Effect of age and treatment on predictive value of measurable residual disease: implications for clinical management of adult patients with acute myeloid leukemia

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Received: March 24, 2023.
Accepted: June 15, 2023.
Early view: June 22, 2023.

https://doi.org/10.3324/haematol.2023.283196

Supplemental data

The effect of age and treatment on the predictive value of measurable residual disease: implications for the clinical management of adult patients with acute myeloid leukemia

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Supplemental Materials and Methods

S1. Treatment protocols

Protocol-1: since April 2004 to March 2007, patients received induction according to standard-dose cytarabine (SDAC) based course, namely "3+7" (Cytarabine 100 mg/sqm bid on days 1-7; Idarubicin 12 mg/sqm on days 1-3). From 2006 on, etoposide 100 mg/sqm on days 1-5 was added (ICE course). High-dose cytarabine (HDAC) 1, 3, 5 (3000 mg/sqm bid on days 1, 3, 5) was used as first consolidation in patients aged < 61 years attaining complete remission (CR) after ICE. Patients with persistent disease (*i.e.*, > 5% BM blasts at hematopoietic recovery) after first course received a salvage regimen (Ida-HDAC). In an intention-to-treat approach, patients aged < 55 years with high-risk karyotype, *FLT3*-ITD or adverse clinical features (secondary AML, CR after second course, hyperleukocytosis) were assigned to undergo allogeneic stem cell transplantation (SCT) from matched related or unrelated donor. Patients with intermediate cytogenetic risk in the absence of *FLT3*-ITD and adverse clinical features were allocated to allogeneic SCT if a related donor was available. Autologous SCT was offered to patients aged < 61 y with low-risk cytogenetics, intermediate-risk cytogenetics without sibling donor and high-risk disease not eligible to allogeneic SCT. Peripheral blood (PB) stem cells for autologous SCT were collected after a mobilization course (Cytarabine 500 mg/sqm bid on days 1-6; Daunorubicin 50 mg/sqm on days 4-6). Patients who failed mobilization received two additional courses with high dose cytarabine.

Protocol-2: since April 2007 to April 2014, patients were treated according to Northern Italy Leukemia Group (NILG) AML 02-06 protocol. Until March 2012, patients were recruited within the NILG AML 02/06 trial [(ClinicalTrials.gov Identifier: NCT00495287; reference: Bassan R, et al; Blood Adv. 2019;3(7):1103–1117)]. From April 2012, after closure of NILG AML 02/06 trial, patients were treated according to the standard arm provided by the protocol. The protocol provided a randomization at induction between a standard ICE induction *versus* an experimental intensified one. Patients aged > 65 y were treated according to standard arm. Upon CR achievement, patients received standard doses cytarabine consolidation and were divided into standard and high-risk cases (SR, HR): SR: favorable or intermediate risk cytogenetics (according to SWOG criteria) without any adverse clinical factor (secondary AML, *FLT3*-ITD, CR after cycle 2, persistence of pre-existing cytogenetic abnormality despite morphological CR; total WBC count >50 $x10^9$/L); HR: all non-SR cases. HR patients were assigned to undergo allogeneic SCT. Provided sufficient CD34+ cells were previously collected (>$2x10^6$/kg) upon recovery from high doses cytarabine, SR patients and HR patients excluded from allo-SCT and aged 65 years or less were randomized between autologous SCT and high doses consolidation therapy (R2). HR/SR patients unable to be randomized in R2 because of inadequate blood stem cell yield received intermediate-dose consolidation. Patients randomized to experimental arm were excluded from outcome analysis.

Protocol-3: since May 2014 to April 2017, patients received induction according to Ida-FLA course, (Cytarabine 2000 mg/sqm on days 1-4; Fludarabine 30 mg/sqm on days 1-4; Idarubicin 10 mg/sqm on days 2-4). High-dose Cytarabine (3000 mg/sqm bid days 1, 3, 5) was used as first consolidation in patients aged < 61 years attaining complete remission (CR). Patients with persistent disease (*i.e.* > 5% BM blasts at hematopoietic recovery) after first course received a salvage regimen (Clofarabine-based). In post CR phase, patients were stratified according to European Leukemia Net 2010 guidelines [reference: Döhner H, et al; Blood. 2010;115(3):453–474]. Patients in adverse-risk

category were allocated to allogeneic HSCT from matched related or unrelated donor. Patients in intermediate category were allocated to allogeneic SCT if a related donor was available. Patients in favorable-risk ELN category and high-risk disease not eligible to allogeneic SCT received up to two additional courses with high dose cytarabine.

Protocol-4: since 2017, patients harboring *FLT3* mutations received induction according to "3+7" scheme (Cytarabine 200 mg/sqm intravenous continuous infusion on days 1-7; Daunorubicin 60 mg/sqm on days 1-3) + Midostaurin 50 mg bid orally on days 8-21. High-dose Cytarabine (3000 mg/sqm bid days 1, 3, 5) + Midostaurin 50 mg bid orally on days 8-21 was used as first consolidation in patients aged < 61 years attaining complete remission (CR) [reference: Stone R, New Engl J Med. 2017;377, 454]. In post CR phase, patients were stratified according to European Leukemia Net 2017 guidelines [reference: Döhner H, et al; Blood. 2022;140 (12): 1345]. Patients in adverse-risk category were allocated to allogeneic HSCT from matched related or unrelated donor. Patients in intermediate category were allocated to allogeneic SCT if a related donor was available. Patients in favorable-risk ELN category and high-risk disease not eligible to allogeneic SCT received up to two additional courses with high dose cytarabine.

Protocol-5: since 2017, elderly patients (>60 y) diagnosed with AML with myelodysplasia-related changes received induction with CPX-351 100 U/sqm intravenously on days 1, 3, 5. For patients in CR after induction, consolidation treatment provided up to two cycles of CPX-351 65 U/sqm intravenously on days 1, 3 [reference: Lancet J, et al; JCO. 2016; 36: 2684]. If eligible, patients were allocated to allogeneic HSCT from matched related or unrelated donor.

Protocol-6: since 2017, patients diagnosed with core binding factor (CBF) related AML received induction according to "3+7" scheme (Cytarabine 200 mg/sqm intravenous continuous infusion on days 1-7; Daunorubicin 60 mg/sqm on days 1-3) + Gemtuzumab Ozogamicin intravenously 3 mg/m² (dose capping at 5 mg] on days 1, 4, and 7. Patients in CR received two consolidation courses of intravenous daunorubicin (60 mg/m² for 1 day or 2 days) in combination with intravenous ARA-C (1000 mg/sqm iv bid on days 1–4) + Gemtuzumab Ozogamicin 3 mg/m² (dose capping at 5 mg] on days 1 [reference: Castaigne S, et al; Lancet. 2012; 379: 1508]. Patients with CBF-related AML were not allocated to allogeneic HSCT in first CR.

S2. Multiparametric flow cytometry (MFC) methods for detection of aberrant Leukemia-Associate Immuno-Phenotypes (LAIP).

MFC study files reporting individual leukemia-aberrant immune-phenotype (LAIP) profiles were acquired locally according to pre-defined standard operating procedures. The same LAIP quantification was applied to BM samples for MRD assessment after induction and consolidation cycles. This evaluation was carried out at hematopoietic recovery and within 28 days after the end of chemotherapy in any instance. Acquisition through an SSC-antigen live-gate was performed and at least 8 x 10^5 BM nucleated cells were collected. LAIP profiles for measurable residual disease (MRD) study were detected using multiple combinations including CD45 conjugated with peridinin chlorophyll protein (PerCP or PerCP-Cy5.5). The panel of diagnostic monoclonal antibodies (MoAb) was previously established and reported elsewhere[a]. A FACSCanto II flow cytometer (Becton Dickinson, BD, San Jose, CA) was used equipped with FACSDiva Software (BD) for data

acquisition. Instrument setup, calibration and quality control were performed to ensure measures' stability[b]. Consistency of fluorescence intensity was monitored weekly by running fluorochrome-conjugated beads (CS&T, BD). Fluorescence photomultiplier voltages were adjusted until the mean channel values for the unlabelled beads corresponded to predetermined target values. Overtime stability of bead mean fluorescence intensity (MFI) profile was checked by Levey-Jennings diagrams; changes of up to ±15% of the mean target MFI were tolerated. The mixed-bead suspension was used to determine the appropriate compensation settings. Each combination of MoAbs was added to 50 µl of a suspension of BM cells adjusted to 20,000 nucleated cells/µl; a stain-lyse-and-then-wash procedure was adopted.

References

a) Mannelli F, Gianfaldoni G, Bencini S, et al. Early peripheral blast cell clearance predicts minimal residual disease status and refines disease prognosis in acute myeloid leukemia. Am J Hematol 2020;95(11):1304–1313.

b) Owens MA, Vall HG, Hurley AA, Wormsley SB. Validation and quality control of immunophenotyping in clinical flow cytometry. Journal of Immunological Methods. 2000;243(1–2):33–50.

S3. PCR-based MRD

Sensitive Real-time quantitative-polymerase chain reaction assays (RQ-PCR) was used for detection of MRD in patients with a suitable molecular probe. RQ-PCR was performed following the Europe Against Cancer (EAC) program recommendations[a] with a sensitivity of 10^{-5}. Level of *RUNX1-RUNX1T1* and *CBFB-MYH11* transcripts and *NPM1* gene mutations were detected by Ipsogen commercial kits: Ipsogen *RUNX1-RUNX1T1* Kit, Ipsogen *CBFB-MYH11* A Kit, Ipsogen *NPM1* mutA MutaQuant Kit and Ipsogen *NPM1* mut B&D MutaQuant Kit (Qiagen, Courtaboeuf, France). One microgram of RNA was reverse transcribed according to EAC protocol. RQ-PCR was performed according to the manufacturer's instructions on a 7900 ABI platform (Applied Biosystems, Foster City, USA). Amplification conditions were: 2 min at 50 °C, 10 min at 95 °C followed by 50 cycles at 95 °C for 15 s and at 6 °C for 1 min. The *NPM1* mutations, *RUNX1-RUNX1T1* and *CBFB-MYH11* transcript values were normalized on the number of housekeeping gene Abelson (*ABL*) transcripts and were expressed as the number of target gene copies per 10^4 copies of *ABL*. Using standards with a known number of molecules, it was possible to establish a standard curve and determine the precise amount of target in the test sample. The Ipsogen standard curves are plasmid-based: 3 plasmid standard dilutions for the control gene, and 5 standard dilutions for the mutated gene, to ensure accurate standard curves. All samples were analyzed in duplicate. A threshold value of 0.1 was used and baseline was set to 3–15 either for *ABL* or target genes[b].

References

a) Gabert J, Beillard E, Velden VHJ van der, et al. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia – A Europe Against Cancer Program. Leukemia 2003;17(12):2318–2357.

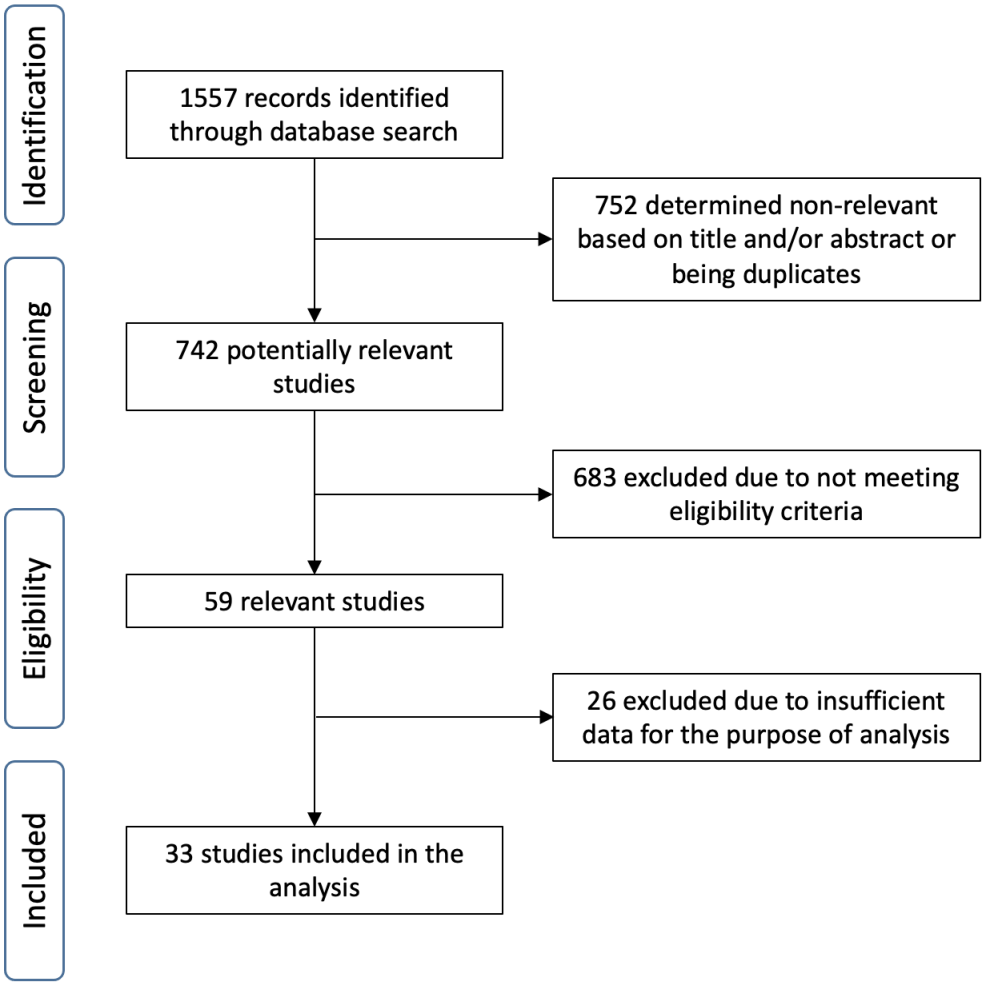
b) Gorello P, Cazzaniga G, Alberti F, et al. Quantitative assessment of minimal residual disease in acute myeloid leukemia carrying nucleophosmin (NPM1) gene mutations. Leukemia 2006;20(6):1103–1108.

