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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 identity 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 (clincialtrials.gov 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 published[26] 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 $x10^9$/L (range 0.3 - 546.6 $x10^9$/L). Bone marrow blasts were ≥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-ITD*s 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 signification 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 *ASLX1*, 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 $P=2\text{x}10^{-5}$, **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=2x10^{-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 *DNTM3A* mutations remains debated,[11,26,46] and we found similar prognosis between R882 hotspot substitutions and other mutations (*not*

shown). *DNTM3A*, 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.

References

1. Dohner H, Weisdorf DJ, Bloomfield CD. Acute Myeloid Leukemia. *N Engl J Med*. 2015;373(12):1136-1152.
2. Juliusson G, Lazarevic V, Horstedt AS, Hagberg O, Hoglund M. Acute myeloid leukemia in the real world: why population-based registries are needed. *Blood*. 2012;119(17):3890-3899.
3. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424-447.
4. Dombret H, Itzykson R. How and when to decide between epigenetic therapy and chemotherapy in patients with AML. *Hematology Am Soc Hematol Educ Program*. 2017;2017(1):45-53.
5. Grimwade D, Walker H, Harrison G, et al. The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. *Blood*. 2001;98(5):1312-1320.
6. Patel JP, Gonen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med*. 2012;366(12):1079-1089.
7. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *N Engl J Med*. 2016;374(23):2209-2221.
8. TCGA TCGAC. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 2013;368(22):2059-2074.
9. Appelbaum FR, Gundacker H, Head DR, et al. Age and acute myeloid leukemia. *Blood*. 2006;107(9):3481-3485.
10. Pastore F, Dufour A, Benthaus T, et al. Combined molecular and clinical prognostic index for relapse and survival in cytogenetically normal acute myeloid leukemia. *J Clin Oncol*. 2014;32(15):1586-1594.
11. Metzeler KH, Herold T, Rothenberg-Thurley M, et al. Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. *Blood*. 2016;128(5):686-698.
12. Straube J, Ling VY, Hill GR, Lane SW. The impact of age, NPM1(mut), and FLT3(ITD) allelic ratio in patients with acute myeloid leukemia. *Blood*. 2018;131(10):1148-1153.
13. Tsai CH, Hou HA, Tang JL, et al. Genetic alterations and their clinical implications in older patients with acute myeloid leukemia. *Leukemia*. 2016;30(7):1485-1492.
14. Silva P, Neumann M, Schroeder MP, et al. Acute myeloid leukemia in the elderly is characterized by a distinct genetic and epigenetic landscape. *Leukemia*. 2017;31(7):1640-1644.
15. Lindsley RC, Mar BG, Mazzola E, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood*. 2015;125(9):1367-1376.
16. DiNardo CD, Jonas BA, Pullarkat V, et al. Azacitidine and Venetoclax in Previously Untreated Acute Myeloid Leukemia. *N Engl J Med*. 2020;383(7):617-629.
17. Rollig C, Thiede C, Gramatzki M, et al. A novel prognostic model in elderly patients with acute myeloid leukemia: results of 909 patients entered into the prospective AML96 trial. *Blood*. 2010;116(6):971-978.
18. Becker H, Marcucci G, Maharry K, et al. Favorable prognostic impact of NPM1 mutations in older patients with cytogenetically normal de novo acute myeloid leukemia and associated gene- and microRNA-expression signatures: a Cancer and Leukemia Group B study. *J Clin Oncol*. 2010;28(4):596-604.
19. Whitman SP, Maharry K, Radmacher MD, et al. FLT3 internal tandem duplication associates with adverse outcome and gene- and microRNA-expression signatures in patients 60 years of age or older with primary cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. *Blood*. 2010;116(18):3622-3626.
20. Ostronoff F, Othus M, Lazenby M, et al. Prognostic significance of NPM1 mutations in the absence of FLT3-internal tandem duplication in older patients with acute myeloid leukemia: a SWOG and UK National Cancer Research Institute/Medical Research Council report. *J Clin Oncol*. 2015;33(10):1157-1164.
21. Dickson GJ, Bustraan S, Hills RK, et al. The value of molecular stratification for CEBPA(DM) and NPM1(MUT) FLT3(WT) genotypes in older patients with acute myeloid leukaemia. *Br J Haematol*. 2016;172(4):573-580.
22. Prassek VV, Rothenberg-Thurley M, Sauerland MC, et al. Genetics of acute myeloid leukemia in the elderly: mutation spectrum and clinical impact in intensively treated patients aged 75 years or older. *Haematologica*. 2018;103(11):1853-1861.
23. Eisfeld AK, Kohlschmidt J, Mrozek K, et al. Mutation patterns identify adult patients with de novo acute myeloid leukemia aged 60 years or older who respond favorably to standard chemotherapy: an analysis of Alliance studies. *Leukemia*. 2018;32(6):1338-1348.

