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Genetic Identification of Acute Myeloid Leukemia Patients Older than 60 years Achieving Long-term Survival with Intensive Chemotherapy

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Running head: genetic decision model for fit older AML patients

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Key Points:

- Mutations in 7 genes independently predict OS in distinct cytogenetic groups of AML patients older than 60 years treated intensively.
- We report and validate a simple genetic model to identify older AML patients with very good, intermediate or very poor outcome with 7+3.

Abstract

To design a simple and reproducible classifier predicting the overall survival (OS) of AML patients ≥ 60 years old treated with 7+3, we sequenced 37 genes in 471 patients from the ALFA1200 study (NCT01966497, median age 68 years). Mutation patterns and OS differed between the 84 patients with poor-risk cytogenetics and the 387 patients with good (N=13), intermediate (N=339) or unavailable (N=35) cytogenetic risk. *TP53* (HR=2.49; P=0.0003) and *KRAS* (HR=3.60; P=0.001) mutations independently worsened OS of patients with poor-risk cytogenetics. In those without poor-risk cytogenetics, *NPM1* (HR=0.57; P=0.0004), *FLT3*-ITDs with low (HR=1.85; P=0.0005) or high (HR=3.51; P<10⁻⁴) allelic ratio, *DNMT3A* (HR=1.86; P<10⁻⁴), *NRAS* (HR=1.54; P=0.019) and *ASXL1* (HR=1.89; P=0.0003) mutations independently predicted OS. Combining cytogenetic risk and mutations in these 7 genes, 39.1% of patients could be assigned to a 'go-go' tier with a 2-year OS of 66.1%, 7.6% to the 'no-go' group (2-year OS 2.8%) while the 53.3% 'slow-go' patients had a 2-year OS of 39.1% (P<10⁻⁵). Across three independent validation cohorts, 31.2-37.7% and 11.2-13.5% of patients were assigned to the 'go-go' and the 'no-go' tiers respectively, with significant differences in OS between tiers in all 3 cohorts (HDF, N=141, P=0.003, SAL N=466 and AMLSG N=223, both P<10⁻⁵). The ALFA decision tool is a simple, robust and discriminant prognostic model for AML patients older than 60 years treated with intensive chemotherapy. This model can instruct the design of trials comparing the 7+3 standard of care with less intensive regimens.

Introduction

Acute Myeloid Leukemia (AML) is mostly diagnosed in patients older than 60 years.¹ Recent improvements in survival have mostly been confined to younger adults with AML.² Intensive chemotherapy, with or without allogeneic stem cell transplantation (HSCT) remains the standard of care of AML, including in older fit patients.^{3,4}

Recurrent cytogenetic and genetic lesions are key prognostic factors in AML patients treated intensively, but the prognostic value of oncogenetic lesions has mostly been studied in younger adults.⁵⁻⁸ Yet major interactions occur between age, oncogenetics and treatment outcome.⁹⁻¹² The genomic landscape of AML in older patients also differs from younger adults.^{9,13-16} Following earlier studies focusing on the prognostic value of *NPM1* or *FLT3* mutations in older AML patients,¹⁷⁻²¹ several studies, including from our group, have interrogated the prognostic value of a broader spectrum of recurrent genetic lesions in this population.²²⁻²⁴ However, none of these studies reproducibly identified patients subsets with outcomes contrasted enough to guide upfront decisions between intensive chemotherapies and alternative investigational approaches.

In recent years, 7+3 based induction chemotherapy has been increasingly challenged by less intensive options, notably the combination of hypomethylating agents and venetoclax.¹⁶ To design future randomized studies of intensive and less intensive therapies in fit older AML patients, specific decision tools must be developed to identify the minority of patients in whom 7+3 is unequivocally beneficial ('go-go') or futile ('no-go') amongst the majority of older fit AML patients ('slow-go' group).

Here we leverage the results of a 37-gene panel in 471 AML patients 60 years or older and treated with intensive chemotherapy in the prospective, multicentric, ALFA-1200 study ([clinicaltrials.gov NCT01966497](https://clinicaltrials.gov/ct2/show/study/NCT01966497))²⁴ to design a very simple 3-tier decision tool which we validate in three independent cohorts.

Patients and Methods

Patients

Patients aged 60 years or older with newly diagnosed de novo AML (excluding acute promyelocytic leukemia and Ph-positive AML) or AML secondary to myelodysplastic syndromes (but not myeloproliferative neoplasms) or therapy-related AML (with ≥ 2 years remission) with an ECOG performance status ≤ 3 , eligible for intensive chemotherapy, from 30 ALFA centers were prospectively enrolled from September 2012 to June 2016 after informed consent. The study was conducted according to current ethical regulations, after approval of the study design by the French Ministry of Health Ethic Committee ("*Comité consultatif sur le traitement de l'information en matière de recherche dans le domaine de la santé*") and of data management by the "*Commission nationale de l'informatique et des libertés*".

