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A Revised Prognostic Model for Patients with Acute Myeloid Leukemia and First Relapse

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Abstract:
Most patients with acute myeloid leukemia (AML) may obtain remission upon induction chemotherapy, but relapse is frequent and associated with poor survival. Previous prognostic models for outcomes after relapse lacked analysis of comprehensive molecular data. A validated prognostic model integrating clinical, cytogenetic, and molecular variables may support treatment decisions. We studied 943 AML patients who relapsed after first-line intensive induction treatment in a development cohort (HOVON-SAKK). A random survival forest algorithm was used to evaluate the association of clinical parameters, cytogenetic abnormalities, and molecular variables at diagnosis with overall survival (OS). Relapsing patients (n=377) who were enrolled in the NCRI-AML18 trial were used for model validation. In the development cohort, the median age at relapse was 58 years, and patients were classified as 2022 ELN favorable (22%), intermediate (31%), and adverse (48%) risk. One-third underwent allogeneic transplantation in first complete remission. Variable selection yielded nine variables significantly associated with 1-year OS, including relapse-free interval, age, white blood cell count, mutated TP53, FLT3-ITD, core-binding factor abnormalities, t(v;11q23)/KMT2A-rearranged and complex/monosomal karyotype, which were assigned points according to their estimated hazard ratios. Three prognostic groups were defined with distinct 1-year OS in both development (favorable: 51±3%, intermediate: 29±3% and poor: 14±2%, respectively) and validation cohorts (51±4%, 26±5% and 14±3%, respectively). Independent validation confirmed the improved accuracy in predicting outcomes for AML patients in first relapse. The revised AML relapse model improved on previous classification systems for prognostication of outcomes after first AML relapse. It provides stratification which might support tailoring second line treatment.

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KEY POINTS

- The revised AML relapse model includes nine predictors, grouped into three risk categories which are each associated with distinct OS
- The model outperformed prior scoring systems and was validated in an independent cohort of AML relapse patients

ABSTRACT

Most patients with acute myeloid leukemia (AML) may obtain remission upon induction chemotherapy, but relapse is frequent and associated with poor survival. Previous prognostic models for outcomes after relapse lacked analysis of comprehensive molecular data. A validated prognostic model integrating clinical, cytogenetic, and molecular variables may support treatment decisions. We studied 943 AML patients who relapsed after first-line intensive induction treatment in a development cohort (HOVON-SAKK). A random survival forest algorithm was used to evaluate the association of clinical parameters, cytogenetic abnormalities, and molecular variables at diagnosis with overall survival (OS). Relapsing patients (n=377) who were enrolled in the NCRI-AML18 trial were used for model validation. In the development cohort, the median age at relapse was 58 years, and patients were classified as 2022 ELN favorable (22%), intermediate (31%), and adverse (48%) risk. One-third underwent allogeneic transplantation in first complete remission. Variable selection yielded nine variables significantly associated with 1-year OS, including relapse-free interval, age, white blood cell count, mutated *TP53*, *FLT3*-ITD, core-binding factor abnormalities, t(v;11q23)/*KMT2A*-rearranged and complex/monosomal karyotype, which were assigned points according to their estimated hazard ratios. Three prognostic groups were defined with distinct 1-year OS in both development (favorable: 51±3%, intermediate: 29±3% and poor: 14±2%, respectively) and validation cohorts (51±4%, 26±5% and 14±3%, respectively). Independent validation confirmed the improved accuracy in predicting outcomes for AML patients in first relapse. The revised AML relapse model improved on previous classification systems for prognostication of outcomes after first AML relapse. It provides stratification which might support tailoring second line treatment.

INTRODUCTION

Disease recurrence is the most common cause of treatment failure in patients with acute myeloid leukemia (AML). Treatment of relapsed AML remains challenging and outcome has been traditionally poor with median overall survival (OS) of less than 6 months.[1-5] Although outcome has been improved with novel targeted treatments,[6-9] allogeneic hematopoietic cell transplantation (allo-HCT) remains the only curative treatment for relapsing patients.[10,11] Risk models for these patients might support decision making for reinduction treatment and consolidation treatment with allo-HCT, whereas alternative, experimental strategies might be considered for high-risk patients.

Prognostic factors associated with survival after relapse in AML have previously been identified.[1,2,5,12-14] These include the time between the first complete remission (CR1) and relapse, age at relapse, the presence of favorable and unfavorable karyotypes, the presence of *FLT3* internal tandem duplications (ITD), and prior allo-HCT.[1,2,5,12-14] Earlier developed prognostic models[1,2] groups have highlighted these factors as significant predictors associated with survival after first AML relapse. Since the development of these risk models, an increasing number of molecular and cytogenetic alterations has been recognized impacting outcome in newly diagnosed AML patients,[11,15] which might also affect survival after first relapse. Models that allow for risk stratification after first relapse using cytogenetic and molecular variables established at initial diagnosis in a recently treated AML patient cohort are currently lacking.

We set out to study a large cohort of AML patients who relapsed after intensive induction treatment and for whom baseline comprehensive clinical, cytogenetic and molecular variables were available. We developed a revised stratification system which was validated in an independent cohort of older AML patients.

METHODS

Clinical cohorts

The complete cohort consisted of 5086 patients aged 18 years and older enrolled in eight consecutive HOVON-SAKK (HO42, HO42A, HO43, HO81, HO92, HO102, HO103, HO132, Supplementary Materials) clinical trials between 2000 and 2018 with an intensive induction chemotherapy backbone for newly diagnosed AML and high-risk myelodysplastic syndrome (MDS) with IPSS ≥1.5, IPSS-R risk score >4.5 or excess blasts of ≥10% (see Figure S1A-H for trial details).[16-23] Post-remission treatment with either additional chemotherapy, high-dose chemotherapy followed by autologous HCT, or allo-HCT was based on AML risk in evolving classifications.[11,24] Patients were excluded because of no next-generation sequencing data available at diagnosis (n=2543), no CR or CR with incomplete count recovery (CRi) following two cycles of induction chemotherapy (n=416), and no relapse (n=1184). The final development cohort consisted of 943 patients who developed a first relapse (Figure 1). The validation cohort included newly diagnosed AML and high-risk MDS patients with excess blasts of ≥10% aged 60 years and older enrolled in the NCRI-AML18 trial who were treated with intensive induction chemotherapy between 2014 and 2018 (Figure S1I).[25] Patients were excluded because of no CR or CR with incomplete count recovery (CRi) following two cycles of induction chemotherapy (n=245), and no relapse (n=354). The final validation cohort consisted of 377 patients who developed a first relapse (Figure 1). All trial participants provided written informed consent in accordance with the Declaration of Helsinki.

