Next-generation sequencing-based measurable residual disease detection predicts outcomes in patients with acute myeloid leukemia undergoing allogeneic stem cell transplantation

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Supplemental appendix

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Supplementary information on Patients & Methods

Information on treatment protocols

Information on induction therapy protocols is shown in Table S3. Three patients received post-transplantation maintenance therapy: one *IDH2* mutated patient received enasidenib, and two patients with *FLT3*-ITD mutation received quizartinib or placebo on a clinical trial.

Sequencing library preparation and data analysis

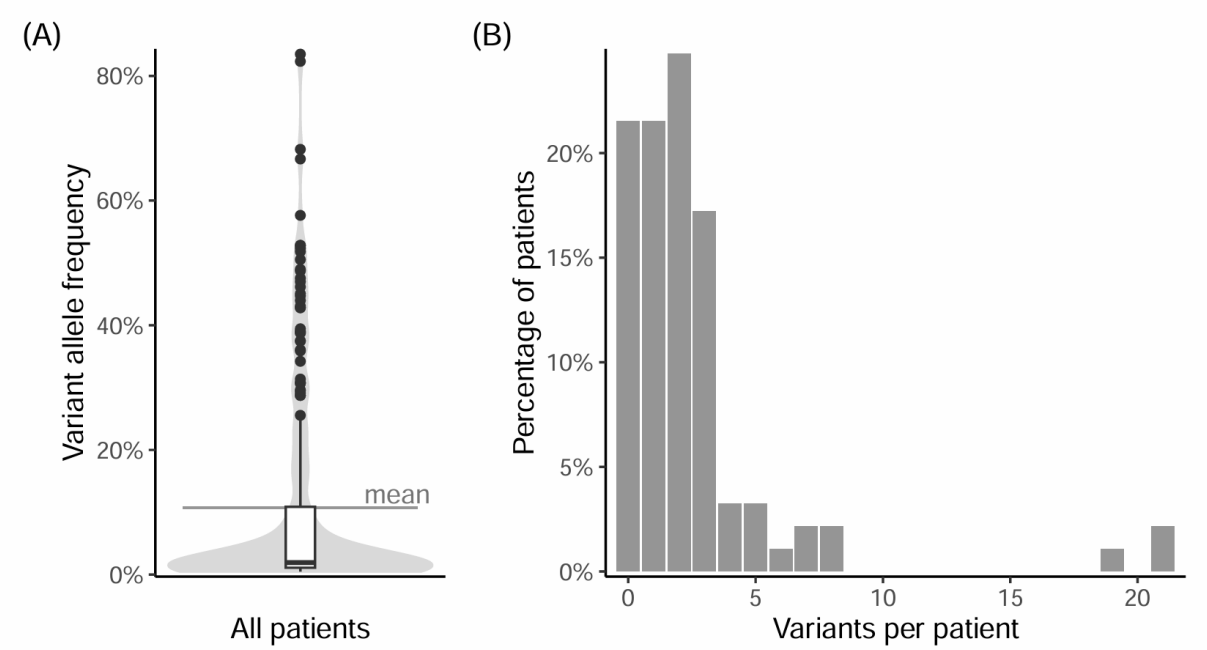
Library preparation, data processing and mutation calling were performed as described previously except for changes described below[1]. DNA target enrichment was performed using 50-100 ng of genomic DNA. Samples with more than 200 ng available DNA were sequenced in duplicates. Paired-end sequencing (2x 150 bp) was performed on the Illumina NovaSeq 6000 platform. All variants were filtered in R[2] (version 4.4.1) using functions from tidyverse packages[3] (2.0.0) as described previously, except for using an updated version of Cosmic[4] (version 97). Furthermore, we developed a custom R script to formalize the last step of variant filtering, replacing manual inspection in IGV. Variants supported by the majority of smMIPs covering their positions were more likely to be true-positive variant calls than mutations only detected by a minority or a single smMIP. Because Mutect2[5] does not distinguish whether reads originate from one or multiple smMIPs, we implemented this step during variant filtering. Each unique read covering a variant position was assigned to its parent smMIP using the trimmed hybridization arm sequences. smMIPs were considered “reliable” if (i) the variant position was ≥4 bases from the smMIP scan start and stop, and (ii) ≥100 unique reads could be assigned. Each variant was assigned a score according to smMIP reliability and detection outcome: +1 for detection by reliable smMIPs, +0.5 for detection by unreliable smMIPs, –1 for lack of detection by reliable smMIPs, and 0 otherwise. Variants with a total score >0 were considered true positive variants.