S4. Analysis of literature: description of methods and flow diagram of the study selection process

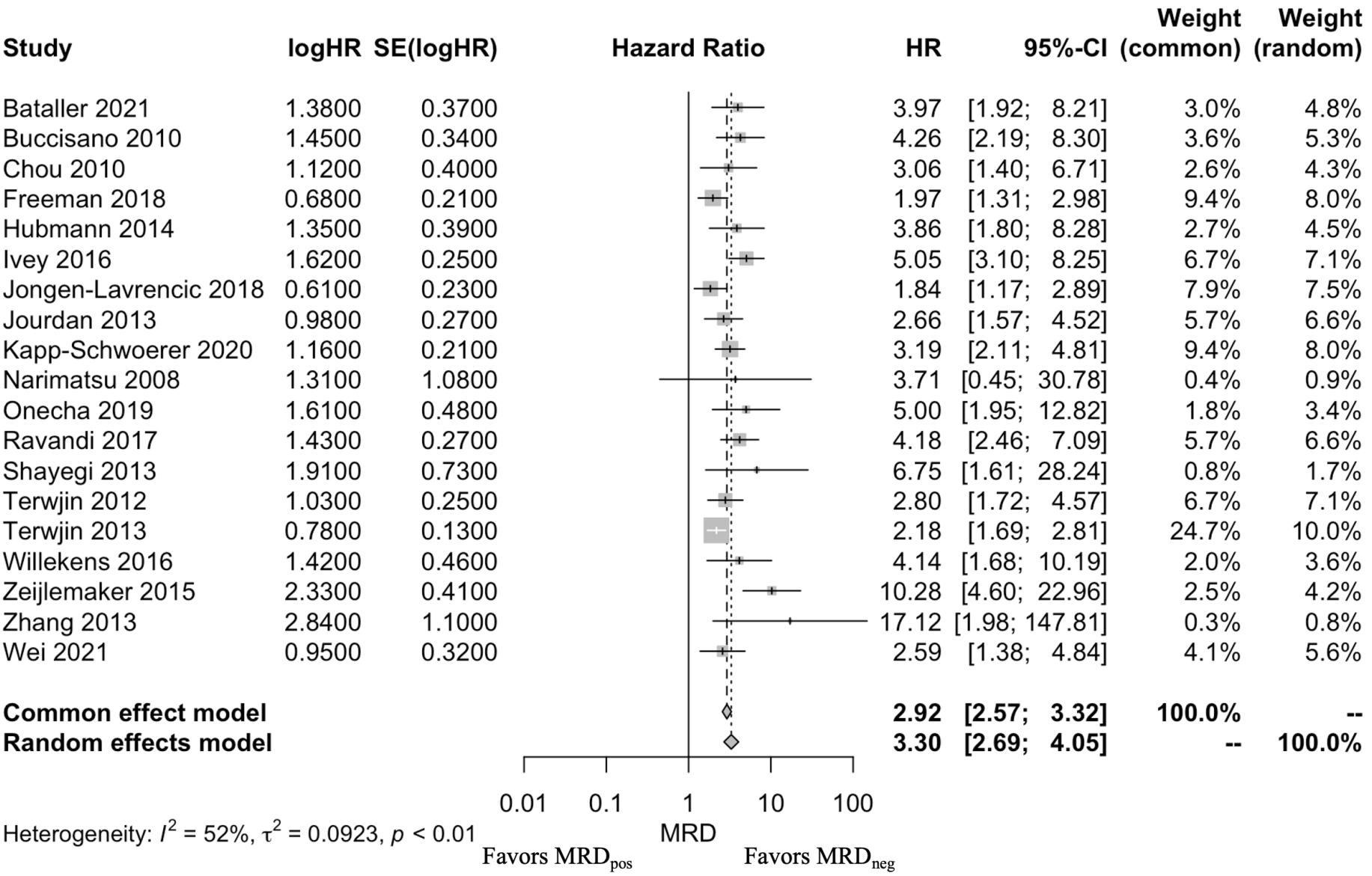
We carried out a search in PubMed for articles published between 2000 and 2021 by filtering for keywords (AML, acute myeloid leukemia, or acute myelogenous leukemia, and MRD, minimal residual disease, or measurable residual disease). The results are summarized in the Figures below. Reports were screened and filtered according to the following criteria: sufficiently detailed MRD data, sufficiently detailed treatment information, availability of Kaplan-Meier curves for DFS in the paper. Based on this assessment, a total of 33 articles were selected. The extracted data are detailed in Table S1. We extracted survival data from Kaplan-Meier curves by using the commercial graph digitizer software (Digitizelt, version 2.1; Bormisoft) and applying a previously published algorithm to reconstruct survival data for MRD_{pos} and MRD_{neg} cases[a]. The main characteristics of treatment in the first two cycles (drugs, ARA-C dosage, schedule) were obtained for each report and tabulated as in Supplemental Table S2. Moreover, each extracted case was annotated for the following variables: genetic subset, method for MRD detection, number of chemotherapy cycles pre-MRD assessment, cumulative dosage of ARA-C pre-MRD assessment, chemotherapy schedule pre-MRD, MRD status. In case of multiple MRD time-points, results were extrapolated and annotated accordingly. Studies selected for analysis of DFS in $MRD2_{neg}$ cases based on treatment intensity were processed as in conventional meta-analyses and extracted data are summarized in a Forest plot (see below S5).

References

a) Guyot P, Ades A, Ouwens MJ, Welton NJ. Enhanced secondary analysis of survival data: reconstructing the data from published Kaplan-Meier survival curves. Bmc Med Res Methodol 2012;12(1):9


Identification
Screening
Eligibility
Included
1557 records identified through database search
752 determined non-relevant based on title and/or abstract or being duplicates
742 potentially relevant studies
683 excluded due to not meeting eligibility criteria
59 relevant studies
26 excluded due to insufficient data for the purpose of analysis
33 studies included in the analysis

S5. Forest plot summarizing the effects of MRD as assessed by hazard ratio (HR), standard error (SE), and the relative weight of each study included in the analysis of MRD_{neg} patients according to the intensity of treatment.

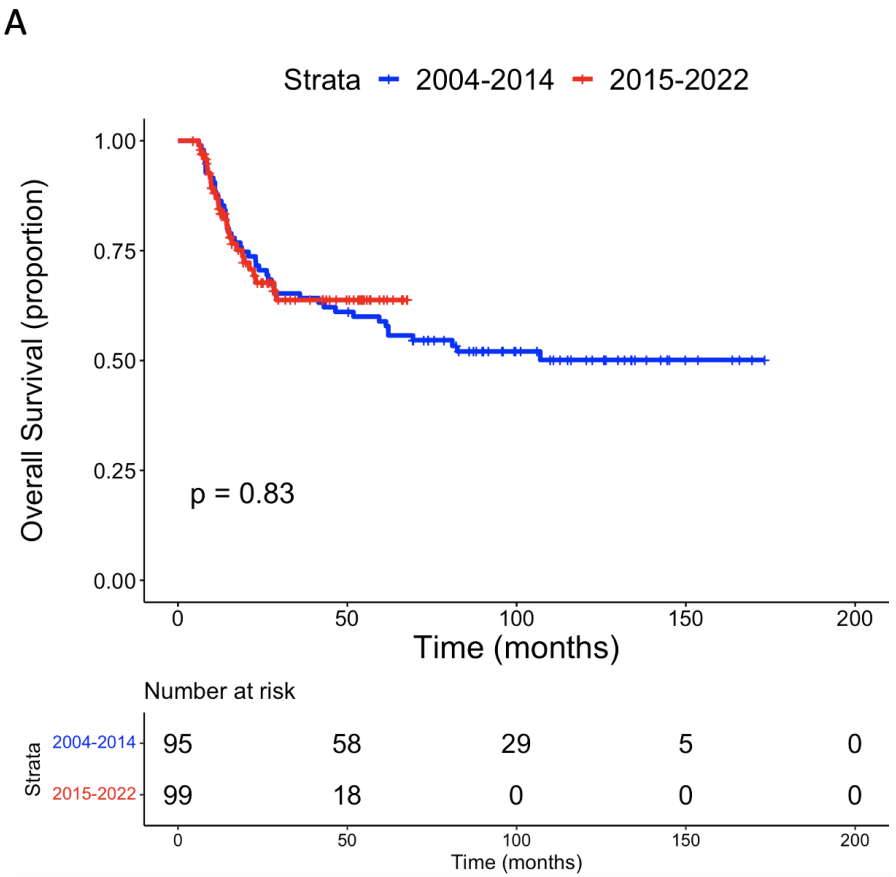


S6. Statistical methods

Pairwise comparisons of patient characteristics between groups, as defined by MRD, baseline features and treatment intensity, were performed using the Mann-Whitney test or the Kruskal-Wallis test for continuous variables and Pearson's chi-squared test or Fisher's exact test for categorical variables. Survival was estimated with the Kaplan-Meier method and long-term outcomes were compared with the log-rank test. The Cox proportional-hazards model was applied to estimate hazard ratios with 95% confidence intervals (CI) for DFS (the interval from CR to relapse or death), and OS (the interval from study entry to death) in both univariate and multivariate contexts. Comparison among longitudinal MRD assessments was done through the Harrells' concordance index (C-index) and 95% CIs, to evaluate the ability of the individual MRD time-point to predict outcome. To rule out an impact by allogeneic SCT, we censored patients receiving allogeneic SCT at the date of transplant in a further analysis. All P values were two-sided, and a 5% significance level was set.

Supplemental Figures and Legends

Figure S1. Overall survival according to year of diagnosis, separating the patient series in two (A), and three (B) consecutive time periods.



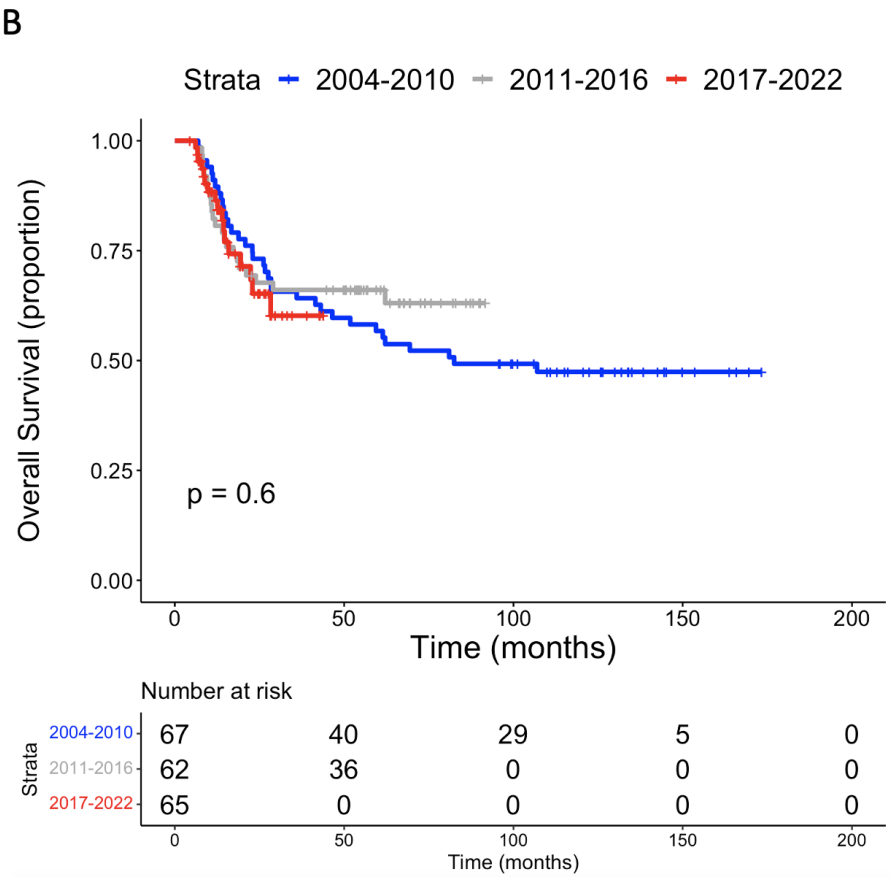


Figure S2. Disease-free (A), and overall (B) survival according to MRD1 status in the overall cohort.

