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Impact of Minimal Residual Disease on the Outcome of Hematopoietic Stem Cell Transplantation for Childhood Acute Lymphoblastic Leukemia within the FORUM Trial

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The FORUM trial (Full title: ALL SCTped 2012 FORUM - Allogeneic Stem Cell Transplantation in Children and Adolescents with Acute Lymphoblastic Leukaemia; EudraCT: 2012-003032-22; EU CT: 2024-512657-24-00; ClinicalTrials.gov: NCT01949129) was sponsored by the St. Anna Children's Research Institute.

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ABSTRACT

In the randomized cohort of the international phase-III FORUM trial, which showed the superiority of total-body irradiation (TBI) over chemotherapy-based conditioning prior to hematopoietic stem cell transplantation (HSCT) for pediatric acute lymphoblastic leukemia (ALL), type of conditioning and remission phase, but not pre-HSCT minimal residual disease (MRD), were associated with outcome. We report the impact of MRD within the extended FORUM cohort.

Patients (n=1014), 4–21 years old, transplanted from a matched donor who had ≥1 MRD measurement prior to and/or 100 days and/or 1 year after HSCT were eligible. A threshold of 0.01% defined MRD positivity versus negativity.

Prior to HSCT, 21% of patients were MRD_{pos}. Three-year event-free survival (EFS) was 0.73 and 0.59 ($p<0.001$), and 3-year cumulative incidence of relapse (CIR) was 0.20 and 0.33 ($p<0.001$) in MRD_{neg} and MRD_{pos} patients, respectively. The level of MRD positivity pre-HSCT (<0.1% versus ≥0.1%), did not significantly affect outcome. Pre-HSCT MRD_{neg} and TBI/etoposide conditioning were associated with a 2-fold lower risk of relapse, whereas MRD_{pos} had a 2-fold higher risk of any failure and/or death. No detrimental effect of MRD_{pos} pre-HSCT could be demonstrated in patients with T-cell ALL. MRD_{pos} versus MRD_{neg} patients at day 100 had an EFS of 0.47 versus 0.77 ($p<0.001$) and a CIR of 0.51 versus 0.17 ($p<0.001$), respectively, but post-HSCT MRD_{pos} did not necessarily imply relapse. In conclusion, the MRD status pre-HSCT and at day 100 post-HSCT was a strong prognostic factor for children transplanted for ALL in the extended FORUM cohort.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is considered beneficial in first complete remission (CR1) for approximately 5% of children, adolescents and young adults (CAYA) with very-high-risk acute lymphoblastic leukemia (ALL) and for the majority experiencing disease recurrence.[1-6] ALL relapse is the primary cause of treatment failure after HSCT, since transplant-related mortality (TRM) in CAYA has been significantly reduced to <10%, mainly due to refined HLA-typing, improved graft-versus-host disease (GVHD) management and better infection prophylaxis and treatment.[6-9]

The prognostic role of minimal residual disease (MRD) for patients with acute leukemia has been consistently demonstrated in the HSCT setting across studies of multiple cooperative groups or single institutions.[3,6,10-14]

The randomized, open-label, international, multicenter, phase III trial ALL SCTped 2012 FORUM - Allogeneic Stem Cell Transplantation in Children and Adolescents with Acute Lymphoblastic Leukaemia - was designed to test for the non-inferiority of chemotherapy-based regimens versus total body irradiation plus etoposide (TBI/VP16) in patients ≥4 years and <21 years old at time of HSCT with an indication for transplantation for ALL in morphological CR from a compatible donor. A futility stopping rule was breached and random assignment halted because patients receiving chemo-conditioning with fludarabine, thiotepa, and either busulfan or treosulfan had inferior overall survival (OS) compared with those who received TBI/VP16, due to a higher cumulative incidence of relapse (CIR) (0.33; 95% confidence interval 0.25–0.40 versus 0.12; 95% confidence interval 0.08–0.17, $p<0.0001$).[15] In the randomized cohort, conditioning and remission phase were the only variables associated with risk of relapse, whereas - unexpectedly - no association between pre-HSCT MRD and CIR or event-free survival (EFS) was detected.[15]

The primary aim of this analysis was to assess the impact of MRD status before and after HSCT in the extended FORUM cohort, which included patients enrolled prior to and after randomization was stopped.

METHODS

Eligibility criteria

Patients ≥4 years old in the FORUM trial (EudraCT: 2012-003032-22; EU CT: 2024-512657-24-00; ClinicalTrials.gov: NCT01949129) enrolled between April 2013 and February 1, 2024, and transplanted from an HLA-identical sibling or a 10/10 or a 9/10 HLA-matched related or unrelated donor were eligible, as long as they had ≥1 MRD measurement either prior to HSCT and/or 100 days after and/or 1 year after HSCT (±45 days), assessed by polymerase chain reaction (PCR) or flow cytometry in the bone marrow, recorded in the study database (Marvin).

The cohort included patients from 97 centers in 21 countries where the randomization took place (randomizing countries) and 8 countries in which the randomization was not planned that could provide MRD data. Within the randomizing countries, non-randomized patients were also included (i.e., those considered ineligible for randomization due to patient-related factors or family/physician's decision, or because they were enrolled after March 2019, when the randomization was stopped). Thus, the cohort of this analysis comprises a much broader population of patients than the previously reported FORUM analysis.[15]

Transplant procedure

Details regarding transplant procedures were previously published[15] and are outlined in the Supplementary Material (including conditioning regimens – Figure S1).

Changes in immunosuppression or administration of donor lymphocyte infusion (DLI) due to MRD levels were beyond the purpose of this investigation.

MRD analysis

MRD status reported within the 45 days prior to HSCT and at days +100 and day +365 (±45 days) was analyzed (see Supplementary Materials). A threshold of $1x10^{-4}$ was selected to stratify patients according to their MRD level. Patients with undetectable MRD, detectable but unquantifiable MRD, and those who were MRD positive but $<1x10^{-4}$ by PCR or <0.01% by flow cytometry were defined as MRD_{neg}, whereas patients with positive MRD $\geq 1x10^{-4}$ by PCR or ≥0.01% by flow cytometry were defined as MRD_{pos}, consistent with previous reports.[3,13,16] For additional sub-analyses, patients with positive MRD were further divided in subgroups of patients with MRD $\geq 1x10^{-4}$ but $<1x10^{-3}$ ($MRD_{pos\text{-}low}$), and $\geq 1x10^{-3}$ ($MRD_{pos\text{-}high}$).

The Protocol and statistical analysis plan were approved by the Vienna Investigational Review Board (IRB) and Ethics Committee (EC), besides IRB and EC for each center. The trial was performed in accordance with the Declaration of Helsinki principles. Informed consents were obtained from each participant or each participant's guardian.

Statistical methods are detailed in the specific Supplemental paragraph.

RESULTS

Enrollment

Of 1619 patients enrolled up to February 1, 2024, the first was transplanted in April 2013 and the last in November 2023. Of 1247 patients 4 years or older and transplanted from a compatible donor, 1014 (81%) met the inclusion criteria regarding MRD data (MRD levels were recorded for at least one timepoint, namely for 852 before HSCT, 714 at 100 days and 504 at 1 year after HSCT), as shown in the Consort diagram (Figure 1). Of 2070 MRD assessments, 1385 (67%) were by PCR and 685 (33%) by flow cytometry.

Patient outcomes were last updated on January 1, 2024, resulting in a median follow-up of 3.0 years (range, 0.03–9.35).

Patient characteristics are listed in Table S1. In brief, median age was 10.5 years (1st and 3rd interquartile, 7.7 and 14.1 years). The conditioning regimen consisted of TBI/VP16 in 719 (71%) and chemo-conditioning in 295 (29%) patients (busulfan-based, 16%; treosulfan-based 13%). ALL immunophenotype was B lineage in 76% and T in 23%. In terms of disease risk, 47% were transplanted in CR1, 45% in CR2, and 7% ≥CR3; 29% were higher risk (see Supplementary Material). Grafts were from MD in 71% and from MSD in 29%; 71% received BM stem cells.

MRD status pre-HSCT

Patient distribution: Of the 852 patients with pre-HSCT MRD status reported, 79% were MRD_{neg} and 21% were MRD_{pos}. As detailed in Table 1, the proportion of MRD_{pos} patients was higher among recipients conditioned with TBI/VP16 (81%) or busulfan-based chemo-conditioning (82%) versus treosulfan-based chemo-conditioning (65%).

Engraftment: 832 of 852 patients with pre-HSCT MRD status achieved myeloid engraftment (absolute neutrophil count $>0.5x10^9$/L) at median 21 days after HSCT, 821 achieved platelet engraftment (unsupported platelet count $>50x10^9$/L) at median 32 days after HSCT, and 20 patients did not achieve myeloid engraftment by day 100. Cumulative incidence of myeloid and platelet engraftment at 30 days was 0.93 (SE, 0.01) and 0.86 (SE, 0.01), respectively. Engraftment kinetics did not differ by MRD status pre-HSCT (Figure S2).

Outcomes: 3-year OS was 0.82 (SE, 0.02) and 0.71 (SE, 0.04) for the MRD_{neg} and MRD_{pos} patients pre-HSCT, respectively (p=0.003), and 3-year EFS was 0.73 (SE, 0.02) and 0.59 (SE, 0.04), respectively (p<0.001) (Figure 2, panels A and B; Table S2).

Three-year CIR was 0.20 (SE, 0.02) and 0.33 (SE, 0.04) for the pre-HSCT MRD_{neg} and MRD_{pos} groups, respectively (p<0.001), whereas 3-year TRM was 0.06 (SE, 0.01) and 0.07 (SE, 0.02), respectively, (p=0.53) (Figure 2, panels C and D; Table S2).

According to disease risk profile, 3-year CIR was 0.17 (SE, 0.02) and 0.27 (SE, 0.04) for the lower risk patients who had pre-HSCT MRD_{neg} and MRD_{pos}, respectively (p=0.020), and 0.26 (SE, 0.03) and 0.57 (SE, 0.09) for the higher risk patients who had pre-HSCT MRD_{neg} and MRD_{pos}, respectively (p=0.036) (Table S2).

Differentiated outcomes for MRD positive patients according to level of positivity are detailed in Figure S4 and no differences could be detected between pre-HSCT $MRD_{pos-low}$ and $MRD_{pos-high}$ patients, respectively.

Detailed outcomes for pre-HSCT MRD_{neg} and MRD_{pos} patients receiving TBI/VP16 versus those receiving chemo-conditioning were shown in Figure 2, panels G–H and Supplemental Table S2.

For patients with B-ALL, 3-year EFS was 0.73 (SE, 0.02) and 0.54 (SE, 0.05) (p<0.001) and 3-year CIR was 0.20 (SE, 0.02) and 0.38 (SE, 0.05) (p <0.001), for the pre-HSCT MRD_{neg} and MRD_{pos} groups, respectively. For patients with T-ALL, 3-year EFS was 0.75 (SE, 0.04) and 0.73 (SE, 0.07) (p=0.747) and 3-year CIR was 0.18 (SE, 0.03) and 0.21 (SE, 0.07) (p=0.796) for pre-HSCT MRD_{neg} and MRD_{pos} groups, respectively (Figure 3; Table S2). The test for interaction between immunophenotype and MRD for the purpose of EFS was not significant (p=0.349).

The impact of MRD within B- and T-lineage ALL, according to disease risk profile and conditioning regimen, is shown in Table 3.

Outcome results by univariable analyses are summarized in Table S2.

Acute and chronic GVHD: acute GVHD (aGVHD) grade and incidence overall and by pre-HSCT MRD status and by conditioning regimen were reported for 799 patients (94%) with MRD status reported pre-HSCT. The test for interaction between aGVHD and MRD for the purpose of EFS was not significant (p=0.914).

Cumulative incidence (CI) of chronic GVHD (cGVHD) was 0.12 (SE, 0.01) for MRD_{neg} and 0.20 (SE, 0.03) for MRD_{pos} patients pre-HSCT (p= 0.017) (Figure S3,

Panel A). The CI of limited and extensive cGVHD were also reported in Figure S3, panels B and C.

In patients with MRD status pre-HSCT, 3-year EFS was 0.74 (SE, 0.02), 0.82 (SE, 0.03) and 0.61 (SE, 0.06) for those with no or grade I aGVHD, grade II aGVHD, or grade III–IV aGVHD, respectively (p=0.027). Three-year CIR was 0.23 (SE, 0.02), 0.15 (SE, 0.03) and 0.21 (SE, 0.06) in patients with no or grade I aGVHD, grade II aGVHD or grade III–IV aGVHD, respectively (p=0.105). Three-year TRM was 0.03 (SE, 0.01), 0.02 (SE, 0.01) and 0.14 (SE, 0.05) in patients with no or grade I aGVHD, grade II aGVHD or grade III–IV aGVHD, respectively (p=0.002) (Figure S5).

Multivariable analyses: the hazard ratio (HR) of relapse in the pre-HSCT MRD_{pos} versus MRD_{neg} groups was 2.039 (p<0.0001) in the multivariable analysis, adjusting for type of donor (MD versus MSD, not significant [n.s.]), remission phase and risk profile (higher risk versus lower risk, HR 2.209, p<0.0001), type of conditioning (chemo-conditioning versus TBI/VP16, HR 2.291, p<0.0001), age (>10 years versus ≤10 years, n.s.), and immunophenotype (B-lineage versus others, n.s.). Results of multivariable analyses are reported in Table 4, in which the HR of failure from any cause (1-EFS), associated with MRD status, risk profile and type of conditioning, and the HR of death (1-OS) were also shown.

The HR of non-leukemic death was not significantly associated with pre-HSCT MRD status or conditioning regimen, but was associated with remission phase (higher versus lower risk, HR 2.026, p=0.035) and older age (>10 years versus ≤10 year, HR 2.793, p=0.002), and there was a trend for an association with donor type (MD versus MSD, HR 2.041, p=0.059).