Genetic analysis

High molecular weight genomic DNA was extracted from bone marrow samples or peripheral blood. Diagnostic samples at first AML diagnosis underwent gene re-sequencing using either a

97-gene panel (Oxford for UK-AML18) or a 54-gene panel (Erasmus MC, Rotterdam for HOVON-SAKK), containing the most frequently mutated genes in myeloid malignancies. Targeted next-generation sequencing (NGS) used liquid-phase capture of molecule-barcoded libraries, with custom-designed probes targeting AML-associated genes for UK-AML18 (Roche, Basel, Switzerland) or the Illumina TruSight Myeloid panel for HOVON-SAKK (Illumina, San Diego, CA). Genomic libraries were sequenced on the Illumina platform, ensuring a minimum mean target coverage of 500x. Details of cytogenetic analysis, NGS, and additional molecular analyses have been previously published.[15]

Statistical methods

The analysis adhered to the TRIPOD guideline for prediction model development and validation.[26] A total of 45 variables (Table S1) were considered for the association with 1-year OS in model development: age at relapse (≥60 vs. <60 years), relapse free interval (≤12 months vs. >12 months) defined as the time between CR1 and relapse, number of treatment cycles needed to obtain CR1 (1 vs. >1), sex (male vs. female), white blood cell count (WBC) at diagnosis (≥10 vs. <10 [$x10^9$/L]), previous allo-HCT (yes vs. no), previous autologous HCT (yes vs. no), 29 different gene mutations (presence vs. absence), and nine different cytogenetic abnormalities (presence vs. absence) (see Table S1 for details of mutations and cytogenetic variables). Complex and monosomal karyotype were grouped due to their frequent co-occurrence in this cohort (94% concordant). During variable selection, we evaluated different thresholds for age, relapse free interval and WBC, and found that the most optimal cut-offs were 60 years, 12 months and 10 $x10^9$/L, respectively. Gene mutations and cytogenetic abnormalities were only evaluated if present in 10 or more patients (Table S1). OS was defined as the time from the date of first relapse to either the date of death from any cause or the date of last contact while still alive. OS curves were estimated using the Kaplan-Meier method and

compared using the log-rank test. Model development consisted of three sequential stages which included: (1) variable selection using a random survival forest; (2) Cox regression analysis with backward selection ($P<0.10$) to define the optimal model; and (3) assignment of weights and defining groups based on significantly different OS compared to a lower score to construct the final prognostic model. Further details are available in the Supplementary Materials. The impact of allo-HCT after relapse was assessed using a time-dependent Cox regression allowing the allo-HCT covariate to change the state at the time of allo-HCT. Fine-Gray models were used to estimate the probability of an event accounting for competing risks, non-relapse mortality (NRM) and relapse. Prognostic accuracy of existing models and the revised model was evaluated using Harrels' C-index. Cytogenetic data were not available in 38 patients (4.0%) due to failed karyotyping and 3 patients (0.3%) had missing WBC at diagnosis in the development cohort. Missing cytogenetics data were uniformly imputed as absent, consistent with real-world clinical practice, and with the median WBC count for missing WBC. All analyses were performed in R version 4.4.1. The R script of the analyses can be found online (https://github.com/niekvandermaas/AML-relapse-model).

RESULTS

Patient characteristics

The development cohort consisted of 943 patients who relapsed after having obtained CR1 upon initial intensive treatment (Table 1). In this cohort, 1-year OS was similar across treatment periods (<2010 vs. ≥2010, Figure S2) and disease type (AML vs high-risk MDS, Figure S3). In addition, no difference was observed for 1-year OS between missing and non-missing NGS data (Figure S4). The median age of patients at relapse in this cohort was 58 (range 18-81 years, Table 1). At diagnosis, 22% of the patients were classified as having a favorable risk according to the European LeukemiaNET (ELN) 2022 AML classification, 31% as intermediate risk, and 48% as adverse risk (Table 1).[11] In this cohort, 33% of patients received post-remission treatment with allo-HCT, while 14% underwent autologous HCT in first CR (Table 1). A total of 95% relapsing patients had at least one cytogenetic abnormality or genetic mutation at diagnosis (median 3), with 62% of patients having molecular mutations only, 3% having karyotype alterations only, and 32% having both. Most mutations at diagnosis were found in the following genes: *DNMT3A* (30%), *NPM1* (27%), and *FLT3*-ITD (22%). Mutated *TP53* was found in 12% of patients. Complex and/or monosomal karyotype according to ELN 2022 was the most frequent cytogenetic abnormality in these patients (19%), whereas core binding factor (CBF) abnormalities (t(16;16)/inv(16) [3%] and t(8;21) [2%]) were relatively infrequent (Figure 2).[27] The development cohort had a median follow up from relapse of 49 months for patients alive (range: 0-128 months, Table 1). OS at 1 and 4 years after first relapse in the development cohort were 33±2% and 16±1%, respectively.

Patient outcomes by previous prognostic models

Previous prognostic models were assessed for their predictive performance in the development cohort. The HOVON-SAKK model[1] was associated with a C-index of 0.65±0.016 for 1-year OS. The majority of patients were classified as adverse risk in that model (684 out of 943, 73%). 1-year OS after relapse was clearly distinct estimating 74±7% for the favorable group, 54±3% for the intermediate group, and 24±2% for the adverse group, respectively (Figure 3A). Similarly, the GOELAMS model[2] was associated with a C-index of 0.64±0.017, which outcomes were distinct in the three risk groups associated with survival probabilities at 1-year 49±3% for the favorable group, 29±2% for the intermediate group, and 11±3% for the adverse group (Figure 3B).