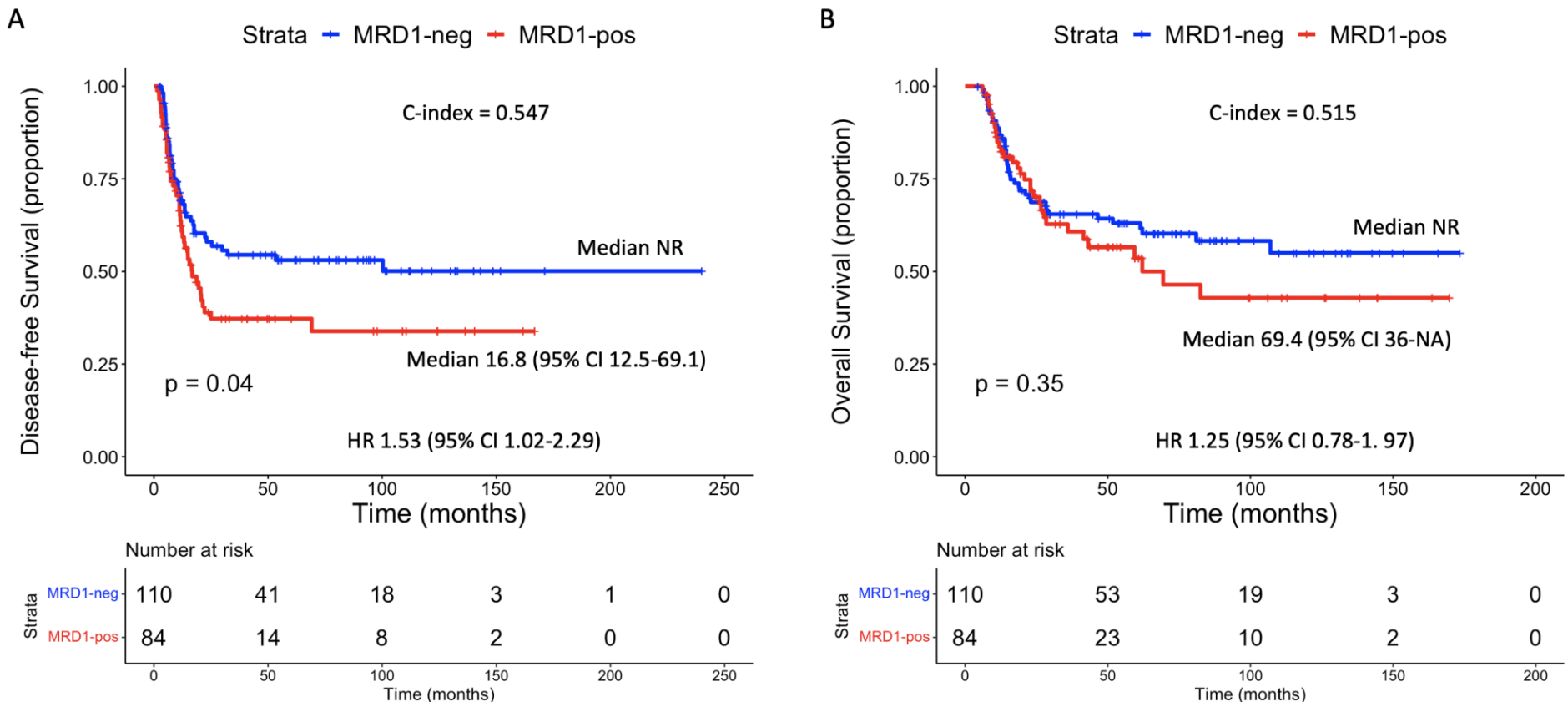


Figure S3. Disease-free (A), and overall (B) survival according to MRD2 status in the overall cohort.

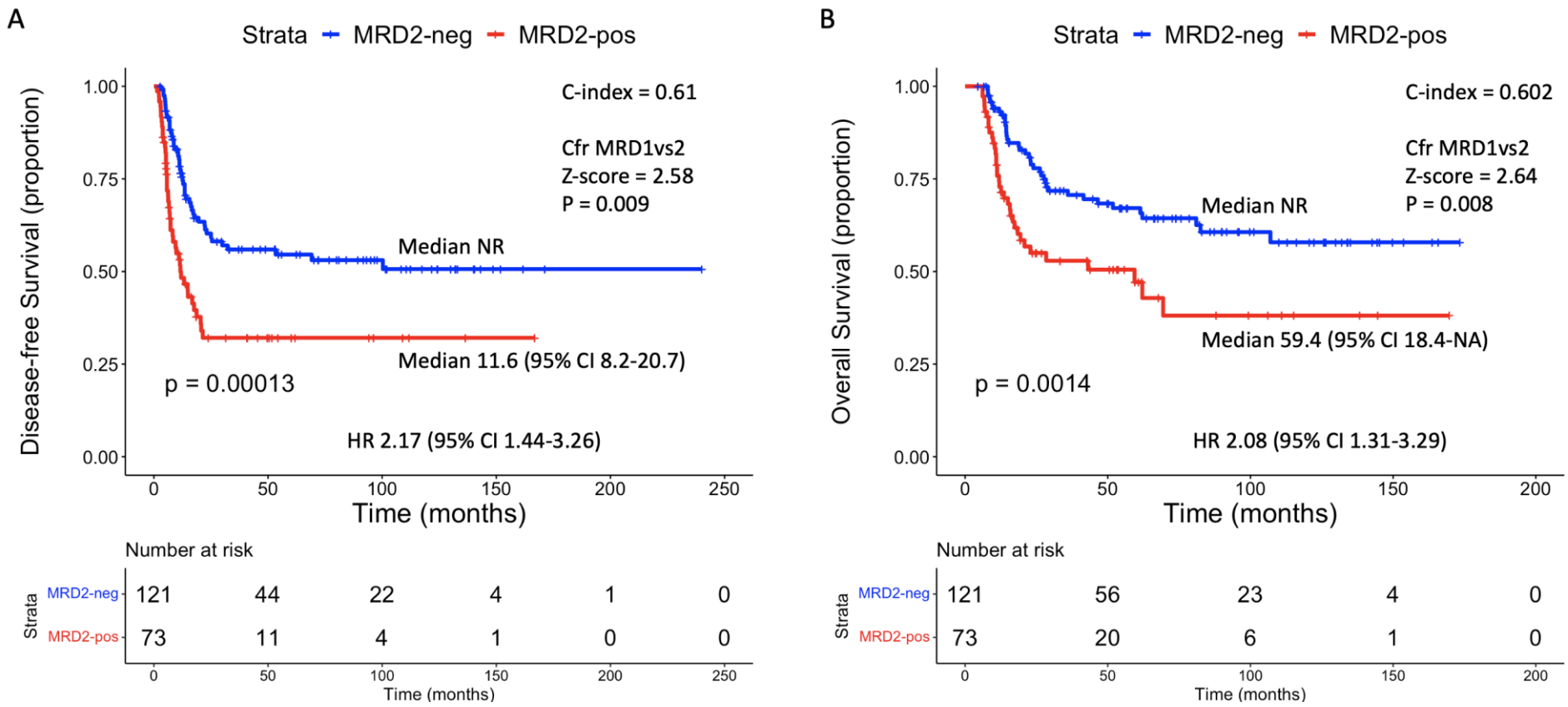


Figure S4. Disease-free (A-C), and overall (B-D) survival according to MRD2 status in age-related strata: patients aged < 55y (A-B) and ≥ 55y (C-D).

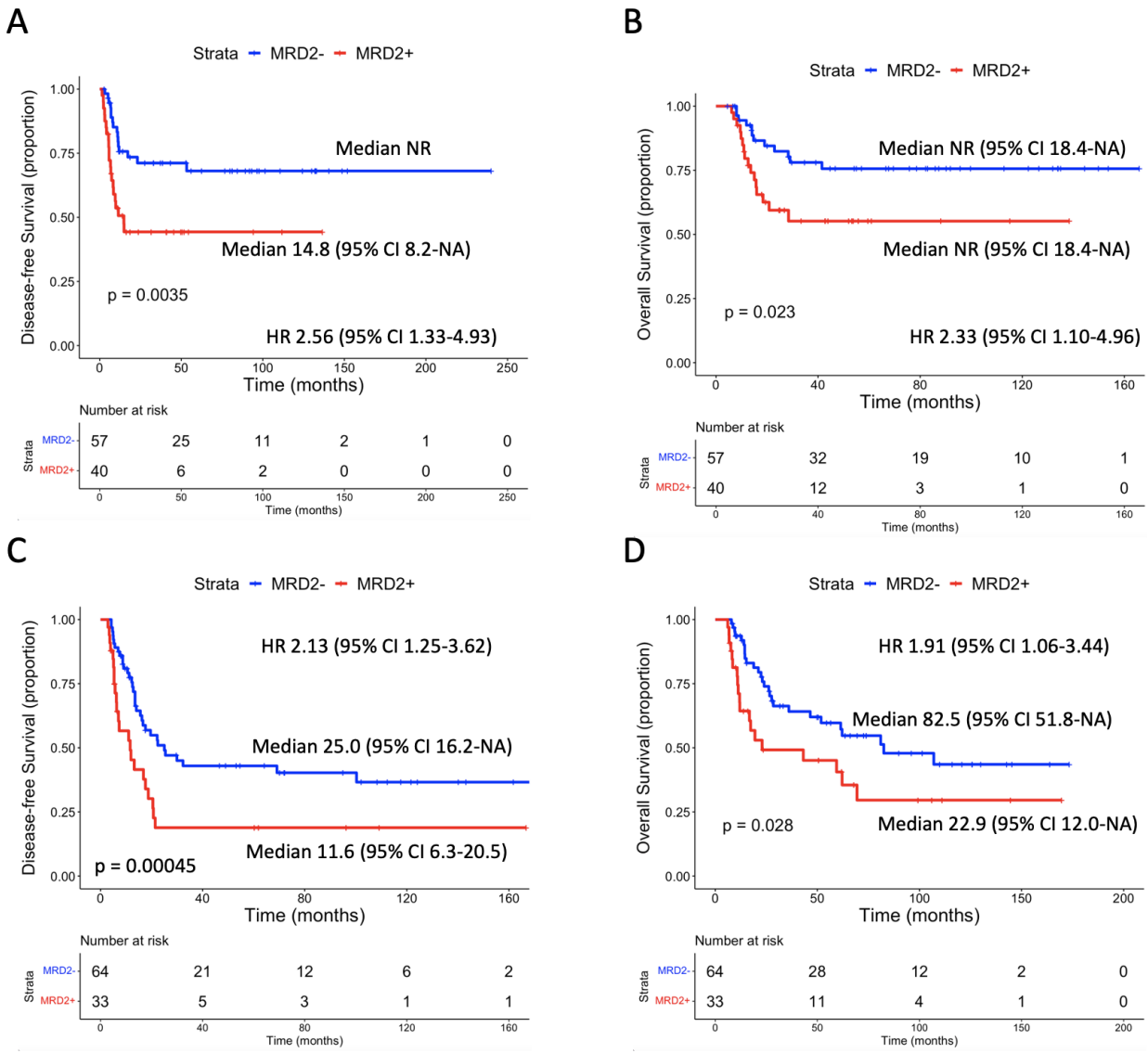


Figure S5. Disease-free (A-C), and overall (B-D) survival according to MRD2 status in WBC-related strata: WBC < $30x10^9$/L (A-B) and ≥ $30x10^9$/L (C-D).