In the model accounting for aGvHD and cGvHD, as time-dependent covariates, the occurrence of aGVHD grade II compared with grade 0 (absent) or I, was associated with a lower risk of death from any cause (HR 0. 587, p=0.031), of any failure (HR 0.613, p=0.010), and of relapse (HR 0.574, p=0.011). The occurrence of grade III–IV,compared with grade 0–I aGVHD, was associated with a higher risk of death from

any cause (HR 1. 741, p= 0.026, and of TRM (HR 3.543, p=0.001) (Table 4). Extensive cGvHD was associated with a significantly higher risk of TRM (HR 4.913, p-value 0.001).

MRD status after HSCT

Of the 714 patients alive and in continuous morphological CR who were assessed for MRD 100 days after HSCT, 92% were MRD_{neg} and 8% MRD_{pos}.

Three-year OS was 0.88 (SE, 0.01) for MRD_{neg} and 0.63 (SE, 0.07) for MRD_{pos} 100 days after HSCT ($p<0.001$), whereas 3-year EFS was 0.77 (SE, 0.02) and 0.47 (SE, 0.07) ($p<0.001$), respectively (Figure S6, panels A and B). Three-year CIR was 0.17 (SE, 0.02) and 0.51 (SE, 0.07) for MRD_{neg} and MRD_{pos} 100 days after HSCT, respectively ($p<0.001$), whereas 3-year TRM was 0.04 (SE, 0.01) and 0.02 (SE, 0.02), respectively (p=0.370) (Figure S6).

Similarly, in Figure S7, the outcome of the 504 patients alive and in continuous CR whose MRD status was assessed 1 year after HSCT was shown, with 95% being MRD_{neg} and 5% MRD_{pos}.

DISCUSSION

This study demonstrated the impact of MRD pre-HSCT on outcome in the extended FORUM cohort of CAYA with ALL in CR aged 4–21 years receiving an HSCT from a compatible donor after myeloablative conditioning. According to previous reports,[3,13,16] the MRD_{neg} group included patients with MRD positivity that was either unquantifiable or $<1x10^{-4}$.

The majority of the patients (79%) were MRD_{neg} pre-HSCT, alias negative/non-quantifiable/$<1x10^{-4}$, as assessed either by PCR or flow cytometry. MRD status pre-HSCT was highly predictive of outcome. The finding of higher 3-year EFS (0.73 versus

0.59; p<0.001) and OS (0.82 versus 0.71; p=0.003) and lower CIR (0.20 versus 0.33; p <0.001) in MRD_{neg} versus MRD_{pos} patients is consistent with previous reports in CAYA patients.[3,6,10-14]

Multiple reasons may explain the discrepancy between the present analysis and the randomized FORUM cohort published in 2021, in which no significant association between MRD pre-HSCT and outcome was detected.[15] Namely: i) the sample size of the current analysis is greater (852 versus 336 patients); ii) a different cut-off was used to define MRD positivity by flow cytometry (0.01%, consistent with $1x10^{-4}$ used in PCR analyses, instead of 0.1%); and iii) median follow-up was longer (3.0 versus 2.1 years).[15] The proportion of MRD_{neg} patients in the current analysis was 79% versus 57% on the previous analysis, which may reflect better disease control pre-HSCT, although the proportion of patients who had received further intensified treatment is unknown.

The prognostic value of MRD status did not differ by method of determination (PCR versus flow cytometry, data not shown).

The level of MRD positivity pre-HSCT, i.e. $MRD_{pos\text{-}low}$ $<1x10^{-3}$ and $MRD_{pos\text{-}high}$ $\geq 1x10^{-3}$, was not relevant to outcome, in particular did not significantly affect EFS (0.60 versus 0.58, p=0.780) and CIR (0.31 versus 0.37, p=0.468), even after stratification by conditioning regimen.

In the multivariable analysis, MRD negativity pre-HSCT, conditioning with TBI/VP16, and lower risk profile (CR1 and CR2 after late relapse) were associated with approximately half the risk of relapse, whereas MRD positivity pre-HSCT was associated with twice the risk of any failure (1-EFS) and death (1-OS). Observations regarding the conditioning regimen should be interpreted with a *caveat*, due to the low rate of randomization (32%); non randomized patients were allocated a regimen based on physician preference. Prior to the stopping rule being breached in March 2019, regimen assignment might have been based on disease risk (with patients with more refractory disease more likely to receive TBI/VP16 and patients with comorbidities more likely to receive chemo-conditioning). After this, the vast majority of the patients

were conditioned with TBI/VP16. Furthermore, no differences in outcome were detected between the randomized and the non-randomized patients allocated TBI/VP16.

The disparity in the outcome between MRD_{pos} and MRD_{neg} patients pre-HSCT was apparently greater among recipients receiving chemo-conditioning versus TBI/VP16, suggesting that TBI/VP16 could partially compensate for poorer disease control, thus rescuing some MRD_{pos} patients. In fact, the 3-year CIR of 0.26 in MRD_{pos} patients given TBI/VP16 is remarkable, suggesting that TBI/VP16 can be effective in CAYA patients with MRD_{pos}. Such estimate compares favorably with a 2-year CIR of 0.54 (±0.14), reported in a recent pediatric series conditioned without TBI in The Netherlands.[17]

TRM in our overall cohort was 0.06, similar across MRD groups, which is remarkably low in the context of previous reports, especially given the international setting. Besides a trend for higher mortality in MD versus MSD recipients, as expected, older age (>10 years) was associated with an almost 3-fold higher risk of TRM compared with younger age.

Despite immunophenotype was not associated with outcome, the impact of the MRD status pre-HSCT was striking for B-cell precursor ALL, whereas it was virtually absent for patients with T-ALL, in whom EFS, OS, and CIR were superimposable for MRD_{neg} and MRD_{pos} patients, suggesting that the graft-versus-leukemia effect may be stronger in T-ALL than B-cell precursor ALL. Based on these findings, intensifying treatment and postponing transplant in patients with T-ALL who are MRD_{pos} pre-HSCT, in the attempt to reduce MRD levels, might be unwarranted.

Furthermore, the relatively large gap between OS and EFS in B-lineage ALL, both overall (0.81 versus 0.69) and according to pre-HSCT-MRD status (0.84 versus 0.77 in pre-HSCT MRD_{neg} patients; 0.70 versus 0.59 in pre-HSCT MRD_{pos} patients), reflects the availability of further treatments, such as immunotherapy, after post-HSCT relapse. In contrast, in T-lineage ALL, EFS is very similar to OS (0.77 versus 0.74), as very few chances would be available to treat a post-HSCT relapse, if not a subsequent HSCT,

possibly with the additional value of TBI, if not used previously, or the alloreactive effect of a partially incompatible donor.

The overall risk of aGVHD was low in our cohort (69% grade 0–I aGVHD, and 9% grade III–IV aGVHD). Cumulative incidence of overall cGVHD was higher in pre-HSCT MRD_{pos} compared with MRD_{neg} patients (0.20 versus 0.12; p=0.017), but extensive cGVHD was similar. AGVHD grade II versus 0–I was associated with lower risk of relapse (HR 0.57, p=0.008), whereas grade III–IV aGVHD was associated with a 4-fold higher risk of TRM. Nevertheless, there was no evidence of interactions between MRD and aGVHD; therefore, whether the occurrence of mild GVHD could have been protective against the risk of any failure with a different pattern in MRD_{pos} compared with MRD_{neg} patients could not be demonstrated. This finding is in line with those from pooled data of several consortia, as reported by Bader et al.,[13] but is in contrast to those of Pulsipher et al., who described that pre-HSCT MRDpos patients who developed no degree of GVHD had higher rates of relapse versus MRDpos patients with mild aGVHD.[18]

Positive MRD at day 100 post-HSCT, seen in 8% of the evaluable patients, was associated with dismal outcome, even though MRD detection did not necessarily imply relapse; MRD_{pos} at 100 days post-HSCT had an EFS of 0.47 and a CIR of 0.51 at 3 years, compared with 0.77 and 0.17 of MRD_{neg} patients, respectively.

At 1 year 5% of patients were MRD_{pos} and this resulted in overt relapse in approximately half. EFS in MRD_{pos} patients at 1-year post-HSCT was relatively high.

Intrinsic limitations of this study include that MRD analyses are standardized within most European countries, but not worldwide, and that the range of 45 days for the timing of MRD assessments may affect the pre-HSCT analyses more than analyses at 100 days or 1 year. Moreover, treatment prior to HSCT was performed according to local policies and was not accounted for in the study. However, no significant differences in outcome were observed among countries, even when accounting for center size (data not shown). Furthermore, MRD levels were not recorded for one third

of the FORUM cohort and assessment of the impact of interventions to manage MRD positivity (including early immunosuppression discontinuation, DLI, infusion of immune effector cells or second HSCT) was beyond the purpose of this study.

The high proportion of MRD_{neg} patients pre-HSCT (79%) provides only partial clues regarding the responsiveness or refractoriness of disease, as MRD recorded pre-HSCT may have been achieved by treatment per protocol or treatment intensification. Unfortunately, exploring the role of additional pre-HSCT chemo- or immune-therapy, to achieve deeper remission in patients who otherwise would have received HSCT with higher MRD levels, was beyond scope and could not be assessed. Moreover, the predictive value of pre-HSCT MRD achieved with immunotherapy versus conventional chemotherapy is still to be assessed.

Specific recommendations might be provided by a panel of experts involved in prospective studies dealing with HSCT in ALL. Based on our data, confirming the crucial role of the MRD status at HSCT on outcome, we conclude that a pre-HSCT MRD level <1x10-4 is recommended overall and, as demonstrated, in B-lineage ALL. Such a finding would suggest to intensify treatment and/or add immunotherapy to achieve MRD negativity prior to HSCT in patients who were still MRD positive prior to HSCT otherwise. Nevertheless, we were unable to determine whether achieving MRD negativity before HSCT was attributable to the disease response to standard treatment or was achieved by treatment intensification, possibly with immunotherapy. Whether a MRD negative status achieved by means of immunotherapy had the same favorable prognostic impact of MRD negativity achieved with standard treatment could not be assessed by this study, but multiple reports would confirm it.

In this study, MRD data analyzed before transplantation and subsequently at 100 days and 1 year (±45 days) were reported. In terms of potential interventions, a graft-versus-leukemia effect could be enhanced by an early tapering of immunosuppression, based on either pre- or post-HSCT MRD positivity. On this regard, an additional timepoint of assessment, approximately in the second month, could drive the decision making process of immunotapering, at least in patients who do not experience GVHD.

Additional timepoints should be considered in case of MRD positivity. Furthermore, more frequent assessments could drive post-HSCT immunotherapy, based on the detection of post-HSCT MRD positivity. The prescription of monoclonal antibodies or CAR T-cell therapy are beyond the purpose of this study and are also limited by regulatory/contract restrictions which varies amongst Countries.

A subsequent analysis assessing the role of immunotherapy pre- and post-HSCT is ongoing and recommendations might followed based on those findings.

In conclusion, in a large, prospective, international trial of CAYA undergoing HSCT for ALL, we could demonstrate that: i. pre-HSCT MRD level $\geq 10^{-4}$ was associated with worse outcome, due to higher relapse incidence, ii. the detrimental effect of pre-HSCT MRD positivity could not be demonstrated in T-ALL, iii. the level of positivity did not significantly affect outcome, and iiii. post-HSCT MRD positivity has a dismal prognosis but does not necessarily imply relapse. Furthermore, the superiority of TBI over chemo-conditioning was confirmed, grade II aGVHD was associated with better OS and EFS and lower CIR, patients $\geq$10 years or developing grade III-IV aGVHD had higher risk of any failure and experienced higher mortality.

REFERENCES

1. Schrappe M, Hunger SP, Pui CH, et al. Outcomes after induction failure in childhood acute lymphoblastic leukemia. N Engl J Med. 2012;366(15):1371-1381.
2. Pulsipher MA, Peters C, Pui CH. High-risk pediatric acute lymphoblastic leukemia: to transplant or not to transplant? Biol Blood Marrow Transplant. 2011;17(1 Suppl):S137-148.
3. Balduzzi A, Di Maio L, Silvestri D, et al. Minimal residual disease before and after transplantation for childhood acute lymphoblastic leukaemia: is there any room for intervention? Br J Haematol. 2014;164(3):396-408.
4. Conter V, Valsecchi MG, Parasole R, et al. Childhood high-risk acute lymphoblastic leukemia in first remission: results after chemotherapy or transplant from the AIEOP ALL 2000 study. Blood. 2014;123(10):1470-1478.
5. Pui CH, Mullighan CG, Evans WE, Relling MV. Pediatric acute lymphoblastic leukemia: where are we going and how do we get there? Blood. 2012;120(6):1165-1174.
6. Balduzzi A, Dalle JH, Wachowiak J, et al. Transplantation in Children and Adolescents with Acute Lymphoblastic Leukemia from a Matched Donor versus an HLA-Identical Sibling: Is the Outcome Comparable? Results from the International BFM ALL SCT 2007 Study. Biol Blood Marrow Transplant. 2019;25(11):2197-2210.
7. Peters C, Schrappe M, von Stackelberg A, et al. Stem-cell transplantation in children with acute lymphoblastic leukemia: A prospective international multicenter trial comparing sibling donors with matched unrelated donors-The ALL-SCT-BFM-2003 trial. J Clin Oncol. 2015;33(11):1265-1274.
8. Majhail NS, Chitphakdithai P, Logan B, et al. Significant improvement in survival after unrelated donor hematopoietic cell transplantation in the recent era. Biol Blood Marrow Transplant. 2015;21(1):142-150.
9. Bader P, Pötschger U, Dalle JH, et al. Low rate of nonrelapse mortality in under-4-year-olds with ALL given chemotherapeutic conditioning for HSCT: a phase 3 FORUM study. Blood Adv. 2024;8(2):416-428.
10. Bader P, Kreyenberg H, Henze GH, et al. Prognostic value of minimal residual disease quantification before allogeneic stem-cell transplantation in relapsed

childhood acute lymphoblastic leukemia: the ALL-REZ BFM Study Group. J Clin Oncol. 2009;27(3):377-384.