Next generation sequencing at initial diagnosis

Of the 46 patients analyzed via next generation sequencing (NGS) at initial diagnosis, 21 had been analyzed inhouse via the TruSight Myeloid Sequencing Panel (Illumina Inc., San Diego, CA, USA) as previously published[6], 16 had been analyzed at the MLL Müchner Leukämielabor (Munich, Germany), 3 had been analyzed inhouse via a custom Haloplex sequencing panel (Agilent Technologies, Santa Clara, CA, USA) used for routine cancer diagnosis[7]. For 6 patients the origin of NGS data was unknown.

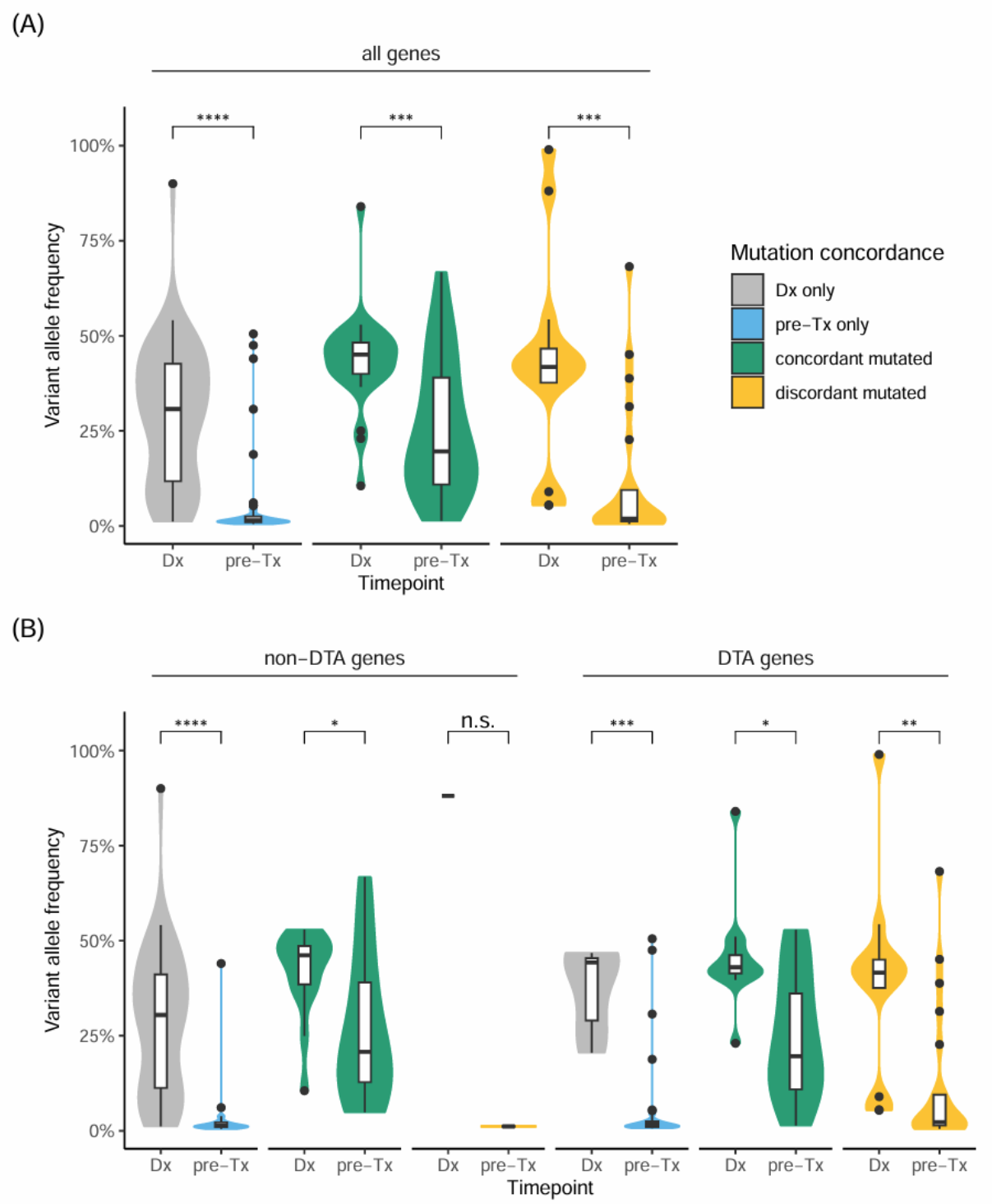
Supplementary figures

Figure S1: Overall VAF and variant count



Overview of all detected mutations. **A)** Box-violin plot showing the distribution of variants allele frequencies (VAFs) of all detected mutations (median is 1.9%, mean is 10.8%). **B)** Barplot showing the distribution of the number of detected variants per patient (median = 2, calculated both including and excluding patients without detectable mutations).