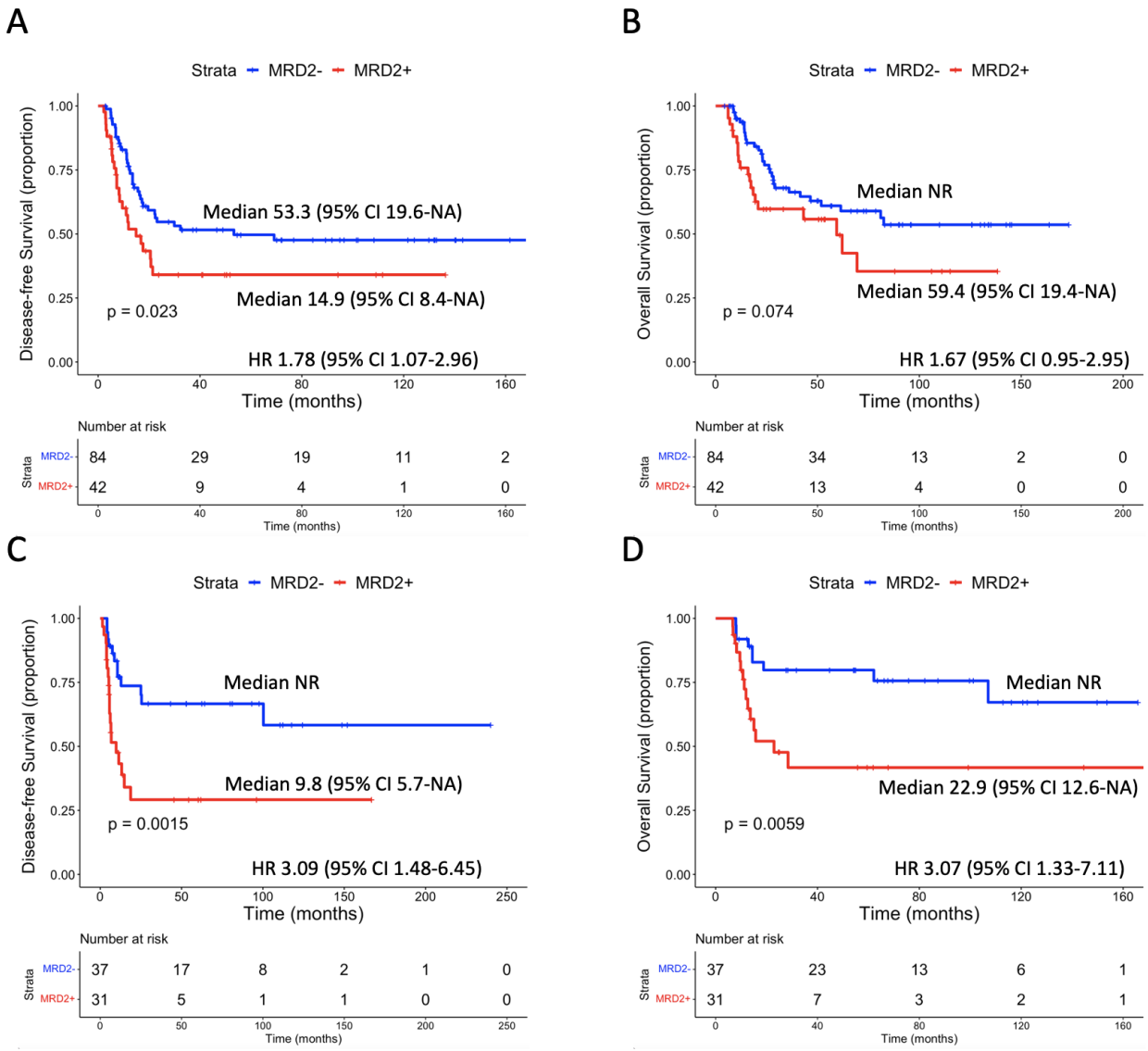


Figure S6 Disease-free (A-C), and overall (B-D) survival according to MRD2 status in ELN-related strata: ELN favorable (A-B) and ELN intermediate (C-D).

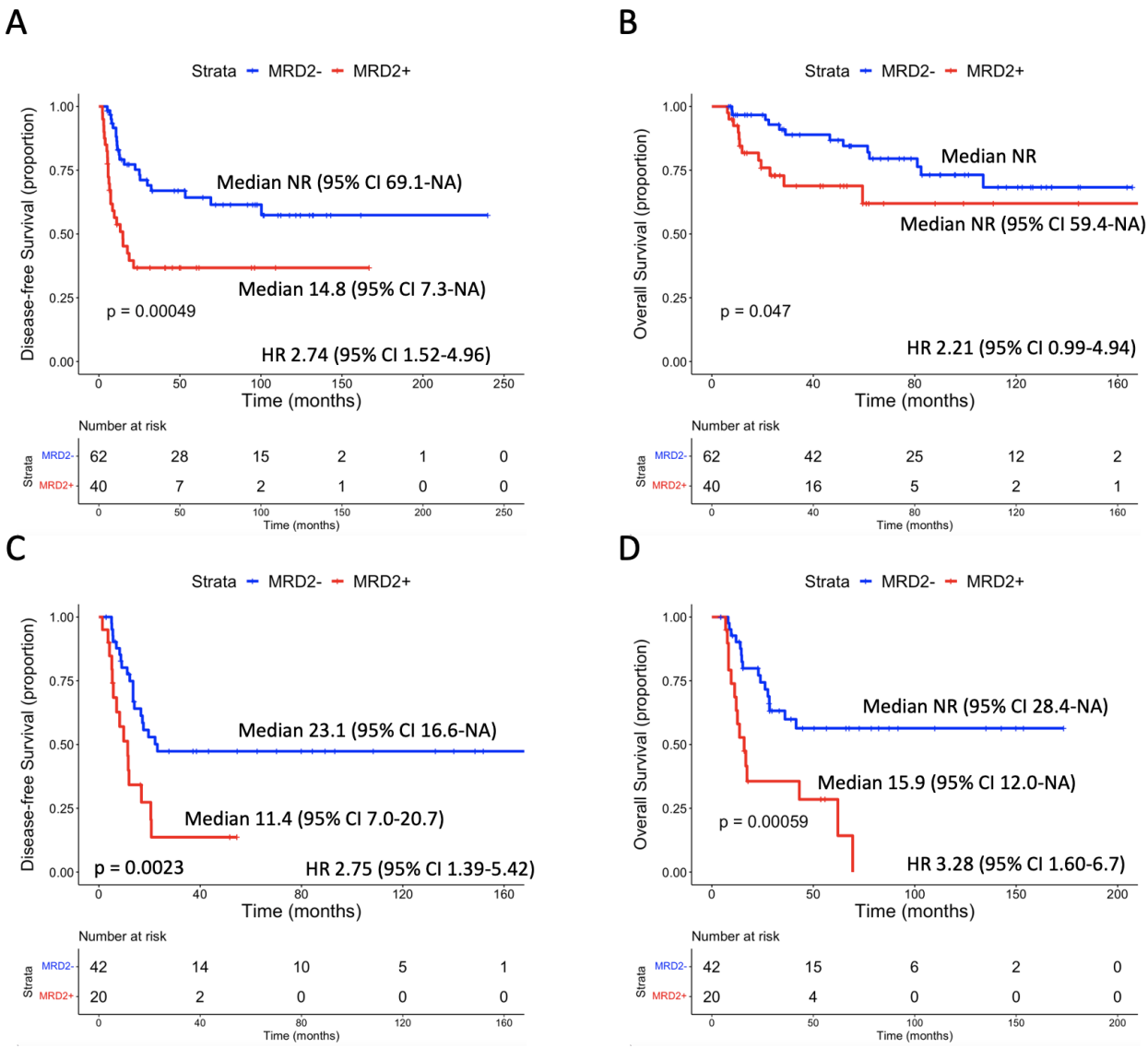


Figure S7. Disease-free (A-C), and overall (B-D) survival according to MRD2 status in gender-related strata: female (A-B) and male (C-D) patients.

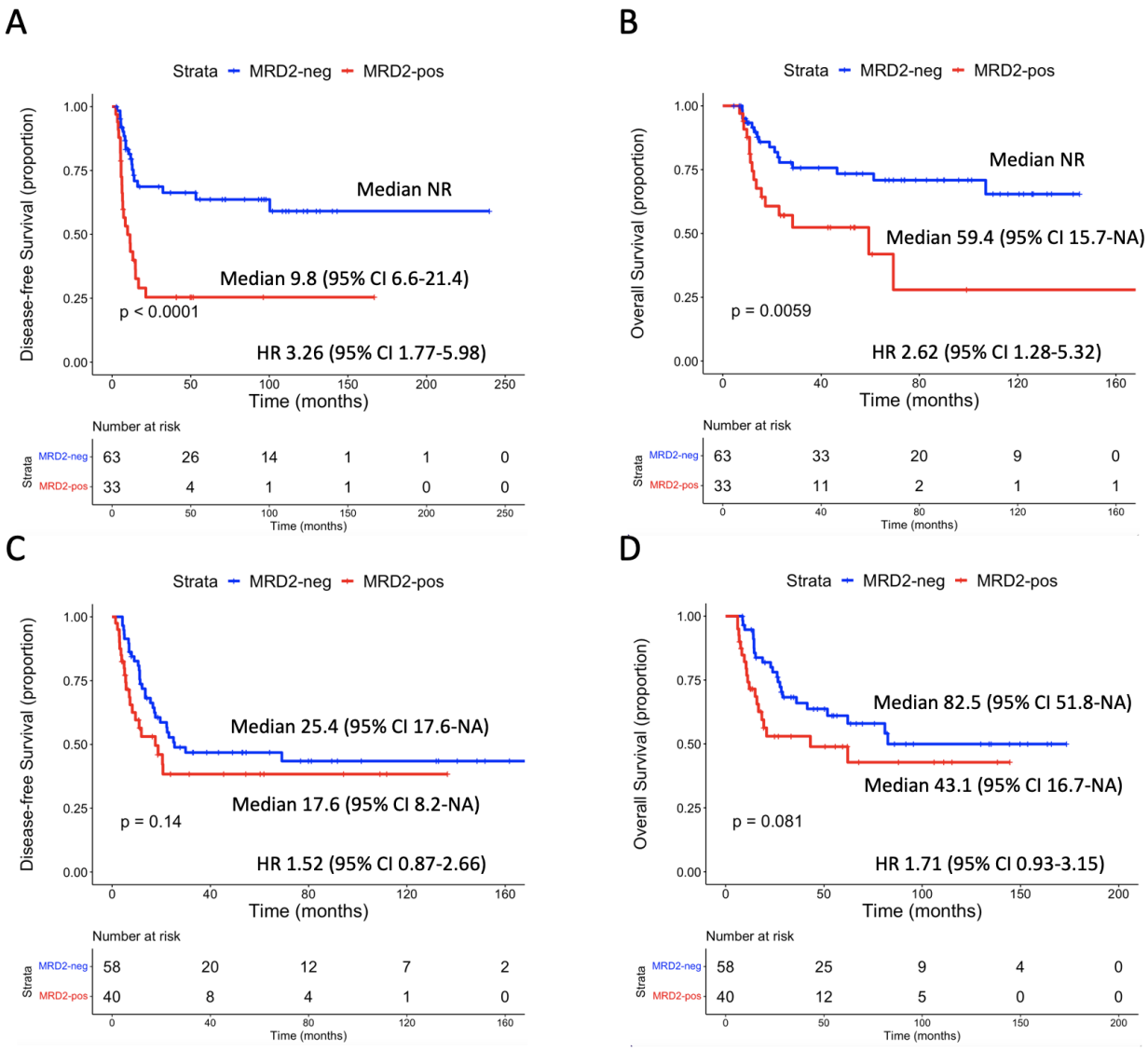


Figure S8. Disease-free survival of $MRD2_{neg}$ patients according to age- (A), WBC- (B), ELN- (C) and gender- (D) related categories.