Here we report the extended analysis of a 37-gene panel of the 471 ALFA-1200 patients for whom samples were sent at inclusion to the ALFA central lab (Lille University Hospital, Pr Claude Preudhomme) for gene sequencing. Detailed information on the global cohort and CONSORT diagram and full list of investigators have been previously published.²⁴

Treatment

Patients received a 7+3 based induction course including idarubicin (IDA, 12 mg/m²/d, days 1-3) and cytarabine as continuous infusion (AraC, 200 mg/m²/d, days 1-7). Patients failing to achieve complete remission (CR) or CR with incomplete platelet recovery (CRp)²⁵ could receive a salvage course consisting of intermediate-dose AraC boluses (IDAC, 1.5/m²/12h, days 1, 3 and 5, with dose reduction to 1

mg/m²/12h in patients older than 70, and further dose adaptation based on serum creatinine levels). Patients in CR/CRp received 2 such IDAC courses as consolidation. Details on HSCT eligibility have been previously published.²⁴

AML genetics

Cytogenetics including standard metaphase karyotyping and fluorescent in situ hybridization (FISH) was performed locally and centrally reviewed by the ALFA cytogenetics reference laboratory (Versailles University Hospital, Dr C. Terré) for the present study. Cytogenetic risk was stratified per current ELN guidelines (Supplementary Table 1, available online only). Molecular genetics was performed by targeted sequencing of a 37-gene panel (Supplementary Table 2) on bone marrow (BM) or peripheral blood (PB) samples collected at inclusion. Technical details are provided in the **Supplementary Methods**.

Genotyping of *FLT3* internal tandem duplications (*FLT3*-ITD) was done by fragment analysis as previously published²⁶ and expressed by the ratio ITD/wild type. Due to technical limitations of our sequencing technology, the *ASXL1* (NM_015338) c.1934dup mutational hotspot was investigated for all patients by fragment technique and confirmed by Sanger sequencing.²⁷ *CEBPA* gene mutations were also sought with Sanger sequencing.²⁸

Knowledge-bank Predictions

Of the 100 variables required for the estimation of prognosis based on the multistage model proposed by Gerstung et al.,²⁹ 28 were not available in our cohort, including 4

clinical (Hemoglobin peripheral blasts and LDH baseline values, presence of splenomegaly) and 24 genetic variables (*ATRX*, *BRAF*, *CBLB*, *CDKN2A*, *CREBBP*, *CUX1*, *EP300*, *FBXW7*, *GNAS*, *IKZF1*, *KDM5A*, *KDM6A*, *MLL2*, *MLL3*, *MLL5*, *MYC*, *NF1*, *PRPF40B*, *PTEN*, *RB1*, *SF1*, *SF3A1*, *SH2B3* and *U2AF2*). After imputation of missing data using the 1540-patient AMLSG knowledge bank, 5-year prediction of overall survival was done as previously described.³⁰

Validation cohorts

Information on the three validation cohorts is provided in the **Supplementary Methods** and their baseline characteristics reported in Supplementary Table 3.

Statistical Analyses

Continuous and categorical variables are summarized with medians and ranges or numbers and percentages. For graphical display of proportions, 95% confidence intervals derived from binary logistic regressions are shown. Group comparisons of dichotomic variables and continuous variables are done with Fisher's exact and Mann-Whitney tests respectively. Overall (OS) and relapse-free (RFS) survivals are estimated with the Kaplan-Meier method from the data of trial inclusion until death or last contact (OS), death, relapse or last contact (RFS). Group differences for censored outcomes are done with log-rank tests.

Given the number of genes tested, variable selection for multivariate Cox models was based on lasso penalized regression, using the one standard error rule, with the R package *glmnet*.³¹ In datasets with large numbers of variables, this method is more

robust than conventional sequential methods (eg. backward selection) to select the variables leading to the model with optimal interpretability (ie lower number of variables) and prediction accuracy.³² The final Cox model was inspected for interactions and for collinearity with Variance Inflation Factors (VIF), retaining the conventional VIF threshold of 4 as indicative of unacceptable collinearity.³³ Collinearity, ie a strong correlation between predictors in a model, does not affect the overall predictive power of the model, but may lead to spurious estimations of individual predictor's contribution to the model. The proportional hazard assumption was verified by graphical inspection and testing of scaled Schoenfeld residuals.³⁴ Harrell's concordance indexes from Cox models were computed and tested with the R package *survcomp*.³⁵ The C-index metric allows global assessment of a Cox model prognostic performance, accounting for both occurrence and timing of events, where values of 0.5 and 1 indicate random and perfect predictions, respectively.^{36,37} Agreement between classifications was estimated with Cohen's kappa.³⁸ All analyses were performed with R version 3.5.3 (www.R-project.org).