Figure S2: Clone sizes by concordance status

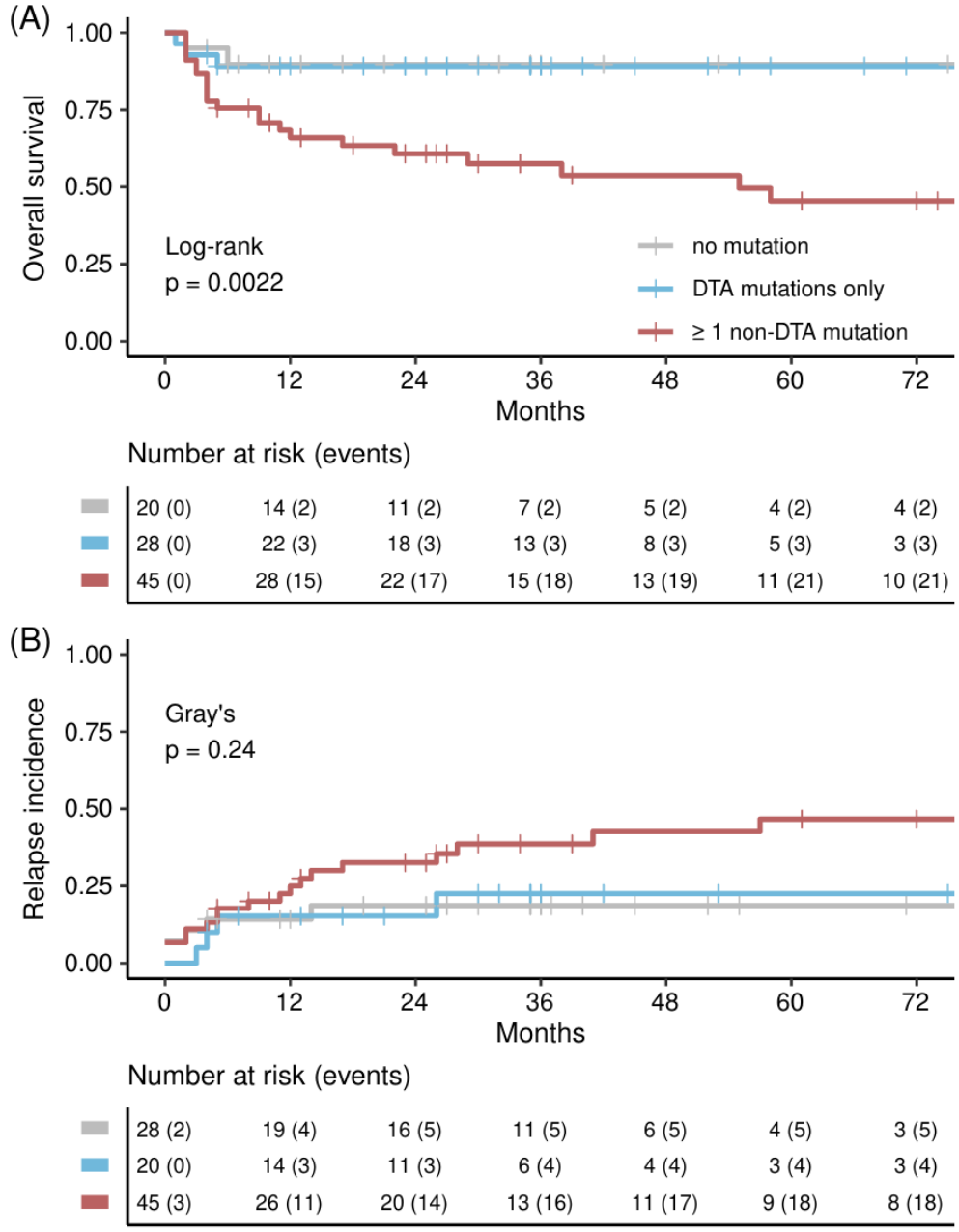


Box-violin plots displaying VAF by concordance status and timepoint. **A)** Across all genes and **(B)** across non-DTA and DTA genes separately. Mutation concordance was defined as described in figure 2. Three concordant mutations were excluded because VAF at Dx is unknown (1x IDH1, 2x TET2). Statistical significance after adjustment for multiple comparisons via the Benjamini-