24. Gardin C, Pautas C, Fournier E, et al. Added prognostic value of secondary AML-like gene mutations in ELN intermediate-risk older AML: ALFA-1200 study results. *Blood Adv*. 2020;4(9):1942-1949.
25. Sievers EL, Larson RA, Stadtmauer EA, et al. Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. *J Clin Oncol*. 2001;19(13):3244-3254.
26. Renneville A, Boissel N, Nibourel O, et al. Prognostic significance of DNA methyltransferase 3A mutations in cytogenetically normal acute myeloid leukemia: a study by the Acute Leukemia French Association. *Leukemia*. 2012;26(6):1247-1254.
27. Gelsi-Boyer V, Trouplin V, Adelaide J, et al. Mutations of polycomb-associated gene ASXL1 in myelodysplastic syndromes and chronic myelomonocytic leukaemia. *Br J Haematol*. 2009;145(6):788-800.
28. Preudhomme C, Sagot C, Boissel N, et al. Favorable prognostic significance of CEBPA mutations in patients with de novo acute myeloid leukemia: a study from the Acute Leukemia French Association (ALFA). *Blood*. 2002;100(8):2717-2723.
29. Gerstung M, Papaemmanuil E, Martincorena I, et al. Precision oncology for acute myeloid leukemia using a knowledge bank approach. *Nat Genet*. 2017;49(3):332-340.
30. Fenwarth L, Thomas X, de Botton S, et al. A personalized approach to guide allogeneic stem cell transplantation in younger adults with acute myeloid leukemia. *Blood*. 2021;137(4):524-532.
31. Friedman J, Hastie T, Tibshirani R. Regularization Paths for Generalized Linear Models via Coordinate Descent. *J Stat Softw*. 2010;33(1):1-22.
32. Tibshirani R. Regression Shrinkage and Selection via the Lasso. *Journal of the Royal Statistical Society Series B (Methodological)*. 1996;58(1):267-288.
33. O'brien RM. A Caution Regarding Rules of Thumb for Variance Inflation Factors. *Quality & Quantity*. 2007;41(5):673-690.
34. Grambsch PM, Therneau TM. Proportional Hazards Tests and Diagnostics Based on Weighted Residuals. *Biometrika*. 1994;81(3):515-526.
35. Schröder MS, Culhane AC, Quackenbush J, Haibe-Kains B. survcomp: an R/Bioconductor package for performance assessment and comparison of survival models. *Bioinformatics*. 2011;27(22):3206-3208.
36. Harrell FE, Jr., Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med*. 1996;15(4):361-387.
37. Pencina MJ, D'Agostino RB. Overall C as a measure of discrimination in survival analysis: model specific population value and confidence interval estimation. *Stat Med*. 2004;23(13):2109-2123.