11. Bader P, Kreyenberg H, von Stackelberg A, et al. Monitoring of minimal residual disease after allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia allows for the identification of impending relapse: results of the ALL-BFM-SCT 2003 trial. J Clin Oncol. 2015;33(11):1275-1284.
12. Sutton R, Shaw PJ, Venn NC, et al. Persistent MRD before and after allogeneic BMT predicts relapse in children with acute lymphoblastic leukaemia. Br J Haematol. 2015;168(3):395-404.
13. Bader P, Salzmann-Manrique E, Balduzzi A, et al. More precisely defining risk peri-HCT in pediatric ALL: pre- versus post-MRD measures, serial positivity, and risk modeling. Blood Adv. 2019;3(21):3393-3405.
14. Ifversen M, Turkiewicz D, Marquart HV, et al. Low burden of minimal residual disease prior to transplantation in children with very high risk acute lymphoblastic leukaemia: The NOPHO ALL2008 experience. Br J Haematol. 2019;184(6):982-993.
15. Peters C, Dalle JH, Locatelli F, et al. Total Body Irradiation or Chemotherapy Conditioning in Childhood ALL: A Multinational, Randomized, Noninferiority Phase III Study. J Clin Oncol. 2021;39(4):295-307.
16. Modvig S, Hallböök H, Madsen HO, et al. Value of flow cytometry for MRD-based relapse prediction in B-cell precursor ALL in a multicenter setting. Leukemia. 2021;35(7):1894-1906.
17. Versluijs AB, de Koning CCH, Lankester AC, et al. Clofarabine-fludarabine-busulfan in HCT for pediatric leukemia: an effective, low toxicity, TBI-free conditioning regimen. Blood Adv. 2022;6(6):1719-1730
18. Pulsipher MA, Carlson C, Langholz B, et al. IgH-V(D)J NGS-MRD measurement pre- and early post-allotransplant defines very low- and very high-risk ALL patients. Blood. 2015;125(22):3501-3508.

Tables

Table 1. Patient distribution by MRD status pre-HSCT and by conditioning regimen in patients for whom MRD status was available pre-HSCT.

	Total		**MRDneg (neg, NQ, <10^{-4})**		**MRDpos (pos ≥10^{-4})**		**p-value**
	n	**%**	**n**	**%**	**n**	**%**	
Total	852		674	*79*	178	*21*	
Sex							
Male	552	*65*	436	*65*	116	*65*	0.930
Female	300	*35*	238	*35*	62	*35*	
Age							
4–10	415	*49*	327	*49*	88	*49*	0.926
10–14	241	*28*	190	*28*	51	*29*	
14–18	166	*19*	134	*20*	32	*18*	
>18	30	*4*	23	*3*	7	*4*	
Age median (Q1, Q3)	10.4 (7.7–14.0)		10.7 (7.9–14.0)		9.8 (7.4–13.5)		0.409
Donor							
MSD	241	*28*	190	*28*	51	*29*	0.926
MD	611	*72*	484	*72*	127	*71*	
Remission status							
CR1	412	*48*	318	*47*	94	*53*	0.236
CR2	376	*44*	305	*45*	71	*40*	
CR3	60	*7*	48	*7*	12	*7*	
>CR3	3	*0*	3	*0*	0	*0*	
Missing	1		0		1		
Risk							
CR1, CR2 late relapse	602	*71*	466	*70*	136	*77*	0.076
CR2 early relapse, ≥CR3	243	*29*	202	*30*	41	*23*	
Missing	7		6		1		
Stem cell source							
BM	595	*70*	465	*69*	130	*73*	0.059
PB	231	*27*	192	*29*	39	*22*	
CB	23	*3*	15	*2*	8	*5*	

Missing	3		2		1			
Conditioning regimen								
TBI/VP16	601	*71*	485	*72*	116	*65*		0.002
FLU/THIO/BU	154	*18*	126	*19*	28	*16*		
FLU/THIO/TREO	97	*11*	63	*9*	34	*19*		
Immunophenotype								
BCP	651	*77*	526	*79*	125	*70*		0.049
T-ALL	195	*23*	142	*21*	53	*30*		
other	2	*0*	2	*0*	0	*0*		
Missing	4		4		0			
BCR-ABL or *t*(9,22)								
Negative	741	*91*	585	*91*	156	*91*		1.000
Positive	73	*9*	58	*9*	15	*9*		
Missing	38		31		7			
TEL-AML or *t*(12,21)								
Negative	693	*89*	545	*88*	148	*92*		0.254
Positive	84	*11*	71	*12*	13	*8*		
Missing	75		58		17			
aff1(aff4)mll or *t*(4,11)								
Negative	755	*97*	595	*97*	160	*97*		0.787
Positive	21	*3*	16	*3*	5	*3*		
Missing	76		63		13			

BCP, B-cell precursor; BM, bone marrow; BU, busulfan; CB, cord blood; CR, complete remission; FLU, fludarabine; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease; PB, peripheral blood; T-ALL, T-cell acute lymphoblastic leukemia; TBI, total body irradiation; THIO, thiotepa; TREO, treosulfan; VP16, etoposide; NQ, non-quantifiable.

Table 2. Incidence of aGVHD by MRD status pre-HSCT and by conditioning regimen in patients for whom MRD status was available pre-HSCT.

	Total							TBI/VP16						FLU/THIO/BU or FLU/THIO/TREO					
	Total		MRD_{neg} (neg, NQ, $<10^{-4}$)		MRD_{pos} ($\geq 10^{-4}$)			Total	MRD_{neg} (neg, NQ, $<10^{-4}$)		MRD_{pos} ($\geq 10^{-4}$)			Total	MRD_{neg} (neg, NQ, $<10^{-4}$)		MRD_{pos} ($\geq 10^{-4}$)		
	N	%	N	%	N	%	p-value	N	N	%	N	%	p-value	N	N	%	N	%	p-value
Total	852		674	*100*	178	*100*		601	485	*100*	116	*100*		251	189	*100*	62	*100*	
aGVHD grade																			
absent	334	*42*	261	*39*	73	*41*		205	163	*34*	42	*36*		129	*98*	*52*	31	*50*	
I	202	*25*	169	*25*	33	*19*		159	136	*28*	23	*20*		43	*33*	*17*	10	*16*	
II	193	*24*	151	*22*	42	*24*		145	116	*24*	29	*25*		48	*35*	*19*	13	*21*	
III	52	*6*	35	*5*	17	*10*		38	25	*5*	13	*11*		14	*10*	*5*	4	*6*	
IV	18	*2*	12	*2*	6	*3*		14	11	*2*	3	*3*		4	*1*	*1*	3	*5*	
Unknown	49		42	*6*	7	*4*		38	32	*7*	6	*5*		11	*10*	*5*	1	*2*	
Death prior to d100 (without aGVHD)	4	*0.5*	4	*1*	0	*0*		2	2	*0*	0	*0*		2	*2*	*1*	0	*0*	
Grade 0-I/II	733	*91*	585	*87*	148	*83*	0.021	511	417	*86*	94	*81*	0.042	222	168	*89*	54	*87*	0.172
Grade III-IV	70	*9*	47	*7*	23	*13*		52	36	*7*	16	*14*		18	11	*6*	7	*11*	
Unknown	49		42	*6*	7	*4*		38	32	*7*	6	*5*		11	10	*5*	1	*2*	
Grade 0-I	540	*67*	434	*64*	106	*60*	0.045	366	301	*62*	65	*56*	0.090	174	133	*70*	41	*66*	0.337
Grade II	193	*24*	151	*22*	42	*24*		145	116	*24*	29	*25*		48	35	*19*	13	*21*	
Grade III-IV	70	*9*	47	*7*	23	*13*		52	36	*7*	16	*14*		18	11	*6*	7	*11*	
Unknown	49		42	*6*	7	*4*		38	32	*7*	6	*5*		11	10	*5*	1	*2*	

aGVHD, acute graft-versus-host disease; BU, busulfan; FLU, fludarabine; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease; NQ, non-quantifiable; TBI, total body irradiation; THIO, thiotepa; TREO, treosulfan; VP16, etoposide.

Table 3. Univariable analysis: Outcomes overall and by MRD status pre-HSCT for the subgroups of patients defined by immunophenotype. OS, EFS, CIR, amd TRM are rerported for patients affected with B- and T-lineage ALL according to conditioning regimen and risk profile (panel A: OS and EFS; panel B: CIR and TRM).

A

Risk factor	Subgroup	OS									EFS								
		MRD_{neg} (neg, NQ, $<10^{-4}$)			MRD_{pos} ($\geq 10^{-4}$)						MRD_{neg} (neg, NQ, $<10^{-4}$)			MRD_{pos} ($\geq 10^{-4}$)					
		N	events	3-year pOS	N	events	3-year pOS	*p-value*	3-year pOS	*p-value*	N	events	3-year pEFS	N	events	3-year pEFS	*p-value*	3-year pOS	*p-value*
TBI	**B-lineage**	379	38	0.88±0.02	76	17	0.75±0.05	*0.007*	0.85±0.02	*0.055*	379	69	0.81±0.02	76	26	0.68±0.06	*0.003*	0.75±0.02	*0.892*
	T- lineage	102	18	0.77±0.05	40	8	0.75±0.09	*0.689*	0.76±0.04		102	22	0.76±0.05	40	8	0.75±0.09	*0.903*	0.76±0.04	
Chemo-conditioning	**B-lineage**	147	35	0.77±0.04	49	17	0.64±0.07	*0.203*	0.74±0.03	*0.905*	147	56	0.62±0.04	49	28	0.45±0.07	*0.016*	0.58±0.04	*0.172*
	T- lineage	40	9	0.77±0.07	13	4	0.69±0.13	*0.493*	0.75±0.06		40	11	0.72±0.07	13	5	0.62±0.13	*0.340*	0.70±0.06	
CR1+CR2 late relapse	**B-lineage**	348	41	0.87±0.02	88	17	0.78±0.05	*0.150*	0.85±0.02	*0.062*	348	75	0.76±0.03	88	31	0.65±0.05	*0.028*	0.74±0.02	*0.633*
	T- lineage	114	19	0.79±0.04	48	10	0.76±0.07	*0.600*	0.78±0.04		114	22	0.78±0.04	48	11	0.75±0.07	*0.634*	0.77±0.04	
CR2 early relapse + ≥CR3	**B-lineage**	173	30	0.79±0.04	37	17	0.51±0.09	*<0.001*	0.73±0.04	*0.746*	173	47	0.67±0.04	37	23	0.27±0.09	*<0.001*	0.60±0.04	*0.998*
	T- lineage	27	7	0.74±0.09	4	2	0.50±0.25	*0.053*	0.70±0.08		27	10	0.67±0.09	4	2	0.50±0.25	*0.150*	0.64±0.09	

B

Risk factor	Subgroup	CI of relapse									CI of TRM							
		MRD_{neg} (neg, NQ, $<10^{-4}$)			MRD_{pos} ($\geq 10^{-4}$)						MRD_{neg} (neg, NQ, $<10^{-4}$)			MRD_{pos} ($\geq 10^{-4}$)				
		N	events	3-year CI of relapse	N	events	3-year CI of relapse	*p-value*	3-year CI of relapse	*p-value*	N	events	3-year CI of TRM	N	events	3-year CI of TRM	*p-value*	3-year CI of TRM	*p-value*
TBI	**B-lineage**	379	47	0.15±0.02	76	20	0.30±0.06	*0.008*	0.18±0.02	*0.956*	379	14	0.04±0.01	76	5	0.07±0.03	*0.283*	0.05±0.01	*0.297*
	T- lineage	102	15	0.17±0.04	40	5	0.19±0.08	*0.787*	0.18±0.04		102	6	0.07±0.03	40	3	0.05±0.04	*0.697*	0.07±0.02	
Chemo-conditioning	**B-lineage**	147	44	0.30±0.04	49	25	0.49±0.07	*0.009*	0.35±0.04	*0.137*	147	11	0.07±0.02	49	3	0.06±0.03	*0.693*	0.07±0.02	*0.936*
	T- lineage	40	8	0.20±0.06	13	4	0.31±0.13	*0.357*	0.23±0.06		40			13				0.08±0.04	
CR1+CR2 late relapse	**B-lineage**	348	51	0.17±0.02	88	26	0.30±0.05	*0.004*	0.20±0.02	*0.646*	348	15	0.05±0.01	88	4	0.05±0.02	*0.945*	0.05±0.01	*0.460*
	T- lineage	114	17	0.17±0.04	48	7	0.19±0.07	*0.937*	0.18±0.03		114	5	0.05±0.02	48	4	0.07±0.04	*0.324*	0.06±0.02	
CR2 early relapse + ≥CR3	**B-lineage**	173	38	0.27±0.04	37	19	0.57±0.09	*<0.001*	0.33±0.04	*0.667*	173	9	0.05±0.02	37	4	0.12±0.06	*0.201*	0.07±0.02	*0.591*
	T- lineage	27	6	0.22±0.08	4	2	0.50±0.25	*0.084*	0.26±0.08		27	3	0.11±0.06	4	0	0.00±0.00	*0.997*	0.10±0.05	

CI, cumulative incidence; CIR, cumulative incidence of relapse; CR, complete remission; EFS, event-free survival; MRD, minimal residual disease; NQ, non-quantifiable; OS, overall survival; TBI, total body irradiation; TRM, treatment-related mortality.

Table 4. Multivariable analyses for any failure (1-EFS), death from any cause (1-OS), relapse, and treatment-related mortality for the cohort according to the MRD status pre-HSCT and by the conditioning regimen after adjusting for age, disease risk profile and immunophenotype, accounting for aGvHD, and cGvHD..

