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Genetic risk classification in acute myeloid leukemia patients treated with hematopoietic cell transplantation and post-transplant cyclophosphamide

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Running Title: Genetic Risk Classification for AML undergoing HCT

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DECLARATIONS

Ethics approval and consent to participate

The hospital's Institutional Review Board approved the protocol, and written informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

Availability of data and material

The dataset supporting the conclusions of this article are available in the Hematology department of the Hospital Universitari i Politècnic La Fe, València, Spain: Jaime.Sanz@uv.es

Competing interests

The authors declare no competing interests.

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Authors' contributions

MV, MAS, and JS designed the study, performed statistical analysis, interpreted the results and wrote the original draft. JM, AB, PC, PA, PG, IG, PS, ES, JC, EB, MS, JVG, BL, AB, AL, and JdlR critically reviewed the study design, maintained the database and reviewed and edited the final manuscript.

ABSTRACT

We analyzed outcomes of 217 AML patients in complete remission who underwent allogeneic HCT with myeloablative conditioning and post-transplant cyclophosphamide-based GVHD prophylaxis, aiming to assess the prognostic significance of genetic risk categories. In the overall cohort, the 2-year overall survival (OS) and event-free survival (EFS) were 77% (95% CI, 71–83) and 72% (95% CI, 66–78), respectively. ELN2022 risk stratification lacked prognostic value in HCT. Instead, we identified four risk categories with distinct impact on OS: standard risk (ELN2022 favorable/intermediate and adverse-risk without high-risk genetic under the defined subcategories), intermediate risk (≥2 myelodysplasia-related gene mutations) (HR 2.23, 95% CI 1.14-4.92), adverse risk (complex karyotype, monosomal karyotype, inv(3)/t(3;3), *KMT2A* rearrangement) (hazard ratio (HR) 4.24, 95% CI 2.00 – 9.02), and very adverse risk (*TP53* mutations) (HR 6.81, 95% CI 3.00 – 15.5). These categories demonstrated similar predictive power for EFS and cumulative incidence of relapse. Moreover, integrating pre-transplant MRD refined risk stratification, identified MRD-negative patients with ≥2 myelodysplasia-related gene mutations whose OS and EFS were comparable to standard-risk patients. This refined classification improves the prognostic value of ELN2022 for AML patients undergoing allogeneic HCT with modern platform by integrating genetic features and MRD status to better guide post-transplant management.

INTRODUCTION

In 2022, the European LeukemiaNet updated its classification of acute myeloid leukemia (AML) (ELN2022) to incorporate advancements in genetic characterization and their prognostic implications (1). Recent studies have validated the predictive value of this classification in large series of patients treated in earlier decades (1986–2013), with a minority receiving allogeneic hematopoietic cell transplantation (HCT) (2–5). One such study excluded HCT patients from analyses of relapse rate, disease-free survival and overall survival (OS) (2), potentially limiting its applicability to current transplant recipients.

To date, three studies have explored the predictive value of the ELN2022 classification in AML patients undergoing HCT. Jentzsch et al. (6) examined a cohort where roughly 20% of patients were not in complete remission (CR), including few haploidentical transplants. Their findings suggested that re-classifying patients with myelodysplasia-related gene (MRG) mutations into the intermediate-risk group, along with incorporating measurable residual disease (MRD) status at HCT, improved risk stratification. In contrast, Jimenez-Vicente et al. (7) studied a smaller single-center series of AML patients in CR and proposed a refinement within the ELN2022 adverse category. They identified a subset with complex karyotype, inv(3)/t(3;3) with *MECOM* (EVI1) rearrangement, and loss of 17p region and/or *TP53* mutation, classifying it as a novel “adverse-plus” category with significantly poorer outcomes than the rest of the ELN2022 adverse risk cohort. Recently, these findings were confirmed in a larger multicenter study by the same group (8), supporting the reproducibility of this genetic refinement. Given the differing characteristics, approaches, and conclusions of these studies, the prognostic value and potential refinement of the ELN2022 classification in the HCT setting remain uncertain. This uncertainty is particularly relevant in the context of modern transplant platforms, which use post-transplant cyclophosphamide (PTCy) for graft-versus-host disease (GVHD) prophylaxis and intensive conditioning regimens. (9,10).

This study aims to evaluate the prognostic value of genetic classification in AML patients undergoing allogeneic HCT with myeloablative conditioning regimens and PTCy-based GVHD prophylaxis. Additionally, we aim to assess the prognostic weight of measurable residual disease (MRD) within genetic groups to explore potential refinements in the prognostic categories in this context.

METHODS

Patients and Transplant Procedures

This retrospective analysis includes all consecutive patients aged ≥18 years from a prospective registry of individuals with AML in CR1 or CR2 who underwent HCT from HLA-matched sibling donor (MSD), matched unrelated donor (MUD), mismatched unrelated donor (MMUD) and haploidentical donors at Hospital Universitari i Politècnic La Fe in València, Spain, between January 2017 and June 2024. The dataset was locked on January 1, 2025. All patients provided informed consent for data collection. The study was registered by the Spanish Agency of Medicines and Health Products with the reference code IIF-SIR-2019-01. According to the Declaration of Helsinki, the protocol was approved by the Research Ethics Board of Hospital Universitari i Politècnic La Fe with reference code 09/2019-465.

Details on eligibility criteria, donor selection, conditioning regimens, and GVHD prophylaxis have been described elsewhere (10). All conditioning regimen used in the present study are considered myeloablative according to the latest definitions (11,12). The regimens consisted of intravenous thiotepa at 5 mg/kg/day on days -7 and -6 (T2), intravenous busulfan at 3.2 mg/kg/day as a single daily dose on days -5 to -3 (B3) or -5 to -4 (B2), and fludarabine at 50 mg/m²/day on days -5 to -3 (F3); as well as a regimen consisting of busulfan on days -5 to -2 (B4) and fludarabine on days -5 to -2 (F4) without thiotepa. Conditioning regimen allocation was based on patient and donor characteristics: patients aged ≤55 years receiving an MSD transplant received B4F4; those receiving an MUD or haploidentical transplant received T2B3F3; and patients >55 years or aged ≥50 years with a Hematopoietic Cell Transplantation–Specific Comorbidity Index (HCT-CI) ≥3 received T2B2F3. GVHD prophylaxis consisted of PTCy, sirolimus, and mycophenolate mofetil for all transplants, regardless of donor type.