Results

Baseline characteristics of the study population

From September 2012 to June 2016, 509 patients were enrolled in the ALFA-1200 study. The 471 (92%) patients whose samples were sent at inclusion for centralized genotyping constitute the study population. Median age was 68 years and 390 (82.8%) had clinically defined *de novo* AML. Median WBC count was $5.3 \times 10^9/L$ (range 0.3 - $546.6 \times 10^9/L$). Bone marrow blasts were $\geq 30\%$ in 380 (80.7%) patients. Three hundred and forty-one (72.4%) achieved CR/CRp after one or two courses ([Table 1](#)). Of 279 patients deemed eligible for transplant, 131 patients were transplanted, including 87 in first CR. With a median follow-up of 44.8 months, there were 207 relapses and 318 deaths, leading to a median RFS and OS of 14.8 and 21.2 months, respectively.

Oncogenetic landscape

Cytogenetic risk was good, intermediate, poor, and not available in 13 (2.8%), 339 (72.0%), 84 (17.8%) and 35 (7.4%) patients, respectively. Details on cytogenetic groups is shown in [Supplementary Table 4](#). Among the 37 sequenced genes, 19 lesions in 17 genes were found in at least 5% of cases (**Supplementary Figure 1A**). The most frequent lesions included *DNTM3A* (28.7%), *NPM1* (27.0%), *TET2* (21.0%) and *FLT3-ITD* (18.7%) mutations. The allele ratio (mutated/wildtype) of *FLT3-ITDs* was ≥ 0.5 in 21/88 (23.9%) *FLT3-ITD* patients. The median number of mutations was 3 (range 1 – 10). Only 290 patients (61.6%) could be assigned unambiguously to oncogene-defined subgroups as defined by Papaemmanuil et al. in an all-ages cohort,⁷ the most frequent being *NPM1*, *TP53*-aneuploidy and chromatin-

spliceosome (**Supplementary Figure 1B**). There were marked differences between the mutation spectrum of patients with poor risk cytogenetics compared to good/intermediate risk cytogenetics, with significant over-representation of *TP53*, *KRAS*, *SETBP1*, *ETV6* and *CALR* mutations (all $P < 0.05$) contrasting with a significant under-representation of *NPM1*, *DNMT3A*, *FLT3-ITD*, *IDH2-R140* and *IDH2-R172* mutations in patients with poor risk cytogenetics (**Figure 1A**). ELN 2017 risk was favorable, intermediate, and adverse in 133 (28.2%), 129 (27.4%) and 195 (41.4%) patients but remained unavailable due to missing cytogenetics and lack of classifying mutation in 14 (3.0%) patients.

Impact of cytogenetics on outcome

Two hundred and sixty eight of 352 (76.1%) patients with good or intermediate cytogenetic risk achieved CR/CRp after one or two courses, compared to 47 of 84 (56.0%) patients with poor risk cytogenetics ($P = 0.0004$, **Figure 1B**). OS and RFS were also markedly different between cytogenetic subgroups, with median OS and RFS of 25.0 and 16.8 months in patients with good/intermediate compared to 9.5 and 7.1 months in patients with poor risk cytogenetics respectively ($P < 10^{-4}$ and $P = 0.0005$, respectively, **Figure 1C-D**). These major differences in mutation spectrum, short-term and long-term outcome between cytogenetic groups prompted us to investigate the prognosis of gene mutations in an oncogenetic hierarchy stemming from cytogenetic groups. The 35 patients with missing cytogenetics had a CR rate of 74.3%, median OS and RFS of 31.0 and 19.3 months, without significant difference with patients with favorable/intermediate cytogenetics (CR $P = 0.84$, OS $P = 0.60$, RFS $P = 1$). We therefore grouped patients into poor ($n = 84$) and non-poor (fav/int/missing, $n = 387$) cytogenetic risk for all further analyses. Of note, although amongst those 387 patients

without poor-risk cytogenetics there was a trend towards better outcome in patients with normal karyotype compared to those with intermediate risk aneuploid karyotype (**Supplementary Figure 2**), further cytogenetic stratification beyond the dichotomic poor-risk vs others classifier did not affect our multivariate survival analyses (*not shown*).