Hochberg method is shown as follows: ns = q > 0.05; * = q ≤ 0.05; ** = q ≤ 0.01; *** = q ≤ 0.001; **** = q ≤ 0.0001.

Abbreviations: VAF, variant allele frequency; DTA, DNMT3A / TET2 / ASXL1; Dx, Diagnosis; pre-Tx, pre-transplantation

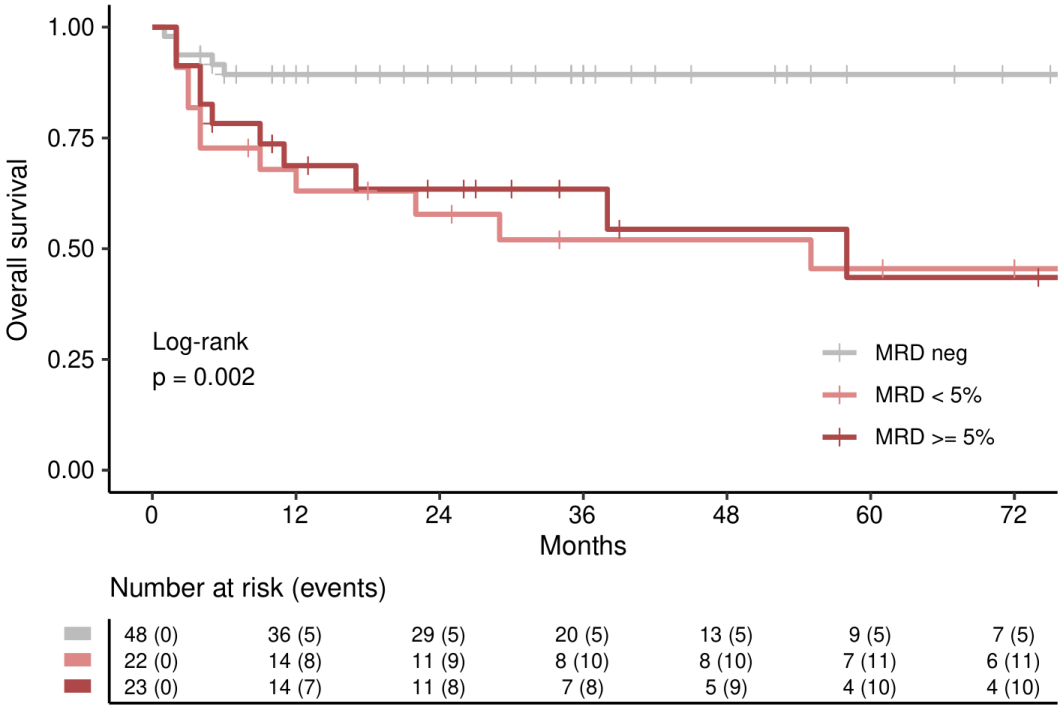
Figure S3: Outcomes by mutation status



Plots displaying the overall survival (A) and cumulative incidence of relapse (B) curves of patients after allo-SCT, stratified by mutation status in pre-transplant bone marrow. Tick marks represent censored observations.

Abbreviations: DTA, DNMT3A / TET2 / ASXL1; allo-SCT, allogeneic stem cell transplantation