Diagnosis and Management of AML Patients

Patients included in the study were retrospectively classified into favorable, moderate, and adverse risk-groups according to the ELN2022 risk classification (1) based on the information available at the time of AML diagnosis.

The molecular analysis consisted in rapid conventional PCR screening for actionable genes (*NPM1, FLT3, IDH1* and *IDH2*) followed by NGS myeloid panel to complete molecular characterization: from 2017-2021, samples were prospectively

analyzed on the Ion S5 platform with the Oncomine Myeloid Research Assay – Chef Ready panel including 40 genes (ABL1, *ASXL1, BCOR, BRAF, CALR, CBL, CEBPA, CSF3R, DNMT3A, ETV6, EZH2, FLT3, GATA2, HRAS, IDH1, IDH2, IKZF1, JAK2, KIT, KRAS, MPL, MYD88, NF1, NPM1, NRAS, PHF6, PRPF8, PTPN11*, *RB1, RUNX1, SETBP1, SF3B1, SH2B3, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRZR2) 29 RNA fusion driver genes covering >800 unique fusions (ABL1, ALK, BCL2, BRAF*, *CCND*, *CREBBP*, *EGFR*, *ETV6*, *FGFR1*, *FGFR2*, *FUS*, *HMGA2*, *JAK2*, *KMT2A*, *MECOM*, *MET*, *MLLT10*, *MLLT3*, *MYBL1*, *MYH11*, *NTRK3*, *RARA*, *NUP214*, *PDGFRA*, *PDGFRB*, *RBM15*, *RUNX1*, *TCF3*, *TFE3*) and 5 gene expression (*BAALC, MECOM, SMC1A, MYC, WT1*); from December 2021 to 2023, we moved to automated Genexus Integrated Sequencer with the Oncomine Myeloid Assay GX v2 that expanded to 45 genes (*ABL*, ***ANKRD26***, *ASXL1*, *BCOR*, *BRAF*, *CALR*, *CBL*, *CEBPA*, *CSF3R*, ***DDX41***, *DNMT3A*, *ETV6*, *EZH2*, *FLT3*, *GATA2*, *HRAS*, *IDH1*, *IDH2*, *IKZF1*, *JAK2*, *KIT*, *KRAS*, *MPL*, *MYD88*, *NF1*, *NPM1*, *NRAS*, *PHF6*, ***PPM1D***, *PRPF8*, *PTPN11*, *RB1*, *RUNX1*, ***SMC1A***, ***SMC3*****,** *SETBP1*, *SF3B1*, *SH2B3*, *SRSF2*, *STAG2*, *TET2*, *TP53*, *U2AF1*, *WT1*, *ZRZR2*) and 30 RNA fusion drivers (*ABL1*, *ALK*, *BCL2*, *BRAF*, *CCND*, *CREBBP*, *EGFR*, *ETV6*, *FGFR1*, *FGFR2*, *FUS*, *HMGA2*, *JAK2*, *KMT2A*, *MECOM*, *MET*, *MLLT10*, *MLLT3*, *MYBL1*, *MYH11*, *NTRK3*, *RARA*, *NUP214*, ***NUP98***, *PDGFRA*, *PDGFRB*, *RBM15*, *RUNX1*, *TCF3*, *TFE3*) and 5 gene expression (*BAALC*, *MECOM*, *SMC1A*, *MYC*, *WT1*). Quality parameter criteria: uniformity (>85%) and mean read depth of 1000X. Consensus criteria for variant report: all pathogenic or probably damaging variants with VAF ≥ 2% in AML key genes were reported (13,14). Cytogenetic analyses were performed locally.

Disease status and minimal residual disease (MRD) at the time of transplant were defined according to the ELN recommendations (15,16). MRD monitoring was performed using multiparameter flow cytometry (MFC) (cut-off: ≥0.1% ($1x10^{-3}$), except when standardized quantitative PCR assays were employed (cut-off: $\geq 10^{-3}$) targeting *NPM1* mutation, RUNX1-RUNX1T1, and CBFB-MYH11 (15,16). Patients classified as intermediate or adverse risk according to the ELN2017 classification were treated with intensive chemotherapy based on PETHEMA protocols (17), primarily consisting of 1-2 courses of remission induction therapy with idarubicin and cytarabine (3 +7). Once CR was achieved, patients received 1-2 consolidation courses followed by intensification with allogeneic HCT. For patients in the favorable risk group, HCT was indicated in CR1 only if MRD remained positive; otherwise, HCT was reserved for those who relapsed and subsequently achieved CR2.

Endpoints and Definitions

The primary objective was to evaluate and refine the ELN2022 classification for predicting OS in patients undergoing myeloablative HCT with a PTCy platform. Secondary objectives included assessing cumulative incidence of relapse (CIR) and leukemia-free survival (EFS). We also aimed to explore the impact of individual genetic mutations and co-mutations on HCT outcomes. Acute and chronic GVHD were defined and graded according to standard criteria (18). Relapse was defined as disease recurrence and appearance of blasts in the peripheral blood or BM (> 5%) after complete response (CR). NRM was defined as death from any cause without evidence of relapse. OS was defined as the time from transplantation to death from any cause, while EFS also included disease relapse. Disease risk index (DRI) and HCT-CI score were calculated as previously described (19,20).