Molecular predictors of complete remission in cytogenetic groups

In univariate analysis, mutations in *NPM1*, *IDH2-R140* and *DNMT3A* were associated to higher CR/CRp rates after one or two courses, and mutations in *TET2*, *NRAS*, *RUNX1*, *ASXL1*, *SETBP1* and *ETV6* to lower CR/CRp rates in the 387 patients with non-adverse cytogenetics (all $P < 0.05$, **Supplementary Figure 3A**). In the 84 patients with adverse cytogenetics, no single gene mutation significantly influenced CR/CRp rate (**Supplementary Figure 3B**). We next performed multivariable logistic regression accounting for all gene mutations, and clinical covariates (age, gender, secondary AML and WBC) in each of the two cytogenetic strata. After variable selection by penalized regression, *NPM1* mutations predicted a high CR/CRp rate, while *NRAS*, *SETBP1*, *RUNX1* and *ASXL1* mutations retained significant detrimental impact on CR/CRp independently of high WBC in patients without poor risk cytogenetics, while only higher WBC count impacted CR/CRp rate in those with poor risk cytogenetics (Table 2).

Univariate prognostic impact of gene mutations in cytogenetic groups

We next studied the prognostic value of gene mutations on OS in patients according to their cytogenetic risk. In patients with non-poor cytogenetics (N=387), *NPM1*

mutations were associated with a lower hazard ratio (HR) for death in univariate analysis (HR= 0.69; 95% Confidence Interval [CI] 0.52-0.92; $P=0.011$), whereas mutations in *DNMT3A*, *NRAS*, *ASXL1*, *RUNX1*, *PHF6*, *CSF3R*, *SETBP1* and *ETV6* all conferred a higher risk of death (all $P<0.05$, **Figure 2A**). In univariate analysis, only the adverse prognostic impact of high allelic ratio (≥ 0.5) *FLT3*-ITDs (HR=2.04; 95%CI 1.23-3.39; $P=0.006$), but not of low allelic ratio (HR=1.31; 95%CI 0.95-1.82; $P=0.09$) reached statistical significance. In patients with poor risk cytogenetics (N=84), both *TP53* (HR=2.31; 95%CI 1.43-3.73; $P=0.0006$) and *KRAS* (HR=3.01; 95%CI 1.42-6.39; $P=0.004$) mutations further worsened OS in univariate analyses (**Figure 2B**).

Multivariate prognostic impact of gene mutations in cytogenetic groups

In the 387 patients with non-poor cytogenetics, presence of *NPM1* mutation predicted prolonged OS (HR=0.57; 95%CI 0.41-0.77; $P=0.0004$), whereas *FLT3*-ITDs with low (HR=1.85; 95%CI 1.31-2.62; $P=0.0005$) or high allelic ratio (HR=3.51; 95%CI 2.03-6.08; $P<10^{-4}$); *DNMT3A* (HR=1.86; 95%CI 1.40-2.47; $P<10^{-4}$); *NRAS* (HR=1.54; 95%CI 1.07-2.20; $P=0.019$) or *ASXL1* (HR=1.89; 95%CI 1.34-2.67; $P=0.0003$) mutations independently predicted a shorter OS in a multivariate Cox model after variable selection by lasso penalized regression (Table 3). The range of hazard ratios in this model allowed to design a simple score (-1 point for *NPM1* mutation, +1 point for each of *FLT3-ITD* low allele ratio, *DNMT3A*, *NRAS* and *ASXL1*, and 2 points for *FLT3-ITD* high allele ratio) that could regroup patients with non-poor cytogenetics into four distinct risk categories with 12-month OS estimates ranging from 96.2% (95%CI 89.0-100.0) to 43.5% (95%CI 31.3-60.5), (Supplementary Table 5, **Supplementary Figure 4**).

After lasso penalized regression in the 84 patients with poor risk cytogenetics, *TP53* (HR=2.49; 95%CI 1.53-4.04; $P=0.0003$) and *KRAS* (HR=3.60; 95%CI 1.68-7.72; $P=0.001$) mutations independently predicted worse OS (Table 3). Patients with poor risk genetics without *TP53* nor *KRAS* mutation had a 12-month OS of 58.3% (95%CI 45.9-74.1) versus 19.4% (95%CI 10.0-37.8) for those with either mutation (log-rang test $P=2\times 10^{-5}$, **Supplementary Figure 4**, Supplementary Table 5).