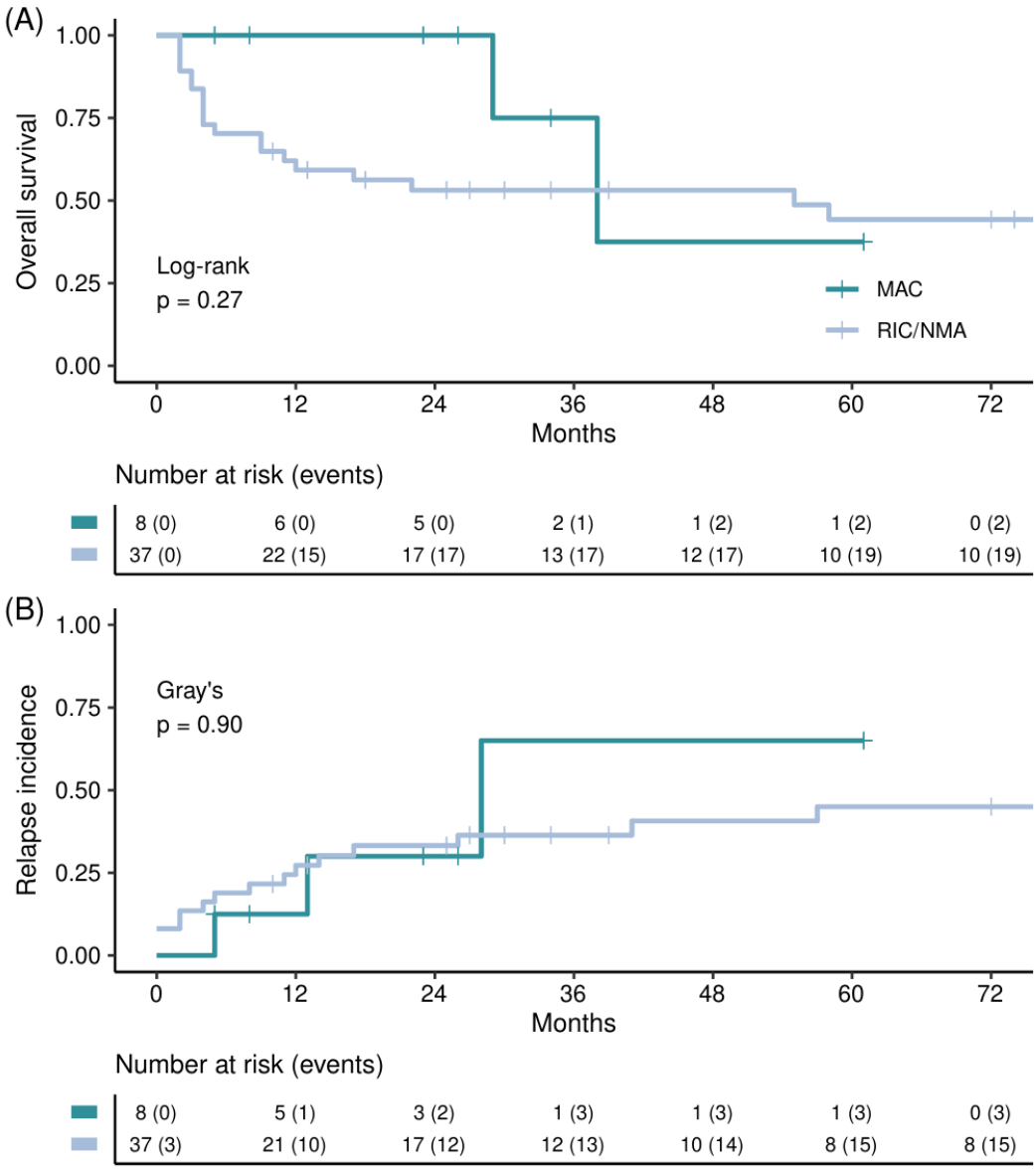
Figure S4: Survival by clone size



Kaplan-Meier plot showing the overall survival of patients after allo-SCT stratified by MRD status. Patients were considered MRD negative if they had no non-DTA mutation. If they carried non-DTA mutations, they were stratified by variant allele frequency of their largest mutation as either MRD < 5% or MRD ≥ 5%. Tick marks represent censored observations