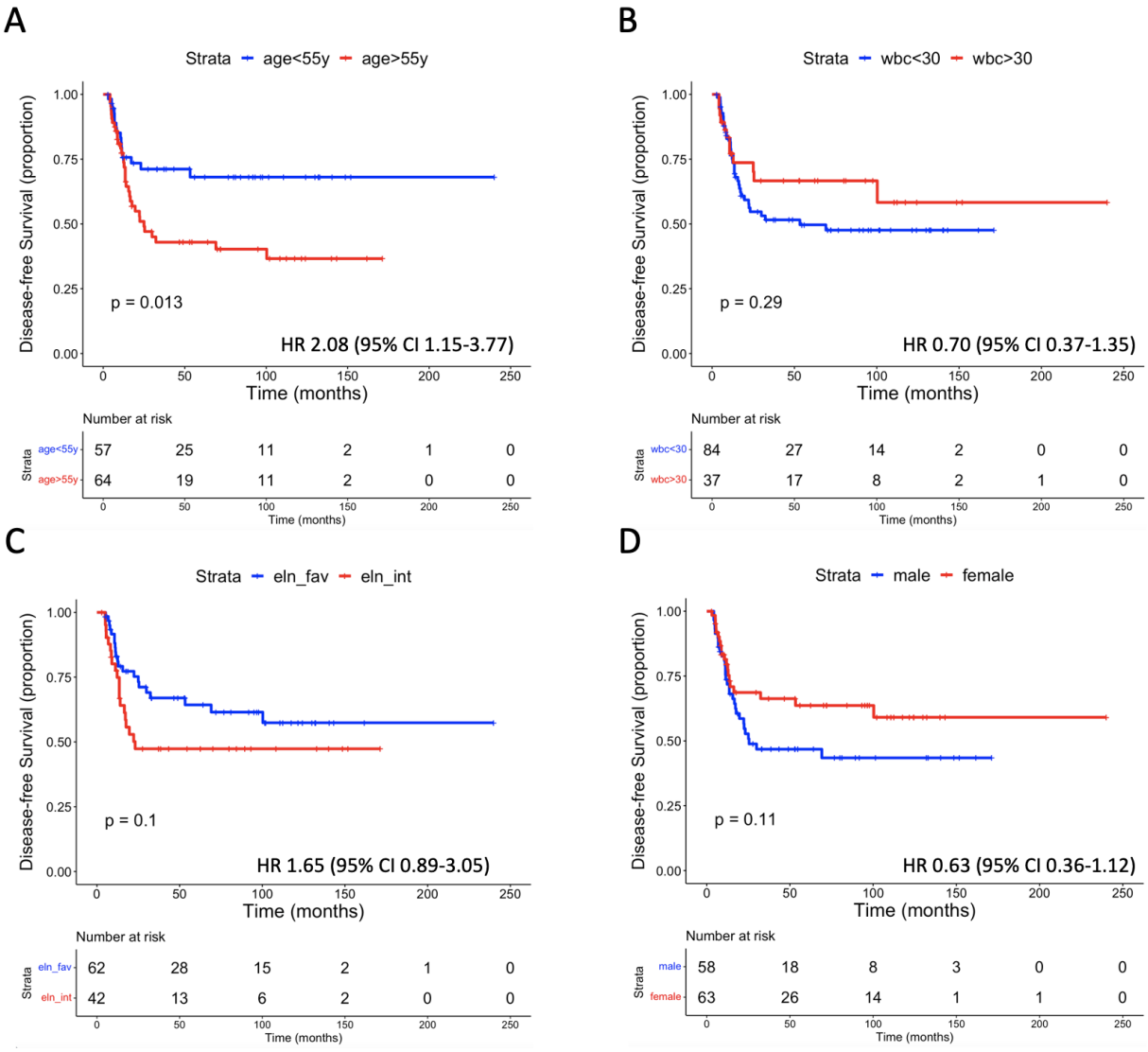


Figure S9. Disease-free (A-C) and overall (B-D) survival according to treatment intensity in first induction cycle (A-B) and in induction + first consolidation cycles (C-D).

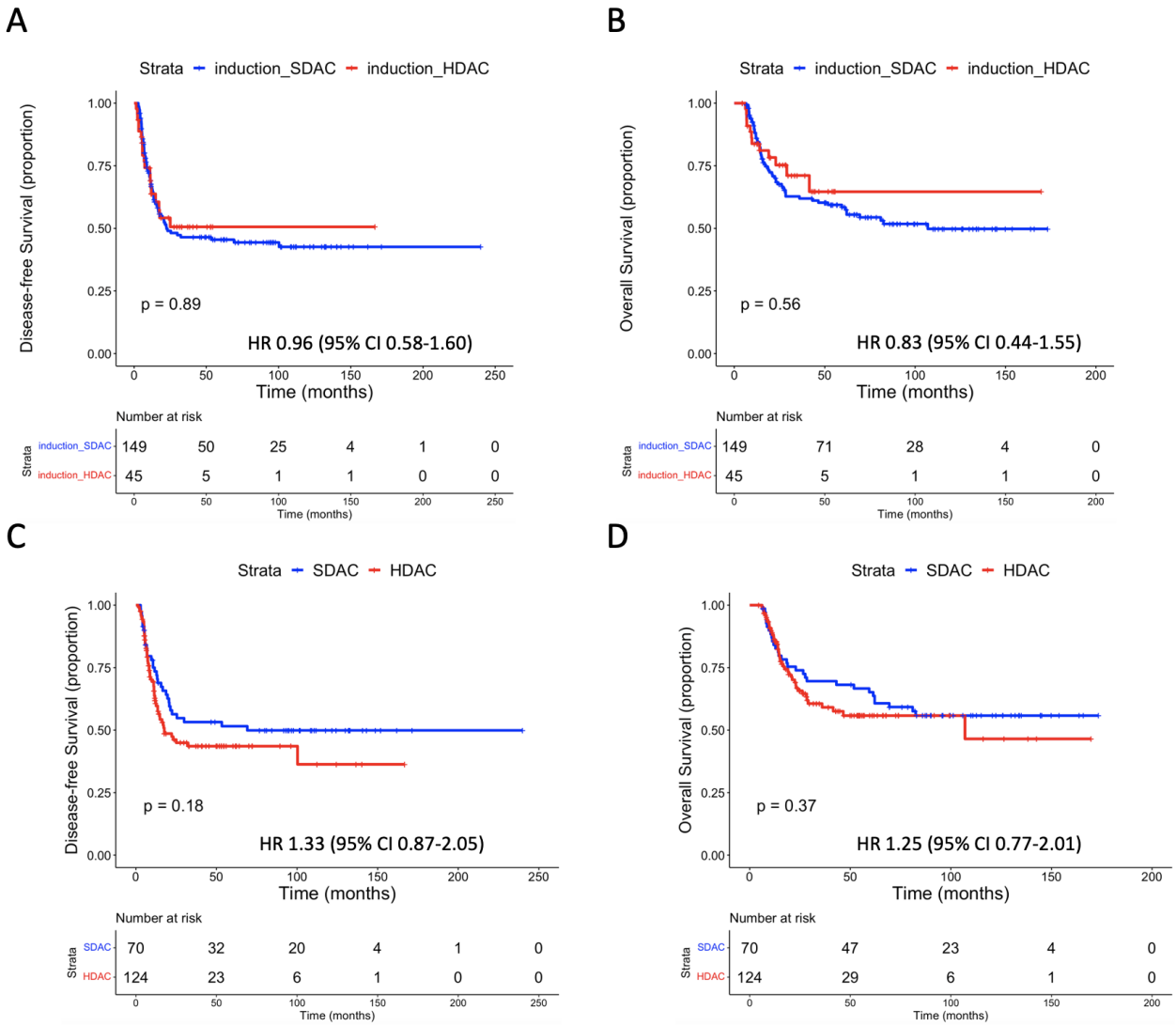


Figure S10. Disease-free (A-C) and overall (B-D) survival according to MRD2 status in treatment intensity categories within intermediate-risk karyotype: standard dose cytarabine (SDAC, panels A-B) and high-dose cytarabine (HDAC, panels C-D).

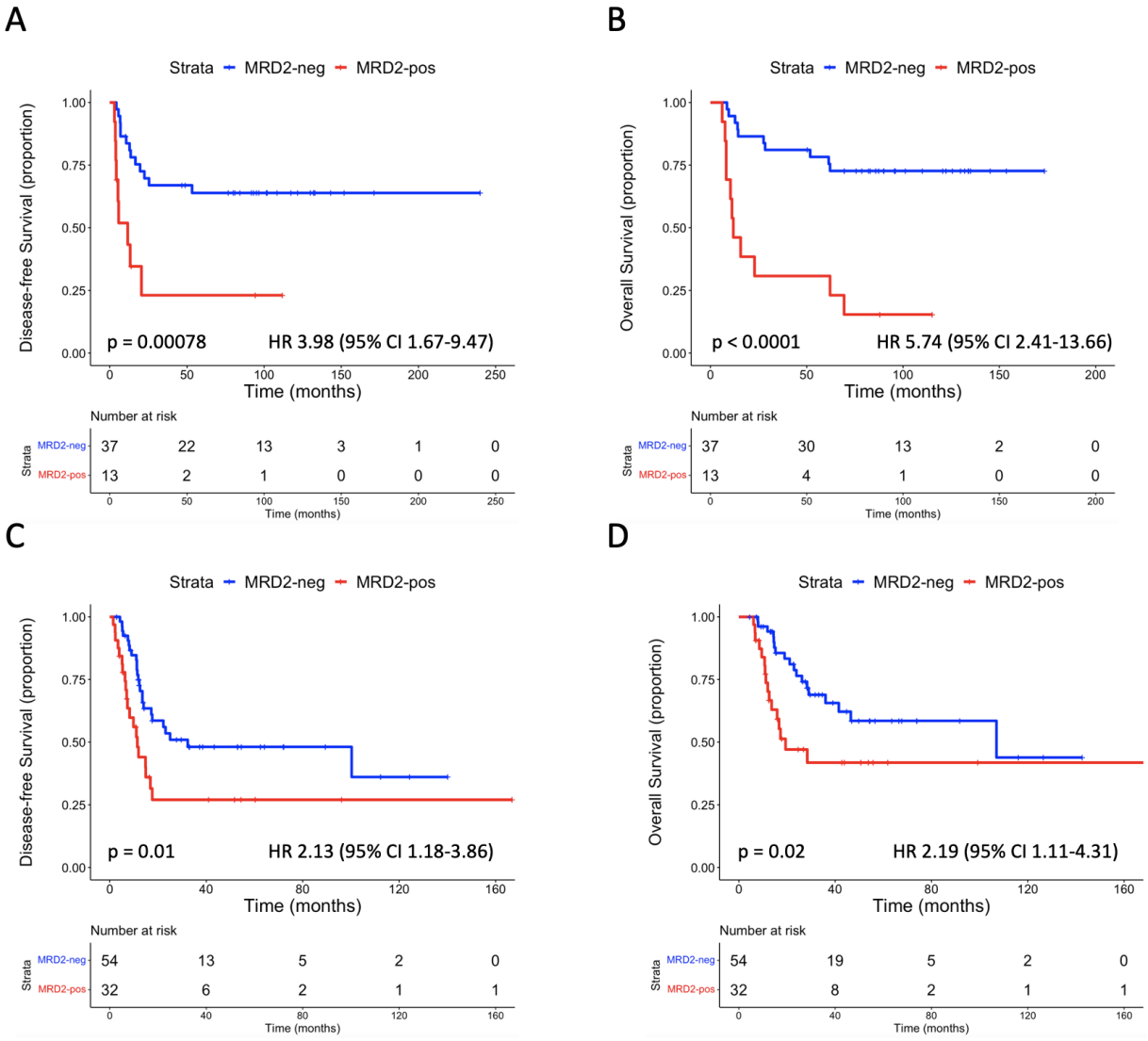


Figure S11. Disease-free (A) and overall (B) survival in $MRD2_{neg}$ patients according to treatment intensity.