38. Cohen J. A Coefficient of Agreement for Nominal Scales. 1960;20(1):37-46.
39. Schlenk RF, Frohling S, Hartmann F, et al. Phase III study of all-trans retinoic acid in previously untreated patients 61 years or older with acute myeloid leukemia. *Leukemia*. 2004;18(11):1798-1803.
40. Stolzel F, Pfirrmann M, Aulitzky WE, et al. Risk stratification using a new prognostic score for patients with secondary acute myeloid leukemia: results of the prospective AML96 trial. *Leukemia*. 2011;25(3):420-428.
41. Bertoli S, Berard E, Huguet F, et al. Time from diagnosis to intensive chemotherapy initiation does not adversely impact the outcome of patients with acute myeloid leukemia. *Blood*. 2013;121(14):2618-2626.
42. Rollig C, Kramer M, Schliemann C, et al. Does time from diagnosis to treatment affect the prognosis of patients with newly diagnosed acute myeloid leukemia? *Blood*. 2020.
43. Vosberg S, Herold T, Hartmann L, et al. Close correlation of copy number aberrations detected by next-generation sequencing with results from routine cytogenetics in acute myeloid leukemia. *Genes Chromosomes Cancer*. 2016;55(7):553-567.
44. Renneville A, Abdelali RB, Chevret S, et al. Clinical impact of gene mutations and lesions detected by SNP-array karyotyping in acute myeloid leukemia patients in the context of gemtuzumab ozogamicin treatment: results of the ALFA-0701 trial. *Oncotarget*. 2014;5(4):916-932.
45. Osterroos A, Bjorklund M, Eriksson A, et al. Integrated transcriptomic and genomic analysis improves prediction of complete remission and survival in elderly patients with acute myeloid leukemia. *Blood Cancer J*. 2020;10(6):67.
46. Gaidzik VI, Schlenk RF, Paschka P, et al. Clinical impact of DNMT3A mutations in younger adult patients with acute myeloid leukemia: results of the AML Study Group (AMLSG). *Blood*. 2013;121(23):4769-4777.
47. Rothenberg-Thurley M, Amler S, Goerlich D, et al. Persistence of pre-leukemic clones during first remission and risk of relapse in acute myeloid leukemia. *Leukemia*. 2018;32(7):1598-1608.
48. Morita K, Kantarjian HM, Wang F, et al. Clearance of Somatic Mutations at Remission and the Risk of Relapse in Acute Myeloid Leukemia. *J Clin Oncol*. 2018;36(18):1788-1797.
49. Jongen-Lavrencic M, Grob T, Hanekamp D, et al. Molecular Minimal Residual Disease in Acute Myeloid Leukemia. *N Engl J Med*. 2018;378(13):1189-1199.