Development of the ALFA molecular decision tool

We empirically designed a three-tier oncogenetic decision model assigning patients groups with 2-year OS estimates > 60% to the ‘go-go’ group, those with 2-year OS estimates < 10% to the ‘no-go’ group, and all others to the ‘slow-go’ tier. Patients with non-poor cytogenetics and either *NPM1* mutation with at most one mutation among *FLT3-ITD* low allelic ratio, *DNMT3A*, *ASXL1* or *NRAS* mutations or those with *NPM1*, *FLT3-ITD*, *DNMT3A*, *ASXL1* or *NRAS* all wildtype (N= 184, 39.1%, ie groups A and B from Supplementary Table 5) were assigned to the very favorable ‘go-go’ group. Conversely, patients with adverse risk cytogenetics and either a mutation in *KRAS* or *TP53* (N=36, 7.6%, ie group F from Supplementary Table 5) were assigned to the “no-go” group, and the remaining 251 (53.3%) to the ‘slow-go’ group (Table 4). Two-year OS estimates were 66.1% (95%CI 59.5-73.3%), 39.1% (95%CI 33.5-45.7%) and 2.8% (95%CI 0.4-19.2%) in the ‘go-go’, ‘slow-go’ and ‘no-go’ groups, respectively (overall log-rank test, $P<10^{-5}$, **Figure 3A**). Censoring OS at the time of HSCT in first CR did not affect those results (**Supplementary Figure 5**). CR rates were 84.2% (95%CI 78.0-89.0%), 66.5% (95%CI 60.3-72.3%) and 52.3% (95%CI 35.7-69.2%) in the ‘go-go’, ‘slow-go’ and ‘no-go’ groups, respectively (overall Fisher test $P<10^{-5}$). Relapse-free survival also markedly differed between these tiers with 2-year

estimates of 49.7% (95%CI 42.4-58.2%), 30.2% (95%CI 24.0-38.1%) and 5.3% (95%CI 0.8-35.5%) respectively (overall log-rank test $P < 10^{-5}$, **Figure 3B**).

Comparison to alternative decision tools

To benchmark our ALFA decision tool, we ranked patients according to their 5-year survival predicted by the knowledge bank (KB) approach.^{29,30} The 184 patients with best predicted outcome were assigned to a 'KB go-go' group, and the 36 with poorest KB predictions to the 'KB no-go' group. Agreement between ALFA and the KB decision tools was moderate (Cohen's kappa 0.47, 95%CI 0.40-0.55, Supplementary Table 6). This KB-guided decision tool also discriminated patients with different outcomes (**Supplementary Figure 6**). However, its concordance index for OS was 0.73 (95%CI 0.68-0.77), comparable to that of the ALFA decision tool (0.72 95%CI 0.68-0.77, $P=0.58$). Thus, the KB approach did not supersede the simpler ALFA decision tool. Similarly, in the 457 patients with evaluable ELN 2017 risk, the concordance index of the ALFA decision tool for OS was still 0.72 (95%CI 0.63-0.72) compared to only 0.63 (95%CI 0.59-0.68) for ELN 2017 risk classification ($P=2 \times 10^{-5}$, Supplementary Table 6).

External validation of the ALFA decision tool

We finally performed external validation of the ALFA decision tool in three distinct cohorts of 141 to 466 older patients treated intensively with older accrual dates and longer follow-up, resulting in lower median OS durations (Supplementary Table 3). The proportion of patients assigned to the 'go-go' group ranged from 31.2 to 37.7% and that of 'no-go' patients from 11.2 to 13.5%. Statistically significant differences in

OS were seen between decision tiers in all three cohorts (overall log-rank tests: HDF $P=0.003$, AMLSG and SAL both $P<10^{-5}$, **Figure 4**), and to a lesser extent on RFS (**Supplementary Figure 7**).

Discussion

This study relies on cytogenetics and targeted sequencing of 37 genes in a uniformly treated, prospective cohort of 471 newly diagnosed AML patients aged 60 years or older. We identify oncogenetic predictors of short-term (remission) and long-term (overall survival) benefit of intensive chemotherapy. We develop and validate a simple decision model accounting for cytogenetics and mutations in 7 genes (*NPM1*, *FLT3-ITD*, *DNMT3A*, *NRAS*, *ASXL1*, *KRAS* and *TP53*) that reproducibly identifies patients with significant differences in overall survival across multiple cohorts.

The results of intensive chemotherapy in older adults with newly diagnosed AML have long remained disappointing, and physicians are increasingly turning to alternative options such as the combination of azacitidine and venetoclax, especially for ‘unfit’ patients.¹⁶ ‘Fitness’ for chemotherapy has two orthogonal dimensions, one related to the patients’ condition, and the other to the disease risk. The characteristics of our cohort, including a majority of patients with no comorbidity and good performance status, resulting in relatively low early death rate (6.8% at day 30), allows the present work to carefully study the role of disease-related factors on chemotherapy outcome. Our cohort including a similar proportion of *de novo* AML and poor-risk cytogenetics (~20%) compared to other populations of older AML patients treated intensively (Supplementary Table 3).^{39,40} This reflects the accepted

notion that treatment decision can be delayed until obtaining the results of cytogenetics in older patients with AML.^{41,42}