Development of a revised prognostic model for patients in first relapse

To determine the optimal patient-specific and genetic variables for risk stratification of OS, we used a random survival forest algorithm, which revealed the hierarchy of each clinical, genetic, and cytogenetic variable for stratifying 1-year OS (details in Supplementary Materials). A total of 45 candidate variables were considered (Table S1), of which 18 were identified as significantly impacting (P<0.01) 1-year OS (Figure S5). These were included in a multivariable Cox regression analysis using a stepwise backward selection which resulted in a final model of 9 predictors. The three most important variables for 1-year OS after relapse were the absence of CBF abnormalities (hazard ratio [HR] 2.07, 95% confidence interval [95%CI] 1.38-3.12, P<0.001), mutated *TP53* (HR 1.99, 95%CI 1.48-2.67, P<0.001) and relapse free interval ≤12 months (HR 1.76, 95%CI 1.46-2.12, P<0.001) (Figure 4A). Notably after adjustment for covariates, including mutated *TP53*, complex and/or monosomal karyotype was independently correlated with adverse OS (HR 1.40 95%CI 1.08-1.82, P=0.01, Figure 4A). Each of the nine variables was assigned points based on their rounded HRs (Figure 4A) resulting in 3 points for either no CBF abnormalities or a *TP53* mutation. Similarly, relapse free interval ≤12 months,

198 previous allo-HCT, t(v;11q23)/KMT2A-rearranged, and age at relapse ≥60 years were each
199 assigned 2 points, whereas WBC ≥10 x10^9/L, complex and/or monosomal karyotype and *FLT3*-
200 ITD were assigned 1 point. These points were subsequently used to derive the total score for
201 each patient. The median total score in the development cohort was 7 points (range 1-14). OS
202 decreased with increasing scores (Figure S6). Based on significantly different OS, we collapsed
203 the development cohort into three groups: favorable (≤6 points; n=389, 42%), intermediate (7
204 points; n=197, 21%), poor (≥8 points; n = 357, 38%, Figure S7).

205 Favorable risk patients in the revised model had a 1-year OS of 51±3% (Figure 4B).
206 Intermediate risk patients had a 1-year OS of 29±3%, which was 14±2% in the poor risk
207 subgroup (Figure 4B). The C-index of the prognostic model in the development cohort was
208 0.71±0.016 with excellent calibration (Figure S8). 4-year OS was also significantly different
209 amongst the three subgroups (29±2%, 11±2%, 5±1%, respectively, Figure S9A). The model
210 restratified 57% and 49% of patients from the previous HOVON-SAKK and GOELAMS risk
211 groups, respectively. The majority of patients in the large HOVON-SAKK poor risk group were
212 reclassified as favorable (26%) or intermediate (24%), whereas 30% and 42% of patients in the
213 large GOELAMS intermediate risk group were reclassified to the favorable and poor risk group
214 of the revised model, respectively (Figures 4C and 4D).

215 *Model validation*

216 The model was validated in an independent dataset derived from the NCRI-AML18 trial,
217 consisting of 976 older newly diagnosed AML patients aged 60 years and older who also
218 received intensive induction chemotherapy. A total of 377 relapsing patients were identified with
219 a median age at relapse of 69 (range 60-81) years (Figure 5A). Sixty-two percent were
220 classified as poor or very poor risk in the AML60+ classification at the time of diagnosis,[15]
221 whereas 60% were adverse according to the 2022 ELN risk classification. Allo-HCT in CR1 was

applied in 14% as post-remission treatment (Figure 5A). A total of 97% of patients had at least one cytogenetic or molecular abnormality (median 4), with 77% of patients having molecular mutations only, 1% having karyotype alterations only, and 21% having both. The most frequently mutated genes identified were *DNMT3A* (37%), *NPM1* (27%) and *ASXL1* (26%). *TP53* was mutated in 11% of patients. According to ELN 2022 criteria, the most common cytogenetic abnormality was a complex and/or monosomal karyotype (14%), while CBF abnormalities, specifically t(16;16)/inv(16) (1%) and t(8;21) (3%), were rare (Figure 5B). Median follow up from relapse was 21 months for patients alive (range: 0-59 months, Figure 5A). OS at 1 and 4 years after first relapse year in the validation cohort were 30±2% and 9±2%, respectively. The three groups defined in the development cohort were used to classify the validation cohort: favorable (≤6 points; n=137, 36%), intermediate (7 points; n=85, 23%), and poor (≥8 points; n=155, 41%), which were associated with distinct 1-year and 4-year OS (1-year OS: 51±4%, 26±5%, and 14±3%, respectively, Figure 5C and Figure S9B). The performance of the revised prognostic model as measured by the C-index was 0.71±0.028 with excellent calibration (Figure S10). The GOELAMS and HOVON-SAKK models had C-indices of 0.69±0.027 and 0.62±0.027 in the validation cohort, respectively. Similar to the development cohort, the revised model restratified 59% from the original HOVON-SAKK risk groups and 35% from the GOELAMS groups (Figures 5D-E). HOVON-SAKK poor risk patients were considered favorable or intermediate in 24% and 26%, respectively, in the revised model.

Treatment after relapse

In the development cohort, reinduction treatment was used in 690 (73%) patients, which resulted in a second CR (CR2) in 348 (50%) of patients (Figure S11, Table S2). Across subgroups, CR2 after re-induction treatment was obtained in 65% and 44% of the favorable and intermediate risk patients, respectively, which was higher compared with the poor risk group

(32%, C-index 0.66, Table S2 and S3). Additionally, the revised model showed a distinct OS and event-free survival (EFS) for patient that received reinduction treatment, and relapse-free survival (RFS) for patients in CR2 (Figure S12 and Table S3). The revised model compared favorably in terms of accuracy to these outcomes compared with the previous risk stratification systems (Table S3). Allo-HCT was used as consolidation treatment of a CR2 in 159 (46%) patients, and 38 (11%) received a donor lymphocyte infusion (DLI) after CR2 (Figure S11, Table S2). A total of 342 (50%) patients did not achieve a CR2 despite re-induction treatment consisting of intensive chemotherapy (n=279, 82%), upfront allo-HCT (n=43, 13%), or DLI (n=20, 6%) (Figure S11, Table S2).