50. Miles LA, Bowman RL, Merlinsky TR, et al. Single-cell mutation analysis of clonal evolution in myeloid malignancies. *Nature*. 2020;587(7834):477-482.
51. Daver N, Schlenk RF, Russell NH, Levis MJ. Targeting FLT3 mutations in AML: review of current knowledge and evidence. *Leukemia*. 2019;33(2):299-312.
52. Ghia P, Hallek M. Management of chronic lymphocytic leukemia. *Haematologica*. 2014;99(6):965-972.
53. Wang M, Lindberg J, Klevebring D, et al. Validation of risk stratification models in acute myeloid leukemia using sequencing-based molecular profiling. *Leukemia*. 2017;31(10):2029-2036.
54. Stone RM, Mandrekar SJ, Sanford BL, et al. Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation. *N Engl J Med*. 2017;377(5):454-464.
55. Stein EM, DiNardo CD, Fathi AT, et al. Ivosidenib or enasidenib combined with intensive chemotherapy in patients with newly diagnosed AML: a phase 1 study. *Blood*. 2020.
56. Cortes JE, Douglas Smith B, Wang ES, et al. Glasdegib in combination with cytarabine and daunorubicin in patients with AML or high-risk MDS: Phase 2 study results. *Am J Hematol*. 2018;93(11):1301-1310.
57. DiNardo CD, Tiong IS, Quaglieri A, et al. Molecular patterns of response and treatment failure after frontline venetoclax combinations in older patients with AML. *Blood*. 2020;135(11):791-803.
58. Maiti A, Qiao W, Sasaki K, et al. Venetoclax with decitabine vs intensive chemotherapy in acute myeloid leukemia: A propensity score matched analysis stratified by risk of treatment-related mortality. 2021;96(3):282-291.
59. Del Galy AS, Marouf A, Raffoux E, et al. Allogeneic hematopoietic stem cell transplantation in elderly patients with acute myeloid leukemia or myelodysplastic syndromes: myth and reality. *Leukemia*. 2021;35(1):225-228.
60. Frohling S, Schlenk RF, Kayser S, et al. Cytogenetics and age are major determinants of outcome in intensively treated acute myeloid leukemia patients older than 60 years: results from AMLSG trial AML HD98-B. *Blood*. 2006;108(10):3280-3288.
61. Schaich M, Parmentier S, Kramer M, et al. High-dose cytarabine consolidation with or without additional amsacrine and mitoxantrone in acute myeloid leukemia: results of the prospective randomized AML2003 trial. *J Clin Oncol*. 2013;31(17):2094-2102.
62. Burnett AK, Hills RK, Russell N. Twenty five years of UK trials in acute myeloid leukaemia: what have we learned? *Br J Haematol*. 2020;188(1):86-100.
63. DiNardo CD, Pratz KW, Letai A, et al. Safety and preliminary efficacy of venetoclax with decitabine or azacitidine in elderly patients with previously untreated acute myeloid leukaemia: a non-randomised, open-label, phase 1b study. *Lancet Oncol*. 2018.
64. Lancet JE, Uy GL, Cortes JE, et al. CPX-351 (cytarabine and daunorubicin) Liposome for Injection Versus Conventional Cytarabine Plus Daunorubicin in Older Patients With Newly Diagnosed Secondary Acute Myeloid Leukemia. *J Clin Oncol*. 2018;36(26):2684-2692.
65. Wei AH, Strickland SA, Jr., Hou JZ, et al. Venetoclax Combined With Low-Dose Cytarabine for Previously Untreated Patients With Acute Myeloid Leukemia: Results From a Phase Ib/II Study. *J Clin Oncol*. 2019:JCO1801600.
66. Vo TT, Ryan J, Carrasco R, et al. Relative mitochondrial priming of myeloblasts and normal HSCs determines chemotherapeutic success in AML. *Cell*. 2012;151(2):344-355.