Statistical Analysis

Categorical variables were presented as percentages and compared using Pearson's chi-square test. Continuous variables were presented as median values with ranges and compared using the Mann-Whitney U or Kruskal-Wallis test, as appropriate. Unadjusted time-to-event survival analyses were performed using the Kaplan–Meier estimate, and comparisons were made using the log-rank tests. Multivariate analyses were performed by Cox regression models. The probabilities of acute and chronic GVHD were estimated using the cumulative incidence method, accounting for death as a competing event. Similarly, the cumulative incidence of relapse and NRM were estimated treating death or death and relapse as competing events, respectively. Fine-Gray subdistribution hazard models were used to evaluate the impact of covariates on the cumulative incidence. Variables with a p value ≤0.1 in univariate analysis were included in the multivariate models. A significance level of 0.05 (two-sided) was applied for all statistical comparisons. Statistical analyses were performed using R Statistical Software (v4.3.1; R Core Team 2023).

RESULTS

Patient, Disease, and Transplantation Characteristics

The patient and disease characteristics of the cohort are summarized in Table 1. This included 217 transplant recipients with AML in CR. The median age was 55

years (range, 18–71), and 57% were male. Most patients (83%) had de novo AML, while 17% had secondary or therapy-related AML (sAML). At transplantation, 71% were in CR1, and 29% in CR2. Pre-transplant MRD was positive in 41% of patients, with data unavailable for 17%. Most patients (79%) had positive cytomegalovirus (CMV) serology. The median time from diagnosis to transplant was 6 months (range, 3–55).

As shown in Table *2*, donor types included MSD in 42%, MUD in 33%, MMUD in 3%, and haploidentical family donors in 22%. Peripheral blood was the graft source for 97% of patients, with 15% receiving cryopreserved grafts. Donors had a median age of 40 years (range, 14–74), with 52% aged ≤40 years. Female donor to male recipient and major ABO mismatch occurred in 26% and 23% of transplants, respectively. Donor CMV serology was positive in 69%. All patients received MAC regimens according to EBMT criteria. Conditioning regimens included T2B2F3 in 53%, T2B3F3 in 31%, and B4F4 in 16%.

The distribution of cytogenetic abnormalities is shown in Table 3. Nearly half of the patients (45%) had a normal karyotype. Favorable-risk abnormalities, such as inv(16) and t(8;21), were present in 4.9% and 1.5% of cases, respectively. Complex (5.4%) and monosomal (6.3%) karyotypes were less frequent but notable. Trisomies were observed in 11% of patients, while other cytogenetic abnormalities appeared at lower frequencies. The distribution of genetic rearrangements and mutations relevant to the ELN2022 classification is presented in **Error! Reference source not found.**.

Transplant outcomes

Two patients died without evidence of myeloid engraftment on days +11 and +14. The remaining 215 patients achieve neutrophil engraftment at a median time of 16 days (range, 13–56). No cases of primary or secondary graft failure were observed. The 100-day cumulative incidence of acute GvHD of any grade was 45% (95% CI, 38-51), with grade II-IV and grade III-IV accounting for 17% (95% CI, 12-22) and 7.9% (95% CI, 4.8-12), respectively (Table 4). The 2-year cumulative incidence of chronic GvHD was 44% (95% CI, 38-51), with moderate-severe and severe being 29% (95% CI, 23-36) and 10% (95% CI, 6.7-15), respectively.

Twenty-four patients died without prior relapse at a median time of 119 days (range, 11–1679). The causes of death were predominantly infection (n = 12) and GVHD (n = 10), with one death attributed to secondary malignancy. Forty-six patients experienced relapse after HCT at a median time of 12.3 months (range, 2.5–70.5). The

2-year cumulative incidence of NRM and relapse were 11% (95% CI, 7–15) and 17% (95% CI, 12–22), respectively (Table 4). The 2-year OS and EFS were 77% (95% CI, 71–83) and 72% (95% CI, 66–78), respectively (Table 4).

We analyzed the impact of patient, disease, and transplant characteristics on outcomes using multivariable analysis (Table 5). Beyond genetic characteristics—categorized according to ELN2022 classification and variants, which will be analyzed in the subsequent section—no variables were associated with OS or EFS. For the CIR, pre-transplant MRD positivity was significantly associated with an increased relapse risk (HR, 2.16; 95% CI, 1.07–4.34), while haploidentical HCT was linked with a reduced relapse risk (HR, 0.30; 95% CI, 0.10–0.93). For the cumulative incidence of NRM, haploidentical donor was the only variable significantly associated with increased risk (HR, 4.43; 95% CI, 1.51–13).

Assessment of Genetic Risk Stratification in the HCT Setting

The 2-year probabilities of OS and EFS based on the ELN2022 risk stratification were 85% (95% CI, 74–98) and 85% (95% CI, 74–98) for the favorable category, 86% (95% CI, 77–96) and 80% (95% CI, 69–92) for the intermediate category, and 70% (95% CI, 62–79) and 65% (95% CI, 57–74) for the adverse category. Similarly, the 2-year CIR were 9.8% (95% CI, 2.4–23), 14% (95% CI, 6–25), and 20% (95% CI, 13–27) for the favorable, intermediate, and adverse categories, respectively.

In multivariable analysis, only adverse risk according the ELN2022 classification was associated with poorer OS (HR, 2.71; 95% CI, 1.03–7.15) with a trend toward significance of EFS (HR, 2.30; 95% CI, 0.98–5.41) compared to the favorable category. Regarding the CIR, there were no significant differences among the ELN2022 categories.

To further refine the predictive value of genetic categories in the context of HCT, we analyzed potential subgroups within the ELN2022 adverse risk category to identify distinct OS patterns based on genetic profiles. Our analysis revealed subgroups with OS comparable to the ELN2022 favorable and intermediate risk categories, as well as three additional subcategories with progressively worse OS in multivariable analysis. Consequently, we stratified the cohort into four subcategories: 1) *Standard risk*: Patients from the ELN2022 favorable and intermediate risk categories, as well as those in the adverse risk category without genetic abnormalities classified under the *intermediate*, *adverse*, or *very adverse* subcategories; 2) *Intermediate risk*: Patients with ≥2 MRG mutations; 3) *Adverse risk*: Patients with one or more of the

following genetic abnormalities: complex karyotype, monosomal karyotype, inv(3)/t(3;3), or *KMT2A* rearrangements; and 4) *Very adverse risk*: Patients harboring a *TP53* mutation. Figure 2 illustrates the reclassification from the ELN2022 risk classification to the proposed HCT-Genetic risk classification. Notably, patients initially classified as ELN2022 favorable (n = 35) and intermediate risk (n =54), along with a substantial subset of those in the adverse-risk category (n = 46), converge into the *standard-risk* group (n = 135). The remaining patients in the ELN2022 adverse-risk category are further stratified into *intermediate* (n = 43), *adverse* (n = 25), and *very adverse* (n = 14) subgroups. The role of MRD status in refining risk stratification, particularly within the *intermediate-risk* subgroup, as indicated by the MRD+ and MRD− division, will be addressed below.