In keeping with many previous reports,^{17,22} our results stress the prominent role of poor risk cytogenetics, mostly complex karyotype and related chromosomal imbalances (del5q/-5, -7, etc.) in predicting induction failure and shorter overall survival. Conventional karyotyping failure remains an obstacle to accurate patient stratification. In our series, the 7.4% of patients in this situation had similar outcome as patients with non-poor cytogenetics, allowing to group them. Routine use of molecular cytogenetic tools in this context will allow to overcome this limitation.^{43,44}

Among patients with non-poor cytogenetics, we identified mutations in *NRAS*, *SETBP1*, *RUNX1* and *ASXL1* to independently predict lower CR rate. These findings differ from other published series,^{23,45} perhaps owing to the assessment after 2 cycles in the present series. Of note, some of these genes (*SETBP1* and *RUNX1*) did not harbor prognostic impact in our analysis of OS. Though this could be due to their overlap with other frequently mutated genes such as *ASXL1*, it raises the possibility that salvage therapies, the details of which were not available in our cohort, could have attenuated their prognostic impact beyond primary induction failure.

In univariate analysis, we found shorter OS among patients without poor risk cytogenetics in those harboring mutations in *DNMT3A*, *NRAS*, *ASXL1*, *RUNX1*, *PHF6*, *CSF3R*, *SETBP1* and *ETV6*, and in poor-risk cytogenetics AMLs with mutations in *TP53* or *KRAS*. Most of these gene mutations have already been associated with poorer outcome in AML cohorts treated intensively.^{6,11,13,23,45}

The prognostic value of *DNMT3A* mutations remains debated,^{11,26,46} and we found similar prognosis between R882 hotspot substitutions and other mutations (*not*

shown). *DNMT3A*, but also *TET2* or *ASXL1* (DTA) are often pre-leukemic hits in AML.⁴⁷⁻⁵⁰ Based on VAFs, most of these mutations appeared as ancestral mutations in our cohort, and our cohort was not powered to assign a different prognostic value to secondary, as opposed to ancestral, DTA lesions (*not shown*).

In our univariate analysis, only high allele ratio of *FLT3*-ITD, as defined by ELN consensus (ratio ≥ 0.5),³ was associated with shorter OS. However, variable selection based on penalized lasso regression uncovered a lesser, yet significant prognostic value to lower *FLT3*-ITD clones in multivariate analysis. This finding both stresses the need to perform stringent variable selection when considering large genotyping datasets,³¹ and the challenges in using allele ratio of *FLT3*-ITDs for prognostic assignment,⁵¹ most of which were overcome here by centralized evaluation. These results confirm the longstanding notion that *FLT3*-ITD is also a high risk lesion in older AML patients.¹⁹ Of 74 *NPM1*-mutated patients without *FLT3*-ITD, 48 (64.9%) had at least one mutation in *DNMT3A*, *NRAS* or *ASXL1*, which had independent poor prognostic value in our final Cox model. The variable incidence of these adverse co-mutations in different cohorts has likely contributed to the divergent reports on the prognostic role of *NPM1* mutations in older AML patients.²⁰⁻²²

Progresses in 'chemo-free' regimens have challenged the role of intensive therapies in other heme malignancies. Decision tools have thus been developed to segregate patients for whom intensive treatment should not be questioned ('go-go') from those where it should be carefully considered ('slow-go') or readily discarded ('no-go').⁵² Combining cytogenetics and the mutational status of 7 genes, we could design and validate such a 3-tier decision tool. With a C-index for OS in the higher range of reported values across genetic classifiers in various AML cohorts,^{29,30,53} the resulting model was more discriminative than ELN 2017 risk stratification, whose limits in this

context have already been reported,^{22,24} and comparable to the more cumbersome knowledge-bank approach, which takes 100 variables as input, including important clinical variables such as age and WBC count, compared to only 8 for our decision tool, and has yet to be fully validated in patients older than 60 years.^{29,30}

Across all tested cohorts, the ALFA decision tool identified 30-35% of patients with superior outcome, including a 2-year OS of 66.1% in the more recent ALFA1200 trial. These 'go-go' patients should be considered candidates for intensive chemotherapies, and future trials should aim at improving its results, eg through the addition of novel therapies, or intervening early using MRD.⁵⁴⁻⁵⁶ Importantly, this 'go-go' group could not be solely identified on the basis of *NPM1* and *FLT3*-ITD status, let alone presence of core-binding factor fusions, which are rarely seen at this age.