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		
de novo	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
NPM1 mutation	2.25	1.15 - 4.51	0.02
NRAS mutation	0.46	0.23 - 0.91	0.02
SETBP1 mutation	0.16	0.02 - 0.87	0.04
RUNX1 mutation	0.43	0.23 - 0.81	0.009
ASXL1 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)			
NPM1 mutation	0.57	0.41 - 0.77	0.0004
FLT3-ITD low ratio	1.85	1.31 - 2.62	0.0005
FLT3-ITD high ratio	3.51	2.03 - 6.08	<0.0001
NRAS mutation	1.54	1.07 - 2.20	0.019
ASXL1 mutation	1.89	1.34 - 2.67	0.0003
DNMT3A mutation	1.86	1.40 - 2.47	<0.0001
Poor risk cytogenetics (N=84)			
KRAS mutation	3.60	1.68 - 7.72	0.001
TP53 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)**	***P*=**	**N (%)**	**2-y OS (95%CI)**	***P*=**	**N (%)**	**2-y OS (95%CI)**	***P*=**	**N (%)**	**2-y OS (95%CI)**	***P*=**
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.

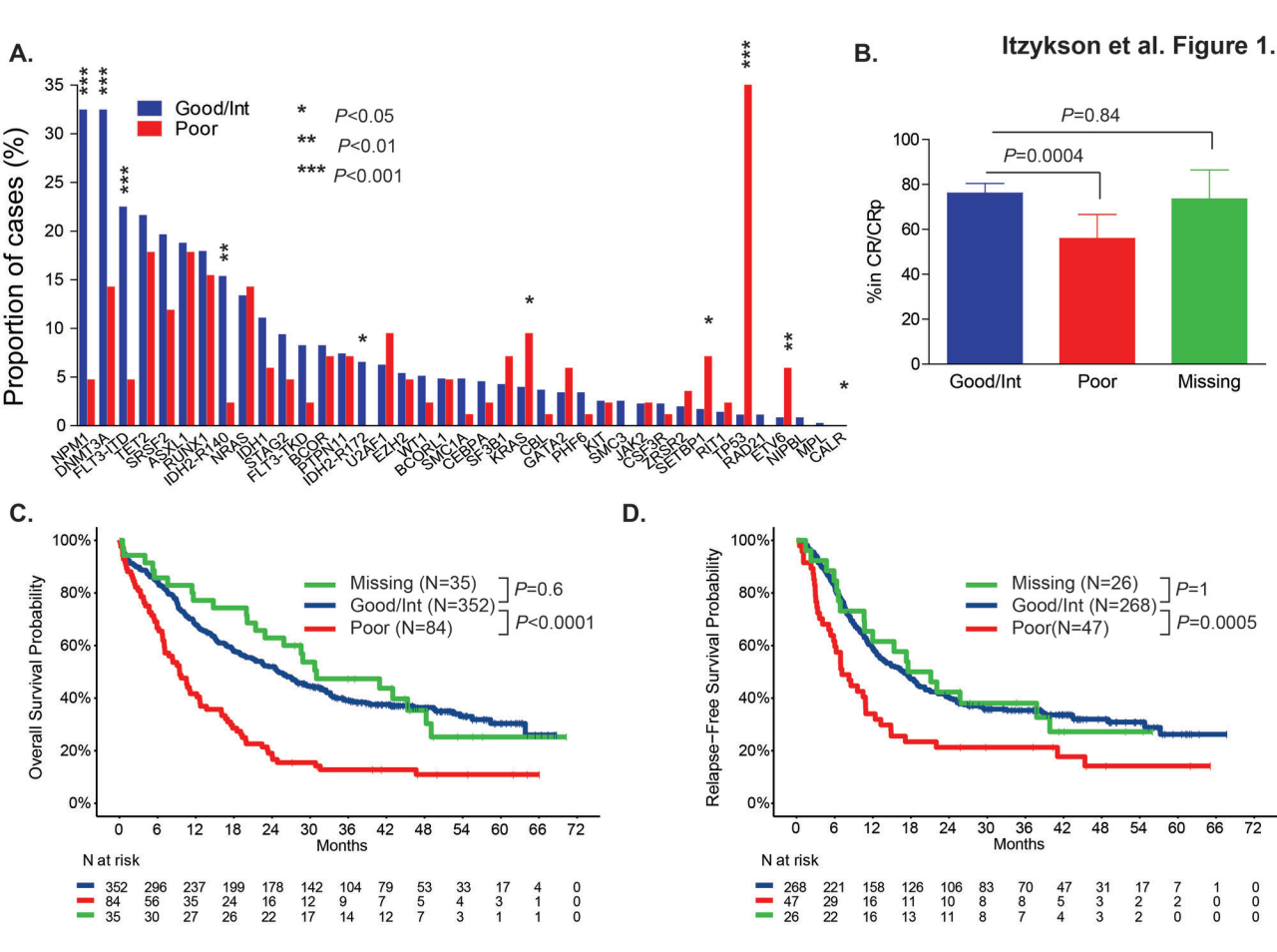
Figure Legends

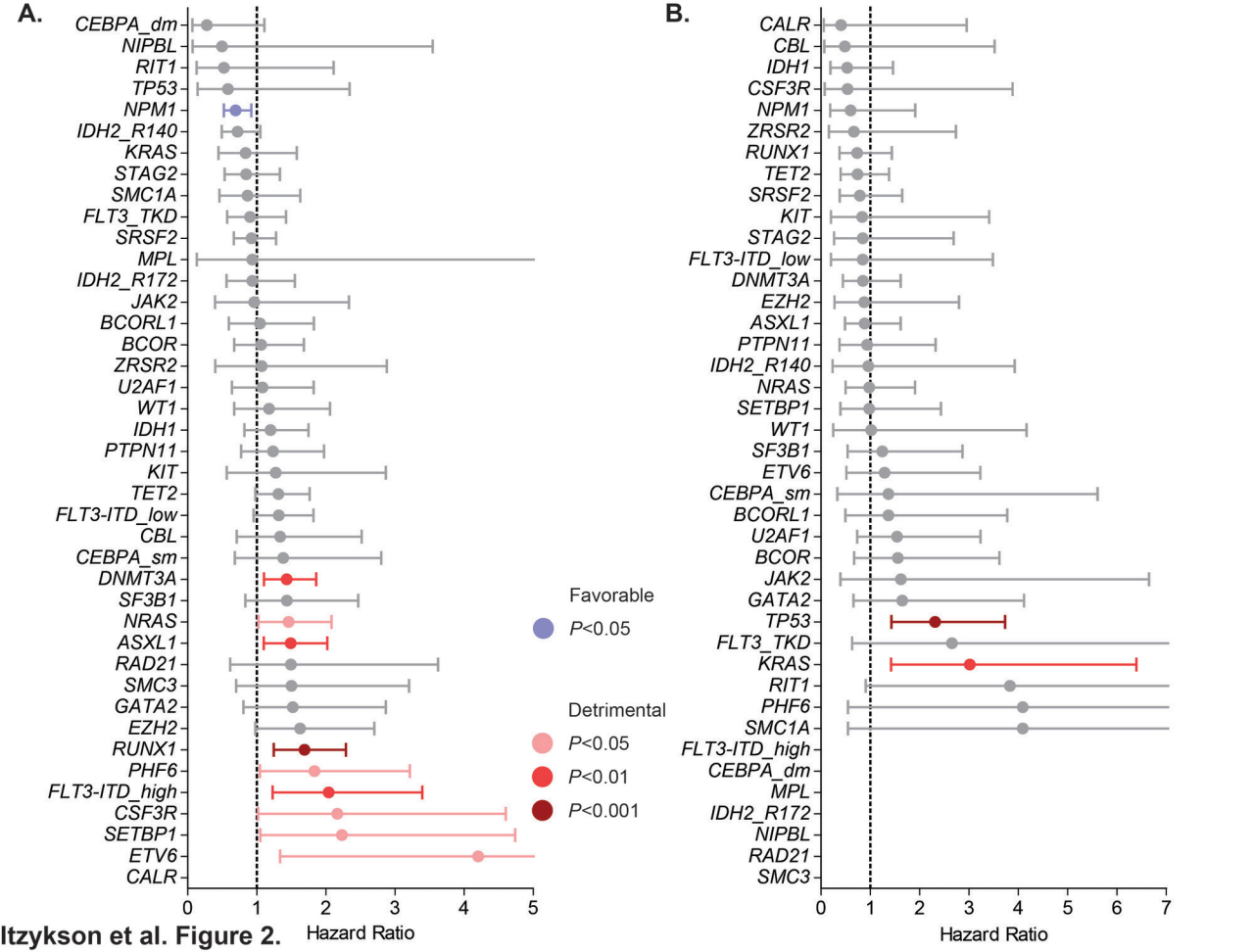
Figure 1. Differences in mutation pattern and outcome according to cytogenetic risk. A. Mutation pattern in patients with favorable/intermediate risk cytogenetics (N=352) versus adverse cytogenetics (N=84). *P* values from Fisher exact tests. **B.** Rates of CR/CRp at two courses in patients with good/intermediate (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.