Compared to the *standard* risk group, the *intermediate* (HR, 2.37; 95% CI, 1.14–4.92), *adverse* (HR, 4.24; 95% CI, 2.00–9.02), and *very adverse* categories (HR, 6.81; 95% CI, 3.00–15.5) were associated with progressively worse OS (Figure 3). These categories demonstrated similar predictive power for EFS and CIR, except for the *intermediate* category, which was not significant for CIR (**Error! Reference source not found.**).

Adjusted risk stratification based on pre-transplant MRD

In the overall cohort, 179 (82%) patients had evaluable MRD, with 88 (49%) showing detectable disease. Despite the smaller sample size, we assessed the impact of pre-transplant MRD status on OS and EFS within the standard (n = 119), intermediate (n = 33), and advanced plus very advanced (n = 28) risk categories. Detectable pre-transplant MRD was significantly associated with worse OS (HR, 8.23; 95% CI, 1.13–60.0) and EFS (HR, 6.51; 95% CI, 1.26–33.7) in the intermediate-risk group. In contrast, no significant association was observed in the standard-risk group [OS (HR, 0.98; 95% CI, 0.41–2.37) and EFS (HR, 1.25; 95% CI, 0.55–2.84)] or the advanced-risk group [OS (HR, 0.87; 95% CI, 0.25–3.06) and EFS (HR, 1.07; 95% CI, 0.29–3.86)]. Among the intermediate risk patients, 2-year OS and EFS probabilities were 85% (95% CI, 69–100) and 79% (95% CI, 61–100) for MRD negative cases, but decreased to 51% (95% CI, 32–83) and 48% (95% CI, 29–79) for MRD positive cases (p = 0.05).

DISCUSSION

This study demonstrates that allogeneic HCT with modern conditioning regimens, and GVHD prophylaxis using PTCY, sirolimus, and MMF offers excellent outcomes for patients with AML. In this setting, the genetic profile significantly influences transplant outcomes; however, the widely used ELN2022 risk classification, which has shown robust predictive value at diagnosis in non-elderly AML patients, was found to lack predictive utility in in this specific HCT context. Notably, patients categorized as favorable, intermediate or a substantial portion of those in the adverse risk category had comparable probabilities of OS and EFS, exceeding 80% at 2 years. As a consequence, we identified four distinct risk categories with progressively declining OS and EFS: 1) *Standard risk*: Patients from the ELN2022 favorable and intermediate risk categories, as well as those in the adverse risk category without genetic abnormalities classified under the intermediate, adverse, or very adverse subcategories defined below; 2) *Intermediate risk*: Patients with ≥2 MRG mutations; 3) *Adverse risk*: Patients with one or more of the following genetic abnormalities: complex karyotype, monosomal karyotype, or *KMT2A* rearrangements; and 4) *Very adverse risk*: Patients harboring a *TP53* mutation. Moreover, the incorporation of pre-transplant MRD further refined risk stratification within patients with ≥2 MRG mutations, identifying a subset of MRD-negative patients with OS and EFS comparable to the standard-risk group. These findings not only enhance our understanding of the prognostic impact of genetic features in the context of modern HCT platforms but also have the potential to guide patient counseling and inform the development of risk-adapted strategies aimed at improving transplant outcomes.

Despite the limitations inherent to the retrospective nature of the study and a sample size smaller than registry-based studies, several strengths should be highlighted. These include a homogenous indication for HCT and standardized transplant management, particularly the consistent use of MAC regimens—primarily based on the combination of busulfan, and fludarabine, with or without thiotepa—and a uniform GVHD prophylaxis regimen combining PTCy, sirolimus, and mycophenolate. These factors enhance the internal validity of our findings and allow for more reliable interpretation of genetic and MRD-related prognostic markers in a contemporary transplant setting. However, it is important to acknowledge that the applicability of our proposed risk classification may be limited in clinical settings that do not utilize myeloablative conditioning or PTCy-based GVHD prophylaxis. Further studies are needed to determine whether similar risk stratification schemes are valid in alternative transplant platforms or in older or less fit patient populations.

Our results confirm the safety and effectiveness of this platform in AML patients undergoing myeloablative allogeneic HCT, as previously reported by our group (9), demonstrating low rates of NRM, and acute and chronic GvHD. Furthermore, we observed encouraging CIR, OS, and EFS outcomes with no significant survival differences across donor types.

Before discussing our findings on the predictive value of the ELN2022 risk classification in HCT, and comparing them with the three studies that have evaluated this classification in the allogeneic HCT setting (6–8), we first outline key aspects of these studies to contextualize similarities and differences. The single-center study by Jiménez-Vicente et al. (7) included 120 AML patients in CR1 or CR2, with only 66 (55%) receiving MAC regimens and 69 (58%) PTCy-based GVHD prophylaxis. Their recently published multicenter study confirmed these findings in a larger cohort, with comparable proportions of MAC regimens (61%) and PTCy use (52%). In contrast, our study, which also focused on AML patients in CR, included 217 patients, all of whom received MAC regiments and PTCy. These differences likely contributed to notable variations in 2-year outcomes between studies. Notably, despite the smaller sample size, the single-center study by Jiménez-Vicente et al. (7) found a significant association between PTCY-based prophylaxis and improved outcomes, which was confirmed in their multicenter validation (8). The universal use of both PTCy and MAC regimens in our cohort may help explain the superior outcomes observed, including overlapping OS and EFS between the ELN2022 favorable- and intermediate-risk groups. This overlap also extended to certain patients within the ELN2022 adverse-risk group—specifically those with a single MRG mutation or with ≥2 MRG mutations and negative pre-transplant MRD status—a pattern not previously reported. Based on these findings, we propose that, in the context of modern transplantation platforms, these patients be considered as part of a standard-risk category, representing approximately three-quarters of transplants in our series, as they achieved OS and EFS rates exceeding 80%.