Conversely, the ALFA decision tool consistently identified ~10% of 'no-go' patients with very-poor short-term survival. Importantly, this group was not only defined by poor-risk cytogenetics, and the short OS of these high-risk patients contrasted with a seemingly acceptable CR/CRp rate of 52.3%. These findings illustrate the need for integrated cytogenetic and genetic risk stratification and stress that CR achievement per se may not always translate into prolonged survival in older AML, questioning its role as clinical trial endpoint in this population. For this minor subset of 'no-go' AMLs, in spite of adequate performance status and lack of comorbidities, it may be considered ethical to evaluate investigational agents in trials without an intensive reference arm. The remaining 50-55% of patients constitute the slow-go group, where one may consider to randomize current intensive regimens versus promising, less intensive combinations.¹⁶ Long-term survival data with these less intensive therapies should be inspected stratifying according to the proposed decision tool to strengthen this hypothesis, accounting for the important differences in age and

comorbidities of cohorts so far treated with intensive chemotherapy versus hypomethylating agents – venetoclax combinations.^{57,58} Our decision tool was robust to censoring at HSCT in first remission, a strategy increasingly accessible to older AML patients.⁵⁹

Finally, the ALFA decision tool was able to identify significant differences in OS in three independent validation cohorts, though the overall lower OS of these cohorts buffered the survival differences between tiers. This likely reflects the fact that patients were mostly accrued to these cohorts before 2010 and thus had longer follow-up,^{17,60,61} but also stresses the progresses made in the supportive care of these patients over the recent years.⁶²

Overall, our ALFA decision model relies on a limited number of lesions, all part of the core oncogenes sequenced in AML cohorts, enabling cross-comparison of different treatment approaches currently explored in non-randomized trials.⁶³⁻⁶⁵ Incorporation of additional genetic and non-genetic biomarkers of chemosensitivity may in the future help further refine prognostic assessment in larger cohorts of older AML patients prior to treatment initiation.⁶⁶ With the acceleration of sequencing turn-around time in most centers, and the increasing acceptance for delayed treatment initiation in older AML patients,^{41,42} our simple, reproducible and discriminant decision tool has the potential to first instruct the design of future clinical trials in ‘fit’ AML patients then guide frontline treatment decisions in routine practice.

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Authors contribution. RI designed the study, performed analyses, and drafted the manuscript. CG and HD were the principal investigators and coordinators of the ALFA-1200 study. CB, TB, CP, EL, JBM, LA, DL, JVM, LGas, ER, PR, XT, SC, TC and NB accrued patients to the ALFA-1200 study. EF, AMR, OB did the sequencing analyses of the ALFA-1200 study under the supervision of CP and ND. CB, CR, HS, LGou and KJW accrued patients to the validation cohorts. CThie, CP and ND supervised molecular analyses of the validation cohorts. CTer centrally reviewed cytogenetics of the ALFA-1200 study. KCL, RI, CG, HD and ND controlled the database. All authors reviewed the manuscript and approved its final version.

Conflicts of interest. The authors have no conflicts of interest to disclose.

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Tables

Table 1. Characteristics of the Study Cohort

Variable	N or median	% or range
Gender		
Female	204	43.3
Male	267	56.7
Age, years	68	60 - 85
ECOG Performance Status		
0	205	43.5
1	200	42.5
2	52	11
3	9	1.9
NA	5	1.1
HCT Comorbidity Index		
0	354	75.2
1	108	22.9
NA	9	1.9
Type of AML		
<i>de novo</i>	390	82.8
post-MDS	68	14.4
tx-related	13	2.8
WBC, x10 ⁹ /L	5.3	0.3 - 546.6
Cytogenetic risk*		
good	13	2.8
intermediate	339	72
poor	84	17.8
NA	35	7.4
ELN2017		
favorable	133	28.2
intermediate	129	27.4
adverse	195	41.4
NA	14	3
Follow-up, months (IQR)	44.8	43.0 - 49.9
CR/CRp		
after one course	311	66
after two courses	30	6.4
no	130	27.6

*defined in [Supplementary Table 1](#). Details in [Supplementary Table 4](#).

Table 2. Multivariate logistic regression for CR/CRp achievement according to cytogenetic risk.

Variable	Odds Ratio	95% CI	P=
Non-poor Cytogenetics (N=387)			
Log(WBC)	0.69	0.58 - 0.82	<0.0001
<i>NPM1</i> mutation	2.25	1.15 - 4.51	0.02
<i>NRAS</i> mutation	0.46	0.23 - 0.91	0.02
<i>SETBP1</i> mutation	0.16	0.02 - 0.87	0.04
<i>RUNX1</i> mutation	0.43	0.23 - 0.81	0.009
<i>ASXL1</i> mutation	0.52	0.28 - 0.98	0.04
Poor Risk Cytogenetics (N=84)			
Log(WBC)	0.61	0.41 - 0.87	0.009

Table 3. Multivariate Cox models for OS in patients according to cytogenetic risk.