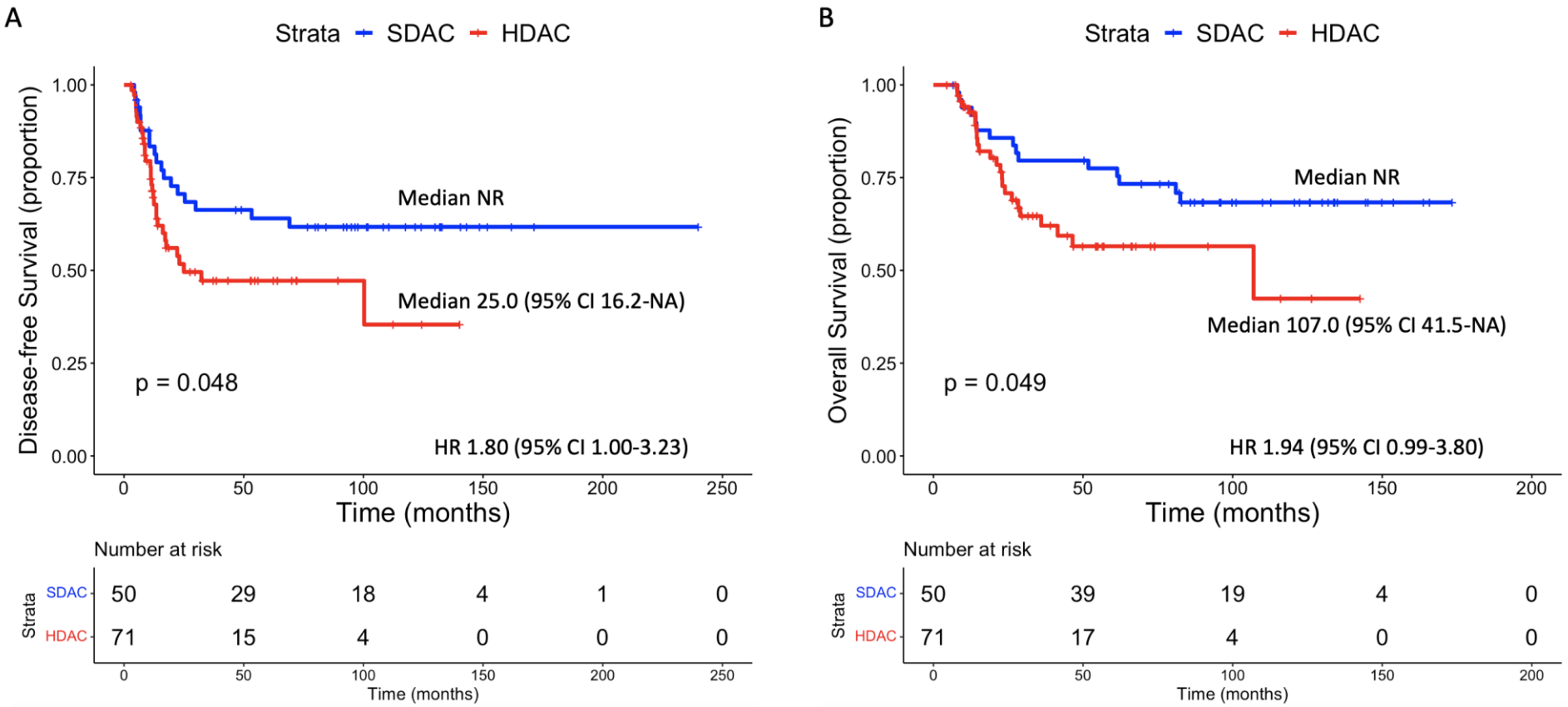


Figure S12. Disease-free (A-C) and overall (B-D) survival in $MRD2_{neg}$ patients according to treatment intensity categories within intermediate-risk karyotype (A-B) and ELN 2017 (C-D) categories.

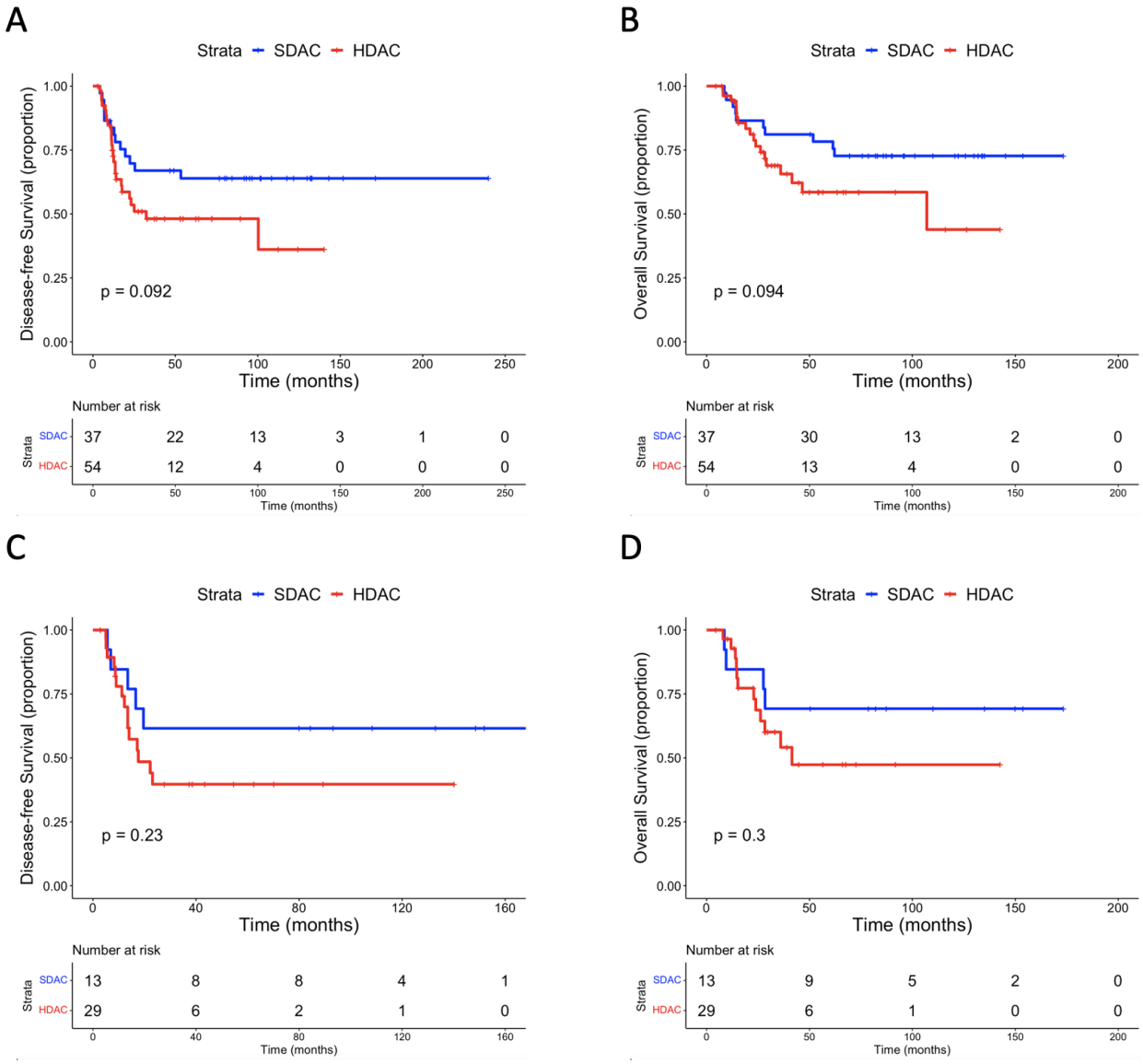


Figure S13. Disease-free (A-C) and overall (B-D) survival in $MRD2_{neg}$ patients according to combined model (age and treatment intensity) within intermediate-risk karyotype (A-B) and ELN (C-D) categories.

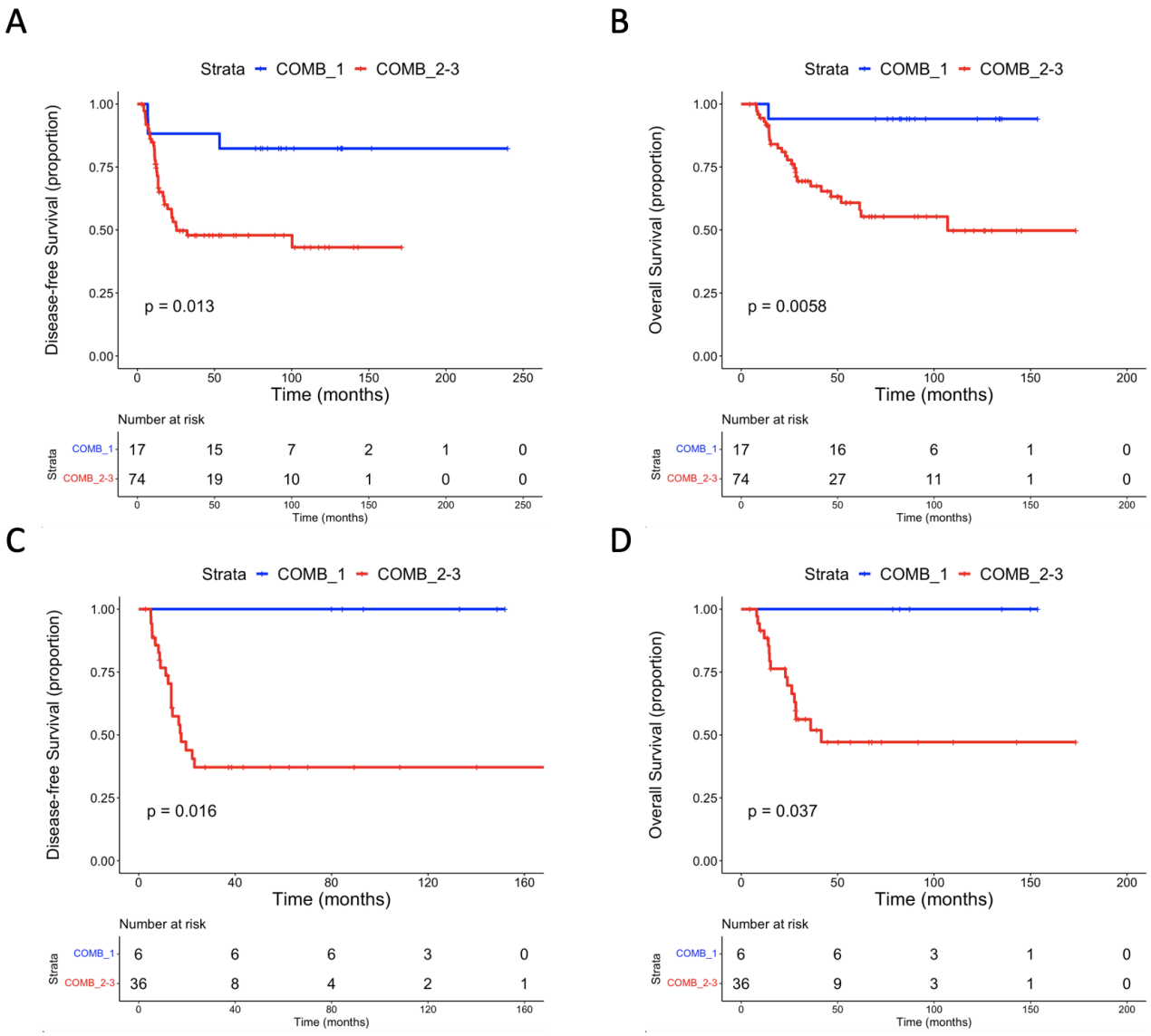
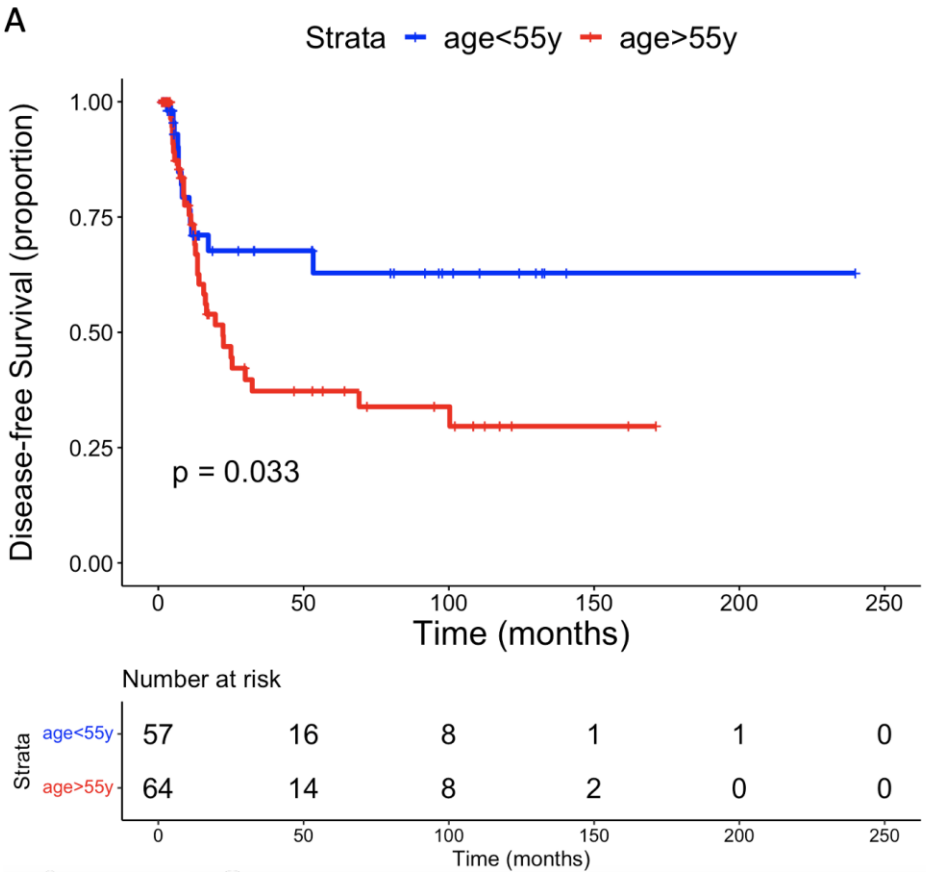


Figure S14. Disease-free survival in $MRD2_{neg}$ patients according to age (A) and treatment (B) categories after censoring at allogeneic transplant.