Among patients in the ELN2022 adverse-risk category, the studies by Jiménez-Vicente et al. (7,8) confirmed poorer outcomes compared with the intermediate-risk group and proposed a novel “adverse plus” (AdvP) subcategory. This group included patients with complex karyotype, inv(3)/t(3;3) with *MECOM*(EVI1) rearrangement, or loss of 17p region and/or *TP53* mutations (7,8). In contrast, after excluding patients with MRG mutations—who had outcomes consistent with our newly defined standard-risk category—we identified two distinct subcategories with adverse outcomes. While these subcategories shared some similarities with the previously defined AdvP, they

also included additional features, such as monosomal karyotype and *KMT2A* rearrangements, which were not considered in the study by Jiménez-Vicente et al. Furthermore, we distinguished the significantly worse prognosis associated with *TP53* mutations, justifying its classification as a separate very adverse-risk group. Given the poor outcomes in this subgroup, future research should focus on strategies to mitigate the impact of *TP53* alterations, such as the use of hypomethylating agents (HMAs) with venetoclax in pre-transplant cytoreduction, targeted agents like APR-246 (eprenetapopt) to restore p53 function (NCT03072043) (21), or post-transplant maintenance approaches, including HMAs or immune-based interventions such as CD47-blocking antibodies (22).

Regarding the contribution of pre-transplant MRD assessment to refining prognostication based on genetic features in AML patients undergoing HCT, this was only evaluated in a study by Jentzsch et al. (6), which included a large series of 229 patients with adequate material available and in remission prior to HSCT. However, only 141 (27%) received MAC regimens, and just 13 (2.5%) underwent haploidentical HCT, making direct comparisons with our study challenging. Notably, MRD in the study by Jentzsch et al. (6) was determined using molecular techniques, specifically digital droplet PCR for *NPM1* mutation, *BAALC/ABL1* and *MN1/ABL1* copy numbers, or quantitative reverse transcriptase PCR for *WT1/ABL1* expression levels. In contrast, MRD in our study was primarily assessed by MFC, with molecular techniques applied in only a minority of patients, targeting *NPM1* mutation, *RUNX1-RUNX1T1*, and *CBFB-MYH11* rearrangements. While Jentzsch et al. found that approximately half of the patients classified as ELN2022 favorable were reclassified as intermediate risk, and *vice versa*, based on the presence or absence of MRD at HCT (6), we did not observe such an impact within these categories, regardless of MRD status. In our study, pre-transplant MRD only stratified distinct prognostic subgroups among patients with ≥2 MRG mutations, a subset of the ELN2022 adverse-risk category. Interestingly, in addition to the study by Jentzsch et al., another large study recently demonstrated that patients with MRG mutations, in the absence of other adverse genetic abnormalities, can achieve outcomes comparable to lower-risk groups after allogeneic HCT (23). This finding, which aligns with our results, supports the notion that the adverse prognostic impact of MRG mutations in AML can be mitigated by transplantation.

In conclusion, our findings underscore the excellent outcomes achieved with modern allogeneic HCT platforms incorporating MAC regimens and PTCy-based GVHD prophylaxis, particularly in patients without high-risk genetic features. In this setting, the ELN2022 classification lacked prognostic utility, prompting the refinement

of risk categories to better stratify transplant recipients. Notably, a newly defined standard-risk category, encompassing approximately three-quarters of patients in our cohort, exhibited survival rates exceeding 80%. Pre-transplant MRD status further refined risk assessment within the subset of ELN2022 adverse-risk patients with MRG mutations in the absence of other adverse genetic abnormalities, identifying an MRD-negative subgroup with outcomes comparable to standard risk. Conversely, distinct subgroups with adverse and very adverse genetic features demonstrated significantly poorer outcomes, emphasizing the need for tailored therapeutic strategies. Future research should focus on optimizing both pre- and post-transplant interventions to improve outcomes in these high-risk groups. While our proposed risk stratification model may provide a more accurate framework for counseling patients undergoing HCT, offering a refined perspective on expected outcomes based on modern transplantation practices, its applicability should be validated in independent cohorts before clinical implementation.

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TABLES

Table 1. Patient and Disease Characteristics

Characteristic	N = 217
Age, median (range)	55 (18-71)
Sex, n (%)	
Male	124 (57)
Female	93 (43)
AML type, n (%)	
AML	180 (83)
sAML	37 (17)
Disease stage at transplant, n (%)	
CR1	155 (71)
CR2	62 (29)
Pretransplant MRD, n (%)	
Positive	88 (49)
Negative	91 (51)
Prior autologous transplant, n (%)	10 (4.6)
CMV serology, n (%)	
Positive	171 (79)
Negative	46 (21)
Time diagnosis-transplant (mo.), median (range)	6 (3-55)

Percentages may not sum to 100 because of rounding.
Abbreviations: AML, acute myeloid leukemia; sAML, secondary/therapy-related AML; CR1, first complete remission; MRD, measurable residual disease; CMV, cytomegalovirus

Table 2. Transplant Characteristics

Characteristic	N = 217
Donor type, n (%)	
MSD	91 (42)
MUD	71 (33)
MMUD	7 (3.2)
Haploidentical	48 (22)
Donor age, median (range)	40 (14-74)
Donor/Recipient Sex, n (%)	
Male - Male	80 (37)
Male - Female	57 (26)
Female - Male	44 (20)
Female - Female	36 (17)
ABO mismatch, n (%)	
None	119 (55)
Minor	49 (23)
Major	49 (23)
Donor CMV serology, n (%)	
Negative	67 (31)
Positive	150 (69)
Stem cell source, n (%)	
Peripheral blood	210 (97)
Bone marrow	7 (3.2)
Cryopreserved graft, n (%)	33 (15)
Conditioning regimen*, n (%)	
T2B3F3	66 (30)
T2B2F3	116 (53)
B4F4	35 (16)

Percentages may not sum to 100 because of rounding,
Abbreviations: MSD, matched sibling donor; MUD, matched unrelated donor; MMUD, mismatched unrelated donor.