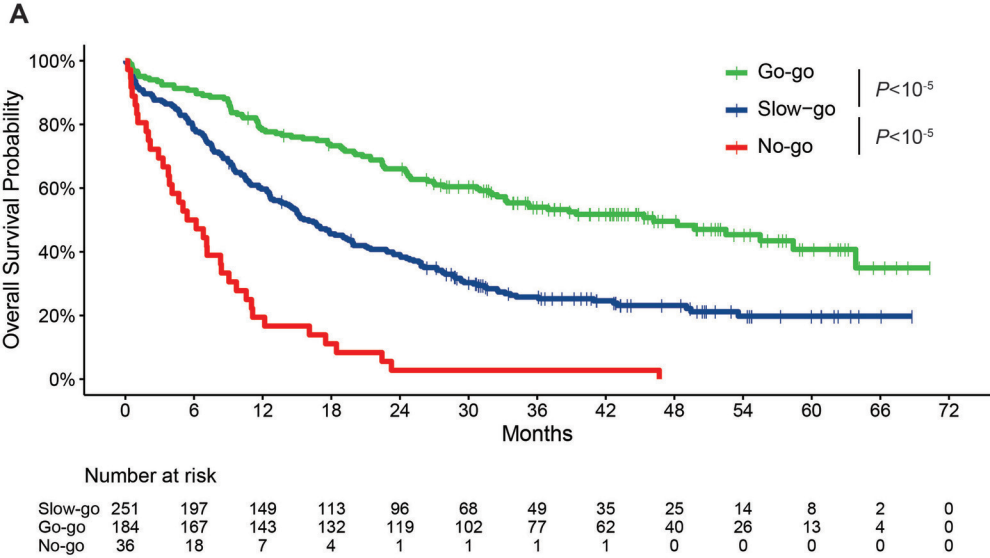
Figure 3. Outcome of the ALFA1200 cohort according to the ALFA Decision Tool. A. Overall Survival and (**B.**) Relapse-Free Survival according to ALFA decision tiers. P values from log-rank tests.

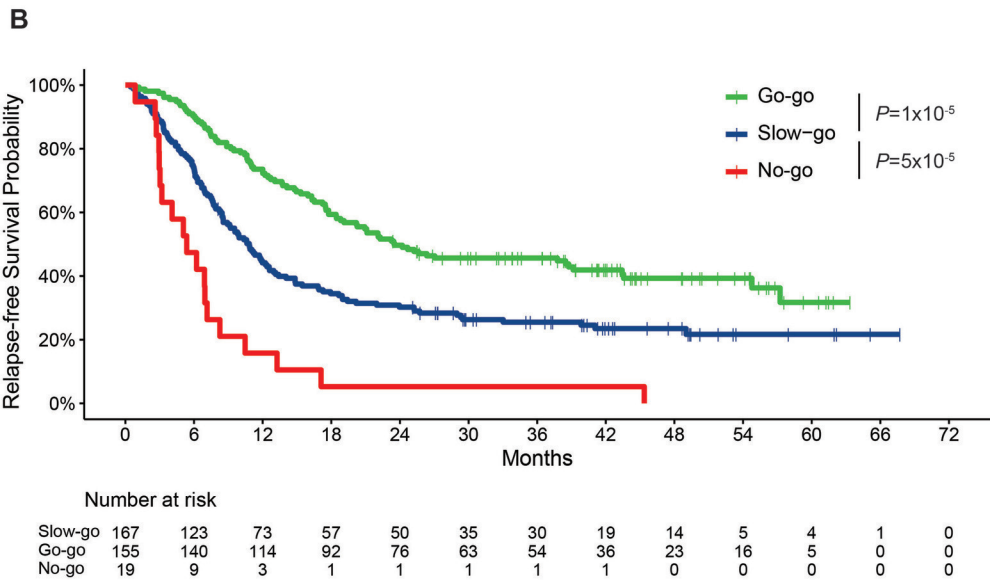
Figure 4. External validation of the ALFA decision tool. Overall Survival according to ALFA decision tiers in A. 141 patients accrued to the Hauts-De-France registry (HDF cohort), B. 466 patients accrued to SAL trials (SAL cohort), C. 223 patients 60 years or older (excluding t(15;17) cases) in the AMLSG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