	Any event (relapse or death)				Death from any cause				Relapse				TRM			
	HR	HR Conf.Lim.		p-value	HR	HR Conf.Lim.		p-value	HR	HR Conf.Lim.		p-value	HR	HR Conf.Lim.		p-value
MRD_{pos} vs MRD_{neg}	1.950	1.447	2.628	*<.0001*	1.855	1.288	2.670	*0.001*	2.182	1.561	3.051	*<.0001*	1.315	0.658	2.629	*0.438*
MD vs MSD	1.103	0.812	1.500	*0.530*	0.941	0.651	1.360	*0746*	0.949	0.677	1.329	*0.758*	2.565	1.166	5.643	*0.019*
CR2 early relapse, ≥CR3 vs CR1, CR2 late relapse	2.355	1.759	3.153	*<.0001*	2.512	1.759	3.588	*<.0001*	2.467	1.775	3.431	*<.0001*	2.436	1.281	4.631	*0.007*
FLU/THIO/BU or FLU/THIO/TREO vs TBI	1.909	1.447	2.517	*<.0001*	1.792	1.270	2.528	*0.001*	2.304	1.687	3.148	*<.0001*	1.484	0.803	2.745	*0.208*
>10years vs ≤10years	1.229	0.937	1.613	*0.137*	1.549	1.101	2.179	*0.012*	1.028	0.755	1.398	*0.862*	2.864	1.484	5.526	*0.002*
Immunophnotype BCP vs no BCP	1.102	0.783	1.551	*0.579*	0.734	0.497	1.085	*0.121*	0.839	0.566	1.242	*0.381*	0.572	0.291	1.124	*0.105*
aGVHD grade II vs grade 0/I	0.613	0.423	0.889	*0.010*	0.587	0.362	0.951	*0.031*	0.574	0.374	0.882	*0.011*	0.900	0.395	2.050	*0.801*
aGVHD grade III/IV vs grade 0/I	1.444	0.949	2.198	*0.086*	1.741	1.070	2.835	*0.026*	1.177	0.702	1.973	*0.536*	3.543	1.645	7.633	*0.001*
cGVHD limited vs no cGvHD	1.303	0.658	2.581	*0.448*	1.081	0.467	2.501	*0.856*	0.842	0.367	1.933	*0.686*	3.444	0.967	12.269	*0.056*
cGVHD extensive vs no cGvHD	1.038	0.549	1.961	*0.909*	1.233	0.604	2.517	*0.565*	0.354	0.111	1.129	*0.079*	4.913	1.907	12.656	*0.001*

aGVHD, acute graft-versus-host disease; BCP, B-cell precursor; BU, busulfan; Conf. limit, confidence limit; cGvHD, chronic graft-versus-host disease; CR, complete remission; FLU, fludarabine; HR, hazard ratio; HSCT, hematopoietic stem cell transplantation; MD, matched donor; MRD, minimal residual disease; MSD, matched sibling donor; TBI, total body irradiation; THIO, thiotepa; TREO, treosulfan; VP16, etoposide; vs, versus; TRM, treatment-related mortality; EFS, event-free survival; OS, overall survival.

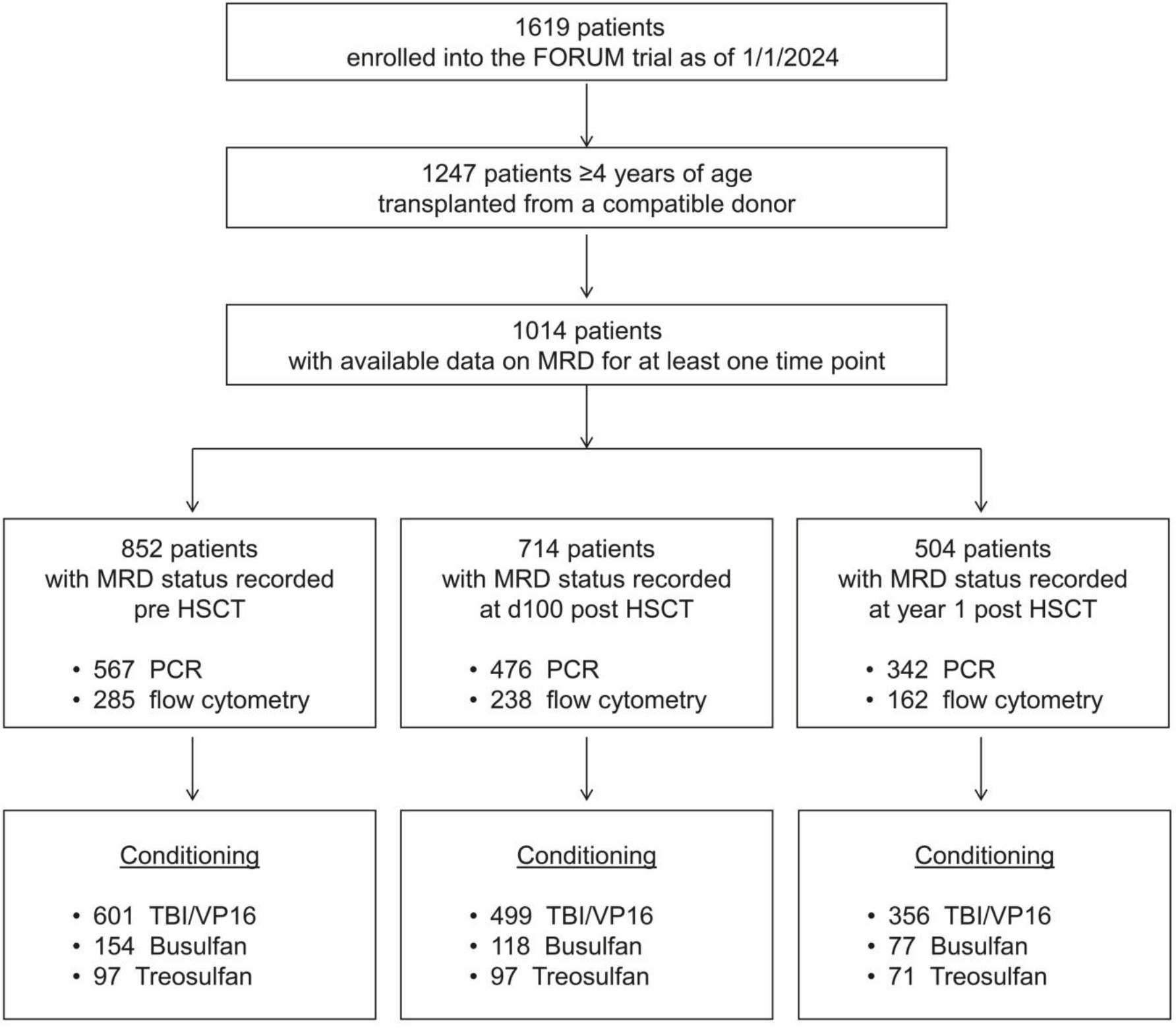
Figure Captions

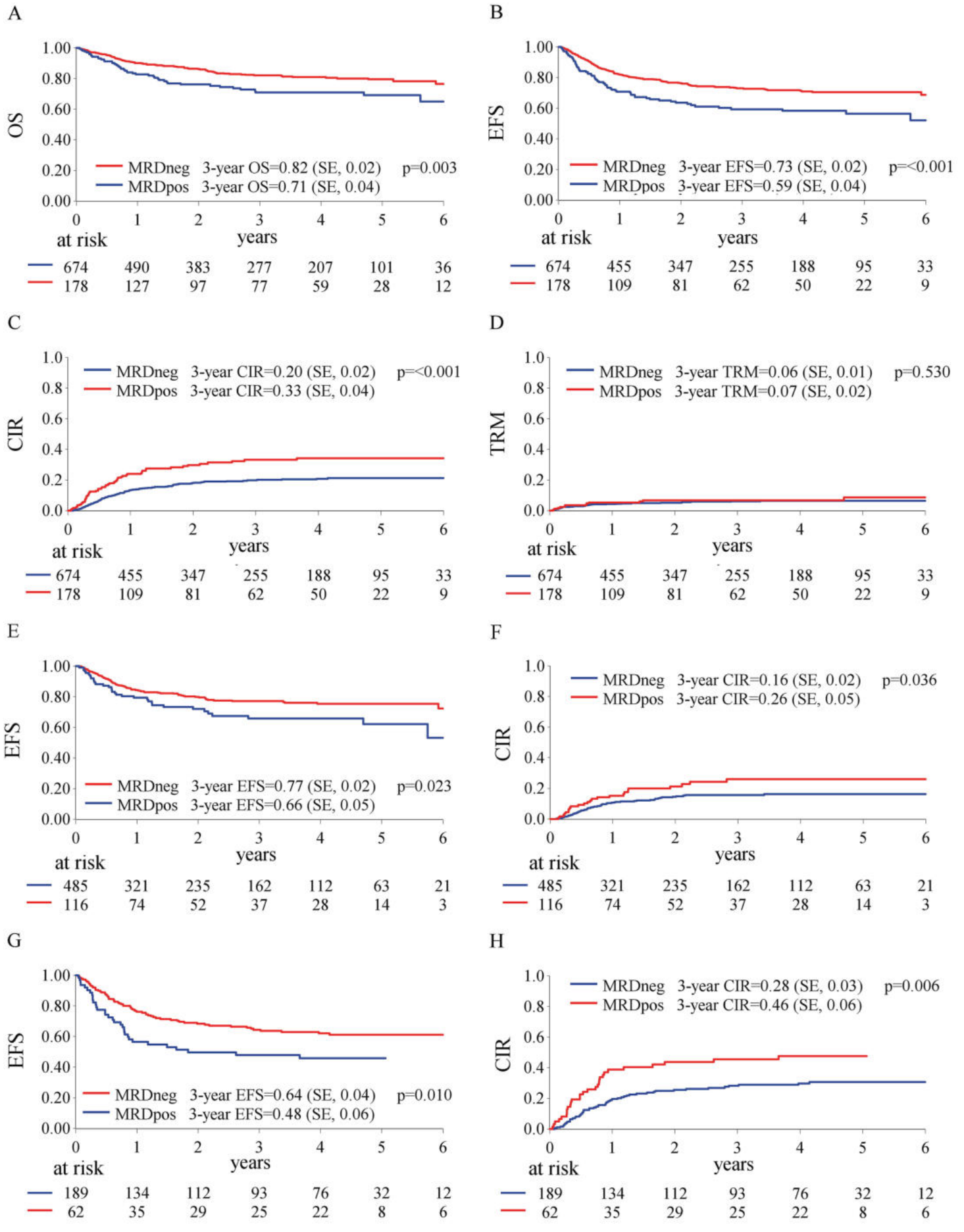
Figure 1. CONsolidated Standards Of Reporting Trials - CONSORT diagram

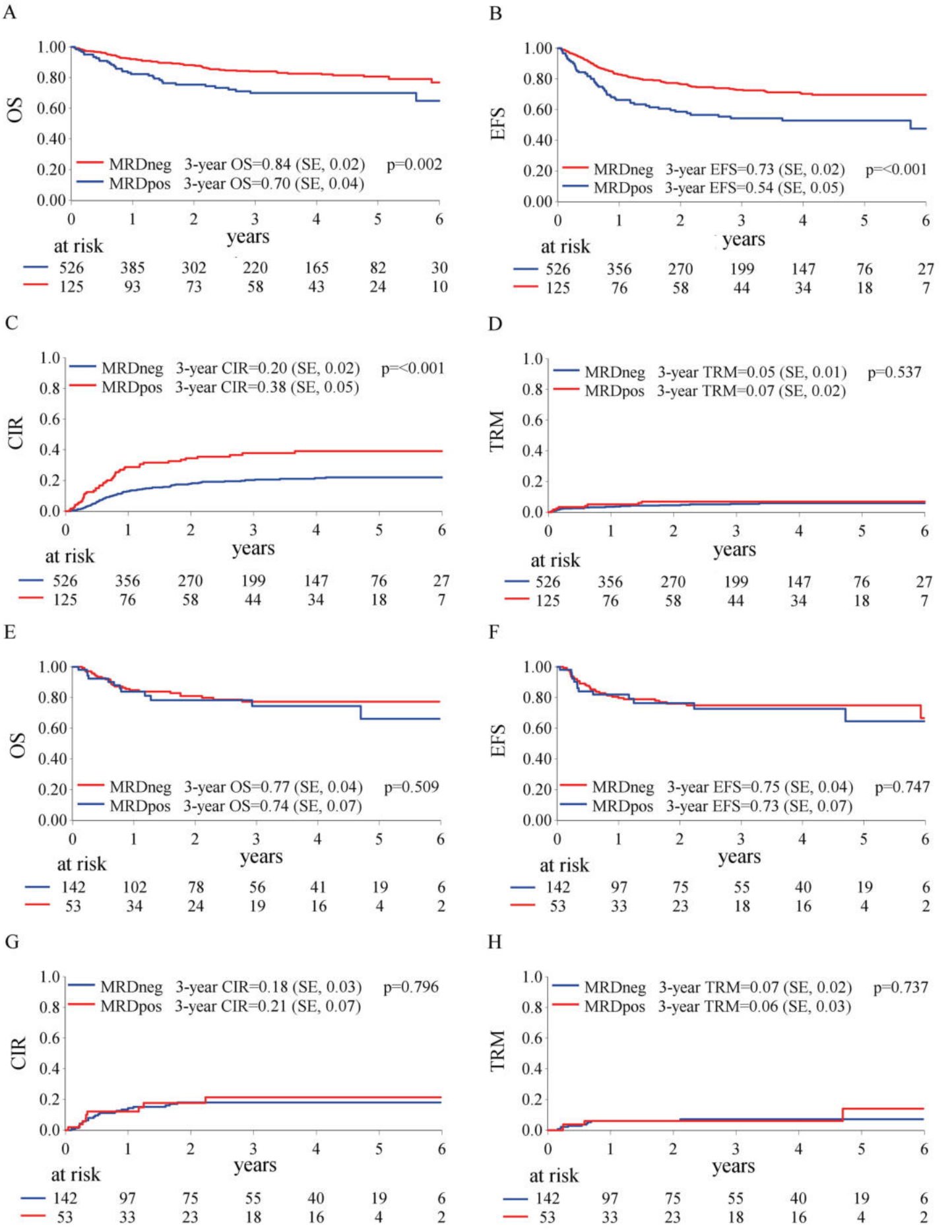
Figure 2. Outcome according to minimal residual disease - MRD status at the time of hematopoietic stem cell transplantation. Overall survival - OS (panel A), event free survival - EFS (panel B), cumulative incidence of relapse - CIR (panel C) and transplant related mortality - TRM (panel D) are shown. EFS and CIR are also shown for the total body irradiation/etoposide - TBI/VP16 subgroup (panel E: EFS; panel F: CIR) and for the chemo-conditioning subgroup (panel G: EFS; panel H: CIR).