The acronyms T, B, and F in the conditioning regimens refer to thiotepa, busulfan, and fludarabine, respectively, while the numbers indicate the number of days each agent was administered.

Table 3. Cytogenetics

Karyotype, n (%)	N = 217
Normal	92 (45)
inv(16)	10 (4.9)
t(8;21)	3 (1.5)
t(x;11)	8 (3.9)
t(9;11)	3 (1.5)
Complex	11 (5.4)
Monosomal	13 (6.3)
inv(3)/t(3;3)	1 (0.5)
Trisomies	22 (11)
t(6;9)	5 (2.4)
t(9;22)	1 (0.5)
t(x;3)	1 (0.5)
-7	8 (3.9)
del5q	8 (3.9)
others	19 (9.3)

Percentages may not sum to 100 because of rounding,

Table 4. Transplant outcomes of the entire cohort

Outcome*	**% (95% CI)**
Acute GVHD, n (%)	
Grade II-IV	17 (12-22)
Grade III-IV	7.9 (4.8-12)
Chronic GVHD	
Moderate-to-severe	29 (23-36)
Severe	10 (6.7-15)
Non-relapse mortality, n (%)	11 (7-15)
Relapse	17 (12-22)
Event-free survival	77 (71-83)
Overall survival	72 (66-78)

*Cumulative incidence probability (95% CI) of acute graft-versus-host disease (GVHD) at 100 days, chronic GVHD, non-relapse mortality (NRM) and relapse at 2 years. Probability of overall survival and event-free survival (95% CI) at 2 years.

Table 5. Multivariate analysis of transplant outcomes for 217 AML HCT patients

	OS		EFS		CIR	
	HR (95% CI)	**p**	**HR (95% CI)**	**p**	**HR (95% CI)**	**p**
Disease stage						
CR1	1		1		1	
CR2	1.12 (0.58-2.19)	0.7	1.00 (0.54-1.85)	>0.9	0.86 (0.43-1.71)	0.7
Pre-HCT MRD						
Negative	1		1		1	
Positive	1.05 (0.57-1.93)	0.7	1.28 (0.73-2.24)	0.4	2.16 (1.07-4.34)	**0.03**
Donor type						
MSD	1		1		1	
MUD	0.98 (0.46-2.09)	>0.9	1.17 (0.57-2.39)	0.7	0.79 (0.31-2.04)	0.6
MMUD	2.26 (0.57-8.96)	0.2	3.45 (0.95-12.06)	0.06	2.05 (0.35-11.9)	0.4
Haploidentical	1.22 (0.56-2.67)	0.6	1.15 (0.53-2.47)	0.7	0.30 (0.10-0.93)	**0.04**
Donor CMV serology						
Negative	1		1		1	
Positive	1.87 (0.97-3.61)	0.06	1.60 (0.90-2.84)	0.1	1.31 (0.63-2.76)	0.5
Conditioning regimen						
T2B3F	1		1		1	
T2B3F	1.22 (0.61-2.43)	0.6	0.93 (0.50-1.76)	0.8	0.68 (0.29-1.58)	0.4
Bu4Flu	1.08 (0.37-3.13)	0.9	1.39 (0.53-3.67)	0.5	1.46 (0.45-4.71)	0.5
ELN2022 risk classification						
Favorable	1		1		1	
Intermediate	1.73 (0.63-4.78)	0.3	1.33 (0.53-3.31)	0.5	1.33 (0.47-3.71)	0.6
Adverse	2.71 (1.03-7.15)	**0.04**	2.30 (0.98-5.41)	0.06	1.83 (0.67-5.02)	0.2
Genetic-based classification						
Standard	1		1		1	
Intermediate	2.37 (1.14-4.92)	**0.02**	2.27 (1.18-4.33)	**0.01**	1.12 (0.44-2.88)	0.8
Adverse	4.24 (2.00-9.02)	**<0.001**	3.80 (1.90-7.61)	**<0.001**	3.82 (1.56-9.31)	**0.003**
Very adverse	6.81 (3.00-15.5)	**<0.001**	6.57 (2.98-14.5)	**<0.001**	7.76 (3.06-19.7)	**<0.001**