A time-dependent analysis with allo-HCT as a time-varying covariate was performed for patients who had attained a CR2. OS was improved by allo-HCT compared with no allo-HCT (HR 0.54; 95%CI 0.37-0.79, P=0.001, Figure S13) and cumulative incidence of relapse was lower after allo-HCT compared with no allo-HCT (HR 0.58; 95%CI 0.38-0.88, P=0.011, Figure S13). In contrast, the cumulative incidence of NRM was higher in the allo-HCT group, although not significantly different (HR 1.30; 95%CI 0.61-2.80, P=0.499, Figure S13). Low patient numbers precluded an analysis of the impact of allo-HCT per risk group.

DISCUSSION

Relapse in AML is frequent with low response rates to reinduction treatment and dismal OS.[1-5,14] Recent prognostic models for relapsed AML patients incorporating comprehensive genetic data are currently lacking. Therefore, it is necessary to reassess the prognostic value of clinical, cytogenetic and molecular AML characteristics in patients with relapsed AML after intensive induction treatment. We analyzed the genomic landscape and clinical outcomes of 943 AML patients with a first relapse aiming to develop a simple prognostic classification system. Using a machine-learning method for variable selection, nine variables were identified which were used to stratify patients into three risk groups. Although age, relapse-free interval, CBF abnormalities, *FLT3*-ITD, and prior allo-HCT were confirmed from previous risk models,[1,2,14] we additionally found WBC count, mutated *TP53*, t(v;11q23)/*KMT2A*-rearranged and complex/monosomal karyotype. The prognostic model was associated with highly distinct OS in three risk groups. The revised prognostic model was validated in an independent cohort of older AML patients treated within the NCRI-AML18 trial indicating similar prognostic accuracy. It classifies patients into other risk groups allowing for better discrimination of OS outcomes for AML patients experiencing their first relapse compared with existing prognostic models, like those from the HOVON-SAKK and GOELAMS groups.[1,2]

Salvage treatment for patients with relapse AML includes high-dose chemotherapy, hypomethylating agents, or targeted treatments for patients with specific mutations followed by allo-HCT as consolidation treatment in patients who obtain CR2.[10,11] High-dose chemotherapy regimens (e.g., intermediate-dose cytarabine with or without anthracycline, or FLAG-Ida) are associated with remission rates of 20-65%, but also considerable toxicity and mortality (6-22%).[28-33] Although the type and intensity of chemotherapy-based reinduction treatment strategies were not available in our dataset, we observed a relatively high CR2 rate of 65% and

44% for favorable and intermediate risk patients, which was 32% for poor risk patients. Alternative salvage treatment approaches might be considered such as targeted treatments with or without hypomethylating agents. Gilteritinib has been approved for patients with relapsed *FLT3* AML with CR rates of 34%.[8] Similarly, both ivosidenib (*IDH1* inhibitor) and enasidenib (*IDH2* inhibitor) offer 23-33% CR rates in refractory or relapsed patients with *IDH*1/2-mutated AML,[6,7] while menin-inhibitors show a CR rate of 30% in *KMT2A*-rearranged or *NPM1* mutated leukemia.[9] Alternatively, combining venetoclax, a BCL2 inhibitor, with hypomethylating agents presents as a potential alternative to attain CR2, despite the absence of trial data in the relapsed setting.[34-37] Combination treatments of targeted drugs, hypomethylating agents and venetoclax, or high-dose chemotherapy with venetoclax are currently investigated and early results have been encouraging.[38-41] Nevertheless, it remains largely unknown whether patient outcomes will significantly improve with these novel treatment modalities. These recent advancements may transform the therapeutic landscape of relapsed AML significantly in the next years, making it a necessity to further validate this prognostic index to reflect emerging treatment modalities in the future.

Historically, allo-HCT in CR2 has been the preferred approach for long-term survival. Our analysis confirms that allo-HCT in CR2 provides a survival benefit with reduced relapse incidence compared with non-allo-HCT treatments. Nonetheless, the benefit of allo-HCT in terms of relapse reduction can be compromised by NRM, particularly in older patients or those with underlying comorbidities.[42-44] Although the risk of a second failure without allo-HCT is high (85% in our study), it needs to be balanced against the risk of NRM as assessed by risk scores.[42-44] Ultimately, the decision to proceed with allo-HCT after AML relapse requires a personalized approach, depending on the specific characteristics of each patient and their disease. Of note, data from the recent ASAP trial suggested that immediate allo-HCT might be an alternative for fit patients with non-proliferative refractory/relapsed AML who have a stem cell

311 donor available.[45] However, the small number of relapsing patients in this study preclude robust
312 conclusions.

313 Our study has several limitations. The optimal external validation cohort may be debated, but
314 TRIPOD guidelines have recommended using a cohort that differs in key characteristics (e.g.,
315 age), treatment approaches, or time period.[26] The revised model was validated in an
316 independent cohort, in which patients received different intensive chemotherapy regimens and
317 were older compared with the development cohort. The model was associated with strong
318 performance across both cohort populations, without any limitations imposed by narrow age
319 restrictions, highlighting broad applicability in relapsing AML patients after first-line intensive
320 treatment. Secondly, intensive chemotherapy and targeted therapies might exert a selective
321 pressure to the clonal landscape of AML, leading to both treatment-sensitive and treatment-
322 resistant cells. For example, RAS mutations that are acquired at relapse may confer a
323 particularly therapy-resistant disease.[46,47] Leukemic transformation by RAS mutations exhibit
324 resistance to the BCL2 inhibitor venetoclax, driving clinical resistance, relapse and worse OS
325 after relapse following venetoclax-based therapy.[37,46] This clonal evolution of the AML is not
326 accounted for as molecular and cytogenetic data were only available from the time of initial
327 diagnosis. The predictive accuracy and discriminatory power of the model, as measured by the
328 C-index, indicate that the model performs relatively good in distinguishing between patients with
329 different risk levels. The C-index quantifies the risk classification performance based on the
330 predicted risk, with values ranging from 0.5 (no better than random chance) to 1.0 (perfect
331 discrimination). A higher C-index indicates better model performance in correctly identifying
332 which patients are more likely to experience an event. The revised relapse model improved
333 upon previous risk classification systems, and its predictive capability may be further enhanced
334 by incorporating molecular and cytogenetic data at the time of relapse. Additionally, the model
335 was developed in cohort of patients who did not receive FLT3 inhibitors as part of the first-line

induction regimen. Although 26% of patients (22 out of 85) with *FLT3*-ITD received quizartinib added to the intensive induction backbone in the validation cohort, the model needs further validation in the era of FLT3 targeted combination therapies. Lastly, there is an inherent bias regarding treatment decision making in the patient population after first relapse. High risk patients might not have received treatment because of their adverse risk factors (e.g., mutated *TP53*, short relapse free interval, older age, other adverse cytogenetics). For example, in the poor risk group, only 215 patients (60%) received reinduction treatment. Among those, response rates were very low (32%), with only 20 patients proceeding to allo-HCT in CR2, suggesting that this is a particularly difficult AML population to treat.