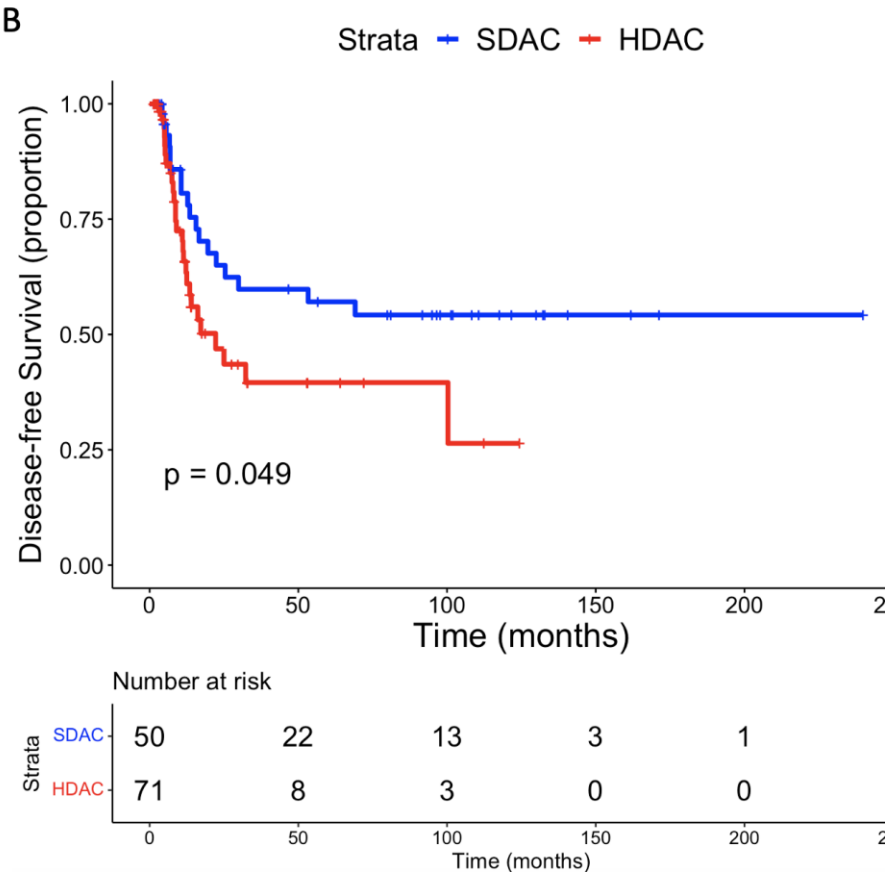


Figure S15 Disease-free (A) and overall (B) survival in $MRD2_{neg}$ patients according to combined model category after censoring at allogeneic transplant: younger, SDAC treated (COMB_1) versus elderly and/or HDAC-treated (COMB_2-3) patients.

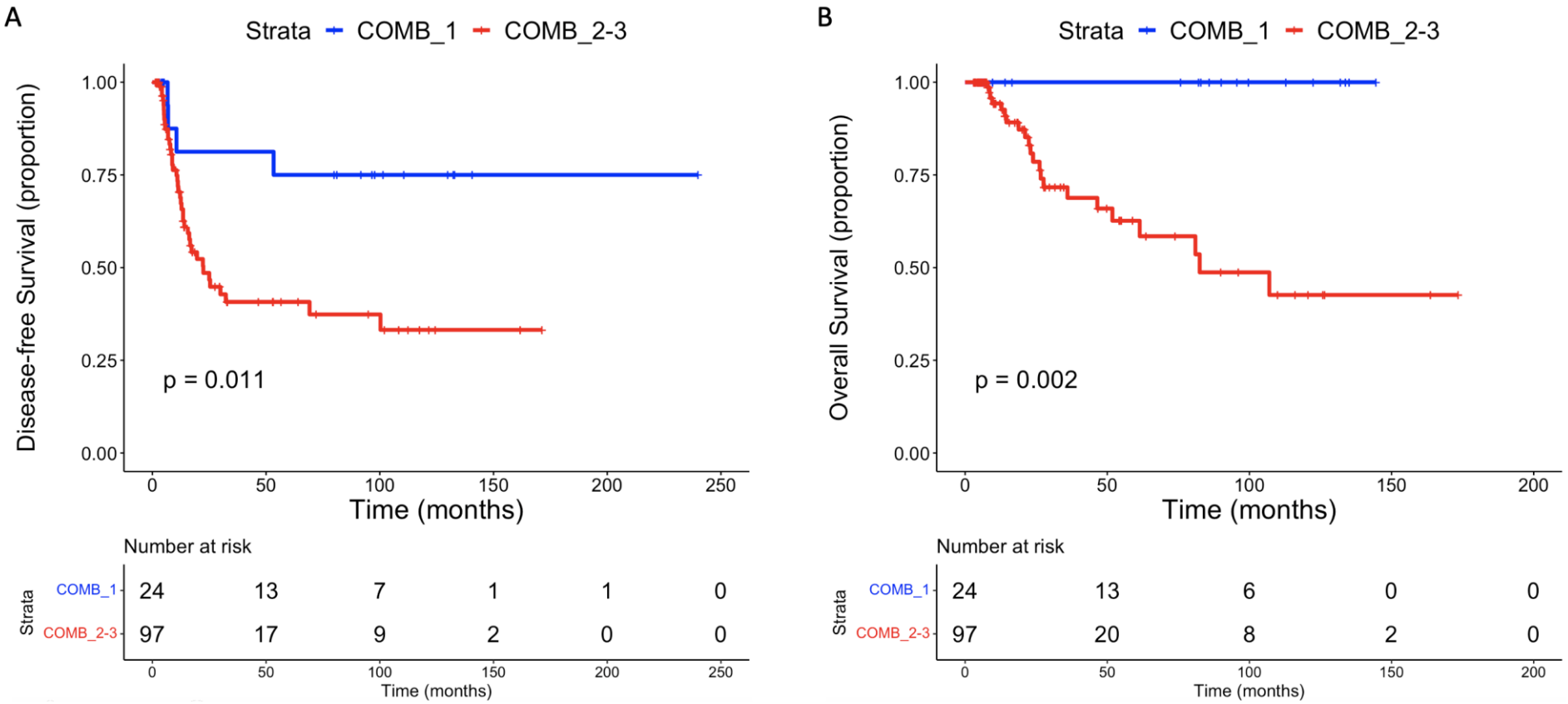
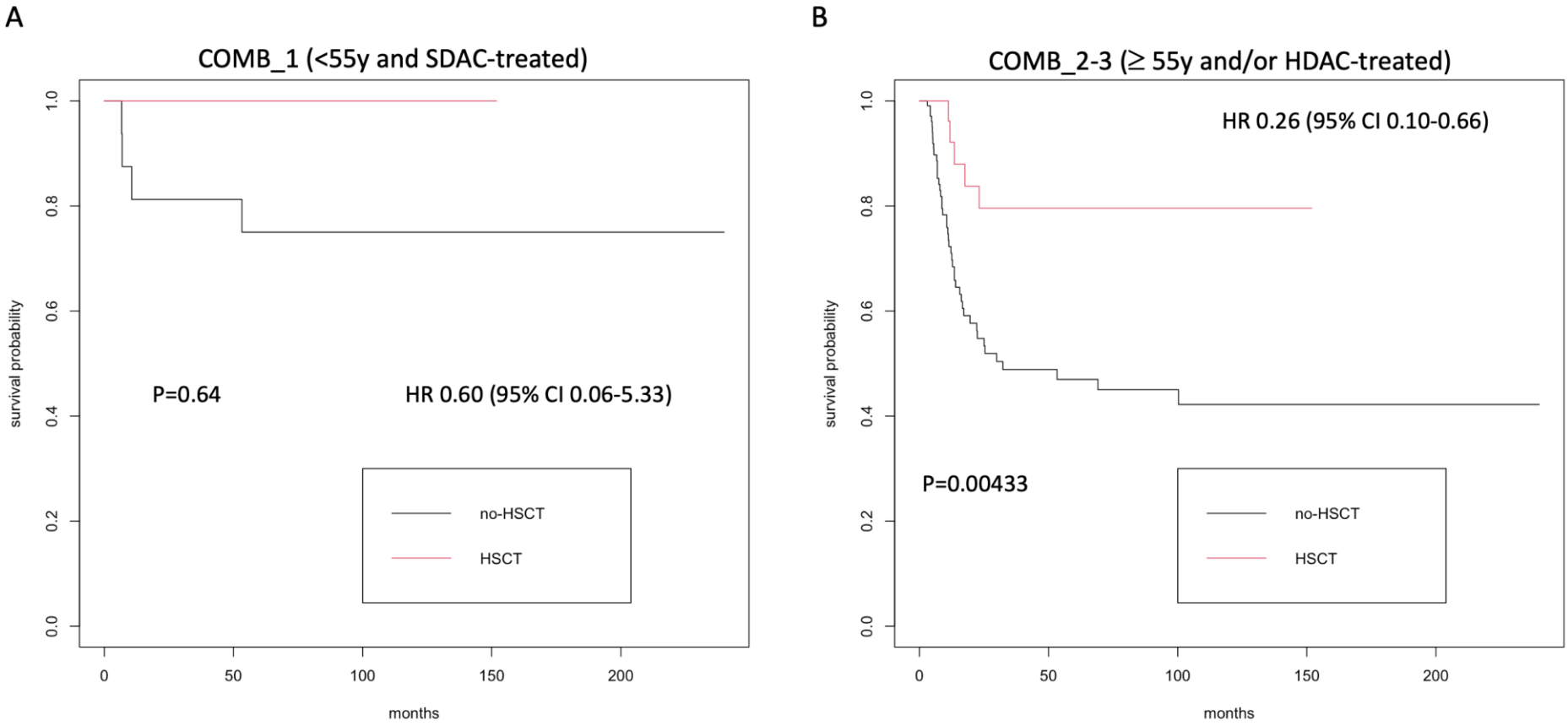


Figure S16. Effect of allogeneic HSCT on disease-free survival as depicted by Simon-Makuch plots in younger, SDAC-treated (A) and elderly and/or HDAC-treated (B) patients.



Supplemental Tables and Results

Table S1. Analysis of literature: summary of the selected clinical trials

Reference	Pts	Subset	Median age (range)	Method	Threshold for MRD status definition	Time-point
Balsat 2017	152	*NPM1*-mut	49 (21-61)	RT-PCR	4-log reduction	Post induction
Bataller 2021	110	*NPM1*-mut	54 (18-71)	RT-PCR	Any positivity; ratio on *ABL*1	Post induction; post consolidation
Boddu 2018	104	*CBF*-AML	53-19-81	RT-PCR	Slope of log-reduction	Post induction
Buccisano 2010	143	Unselected	NA (72% <60 y)	MFC	0.035% of total cells	Post consolidation
Chou 2010	55	*FLT3*-ITD	49 (17-90)	RT-PCR	3-log reduction	Post consolidation
Corbacioglu 2010	84	*CBF::MYH11*	NA (16-60)	RT-PCR	Transcript copies reduction; any positivity	Post consolidation
Ferret 2018	103	*IDH1/2*-mut	54 (22-70)	ddPCR	Detection limit 0.2%	Post induction
Frairia 2017	223	Unselected	56 (19-76)	RT-PCR	*WT1*; 2-log reduction	Post induction
Freeman 2018	286	Unselected	50 (16-71)	MFC	Variable between 0.05-0.2% based on controls	Post induction; post consolidation
Gueieze 2010	59	*CBF::MYH11*	36 (4-77)	RT-PCR	3-log reduction at MRD2; 0.001% at MRD3	Post consolidation
Hubmann 2014	158	*NPM1*-mut	57 (18-80)	RT-PCR	0.01%; 3-log reduction	Post induction; post consolidation
Ivey 2016	346	*NPM1*-mut	50 (6-68)	RT-PCR	Any positivity	Post consolidation
Jongen-Lavrencic 2018	430	Unselected	51 (18-66)	NGS	Any positivity	Post consolidation

