3*-TKD^{mut} did not impact on RFS and OS in multivariable analysis. The prognostic value of *FLT3*-TKD^{mut} that also occurs in patients with favorable cytogenetics, in particular with *inv(16)*, remains to be determined. At present, patients with *FLT3*-ITD, *FLT3*-TKD^{mut} or both are eligible for the inclusion into *FLT3*-inhibitor trials (CALGB 10603; clinicaltrials.gov, NCT00651261). Data from these trials will probably show in the future whether *FLT3*-TKD^{mut} will become a predictive marker for the treatment with these agents.

CEBPA mutations consistently have been associated with a favorable prognosis, either in the subset of patients with intermediate-risk cytogenetics [46,47] or in patients with normal karyotypes [10,33,48]. In the context of other molecular markers, the genotype *CEBPA*^{mut} retained its prognostic importance for RFS and OS; additional mutations did not affect outcome in the *CEBPA*^{mut} subgroup [10]. Actually, even in the largest cohort of patients analyzed so far in CN-AML, the sample size

in the *CEBPA*^{mut} subgroup was too low for meaningful analysis, in particular to compare the different postremission strategies (chemotherapy versus autologous SCT versus allogeneic SCT) [10^{••}]. Therefore, the prognostic marker *CEBPA*^{mut} cannot actually be used as a predictive marker.

Other gene mutations

The partial tandem duplication (PTD) of the myeloid/lymphoid or mixed lineage leukaemia (*MLL*) gene was the first gene mutation shown to affect prognosis in CN-AML patients [49]. *MLL*-PTD is mainly found in CN-AML with an incidence ranging from 5 to 11%. There are no clinical features distinguishing *MLL*-PTD-positive from *MLL* wild-type patients [50,51]. Approximately 30–40% of *MLL*-PTD-positive patients also harbor *FLT3*-ITD mutations, whereas coexistence with *CEBPA* or *NPM1* mutations is rare [10^{••}]. *MLL*-PTD has been associated with shorter complete remission duration or worse RFS; however, in these studies, *MLL*-PTD had no effect on OS. [10^{••},50,51]. Recently, the CALGB [52[•]] reported on the impact of *MLL*-PTD in a large cohort of younger adult patients who received autologous SCT in first complete remission. Clinical outcome did not differ between the *MLL*-PTD-positive and the *MLL* wild-type group. Although the authors suggested that intensive consolidation therapy using autologous SCT might improve outcome in this subgroup of patients, this study did not provide direct evidence for this.

RAS oncogenes represent a family of membrane-associated proteins that adjust signal transduction upon binding of ligands to a variety of membrane receptors. They regulate mechanism of proliferation, differentiation, and apoptosis. Two large studies [53,54] in AML patients described *RAS* mutations in 10.3–13.6% of adult AML patients. Of note, the frequency of *RAS* mutations was highest in patients exhibiting an *inv(16)* at diagnosis. Consistent with previous reports, there was no prognostic impact of *RAS* mutations. More recently, Neubauer *et al.* [55[•]] showed a predictive impact of *RAS* mutations in that patients receiving high-dose cytarabine in consolidation therapy had a significantly lower probability of relapse as compared with patients receiving standard dose cytarabine and this effect holds if adjustment for cytogenetics, in particular *inv(16)*, has been performed. Although this is a retrospective analysis, the randomization of the initial trial valorizes the results. In addition, *RAS* mutations may provide a target for molecular therapy.

Mutations in the Wilms' tumor suppressor 1 gene (*WT1*) in AML were first reported by King-Underwood *et al.* in 1996 [56]. In more recent studies [11^{••},57[•]] (Gaidzik V,

et al., in preparation), *WT1* mutations have been identified with an incidence of 10–12.6% in CN-AML. However, inconsistent results have been reported about the prognostic impact of *WT1* mutations. Both CALGB and MRC studies evaluated the prognostic significance of *WT1* mutations in younger adults with CN-AML. In both studies, patients with *WT1* mutations had inferior RFS and OS, and in multivariable analysis, *WT1* mutation was an independent adverse prognostic factor. This is in contrast to the findings of Gaidzik *et al.* (in preparation) who did not observe any prognostic impact of *WT1* mutations on RFS and OS in either univariable or multivariable analysis. Of note, when performing exploratory subset analysis that takes into account the *FLT3*-ITD status, the *WT1*^{mut}/*FLT3*-ITD^{pos} genotype appeared to be associated with worse clinical course. One major difference between the three studies relates to treatment in that the cumulative dose of cytarabine was significantly higher in the trial reported by Gaidzik *et al.* (in preparation), suggesting that the negative impact of *WT1* mutations reported by others may be overcome by the use of repetitive cycles of high-dose cytarabine. On the basis of the current data, the prognostic impact of *WT1* mutation remains unclear and the potential interrelationship to treatment has to be addressed in future studies.

Conclusion

In AML, novel molecular markers of prognostic and more importantly of predictive significance have been discovered. The link between the leukemogenic importance of these markers and their role as potential targets for old and novel drugs will contribute to the stepwise replacement of risk adapted by genotype-specific treatment strategies. This development will necessitate large collaborative group efforts to perform clinical trials even in small genetic subgroups.

Acknowledgements

Supported by grants from the Bundesministerium für Bildung und Forschung (01GI9981 and 01KG0605), the Deutsche José Carreras Leukämie-Stiftung (DJCLS R06/06v), and the Else Kröner-Fresenius-Stiftung (P38/05//A49/05//F03).

There are no conflicts of interest.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 150).

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