Abbreviations: MRD, measurable residual disease; DTA, *DNMT3A/TET2/ASXL1* genes

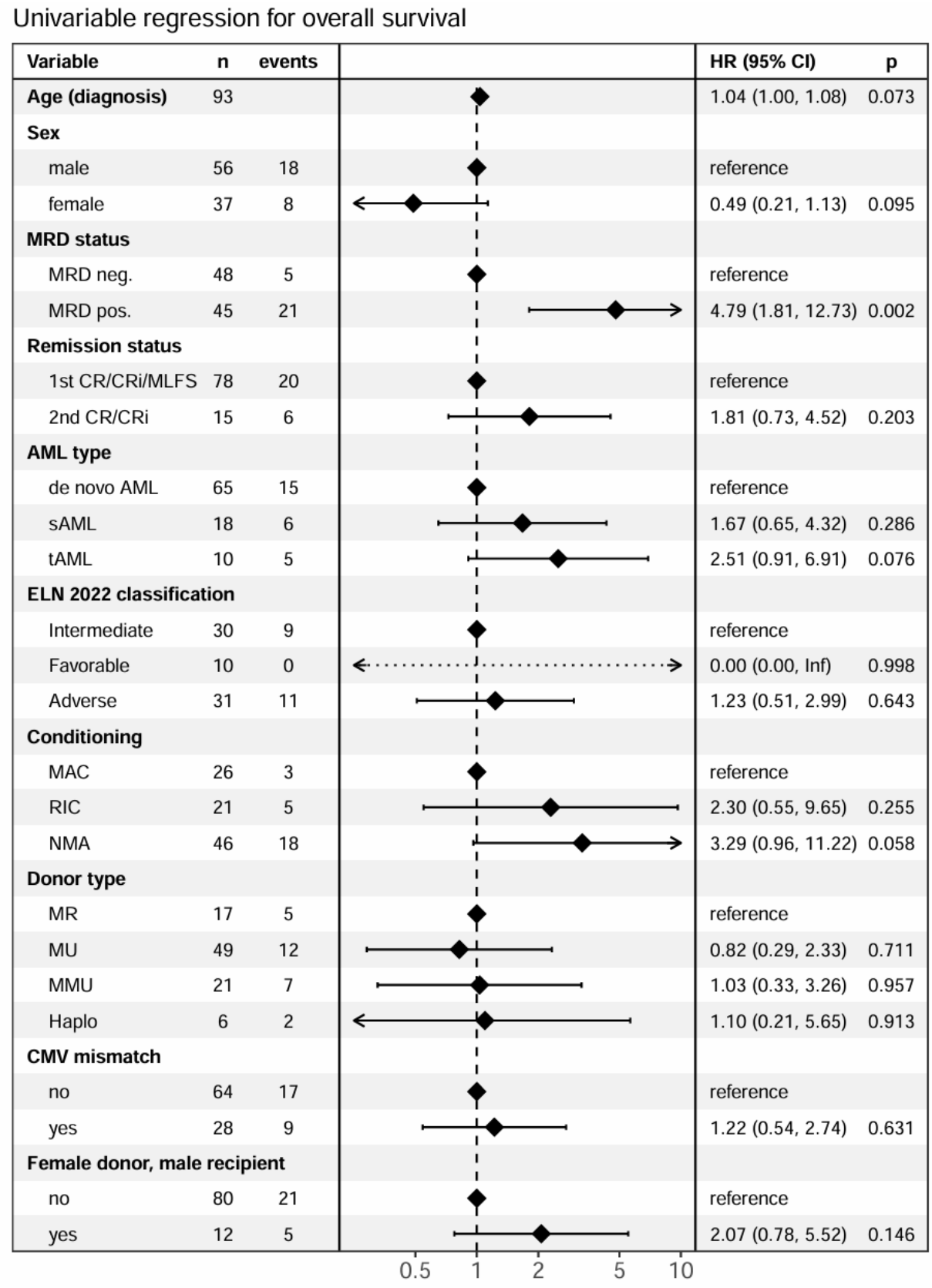
Figure S5: Survival of MRD positive patients by conditioning intensity



Plots showing overall survival **(A)** and cumulative incidence of relapse **(B)** curves of MRD positive patients after allo-SCT stratified by conditioning intensity. Tick marks represent censored observations.

Abbreviations: MAC, myeloablative conditioning; RIC, reduced intensity conditioning; NMA, non-myeloablative conditioning; MRD, measurable residual disease