Variable	HR	95% CI	P=
Non-poor risk cytogenetics (N=387)			
<i>NPM1</i> mutation	0.57	0.41 - 0.77	0.0004
<i>FLT3</i> -ITD low ratio	1.85	1.31 - 2.62	0.0005
<i>FLT3</i> -ITD high ratio	3.51	2.03 - 6.08	<0.0001
<i>NRAS</i> mutation	1.54	1.07 - 2.20	0.019
<i>ASXL1</i> mutation	1.89	1.34 - 2.67	0.0003
<i>DNMT3A</i> mutation	1.86	1.40 - 2.47	<0.0001
Poor risk cytogenetics (N=84)			
<i>KRAS</i> mutation	3.60	1.68 - 7.72	0.001
<i>TP53</i> mutation	2.49	1.53 - 4.04	0.0003

Table 4. Repartition and outcome per ALFA decision tool tier in the ALFA1200 training cohort and validation cohorts. *P* values from the overall log-rank test and from pairwise log-rank tests considering the ‘slow-go’ group as reference.

	Training cohort			Validation cohorts								
	ALFA1200 (N=471)			AMLSG (N=223)			HDF (N=141)			SAL (N=466)		
	N (%)	2-y OS (95%CI)	<i>P</i> =	N (%)	2-y OS (95%CI)	<i>P</i> =	N (%)	2-y OS (95%CI)	<i>P</i> =	N (%)	2-y OS (95%CI)	<i>P</i> =
Go-go*	184 (39.1)	66.1% (59.5-73.3%)	<10 ⁻⁵	84 (37.7)	44.8 (35.3-56.9)	0.0006	44 (31.2)	43.4 (30.7-61.4)	0.06	171 (36.7)	35.5 (28.9-43.5)	0.02
Slow-go	251 (53.3)	39.1% (33.5-45.7%)	ref	113 (50.7)	21.9 (15.4-31.2)	ref	78 (55.3)	29.9 (21.2-42.2)	ref	243 (52.1)	28.2 (20.1 - 31.2)	ref
No-go	36 (7.6)	2.8% (0.4-19.2%)	<10 ⁻⁵	26 (11.6)	3.8 (0.5-26.2)	3x10 ⁻⁵	19 (13.5)	10.5 (2.8 - 39.1)	0.01	52 (11.2)	2.0 (0.2 - 13.9)	<10 ⁻⁵
Overall log-rank test			<10 ⁻⁵			<10 ⁻⁵			0.003			<10 ⁻⁵

*‘Go-go’ tier: non-poor cytogenetics, *NPM1* mutated and at most 1 mutation among *FLT3*-ITD low, *DNMT3A*, *ASXL1* or *NRAS* OR non-poor cytogenetics and *NPM1*, *FLT3*-ITD, *DNMT3A*, *ASXL1* and *NRAS* all wildtype; ‘no-go’ tier: poor-risk cytogenetic with *KRAS* and/or *TP53* mutation; ‘slow-go’: all others.

Figure Legends

Figure 1. Differences in mutation pattern and outcome according to cytogenetic risk. **A.** Mutation pattern in patients with favorable/intermediate risk cytogenetics (N=352) versus adverse cytogenetics (N=84). *P* values from Fisher exact tests. **B.** Rates of CR/CRp at two courses in patients with good/intermediate (N=352), poor (N=84) or missing (N=35) cytogenetics with 95% confidence intervals. *P* values from Fisher exact tests. **C-D.** OS (**C.**) and RFS (**D.**) according to cytogenetic risk. *P* values from log-rank tests.

Figure 2. Hazard ratios of death according to gene mutations. **A.** Patients with non-poor cytogenetic risk (n=387). **B.** Patients with poor cytogenetic risk (n=84). Error bars indicate 95% confidence intervals. *P* values are from univariate Cox models.

Figure 3. Outcome of the ALFA1200 cohort according to the ALFA Decision Tool. **A.** Overall Survival and (**B.**) Relapse-Free Survival according to ALFA decision tiers. *P* values from log-rank tests.

Figure 4. External validation of the ALFA decision tool. Overall Survival according to ALFA decision tiers in A. 141 patients accrued to the Hauts-De-France registry (HDF cohort), B. 466 patients accrued to SAL trials (SAL cohort), C. 223 patients 60 years or older (excluding t(15;17) cases) in the AMLSG public dataset^{7,29} (AMLSG cohort). Characteristics of these cohorts are provided in Supplementary Table 3. *P* values from log-rank tests.