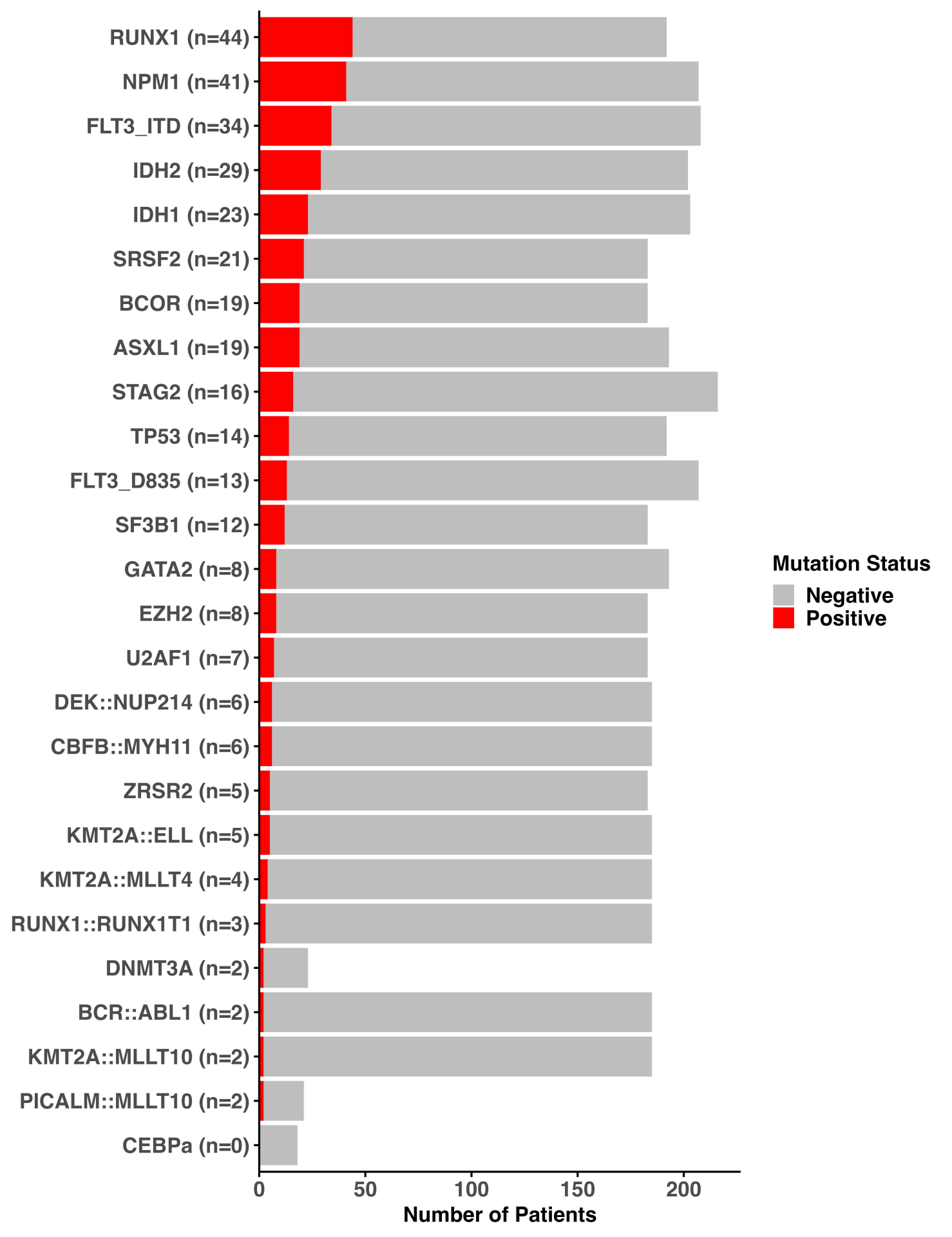
Abbreviations: OS, overall survival; EFS, event-free survival; CIR, cumulative incidence of relapse; HR (95% CI), hazard ratio (95% confidence interval); CR, complete remission; HCT, hematopoietic cell transplantation; MRD, measurable residual disease; MSD, matched sibling donor; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; the acronyms T, B, and F in the conditioning regimens refer to thiotepa, busulfan, and fludarabine, respectively, while the numbers indicate the number of days each agent was administered.