Figure 3. Outcome by minimal residual disease - MRD status at the time of hematopoietic stem cell transplantation according to immunophenotype. Overall survival - OS (panel A), event free survival - EFS (panel B), cumulative incidence of relapse - CIR (panel C) and transplant related mortality - TRM (panel D) are shown for patients affected with B-lineage acute lymphoblastic leukemia (ALL) and OS (panel E), EFS (panel F), CIR (panel G) and TRM (panel H) for those affected with T-lineage ALL.

Abbreviations: HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease; PCR, polymerase chain reaction; TBI, total body irradiation; VP16, etoposide


1619 patients
enrolled into the FORUM trial as of 1/1/2024
1247 patients ≥4 years of age
transplanted from a compatible donor
1014 patients
with available data on MRD for at least one time point
852 patients
with MRD status recorded
pre HSCT
• 567 PCR
• 285 flow cytometry
714 patients
with MRD status recorded
at d100 post HSCT
• 476 PCR
• 238 flow cytometry
504 patients
with MRD status recorded
at year 1 post HSCT
• 342 PCR
• 162 flow cytometry
Conditioning
• 601 TBI/VP16
• 154 Busulfan
• 97 Treosulfan
Conditioning
• 499 TBI/VP16
• 118 Busulfan
• 97 Treosulfan
Conditioning
• 356 TBI/VP16
• 77 Busulfan
• 71 Treosulfan


A
OS
1.00
0.80
0.60
0.40
0.20
0.00
MRDneg 3-year OS=0.82 (SE, 0.02) p=0.003
MRDpos 3-year OS=0.71 (SE, 0.04)
0 1 2 3 4 5 6
at risk
years
674 490 383 277 207 101 36
178 127 97 77 59 28 12
B
EFS
MRDneg 3-year EFS=0.73 (SE, 0.02) p=<0.001
MRDpos 3-year EFS=0.59 (SE, 0.04)
at risk
years
674 455 347 255 188 95 33
178 109 81 62 50 22 9
C
CIR
MRDneg 3-year CIR=0.20 (SE, 0.02) p=<0.001
MRDpos 3-year CIR=0.33 (SE, 0.04)
at risk
years
674 455 347 255 188 95 33
178 109 81 62 50 22 9
D
TRM
MRDneg 3-year TRM=0.06 (SE, 0.01) p=0.530
MRDpos 3-year TRM=0.07 (SE, 0.02)
at risk
years
674 455 347 255 188 95 33
178 109 81 62 50 22 9
E
EFS
MRDneg 3-year EFS=0.77 (SE, 0.02) p=0.023
MRDpos 3-year EFS=0.66 (SE, 0.05)
at risk
years
485 321 235 162 112 63 21
116 74 52 37 28 14 3
F
CIR
MRDneg 3-year CIR=0.16 (SE, 0.02) p=0.036
MRDpos 3-year CIR=0.26 (SE, 0.05)
at risk
years
485 321 235 162 112 63 21
116 74 52 37 28 14 3
G
EFS
MRDneg 3-year EFS=0.64 (SE, 0.04) p=0.010
MRDpos 3-year EFS=0.48 (SE, 0.06)
at risk
years
189 134 112 93 76 32 12
62 35 29 25 22 8 6
H
CIR
MRDneg 3-year CIR=0.28 (SE, 0.03) p=0.006
MRDpos 3-year CIR=0.46 (SE, 0.06)
at risk
years
189 134 112 93 76 32 12
62 35 29 25 22 8 6


A
OS
1.00
0.80
0.60
0.40
0.20
0.00
MRDneg 3-year OS=0.84 (SE, 0.02) p=0.002
MRDpos 3-year OS=0.70 (SE, 0.04)
0 1 2 3 4 5 6
years
at risk
526 385 302 220 165 82 30
125 93 73 58 43 24 10
B
EFS
MRDneg 3-year EFS=0.73 (SE, 0.02) p=<0.001
MRDpos 3-year EFS=0.54 (SE, 0.05)
at risk
526 356 270 199 147 76 27
125 76 58 44 34 18 7
C
CIR
MRDneg 3-year CIR=0.20 (SE, 0.02) p=<0.001
MRDpos 3-year CIR=0.38 (SE, 0.05)
at risk
526 356 270 199 147 76 27
125 76 58 44 34 18 7
D
TRM
MRDneg 3-year TRM=0.05 (SE, 0.01) p=0.537
MRDpos 3-year TRM=0.07 (SE, 0.02)
at risk
526 356 270 199 147 76 27
125 76 58 44 34 18 7
E
OS
MRDneg 3-year OS=0.77 (SE, 0.04) p=0.509
MRDpos 3-year OS=0.74 (SE, 0.07)
at risk
142 102 78 56 41 19 6
53 34 24 19 16 4 2
F
EFS
MRDneg 3-year EFS=0.75 (SE, 0.04) p=0.747
MRDpos 3-year EFS=0.73 (SE, 0.07)
at risk
142 97 75 55 40 19 6
53 33 23 18 16 4 2
G
CIR
MRDneg 3-year CIR=0.18 (SE, 0.03) p=0.796
MRDpos 3-year CIR=0.21 (SE, 0.07)
at risk
142 97 75 55 40 19 6
53 33 23 18 16 4 2
H
TRM
MRDneg 3-year TRM=0.07 (SE, 0.02) p=0.737
MRDpos 3-year TRM=0.06 (SE, 0.03)
at risk
142 97 75 55 40 19 6
53 33 23 18 16 4 2

SUPPLEMENTARY MATERIALS

Balduzzi A, et al. Impact of minimal residual disease on the outcome of hematopoietic stem cell transplantation for childhood acute lymphoblastic leukemia within the FORUM trial

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PATIENTS AND METHODS

Transplant procedure

Complete remission (CR) was defined as <5% bone marrow (BM) blasts and no evidence of extramedullary disease. Relapse was defined as >5% leukemic blasts in BM and/or detection in extramedullary sites (e.g., cerebrospinal fluid, testes, or ovary).

For the purpose of this study, patients transplanted in CR2 after early relapse or in >CR3 were considered at higher risk compared with those transplanted in CR1 or in CR2 after late relapse (defined as relapse ≥6 months after completion of primary therapy or ≥30 months after diagnosis to relapse).

HLA compatibility was defined by high resolution typing for HLA-A, -B, -C, -DR, and -DQ alleles (10 alleles, 4-digit typing). Donors were defined as HLA-identical siblings (MSD) or matched donors (MD), either related or unrelated, when they were fully matched (10/10) or had a single allelic disparity (9/10). In case of cord blood as stem cell source, a 5/6 or 6/6 compatibility for the A and B loci in low resolution and DR locus in high resolution was considered HLA matched. Donors with lower degrees of compatibility with their recipients were not included in this study.

Total body irradiation (TBI)-based conditioning consisted of fractionated TBI, total dose 12 Gy, and etoposide (VP16) 60 mg/kg or 1.8 g/m^2, maximum total dose 3.6 g, whereas the chemotherapy-based conditioning consisted of fludarabine 150 mg/m^2, thiotepa 10 mg/kg, and either treosulfan, 42 g/m^2 or busulfan (AUC 90 mg*h/L) (Figure S1).

The recommended stem cell source was BM or cord blood from a matched sibling donor (MSD), or BM, peripheral blood (PB) stem cells or cord blood from a matched donor (MD).

Graft-versus-host disease (GVHD) prophylaxis was contingent upon donor type and stem cell source. MSD recipients received cyclosporine A only or cyclosporine A plus short methotrexate (MTX, 10 mg/m^2/dose on days +1, +3, +6) if PB was the stem cell source, whereas MD recipients received cyclosporine A, short MTX (10 mg/m^2/dose on days +1, +3, +6, +11) and anti-thymocyte globulin (Thymoglobulin) or anti-T-lymphocyte immunoglobulin (Grafalon).

Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were diagnosed and graded according to Glucksberg criteria.[1] If no aGVHD occurred after hematopoietic stem cell transplantation (HSCT), immunosuppression tapering was initiated at day 90 (MD) or day 60 (MSD).

MRD analysis

Results of the minimal residual disease (MRD) analysis were reported from local authorized laboratories based on either real-time quantitative polymerase chain reaction (PCR) of immunoglobulin and *TCR* gene rearrangements or multicolor flow cytometry in case of identified informative markers.[2-4]

MRD level was reported in log intervals using either method. In cases where both methods were applied, the highest value was used for the current analysis. Standards for MRD analysis were according to the European Study Group on MRD detection (ESG-MRD-ALL) and the Euroflow consortium.[2-4]

Statistical Analysis

Descriptive analyses were reported as medians and ranges. Kaplan-Meier estimators were used to estimate overall survival, event-free survival (EFS) and the Log-Rank test was used for survival comparison.[5] The starting point for the analysis was the date of HSCT for the assessment of the influence of MRD at HSCT on survival, day 100 for the impact of MRD at day 100, and 1 year after HSCT for the impact of MRD at year 1 after HSCT. EFS time was defined as the time until relapse, second neoplasm or death, whichever occurred first, while survival time was defined as the time until death due to any cause. Patients were censored at last follow-up in case no events occurred.

The method of Kalbfleisch and Prentice, and the test of Gray were used to estimate the cumulative incidence of relapse, treatment-related mortality and chronic GVHD, allowing for competing risks.[6,7] Patients alive and in remission 90 days after HSCT were considered at risk for acute and cGVHD. The proportional sub-distribution hazards model of Fine and Gray for censored data subject to competing risks[8] was applied to study the impact of MRD pre-HSCT ($\geq 1x10^{-4}$ versus $<1x10^{-4}$) on survival and relapse incidence, after adjustment for recipient age (>10 years versus ≤10 years), immunophenotype (B lineage versus others), disease phase (CR2 early relapse and ≥CR3, high-risk, versus CR1 and CR2 after late relapse, low-risk), type of donor and HLA compatibility (MD versus MSD).

The Cox proportional hazard model was used to calculate the impact of MRD on survival. The effect of MRD at HSCT on relapse incidence and survival was additionally assessed accounting for aGVHD and cGVHD as time dependent covariates. This was done by means of a separate Cause-specific Cox regression models with time dependent covariates, after adjustment for the variables mentioned above.

For non-time-to-event variables the Chi-Square test or, where appropriate, the Fisher exact test were used to compare groups for categorical variables and the Wilcoxon rank-sum test was used for continuous variables.

Median follow-up was estimated using the inverse Kaplan-Meier method.

A two-sided p-value <0.05 was regarded as significant.

Statistical analyses were performed by means of the SAS version 9.4 (SAS Institute, Cary, NC).

References

1. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation*. 1974;18:295-304.

2. van der Velden VH, Cazzaniga G, Schrauder A, et al. Analysis of minimal residual disease by Ig/TCR gene rearrangements: Guidelines for interpretation of real-time quantitative PCR data. *Leukemia*. 2007;21:604-11.

3.van der Velden VH, van Dongen JJ. MRD detection in acute lymphoblastic leukemia patients using Ig/TCR gene rearrangements as targets for real-time quantitative PCR. *Methods Mol Biol*. 2009;538:115-150.

4. Dworzak MN, Fröschl G, Printz D, et al. Prognostic significance and modalities of flow cytometric minimal residual disease detection in childhood acute lymphoblastic leukemia. *Blood*. 2002;99:1952-1958.

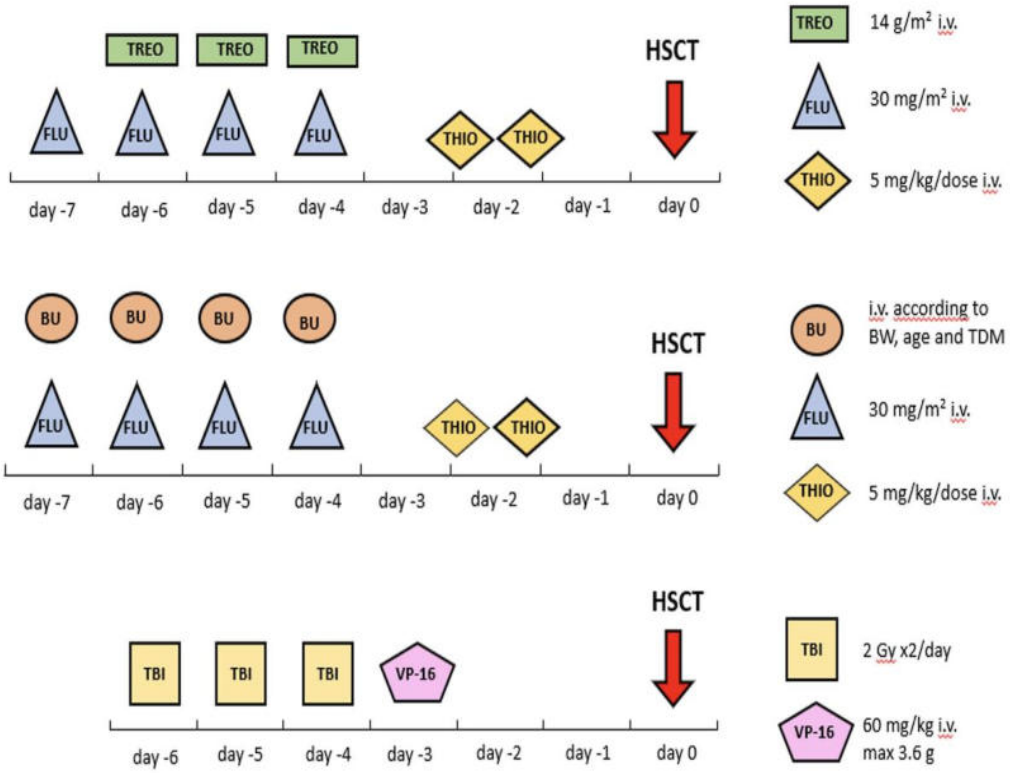
5. Kaplan, E.L. and Meier, P. Nonparametric Estimation from Incomplete Observations. Journal of the American Statistical Association, 1958; 53, 457-481.

6. Prentice RL, Kalbfleisch JD, Peterson AV Jr, Flournoy N, Farewell VT, Breslow NE. The analysis of failure times in the presence of competing risks. Biometrics. 1978 Dec;34(4):541-54.