Figure S6: Univariable regression analysis

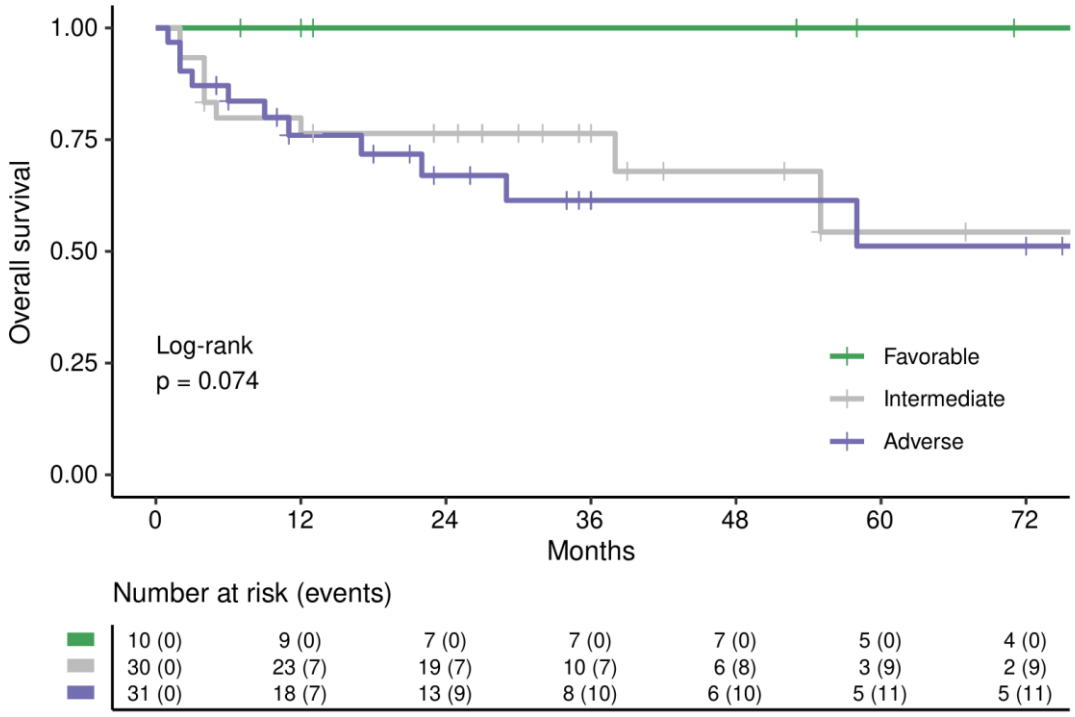


Univariable cox proportional hazards regression analyses for overall survival (OS) after allo-SCT. Results of analysis of the association of various patient-, disease- and transplantation-

related factors. The hazard ratio estimate for the ELN 2022 favorable category is not displayed due to the logarithmic axis scale. Corresponding confidence intervals extending toward zero or infinity are indicated by dotted lines.

Abbreviations: allo-SCT, allogeneic stem cell transplantation; MRD, measurable residual disease; MRD neg., MRD negative; MRD pos., MRD positive; CR, complete remission; CRi, complete remission with incomplete count recovery; MLFS, morphologic leukemia free state; AML, acute myeloid leukemia; sAML, secondary AML; tAML, therapy related AML; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; NMA, non-myeloablative conditioning; MR, matched-related donor; MU, matched-unrelated donor; MMU, mismatched-unrelated donor; Haplo, haploidentical donor; CMV, cytomegaly virus