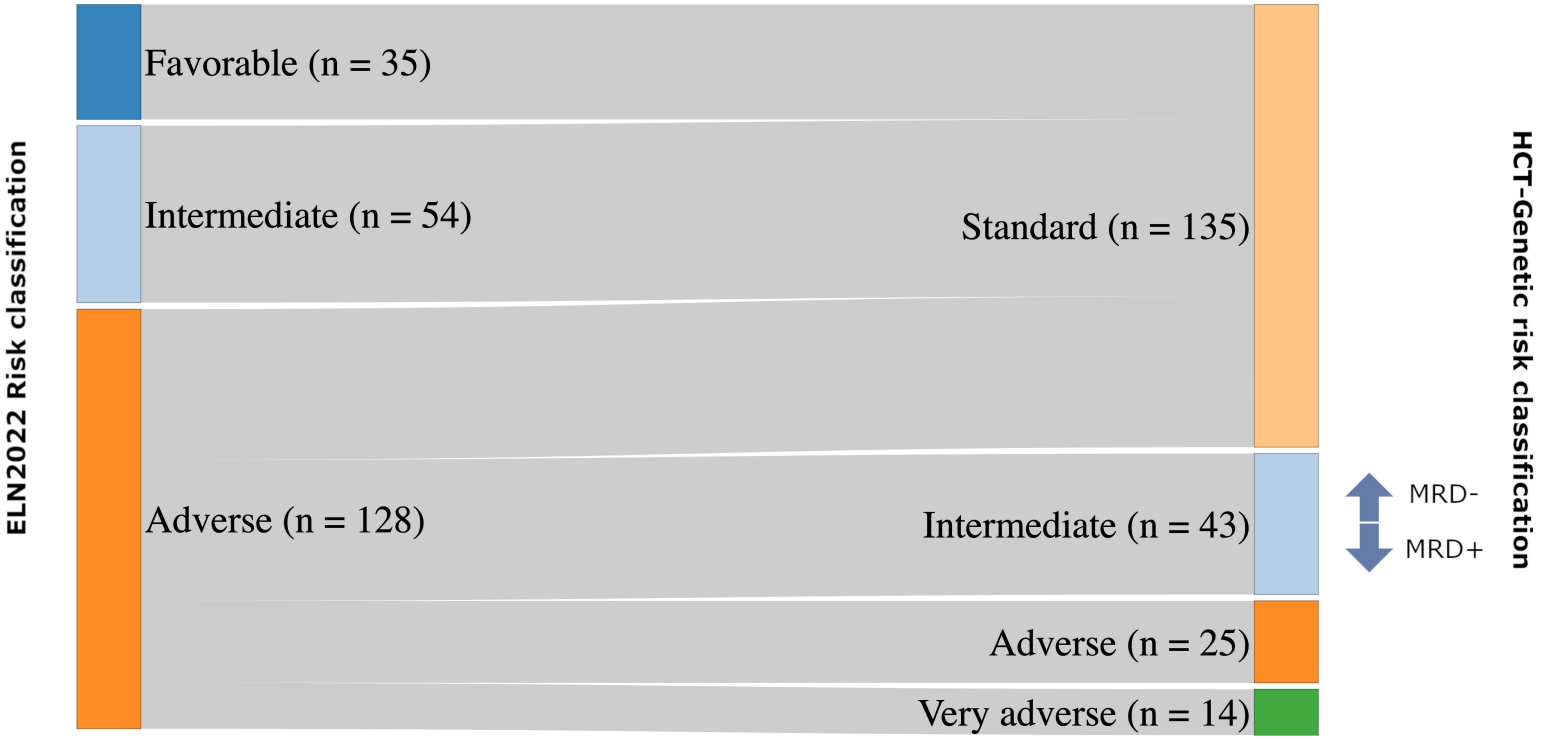
FIGURES LEGENDS

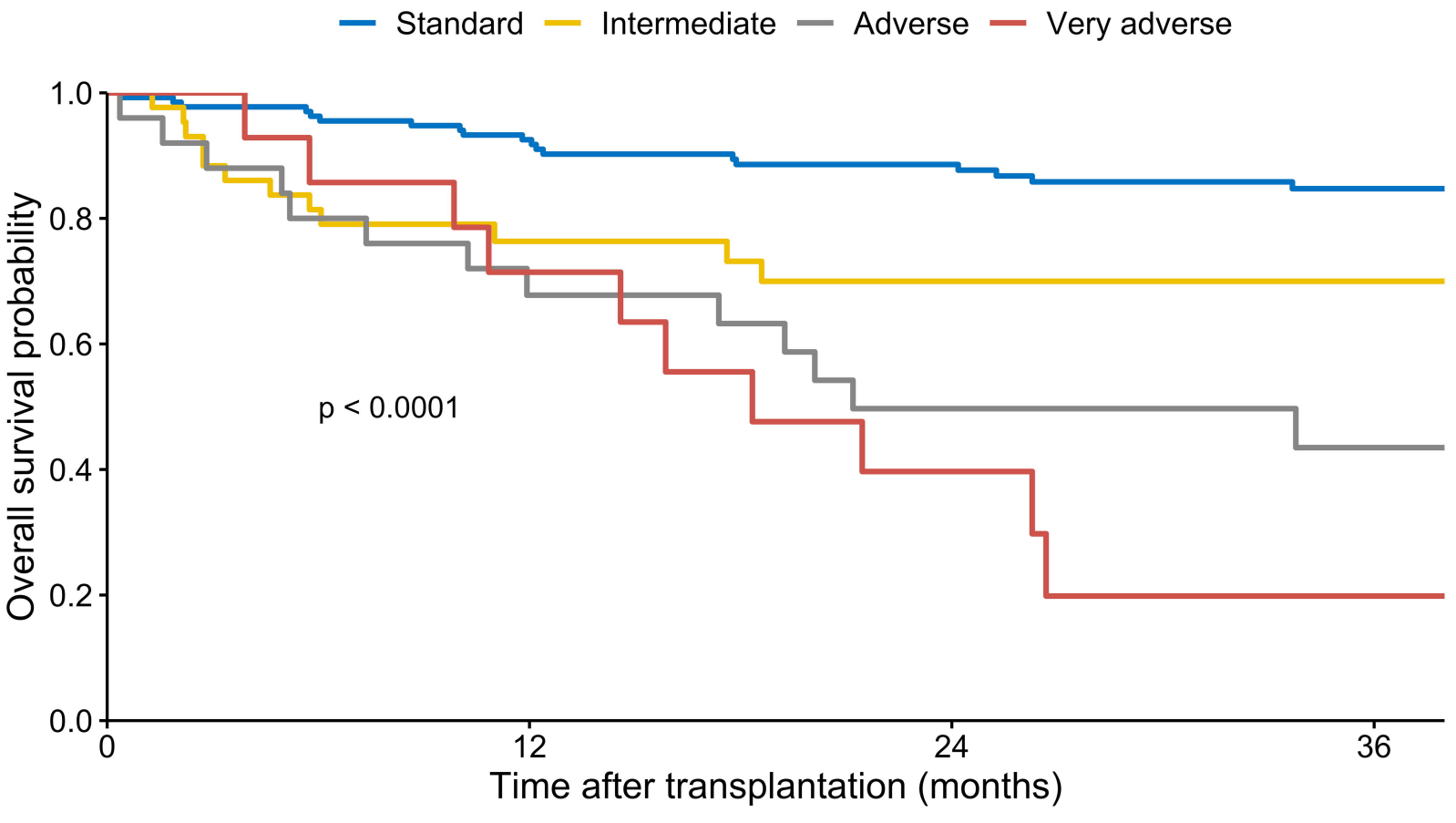
Figure 1. Frequency of genetic rearrangements and mutations involved in the ELN2022 classification

Figure 2. Sankey diagram illustrating the reclassification of AML patients from the ELN2022 risk classification to the proposed HCT-Genetic risk classification

Figure 3. Probability of overall survival according to HCT-genetic risk categories


RUNX1 (n=44)
NPM1 (n=41)
FLT3_ITD (n=34)
IDH2 (n=29)
IDH1 (n=23)
SRSF2 (n=21)
BCOR (n=19)
ASXL1 (n=19)
STAG2 (n=16)
TP53 (n=14)
FLT3_D835 (n=13)
SF3B1 (n=12)
GATA2 (n=8)
EZH2 (n=8)
U2AF1 (n=7)
DEK::NUP214 (n=6)
CBFB::MYH11 (n=6)
ZRSR2 (n=5)
KMT2A::ELL (n=5)
KMT2A::MLLT4 (n=4)
RUNX1::RUNX1T1 (n=3)
DNMT3A (n=2)
BCR::ABL1 (n=2)
KMT2A::MLLT10 (n=2)
PICALM::MLLT10 (n=2)
CEBPa (n=0)
0
50
100
150
200
Number of Patients
Mutation Status
Negative
Positive


ELN2022 Risk classification
Favorable (n = 35)
Intermediate (n = 54)
Adverse (n = 128)
Standard (n = 135)
Intermediate (n = 43)
Adverse (n = 25)
Very adverse (n = 14)
MRD-
MRD+
HCT-Genetic risk classification



Number at Risk

Standard	134	122	98	76
Intermediate	43	26	19	13
Adverse	25	16	11	7
Very adverse	14	10	4	2