In conclusion, the revised prognostic classification system for adult patients with AML in first relapse offers a useful and distinctive model for clinical practice. It identifies favorable or intermediate risk patients who may benefit from reinduction strategies and consolidation with allo-HCT taking into account the risk of NRM. Conversely, patients classified within the poor risk group have dismal survival after first relapse and might be considered for novel treatment strategies, experimental treatments or even best supportive care. To facilitate the integration of this prognostic tool into clinical practice, an online calculator has been developed.[48] Based on a patient-specific hematologic, cytogenetic, and molecular profile, the tool provides a personalized prediction score, assigns an associated risk group, and offers estimated OS projections. This tool may aid clinicians in evaluating the relative benefits of salvage and experimental treatments, balancing these against potential treatment-related risks.

AUTHOR CONTRIBUTION STATEMENT

N.M. and J.V. designed the study, analyzed the data, and wrote the manuscript; D.B., T.P., A.T., B.J.B., J.K., C.E., O.V., M.C.V., C.G., J.M., S.K., M.D., S.F., I.T., G.H., C.C., P.V., N.R., G.O., and B.L. provided patient data; N.M., P.G., H.B.B., P.J.V. and J.V. collected and assembled clinical, laboratory, and genetic data; N.G. and J.V. conceived the statistical plan and performed the statistical analysis; N.G., J.C. and J.V. interpreted data and contributed to research discussion; and all authors reviewed the manuscript and approved the submission. N.G. and J.V. had full access to all data.

CONFLICT-OF-INTEREST DISCLOSURE

None of the authors has a relevant conflict of interest.

DATA SHARING SECTION

Requests for data sharing may be submitted to Jurjen Versluis (j.versluis.1@erasmusmc.nl).

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Table 1. Patient characteristics at first AML diagnosis

	Development cohort (n=943)
Age at relapse	
Median (range)	58.0 (18.0-81.0)
Sex	
Male	523 (55.5%)
Female	420 (44.5%)
WBC (at diagnosis;[x10^9/L])	
Median (range)	8.45 (0-510)
ELN risk at diagnosis	
Favorable	204 (21.6%)
Intermediate	289 (30.6%)
Adverse	450 (47.7%)
Best response within 2 induction cycles	
CR	889 (94.3%)
CRi	54 (5.7%)
HCT in first CR(i)	
No	506 (53.7%)
Allogeneic	310 (32.9%)
Autologous	127 (13.5%)
Relapse free interval (months)	
Median (range)	8 (0-124)
Follow-up of patients alive after first relapse (months)	
Median (range)	49 (0-128)

Abbreviations: ELN, European Leukemia Network; CR, complete remission; CRi, complete

remission with incomplete count recovery; HCT, hematopoietic cell transplantation; WBC, white blood cell count.

FIGURE LEGENDS

Figure 1. Consort diagram of patients included in this study

Abbreviations: AML, acute myeloid leukemia.

Figure 2. Mutational landscape of the development cohort at first AML diagnosis

Driver events found at diagnosis in 943 patients with relapsed AML. Each bar represents a driver lesion, including gene mutations, and chromosomal abnormalities. Abbreviations: abn, abnormality in; ck, complex karyotype (annotated according to the 2022 ELN risk classification); del, deletion in; inv, inversion of; ITD, internal tandem duplication; mk, monosomal karyotype; m, monosomy; t, translocation of; TKD, tyrosine kinase domain.

Figure 3A-B. Overall survival after 1 year by previous prognostic models

Overall survival in the development cohort by (A) HOVON-SAKK and (B) GOELAMS prognostic models.

Figure 4A-D. Revised prognostic model for AML after first relapse

Hazard ratios with 95% confidence interval and P-value of final model in the- development cohort with points for a clinical prediction score (A) and overall survival after 1 year for patients in first relapse according to risk categories of the revised prognostic model in the development cohort (B). Figure S9A shows the 4 year overall survival rates for patients from their first relapse, categorized by the revised prognostic model. Restratification of patients from HOVON-SAKK (C) and GOELAMS (D) model to the revised prognostic model in the development cohort. Abbreviations: allo-HCT, allogeneic hematopoietic cell transplantation; CBF, core-binding factor; ITD: internal tandem duplication; mk, monosomal karyotype; t, translocation of.

Figure 5A-E. Validation of the revised prognostic model in the NCRI-AML18 trial

Patient characteristics (A) with the molecular and cytogenetic landscape at diagnosis of the validation cohort (B) and overall survival after 1 year for patients in first relapse according to risk categories of the revised prognostic model (C). Figure S9B shows the 4 year overall survival rates for patients at their first relapse, categorized by the revised prognostic model. Restratification of patients from HOVON-SAKK (D) and GOELAMS (E) model to the revised prognostic model in the validation cohort. Abbreviations: abn, abnormality in; ck, complex karyotype (annotated according to the 2022 ELN risk classification); CR, complete remission; CRi, complete remission with incomplete count recovery; del, deletion in; ELN, European Leukemia Network; HCT, hematopoietic cell transplantation; inv, inversion of; ITD, internal tandem duplication; mk, monosomal karyotype; m, monosomy; t, translocation of; TKD, tyrosine kinase domain; WBC, white blood cell count.