7. Gray, Robert J. A Class of K-Sample Tests for Comparing the Cumulative Incidence of a Competing Risk. The Annals of Statistics, vol. 16, no. 3, 1988, pp. 1141–54.

8. Fine, J.P. and Gray, R.J. A Proportional Hazards Model for the Subdistribution of a Competing Risk. Journal of the American Statistical Association, 1999; 94:496-509.

Figure S1. Outline of the conditioning regimens included in the FORUM Trial.



BU, busulfan; BW, bodyweight; FLU, fludarabine; HSCT, hematopoietic stem cell transplantation; i.v., intravenous; THIO, thiotepa; TBI, total body irradiation; TDM, therapeutic drug monitoring; TREO, treosulfan; VP-16, etoposide.

Figure S2. Cumulative incidence of myeloid engraftment (panel A; first of 3 consecutive days of ANC $>0.5x10^9$/L) and platelet engraftment (panel B; first of 5 consecutive days of platelet count $>50x10^9$/L without transfusional support) according to MRD status pre-HSCT.

Legends

ANC, absolute neutrophil count; CI, cumulative inc lence; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease; PLT, platelet.

Figure S3. Incidence of cGVHD overall, limited cG HD, and extensive cGVHD by MRD status pre-HSCT (panel A, B, and C) and for the TBI/VP16 subgroup anel D, E, and F), and limited cGVHD and extensive cGVHD for the chemo-conditioning subgroup (pane G and H).

Legends

cGVHD, chronic graft-versus-host disease; CI, cumulative incidence; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease; TBI, total body irradiation; VP16, etoposide.

Figure S4. Outcome by three MRD levels pre-HSCT: negative (non-quantifiable or $<1x10^{-4}$), low-positive (between $1x10^{-4}$ and $<1x10^{-3}$) or high-positive ($1x10^{-3}$ or higher) (panel A: OS; panel B: EFS; panel C: CIR; panel D: TRM), in the TBI/VP16 subgroup (panel E: EFS; panel F: CIR) and in the chemo-conditioning subgroup (panel G: EFS, panel H: CIR).

Legends

CI, cumulative incidence; CIR, cumulative incidence of relapse; EFS, event-free survival; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease; OS, overall survival; TBI, total body irradiation; TRM, treatment-related mortality; VP16, etoposide.

Figure S5. CIR (panel A) and TRM (panel B) according to the occurrence of aGVHD by grade in the patients with MRD status pre-HSCT recorded.

Legends

aGVHD, acute graft-versus-host disease; CI, cumulative incidence; CIR, cumulative incidence of relapse; MRD, minimal residual disease; TRM, treatment-related mortality.

Figure S6. Outcome according to MRD at day 100 (panel A: OS; panel B: EFS; panel C, CIR; panel D: TRM), for the TBI/VP16 subgroup (panel E: EFS; panel F: CIR) and for the chemo-conditioning subgroup (panel G: EFS; panel H: CIR). Observation starts 100 days after infusion.

Legends

CI, cumulative incidence; CIR, cumulative incidence of relapse; EFS, event-free survival; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease; OS, overall survival; TBI, total body irradiation; TRM, treatment-related mortality; VP16, etoposide.

Figure S7. Outcome according to MRD at 1 year (panel A: OS; panel B: EFS; panel C, CIR; panel D: TRM), for the TBI/VP16 subgroup (panel E: EFS; panel F: CIR) and for the chemo-conditioning subgroup (panel G: EFS; panel H: CIR). Observation starts 1 year after infusion.

Legends

CI, cumulative incidence; CIR, cumulative incidence of relapse; EFS, event-free survival; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease; OS, overall survival; TBI, total body irradiation; TRM, treatment-related mortality; VP16, etoposide.

Table S1. Patient characteristics overall and by MRD status recorded at each timepoint, namely pre-HSCT, at 100 days, and at 1 year.

	Total		Patients with MRD data available pre-HSCT		Patients with MRD data available at day 100 post HSCT		Patients with MRD data available at year 1 post HSCT	
	n	%	n	%	n	%	n	%
Total	1014		852		714		504	
Sex								
Male	659	*65*	552	*65*	460	*64*	340	*67*
Female	355	*35*	300	*35*	254	*36*	164	*33*
Age								
4–10	492	*49*	415	*49*	335	*47*	239	*47*
10–14	279	*28*	241	*28*	206	*29*	144	*29*
14–18	212	*21*	166	*19*	155	*22*	107	*21*
>18	31	*3*	30	*4*	18	*3*	14	*3*
Age median (Q1, Q3)	10.5 (7.7-14.1)				11.1 (7.8–14.2)		11.3 (7.9–14.1)	
Donor								
MSD	290	*29*	241	*28*	214	*30*	146	*29*
MD	724	*71*	611	*72*	500	*70*	358	*71*
Remission status								
CR1	481	*47*	412	*48*	353	*49*	259	*51*
CR2	460	*45*	376	*44*	317	*44*	214	*42*
CR3	67	*7*	60	*7*	41	*6*	29	*6*
>CR3	5	*0*	3	*0*	2	*0*	2	*0*
Missing	1		1		1			
Risk								
CR1, CR2 late relapse	709	*71*	602	*71*	521	*73*	369	*74*
CR2 early relapse, ≥CR3	295	*29*	243	*29*	189	*27*	132	*26*
Missing	10		7		4		3	
Stem cell source								
BM	718	*71*	595	*70*	514	*72*	373	*74*
PB	264	*26*	231	*27*	185	*26*	119	*24*
CB	26	*3*	23	*3*	14	*2*	9	*2*
Missing	6		3		1		3	
Conditioning regimen								
TBI/VP16	719	*71*	601	*71*	499	*70*	356	*71*
FLU/THIO/BU	167	*16*	154	*18*	118	*17*	77	*15*
FLU/THIO/TREO	128	*13*	97	*11*	97	*14*	71	*14*
Immunophenotype								
BCP	765	*76*	583	*78*	547	*77*	388	*77*
T-ALL	230	*23*	167	*22*	154	*22*	105	*21*
Other	13	*1*	2	*0*	10	*1*	9	*2*
Missing	6		4		3		2	
BCR-ABL or *t*(9,22)								
Negative	876	*91*	741	*91*	619	*91*	441	*92*
Positive	86	*9*	73	*9*	61	*9*	39	*8*
Missing	52		38		34		24	
TEL-AML or *t*(12,21)								
Negative	812	*89*	693	*89*	573	*87*	408	*88*
Positive	105	*11*	84	*11*	82	*13*	57	*12*
Missing	97		75		59		39	
aff1(aff4)mll or *t*(4,11)								
Negative	893	*97*	755	*97*	636	*97*	452	*97*
Positive	25	*3*	21	*3*	17	*3*	12	*3*
Missing	96		76		61		40	

BCP, B-cell precursor; BM, bone marrow; BU, busulfan; CB, cord blood; CR, complete remission; FLU, fludarabine; HSCT, hematopoietic stem cell transplantation; MD, matched donor; MRD, minimal residual disease; MSD, matched sibling donor; PB, peripheral blood; T-ALL, T-cell acute lymphoblastic leukemia; TBI, total body irradiation; THIO, thiotepa; TREO, treosulfan; VP16, etoposide.

Table S2. Univariable analysis: Outcomes according to MRD status pre-HSCT (panel A: OS and EFS; panel B: CIR and TRM).

A

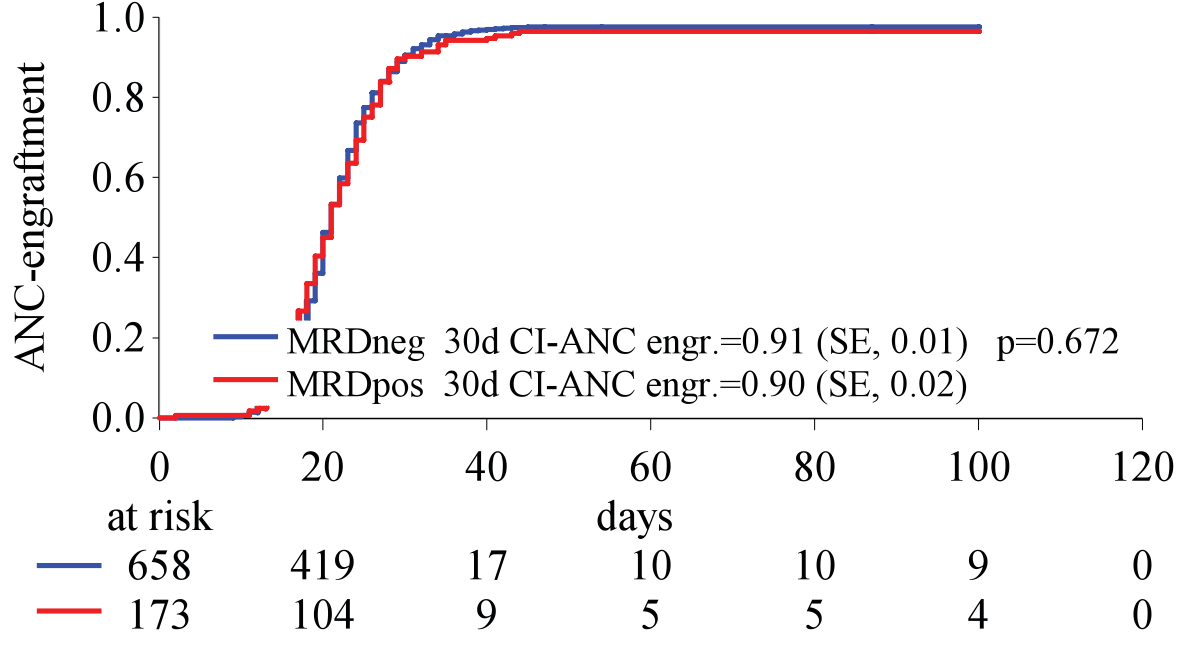
Risk factor	Subgroup	OS								EFS							
		MRD_{neg} (neg, NQ, $<10^{-4}$)			MRD_{pos} ($\geq 10^{-4}$)					MRD_{neg} (neg, NQ, $<10^{-4}$)			MRD_{pos} ($\geq 10^{-4}$)				
		N	events	3-year pOS	N	events	3-year pOS	*p-value*	3-year pOS	*p-value*	N	events	3-year pEFS	N	events	3-year pEFS	*p-value*	3-year pEFS	*p-value*
Total cohort		674	103	0.82±0.02	178	46	0.71±0.04	***0,003***	0.80±0.02		674	161	0.73±0.02	178	67	0.59±0.04	***<0.001***	0.70±0.02	
Age group	**4-10**	327	41	0.85±0.02	88	22	0.75±0.05	***0,019***	0.83±0.02	*0.036*	327	75	0.74±0.03	88	33	0.64±0.05	***0,016***	0.72±0.02	*0.406*
	>10	347	62	0.79±0.03	90	24	0.67±0.06	***0,058***	0.76±0.02		347	86	0.72±0.03	90	34	0.53±0.06	***0,006***	0.68±0.03	
Donor type	**MSD**	190	28	0.83±0.03	51	15	0.62±0.08	***0,016***	0.79±0.03	*0.983*	190	44	0.76±0.03	51	15	0.66±0.07	***0,316***	0.74±0.03	*0.276*
	MD	484	75	0.82±0.02	127	31	0.74±0.04	***0,042***	0.80±0.02		484	117	0.72±0.02	127	52	0.57±0.05	***<0.001***	0.69±0.02	
Remission phase	**CR1**	318	39	0.85±0.02	94	19	0.77±0.05	***0,113***	0.83±0.02	*0.004*	318	57	0.80±0.03	94	23	0.73±0.05	***0,221***	0.78±0.02	*<0.001*
	≥CR2	356	64	0.79±0.02	83	27	0.63±0.06	***0,005***	0.76±0.02		356	104	0.67±0.03	83	44	0.43±0.06	***<0.001***	0.62±0.03	
Risk profile	**CR1+CR2 late relapse**	466	61	0.85±0.02	136	27	0.77±0.04	***0,105***	0.83±0.02	*<0.001*	466	98	0.76±0.02	136	42	0.68±0.04	***0,039***	0.74±0.02	*<0.001*
	CR2 early relapse + ≥CR3	202	39	0.77±0.03	41	19	0.50±0.09	***<0.001***	0.72±0.03		202	59	0.67±0.04	41	25	0.28±0.08	***<0.001***	0.60±0.04	
Stem cell source	**BM**	465	70	0.83±0.02	130	33	0.72±0.04	***0,008***	0.80±0.02	*0.269*	465	116	0.73±0.02	130	47	0.62±0.05	***0,009***	0.71±0.02	*0.255*
	PB	192	31	0.80±0.04	39	11	0.67±0.09	***0,157***	0.77±0.03		192	43	0.71±0.04	39	18	0.43±0.09	***0,004***	0.66±0.04	
	CB	15	2	0.87±0.09	8	2	0.71±0.17	***0,324***	0.82±0.08		15	2	0.87±0.09	8	2	0.71±0.17	***0,324***	0.82±0.08	
Immunophenotype	**B-lineage**	526	73	0.84±0.02	125	34	0.70±0.04	***0,002***	0.81±0.02	*0.200*	526	125	0.77±0.02	125	54	0.59±0.05	***<0.001***	0.69±0.02	*0.375*
	T- lineage	142	27	0.77±0.04	53	12	0.74±0.07	***0,509***	0.77±0.03		142	33	0.76±0.04	53	13	0.76±0.06	***0,747***	0.74±0.03	
Conditional regimen	**TBI**	485	58	0.85±0.02	116	25	0.75±0.05	***0,014***	0.83±0.02	*0.006*	485	93	0.77±0.02	116	34	0.66±0.05	***0,023***	0.75±0.02	*<0.001*
	Chemo	189	45	0.77±0.03	62	21	0.65±0.06	***0,159***	0.74±0.03		189	68	0.64±0.04	62	33	0.48±0.06	***0,010***	0.60±0.03	
Chemo-conditioning	**BU**	126	37	0.71±0.04	28	10	0.64±0.09	***0,625***	0.70±0.04	*0.056*	126	51	0.60±0.05	28	12	0.60±0.09	***0,805***	0.60±0.04	*0.693*
	TREO	63	8	0.87±0.05	34	11	0.66±0.08	***0,019***	0.79±0.04		63	17	0.74±0.06	34	21	0.37±0.08	***<0.001***	0.61±0.05	