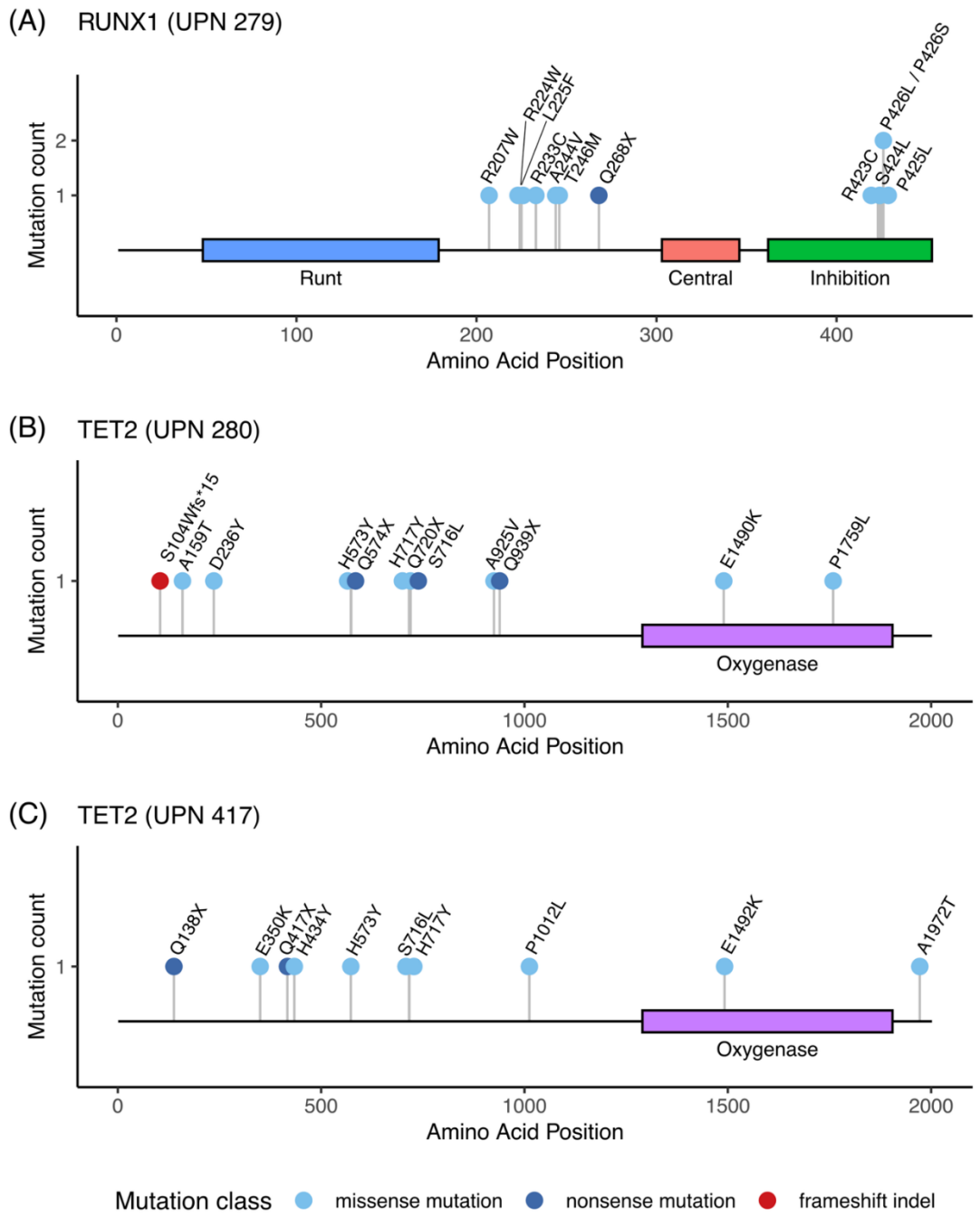
Figure S7: Survival by ELN 2022 classification



Kaplan-Meier plot showing the overall survival of patients after allo-SCT stratified by ELN 2022 classification. ELN 2022 classification was missing for 22 patients. Tick marks represent censored observations.

Abbreviations: ELN, European Leukemia Network

Figure S8: Clustered variants



Lollipop plots displaying distribution and classification of clustered RUNX1 (A) and TET2 (B, C) variants in three patients, all of whom had received high-dose cytarabine treatment. Colored regions indicate protein domains. Amino acid changes are annotated according to the canonical reference transcript. Variants are colored by mutation class, and the stem length represents the mutation count at a given amino acid position. Lollipop heads are jittered for

better visibility and readability. References: RUNX1 - transcript: NM_001754, domains from InterPro Q01196; TET2 - transcript: NM_001127208, domains from InterPro Q6N021.

Supplementary tables

Table S1: List of smMIPs

See supplementary excel file.

Table S2: List of smMIP target loci

See supplementary excel file.

Table S3: Induction therapy protocols

Induction approach	**Patient count**	**Reference**
7+3	20	
7+3 + midostaurin	4	[8]
7+3 + gemtuzumab	5	[9]
CPX-351	5	[10]
Cytarabine + idarubicine/mitoxantrone-based induction therapy ("OSHO" induction); OSHO protocols #061 and #069	44	[11,12]
Azacitidine followed by "OSHO" induction	9	
7+3 + quizartinib/placebo (QuANTUM-First trial)	3	[13]
Azacitidine	1	
Low dose cytarabine + venetoclax	1	[14]
AML BFM-2014 pediatric protocol	1	[15]

Table S4: List of all detected variants prior to allo-SCT

See supplementary excel file.

Table S5: List of all detected variants at diagnosis

See supplementary excel file.

Table S6: smMIP assay costs

Type	**Approximate per-sample library preparation cost**
Reagents	6 €
Plasticware	0.7 €
Filter tips	0.8 €
Quality control	0.5 €
Sum	**8 €**

Costs represent calculated per-sample reagent and consumable expenses and are shown as approximate values to account for institutional and regional price variance.

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