Figure 1

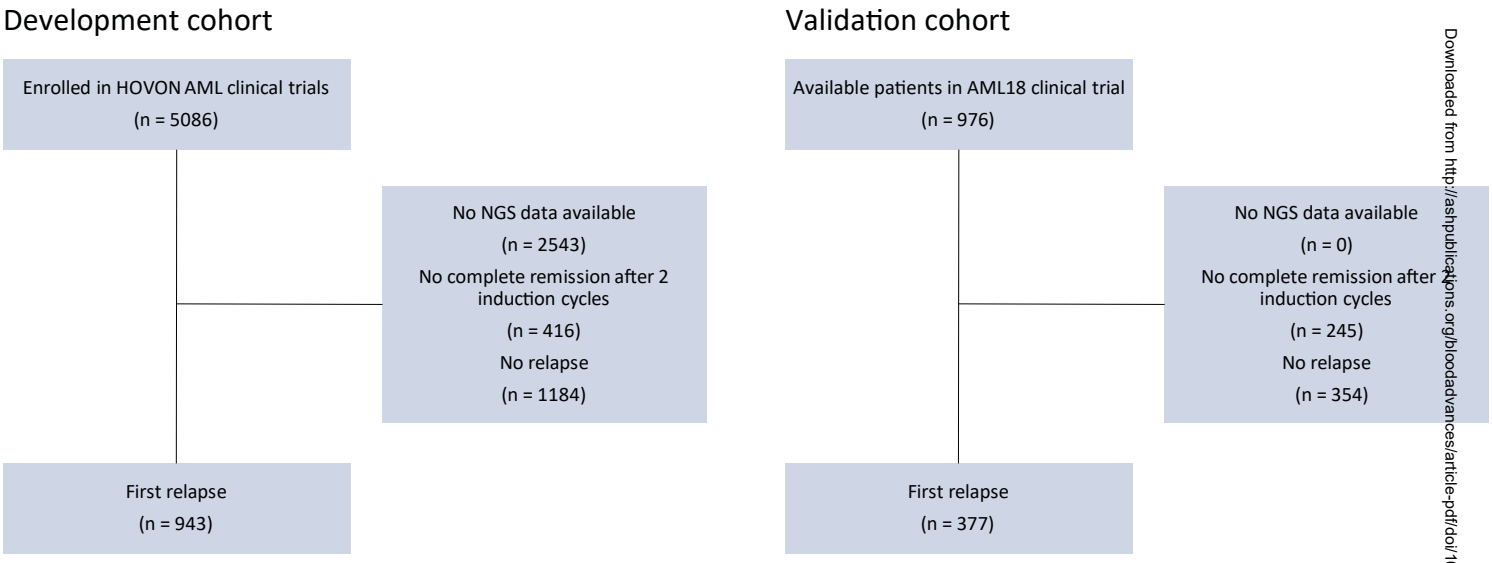


Figure 2

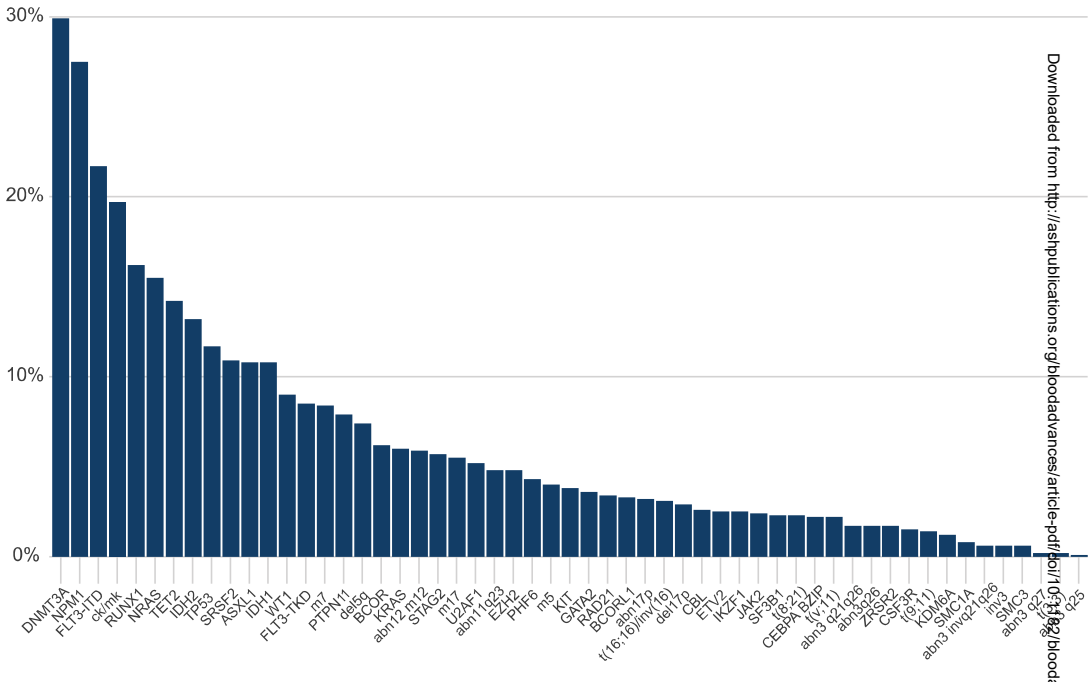


Figure 3A-B

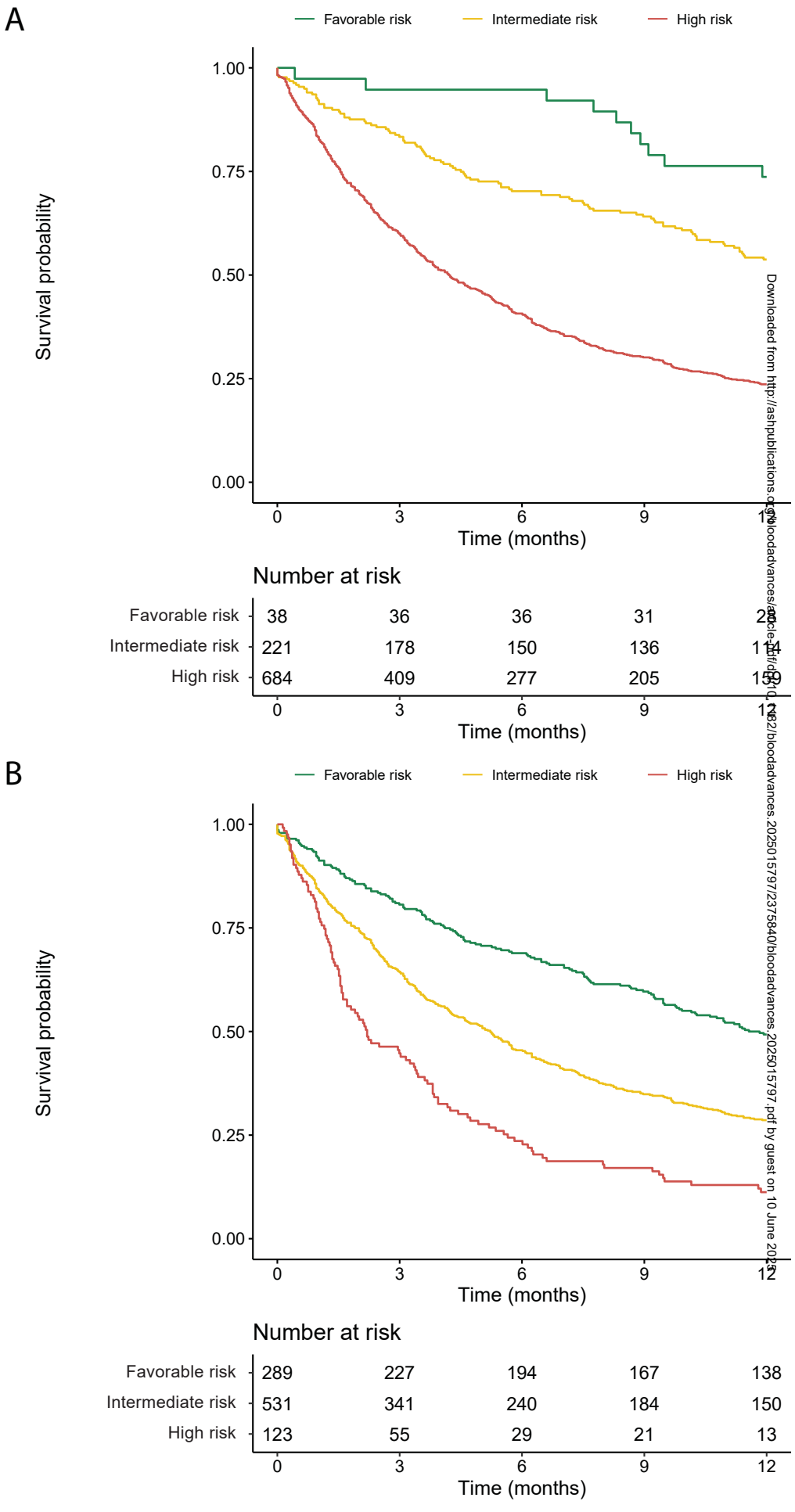
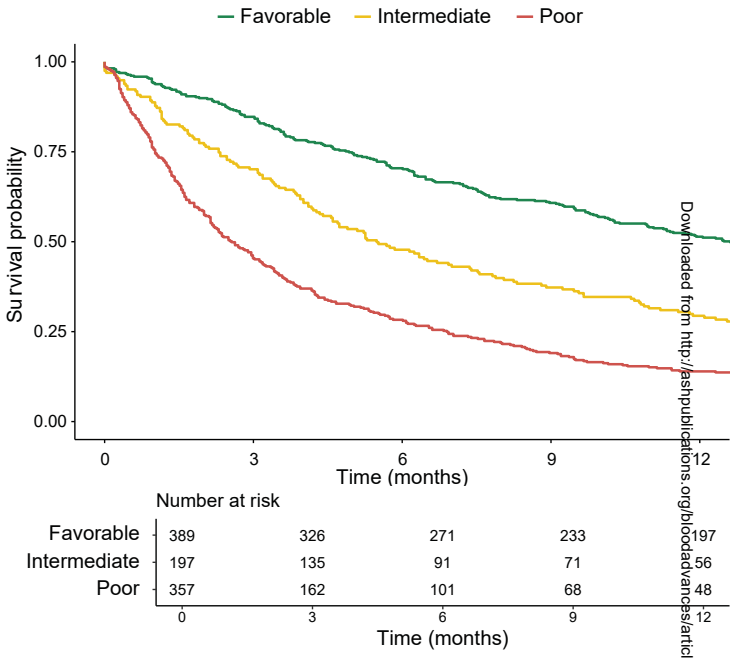


Figure 4A-D

A

Predictor variable	HR	95% CI	P-value	Points
No CBF abnormalities	2.07	1.38-3.12	<0.001	3
Mutated *TP53*	1.99	1.48-2.67	<0.001	3
Relapse free interval ≤12 months	1.76	1.46-2.12	<0.001	2