B

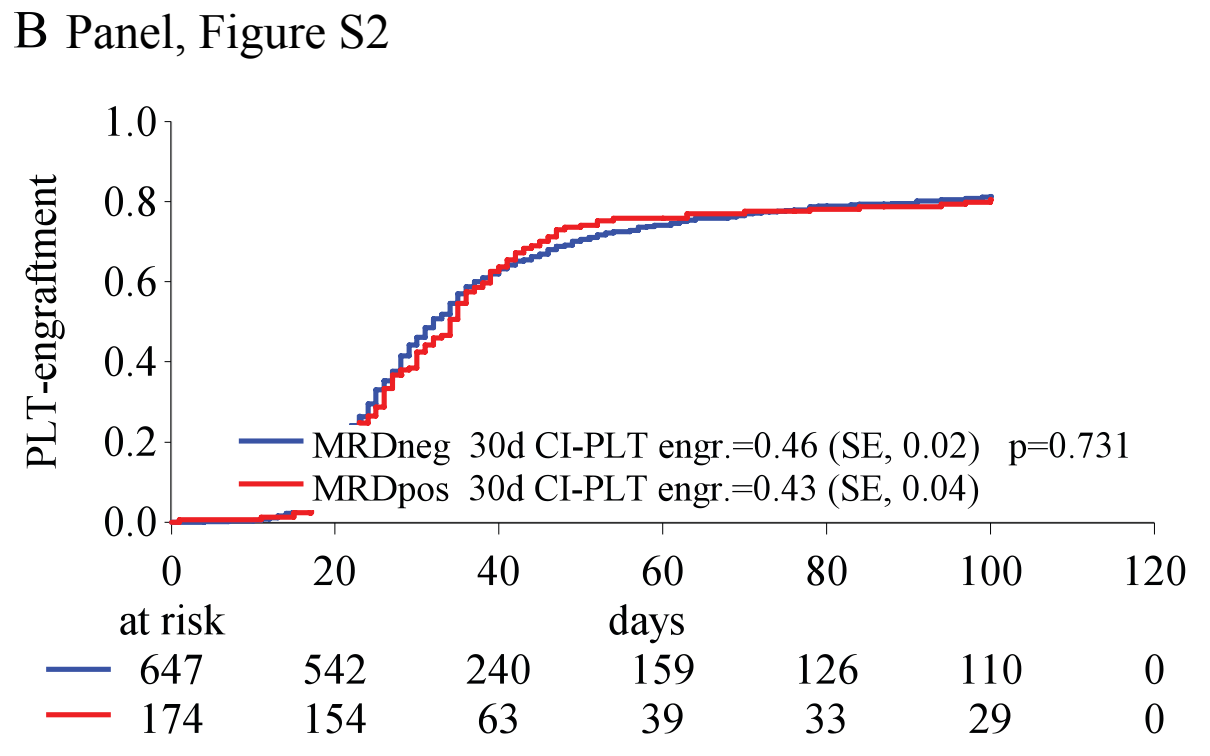
Risk factor	Subgroup	CI of relapse									CI of TRM							
		MRD_{neg} (neg, NQ, $<10^{-4}$)			MRD_{pos} ($\geq 10^{-4}$)						MRD_{neg} (neg, NQ, $<10^{-4}$)			MRD_{pos} ($\geq 10^{-4}$)				
		N	events	3-year CI of relapse	N	events	3-year CI of relapse	*p-value*	3-year CI of relapse	*p-value*	N	events	3-year CI of TRM	N	events	3-year CI of TRM	*p-value*	3-year CI of TRM	*p-value*
Total cohort		674	115	0.20±0.02	178	54	0.33±0.04	*<0.001*	0.23±0.02		36	0.06±0.01	12	0.07±0.02	*0.530*	0.06±0.01		
Age group	**4-10**	327	61	0.22±0.03	88	28	0.32±0.05	*0.020*	0.24±0.02	*0.278*	9	0.03±0.01	4	0.03±0.02	*0,489*	0.03±0.01	*0.002*	
	>10	347	54	0.18±0.02	90	26	0.34±0.06	*0.004*	0.21±0.02		27	0.09±0.02	8	0.10±0.04	*0,696*	0.09±0.02		
Donor type	**MSD**	190	38	0.21±0.03	51	13	0.27±0.07	*0.558*	0.23±0.03	*0.680*	5	0.03±0.01	3	0.07±0.04	*0,236*	0.04±0.01	*0.059*	
	MD	484	77	0.19±0.02	127	41	0.35±0.05	*<0.001*	0.23±0.02		31	0.07±0.01	9	0.07±0.02	*0,855*	0.07±0.01		
Remission phase	**CR1**	318	40	0.15±0.02	94	17	0.21±0.05	*0.239*	0.16±0.02	*<0.001*	11	0.04±0.01	6	0.06±0.03	*0,242*	0.04±0.01	*0.057*	
	≥CR2	356	75	0.24±0.03	83	37	0.47±0.06	*<0.001*	0.29±0.02		25	0.08±0.02	6	0.08±0.03	*0,972*	0.08±0.01		
Risk profile	**CR1+CR2 late relapse**	466	69	0.17±0.02	136	33	0.27±0.04	*0.020*	0.19±0.02	*<0.001*	20	0.05±0.01	8	0.06±0.02	*0,516*	0.05±0.01	*0.071*	
	CR2 early relapse + ≥CR3	202	44	0.26±0.03	41	21	0.57±0.09	*<0.001*	0.31±0.03		14	0.07±0.02	4	0.11±0.05	*0,499*	0.08±0.02		
Stem cell source	**BM**	465	86	0.20±0.02	130	38	0.31±0.04	*0.013*	0.23±0.02	*0.667*	23	0.05±0.01	8	0.06±0.02	*0,596*	0.06±0.01	*0.342*	
	PB	192	27	0.19±0.04	39	15	0.47±0.09	*0.001*	0.24±0.03		13	0.08±0.02	3	0.10±0.05	*0,917*	0.08±0.02		
	CB	15	2	0.13±0.09	8	1	0.14±0.13	*0.902*	0.14±0.07		0	0.00±0.00	1	0.14±0.13	*<0.001*	0.05±0.04		
Immunophenotype	**B-lineage**	526	91	0.20±0.02	125	45	0.38±0.05	*<0.001*	0.24±0.02	*0.237*	25	0.05±0.01	8	0.07±0.02	*0,537*	0.06±0.01	*0.396*	
	T- lineage	142	23	0.18±0.03	53	9	0.21±0.07	*0.796*	0.19±0.03		9	0.07±0.02	4	0.06±0.03	*0,737*	0.07±0.02		
Conditional regimen	**TBI**	485	62	0.16±0.02	116	25	0.26±0.05	*0.036*	0.18±0.02	*<0.001*	22	0.05±0.01	8	0.07±0.02	*0,320*	0.06±0.01	*0.432*	
	Chemo	189	53	0.28±0.03	62	29	0.46±0.06	*0.006*	0.33±0.03		14	0.07±0.02	4	0.07±0.03	*0,759*	0.07±0.02		
Chemo-conditioning	**BU**	126	41	0.33±0.04	28	10	0.33±0.09	*0.755*	0.33±0.04	*0.805*	10	0.08±0.02	2	0.07±0.05	*0,849*	0.07±0.02	*0.654*	
	TREO	63	12	0.19±0.05	34	19	0.57±0.09	*<0.001*	0.33±0.05		4	0.07±0.03	2	0.06±0.04	*0,897*	0.07±0.03		

BM, bone marrow; BU, busulfan; CB, cord blood; CI, cumulative incidence; CIR, cumulative incidence of relapse; CR, complete remission; EFS, event-free survival; MD, matched donor; MRD, minimal residual disease; MSD, matched sibling donor; NQ, non-quantifiable; OS, overall survival; PB, peripheral blood; TBI, total body irradiation; TREO, treosulfan; TRM, treatment-related mortality; VP16, etoposide.

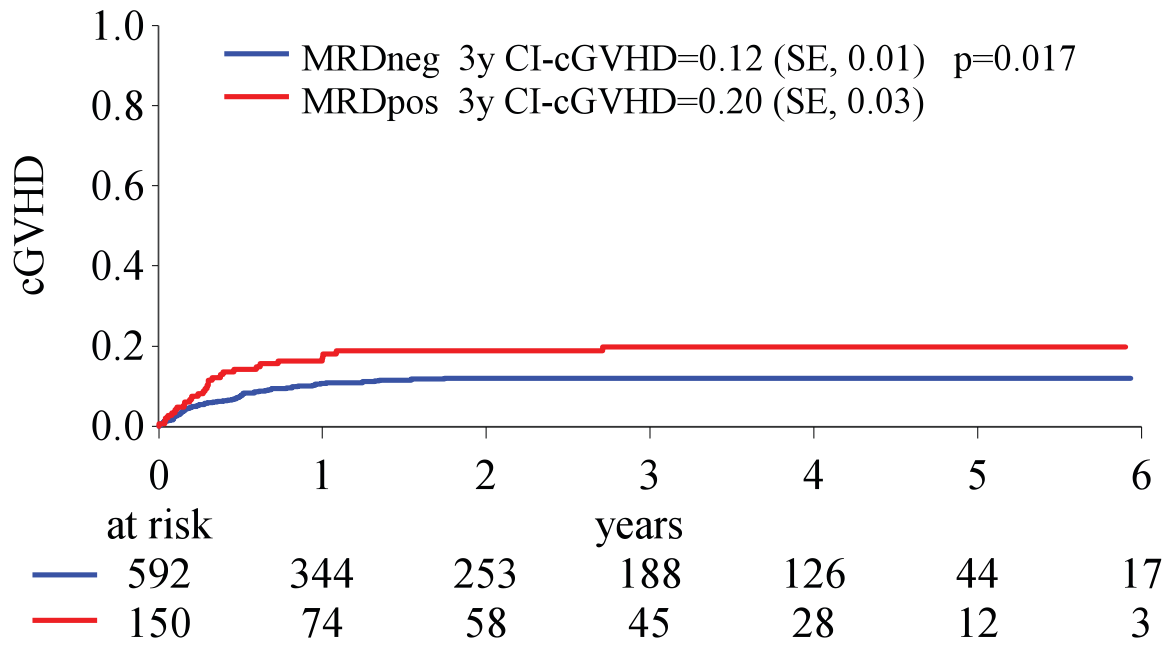
A


ANC-engraftment
1.0
0.8
0.6
0.4
0.2
0.0
MRDneg 30d CI-ANC engr.=0.91 (SE, 0.01) p=0.672
MRDpos 30d CI-ANC engr.=0.90 (SE, 0.02)
0
20
40
60
80
100
120
days
at risk
658 419 17 10 10 9 0
173 104 9 5 5 4 0