Jourdan 2013	198	*CBF*-AML	42 (18-60)	RT-PCR	3-log reduction	Post consolidation
Kapp-Schwoerer 2020	469	*NPM1*-mut	58 (20-78)	RT-PCR	Any positivity	Post induction
Klco 2015	68	Unselected	50 (39-58)	NGS	VAF 2.5%	Post induction
Kronke 2011	245	*NPM1*-mut	49 (19-61)	RT-PCR	Any positivity	Post consolidation
Marani 2013	42	Unselected	54 (17-81)	RT-PCR	*WT1*; 1.5-log reduction	Post induction
Morita 2018	131	Unselected	51 (NA)	NGS	VAF strata	Post induction
Narimatsu 2008	46	*RUNX1::RUNXT1*	50 (25-64)	RT-PCR	1000 copies	Post consolidation
Onecha 2019	63	*NPM1 - IDH1/2-FLT3*-mut	54 (NA)	NGS, RT-PCR, MFC	Dependent on method and time-point	Post induction; post consolidation
Othus 2016	170	Unselected	NA (18-60)	MFC	0.01% of total cells	Post induction
Ravandi 2017	186	Unselected	51 (17-79)	MFC	0.1-0.01% of total cells	Post induction; post consolidation
Rossi 2014	45	Unselected	53 (19-76)	MFC, RT-PCR	Dependent on method; log-reduction and clearance	Post induction; post consolidation
Shayegi 2013	92	*NPM1*-mut	51 (20-79)	RT-PCR	Strata (negative, 0.1-1%, >1%)	Post induction; post consolidation
Terwjin 2012	77	Unselected	NA (NA)	MFC	Dependent on time-point	Post induction; post consolidation
Terwjin 2013	164	Unselected	48 (18-60)	MFC	Dependent on time-point	Post induction; post consolidation
Willekens 2016	94	*RUNX1::RUNXT1*	41 (18-60)	RT-PCR	Ratio on *ABL1* >0.001%	Post consolidation
Yin 2012	278	*CBF*-AML	42 (15-70)	RT-PCR	Log-reduction for *RUNX1::RUNXT1;* number of copies for *CBF::MYH11*	Post induction; post consolidation

Yoon 2014	206	*CBF*-AML	39 (18-89)	RT-PCR	3 log-reduction at MRD1; number of copies at MRD2	Post induction; post consolidation
Zeijlemaker 2015	114	Unselected	59 (25-73)	MFC	Dependent on LAIP and sample type	Post induction; post consolidation
Zhang 2013	52	*RUNX1::RUNXT1*	21 (13-57)	RT-PCR	Ratio on *ABL1* >0.01%	Post consolidation
Wei 2021	187	*RUNX1::RUNXT1*	34 (14-54)	RT-PCR	3 log-reduction	Post consolidation

Table S2. Analysis of literature: treatment details of the selected clinical trials.

Reference	**Induction**					**Consolidation**		
	Trial	**Scheme**	**ARA-C cumulative dose, mg**	**Anthracycline, cumulative dose, mg**	**Third drug, cumulative dose, mg**	**ARA-C dose, mg**	**Anthracycline, dose, mg**	**Third drug, dose, mg**
Balsat 2017	ALFA 07-02	-	7.500	Daunorubicin 285	-	18.000	-	-
Bataller 2021	-	-	1.400	Idarubicin 36	-	18.000	-	-
Boddu 2018	-	-	10.000	Idarubicin 36	Gemtuzumab 3	8.000	-	Gemtuzumab 3
			8.000	Idarubicin 36	-	10.000	-	-
Buccisano 2010	AML-10	DAE	1.000	Daunorubicin 150	Etoposide 500	6.000	Daunorubicin 150	-
	AML-10	ICE	1.000	Idarubicin 30	-	6.000	Idarubicin 30	-
	AML-10	MiCE	1.000	Mitoxantrone 36	-	6.000	Mitoxantrone 36	-
	AML-12	HDAC	24.000	Daunorubicin 150	Etoposide 250	6.000	Daunorubicin 150	-
	AML-13	MiCE	700	Mitoxantrone 21	Etoposide 250	500	Idarubicin 24	-
	AML-17	MiCE	700	Mitoxantrone 21	Etoposide 300	500	Idarubicin 24	Etoposide 300
Chou 2010	-	3+7	700	Idarubicin 36	-	16.000	-	-

Corbacioglu 2010	AML HD93	Double induction (ICE-ICE)	1.400	Idarubicin 72	Etoposide 600	18.000	Mitoxantrone 36	-
	AML HD98A	Double induction (ICE-ICE)	1.400	Idarubicin 72	Etoposide 600	18.000	Mitoxantrone 36	-
	AMLSG 07-04	Double induction (ICE-ICE)	1.400	Idarubicin 72	Etoposide 600	18.000	Mitoxantrone 36	-
Ferret 2018	ALFA 07-01	3+7	1.400	Daunorubicin 180	-	8.000	Daunorubicin 60	-
	ALFA 07-01	3+7+GO	1.400	Daunorubicin 180	Gentuzumab 9	8.000	Daunorubicin 60	Gentuzumab 3
	ALFA 07-02	-	7.500	Daunorubicin 285	-	18.000	-	-
Frairia 2017	NILG 02-06	ICE	1.400	Idarubicin 36	Etoposide 800	1.400	Idarubicin 36	-
	-	Ida-FLA	4.000	Idarubicin 24	Fludarabine 100	4.000	Idarubicin 24	Fludarabine 100
Freeman 2018	MRC AML-17	DA	2.000	Daunorubicin 150	Gemtuzumab 3-6	2.000	Daunorubicin 150	-
	MRC AML-17	ADE	2.000	Daunorubicin 150	Etoposide 500 Gemtuzumab 3-6	2.000	Daunorubicin 150	-
Gueieze 2010	ALFA	3+7	1.400	Daunorubicin 180	-	18.000	-	-

Hubmann 2014	AMLCG	Double induction (TAD-HAM)	19.400	Daunorubicin 180 Mitoxantrone 30	Thioguanine 1.400	1.400	Daunorubicin 180	Thioguanine 1.400
		Double induction (HAM-HAM)	36.000	Mitoxantrone 60	Thioguanine 1.400	1.400	Daunorubicin 180	Thioguanine 1.400
		Sequential induction (S-HAM)	24.000	Mitoxantrone 40	Thioguanine 1.400	1.400	Daunorubicin 180	Thioguanine 1.400
Ivey 2016	MRC AML-17	DA	2.000	Daunorubicin 150	Gemtuzumab 3-6	2.000	Daunorubicin 150	-
	MRC AML-17	ADE	2.000	Daunorubicin 150	Etoposide 500 Gemtuzumab 3-6	2.000	Daunorubicin 150	-
Jongen-Lavrencic 2018	HOVON 42	-	1.400	Idarubicin 36	-	12.000	-	Amsacrine 360
	HOVON 42	-	10.000	Idarubicin 36	-	16.000	-	Amsacrine 360
	HOVON 102	-	1.400	Idarubicin 36	-	12.000	-	Amsacrine 360
	HOVON 102	-	1.400	Idarubicin 36	Clofarabine 50	12.000	-	Amsacrine 360 Clofarabine 50
Jourdan 2013	CBF 2006	3+7	1.400	Daunorubicin 180	-	18.000	-	-
	CBF 2006	-	7.500	Daunorubicin 285	-	18.000	-	-

Kapp-Schwoerer 2020	AMLSG 09-09	3+7	1.400	Idarubicin 36	Etoposide 500 Gentuzumab 3	1.400	Idarubicin 36	Etoposide 500 Gentuzumab 36
Klco 2015	-	3+7	1.400	Daunorubicin 180	-	-	-	-
Kronke 2011	AML HD98A	Double induction (ICE-ICE)	1.400	Idarubicin 72	Etoposide 600	18.000	Mitoxantrone 36	-
	AMLSG 07-04	Double induction (ICE-ICE)	1.400	Idarubicin 72	Etoposide 600	18.000	Mitoxantrone 36	-
Marani 2013	-	FLAI5	10.000	Idarubicin 36	Fludarabine 150	-	-	-
Morita 2018	-	FIA	5.000	Idarubicin 30	Fludarabine 150	-	-	-
	-	CIA	5.000	Idarubicin 30	Clofarabine 100	-	-	-
	-	CLIA	5.000	Idarubicin 30	Cladribine 25	-	-	-
Narimatsu 2008	-	3+7	700	Idarubicin 36	-	18.000	-	-
	-	3+7	700	Idarubicin 36	-	1.400	-	-
Onecha 2019	-	3+7	700	Idarubicin 36	-	18.000	-	-
Othus 2016	SWOG 0106	-	700	Daunorubicin 135	-	-	-	-
	SWOG 0106	-	700	Daunorubicin 135	Gemtuzumab 6	-	-	-
Ravandi 2017	-	CIA	5.000	Idarubicin 30	Clofarabine 100	4.000	Idarubicin 20	
	-	FIA	5.000	Idarubicin 30	Fludarabine 150	4.000	Idarubicin 20	Fludarabine 60

	-	FLAG-Ida	10.000	Idarubicin 30	Fludarabine 150	8.000	Idarubicin 20	Fludarabine 60
	-	CLIA	5.000	Idarubicin 30	Cladribine 25	4.000	Idarubicin 20	-
Rossi 2014	AML 12	-	24.000	Daunorubicin 150	Etoposide 250	6.000	Daunorubicin 150	-
	-	FLAG	10.000	Daunorubicin 30	Fludarabine 100	6.000	Daunorubicin 150	-
Shayegi 2013	SAL	Double induction	2.800	Daunorubicin 360	-	-	-	-
Terwjin 2012	HOVON 42	-	1.400	Idarubicin 36	-	12.000	-	Amsacrine 360
	HOVON 42a	-	10.000	Idarubicin 36	-	16.000	-	Amsacrine 360
	HOVON 29	-	1.400	Idarubicin 36	-	12.000	-	Amsacrine 360
Terwjin 2013	HOVON 42a	-	10.000	Idarubicin 36	-	16.000	-	Amsacrine 360
Willekens 2016	CBF 2006	3+7	1.400	Daunorubicin 180	-	18.000	-	-
	CBF 2006	-	7.500	Daunorubicin 285	-	18.000	-	-
Yin 2012	MRC AML-15	DA	2.000	Daunorubicin 150	Gemtuzumab 3	-	-	-
	MRC AML-15	ADE	2.000	Daunorubicin 150	Etoposide 500 Gemtuzumab 3	-	-	-
	MRC AML-15	FLAG-Ida	10.000	Idarubicin 24	Gemtuzumab 3 Fludarabine 150	-	-	-
Yoon 2014	-	3+7	1.400	Idarubicin 36	-	12.000	-	-

Zeijlemaker 2015	HOVON	3+7	1.400	Idarubicin 36	-	12.000	-	-
Zhang 2013	-	3+7	700	Idarubicin 36	-	12.000	-	-
	-	IDAC	4.400	Idarubicin 36	-	12.000	-	-
Wei 2021	-	3+7	700	Daunorubicin 180	-	9.000	-	-
	-	IDAC	6.440	Daunorubicin 180	-	18.000	-	-

Table S3. Characteristics of patients according to treatment group after induction cycle.

	Overall n = 194	**SDAC n = 149 (66.5%)**	**HDAC n = 45 (63.9%)**	***P* value**	
Age, median (range)	55 (16-73)	57 (16-73)	47 (19-61)	**<0.0001**	
WBC, $x10^9$/L, median (range)	0.6 (14.8-435)	17.8 (0.6-435.0)	11.9 (1.1-289)	0.70	
Hb, g/dL, median (range)	9.2 (3.9-14.9)	9.1 (3.9-14.9)	9.2 (4.2-12.8)	0.25	
Plt, $x10^9$/L, median (range)	53 (1-281)	53 (10-281)	44 (1-216)	0.36	
Peripheral blasts, %, median (range)	53 (0-100)	52 (0-100)	53 (0.98)	0.86	
Secondary AML, n (%)	12 (6.2%)	12 (8.0)	0 (-)		
Karyotype, n (%)					
Favorable	33 (17.0)	23 (15.4)	10 (22.2)	0.36	0.29
Normal	110 (56.7)	87 (58.4)	23 (51.1)	0.40	
Intermediate, non-normal	26 (13.4)	18 (12.1)	8 (17.8)	0.33	
Adverse	15 (7.7)	11 (7.4)	4 (8.9)	0.75	
Lack of growth	10 (5.2)	10 (6.7)	0 (-)	0.12	
Molecular genetics, n (%)					
NPM1-mutated	80 (41.2)	66 (44.3)	14 (31.1)	0.12	
FLT3-ITD	41 (21.1)	36 (24.2)	5 (11.1)	0.06	
CEBPA-bZIP	10 (5.2)	7 (4.7)	3 (6.7)	0.70	
ELN 2010 risk groups, n (%)					
Favorable	90 (46.4)	69 (46.3)	21 (46.7)	1.0	0.76
Intermediate-1	80 (41.2)	63 (42.3)	17 (37.8)	0.61	
Intermediate-2	8 (4.1)	5 (3.4)	3 (6.7)	0.39	
Adverse	16 (8.2)	12 (8.1)	4 (8.9)	0.77	
ELN 2017 risk group, n (%)					
Favorable	102 (52.5)	76 (51.0)	26 (57.8)	0.49	0.74
Intermediate	62 (32.0)	49 (32.9)	13 (28.9)	0.72	
Adverse	24 (12.4)	18 (12.1)	6 (13.3)	0.80	
Not assessable	6 (3.1)	6 (4.0)	0 (-)	0.33	