Previous allo-HCT	1.65	1.38-1.96	<0.001	2
t(v;11q23)/KMT2A-rearranged	1.54	1.10-2.14	0.01	2
Age at relapse ≥60 years	1.53	1.29-1.82	<0.001	2
Complex karyotype (inc. MK)	1.40	1.08-1.82	0.01	1
FLT3-ITD	1.37	1.11-1.69	0.003	1
White blood cell count ≥10 x10^9/L	1.27	1.07-1.52	0.01	1

B



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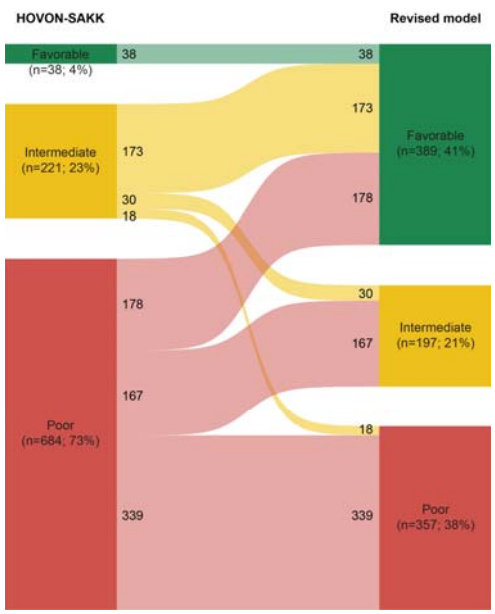