Itzykson et al. Figure 1.
A.
Proportion of cases (%)
Good/Int
Poor
* P<0.05
** P<0.01
*** P<0.001
NPM1
DNMT3A
FLT3-ITD
TET2
SRSF2
ASXL1
RUNX1
IDH2-R140
NRAS
IDH1
STAG2
FLT3-TKD
BCOR
PTPN11
IDH2-R172
U2AF1
EZH2
WT1
BCORL1
SMC1A
CEBPA
SF3B1
KRAS
CBL
GATA2
PHF6
KIT
SMC3
JAK2
CSF3R
ZRSR2
SETBP1
RIT1
TP53
RAD21
ETV6
NIPBL
MPL
CALR
B.
P=0.84
P=0.0004
%in CR/CRp
Good/Int
Poor
Missing
C.
Overall Survival Probability
Missing (N=35)
Good/Int (N=352)
Poor (N=84)
P=0.6
P<0.0001
Months
N at risk
352 296 237 199 178 142 104 79 53 33 17 4 0
84 56 35 24 16 12 9 7 5 4 3 1 0
35 30 27 26 22 17 14 12 7 3 1 1 0
D.
Relapse-Free Survival Probability
Missing (N=26)
Good/Int (N=268)
Poor(N=47)
P=1
P=0.0005
Months
N at risk
268 221 158 126 106 83 70 47 31 17 7 1 0
47 29 16 11 10 8 8 5 3 2 2 0 0
26 22 16 13 11 8 7 4 3 2 0 0 0

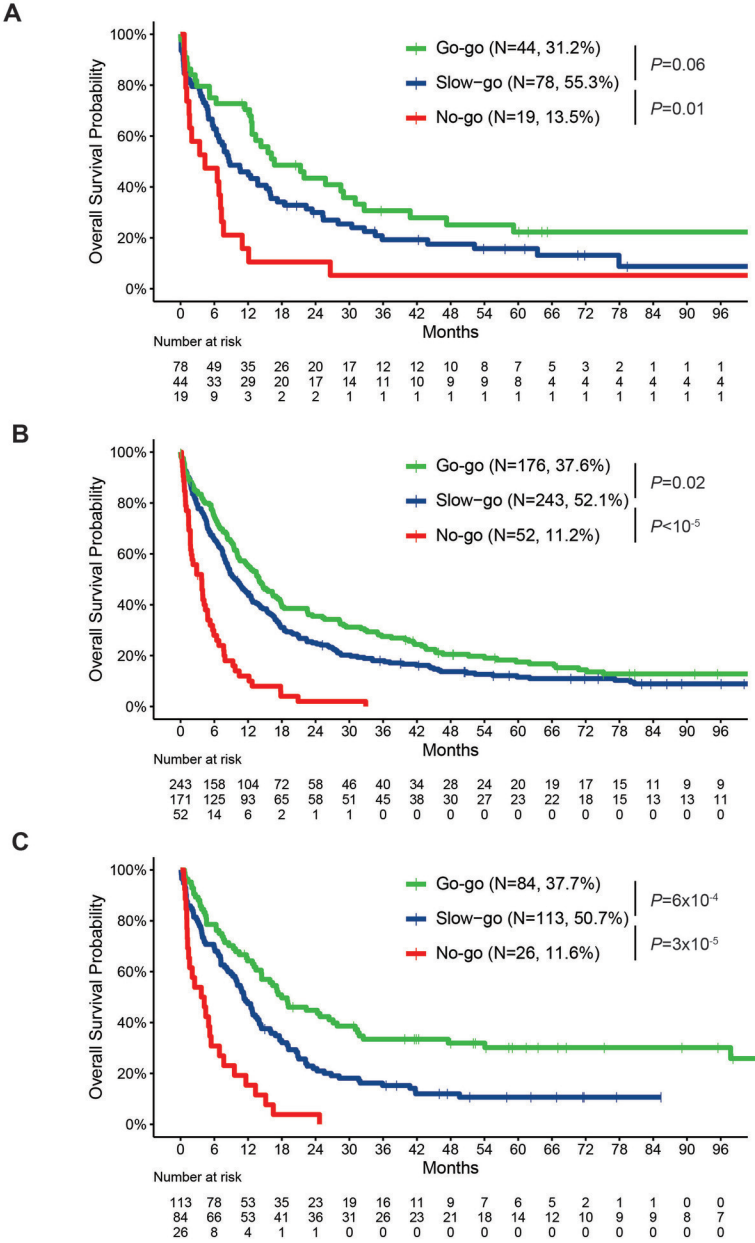


Itzykson et al. Figure 2.





Itzykson et al. Figure 3.



Itzykson et al. Figure 4.