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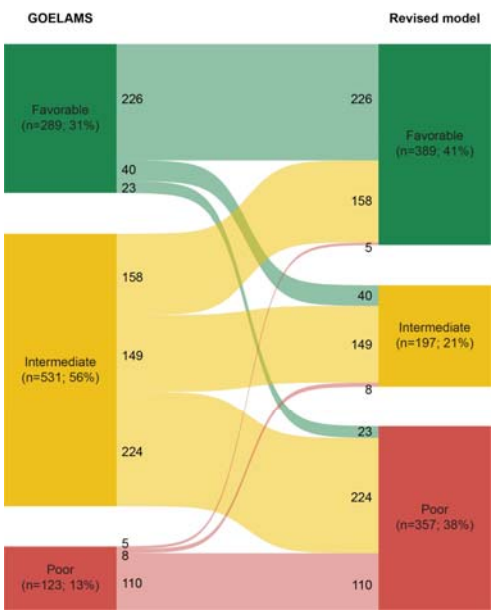
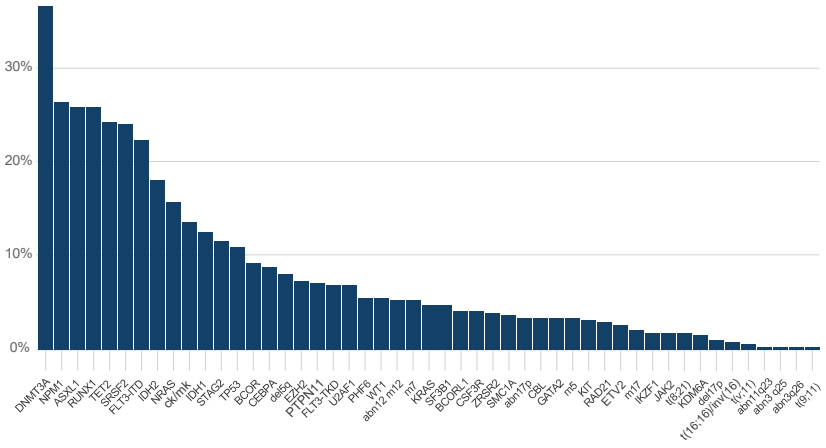


Figure 5A-E

A

	Validation cohort (n=377)
Age at relapse	
(median [range])	69.0 [60.0-81.0]
Sex	
Male	226 (59.9%)
Female	151 (40.1%)
WBC (at diagnosis;[x10^9/L])	
(median [range])	6.4 [0-365]
ELN risk 2022 at diagnosis	
Favorable	58 (15.4%)
Intermediate	92 (24.4%)
Adverse	227 (60.2%)
AML60+ classification at diagnosis	
Favorable	25 (6.6%)
Intermediate	117 (31.0%)
Poor	178 (47.2%)
Very Poor	57 (15.1%)
Best response on protocol treatment	
CR	333 (88.3%)
CRi	44 (11.7%)
SCT in first CR(i)	
No	326 (86.5%)
Allogeneic	51 (13.5%)
Autologous	0 (0%)
Relapse free interval (months)	
(median [range])	12.1 [1-61]
Follow-up of patients alive after first relapse (months)	
(median [range])	21 [0-59]

B



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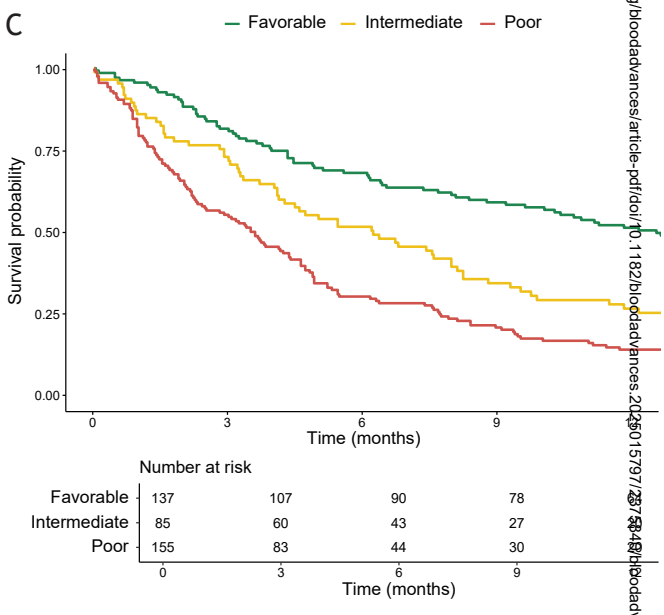

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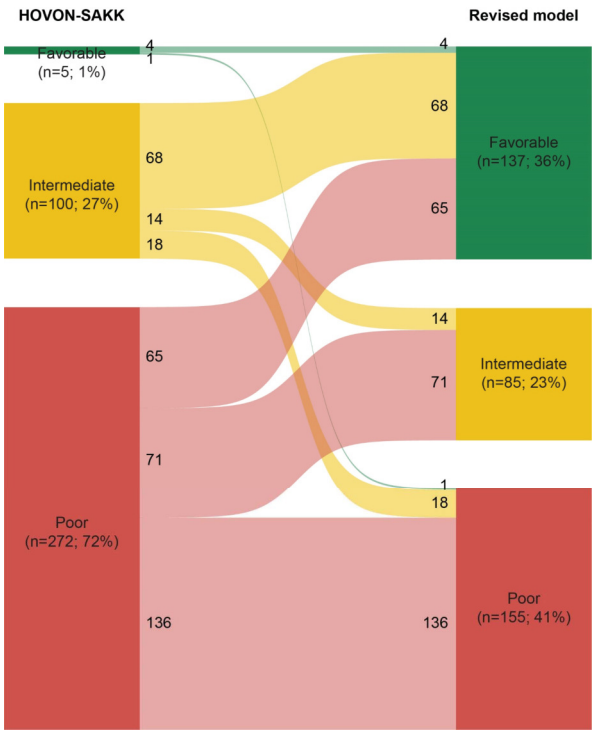

E