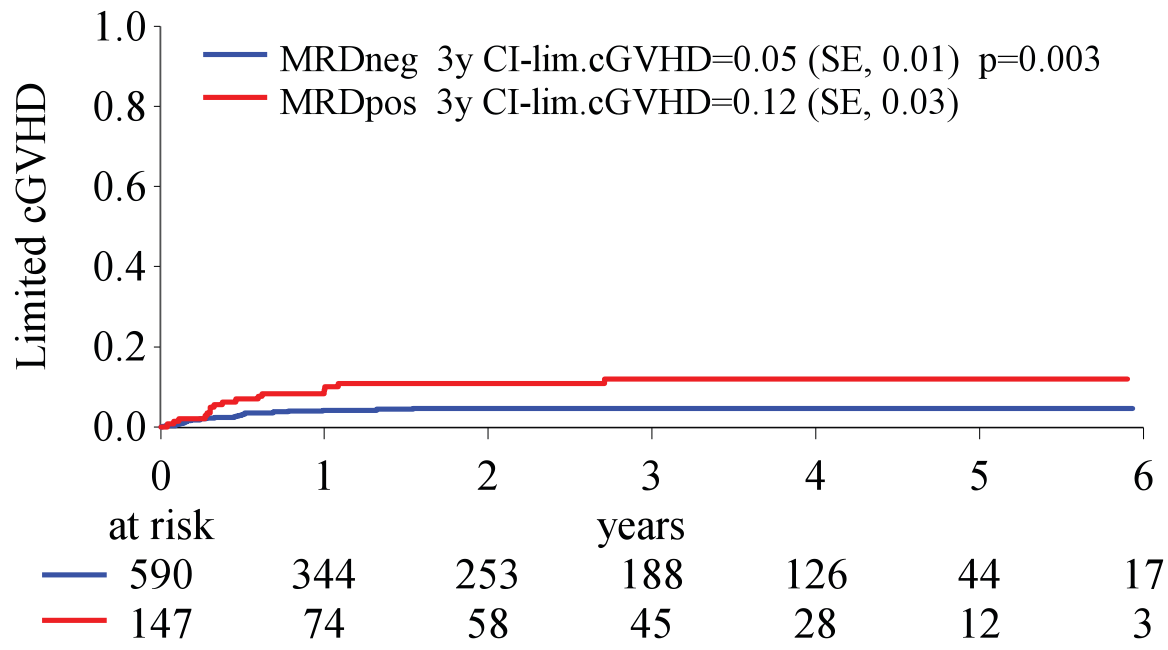
B Panel, Figure S2



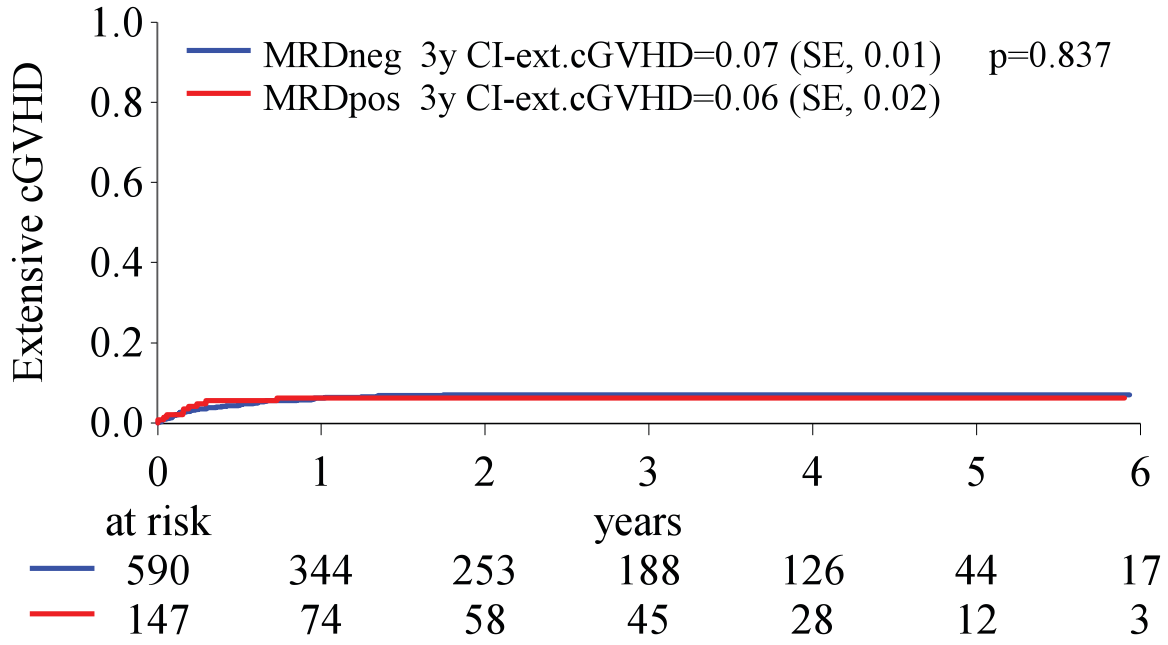
A Panel, Figure S3



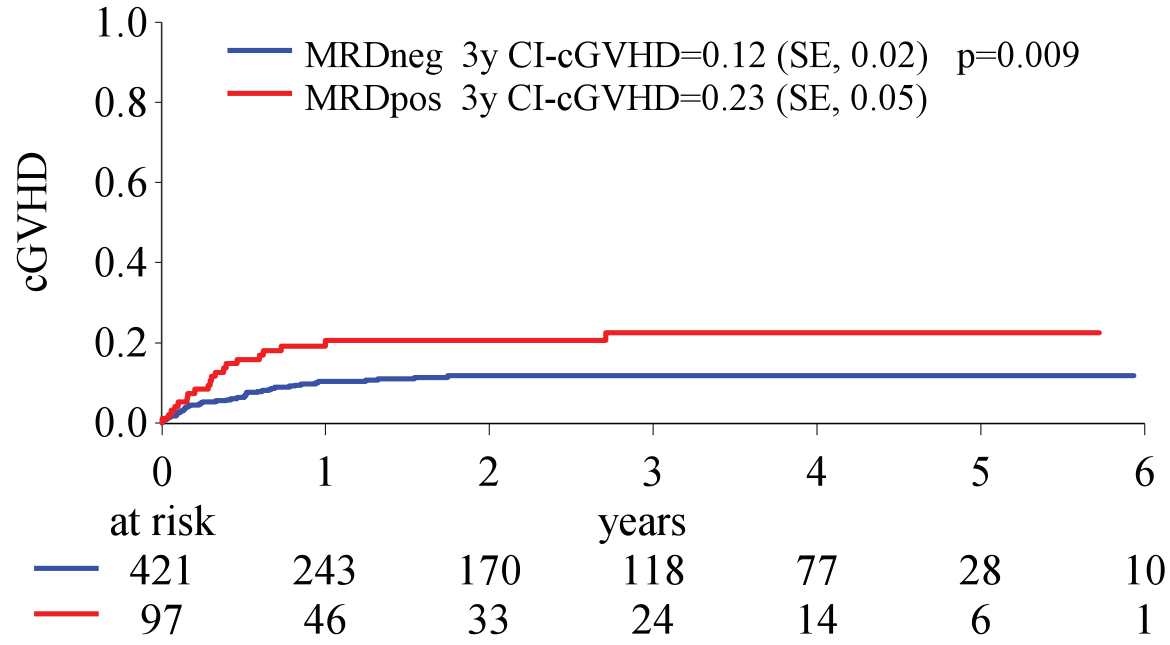
B Panel, Figure S3



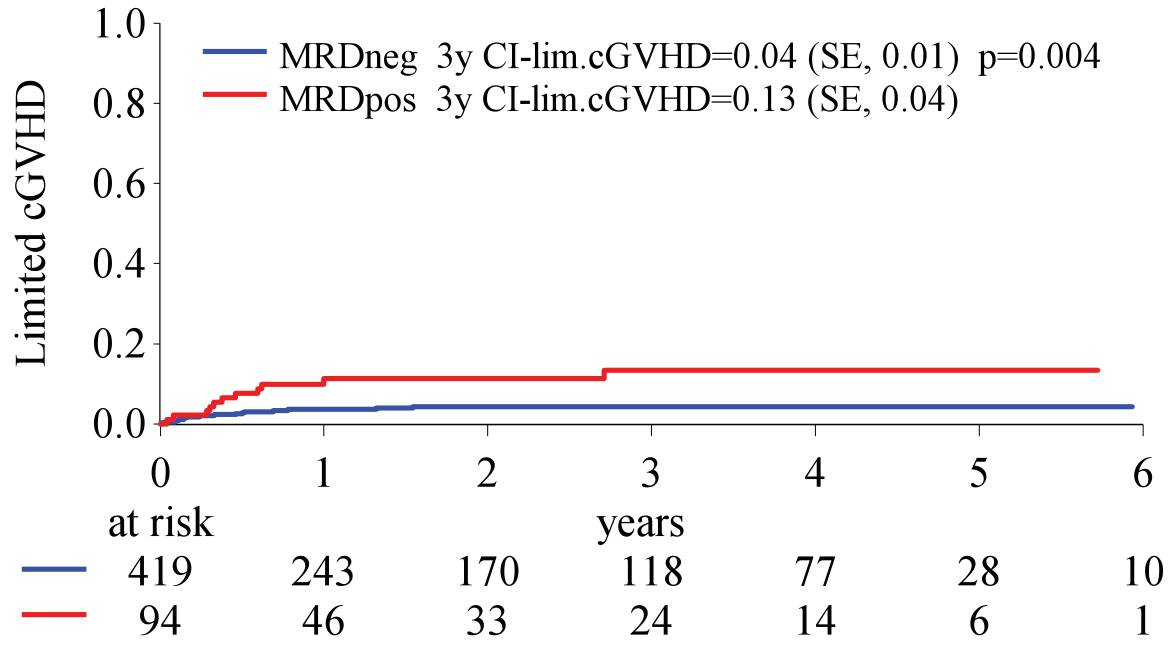
C Panel, Figure S3



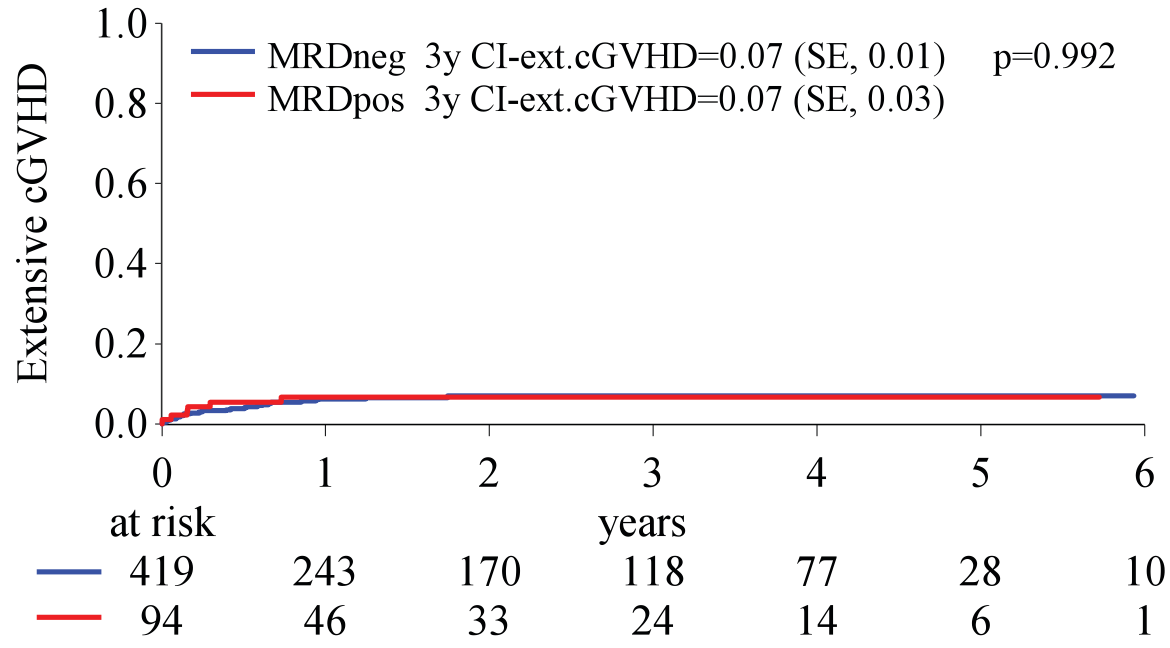
D Panel, Figure S3



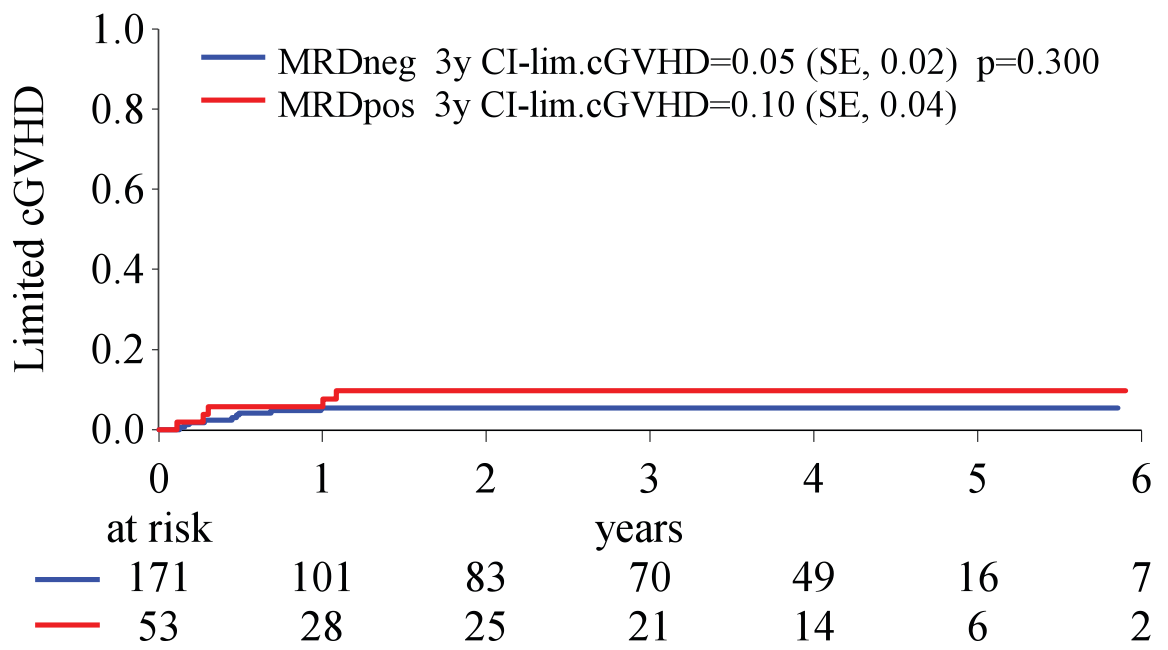
E Panel, Figure S3



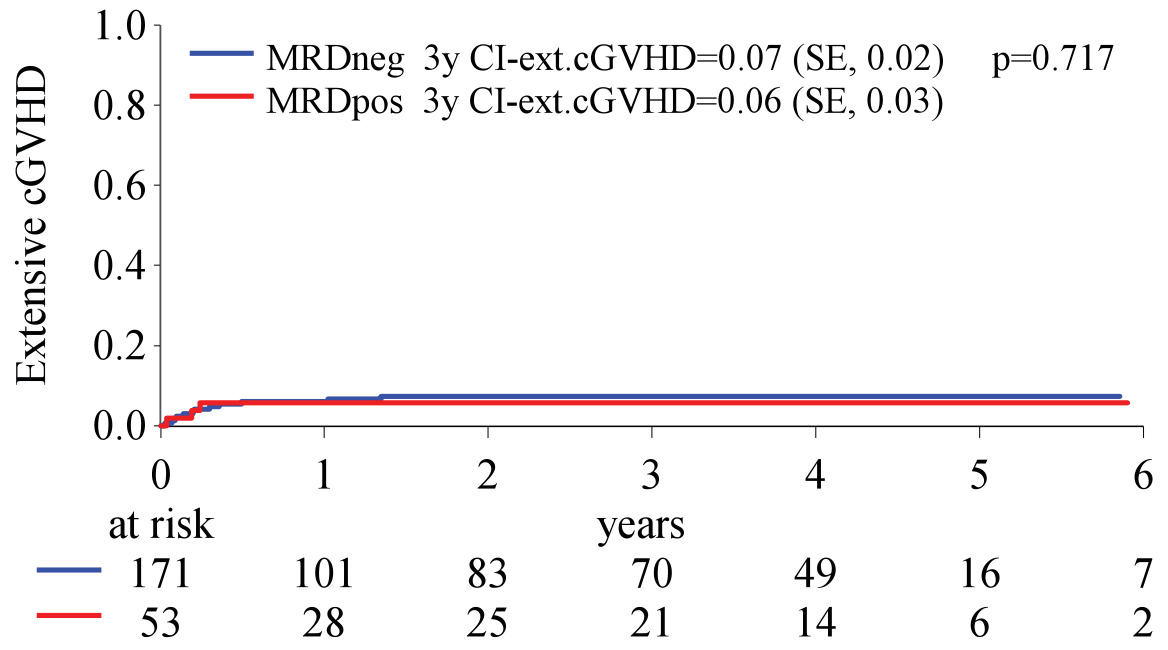
F Panel, Figure S3



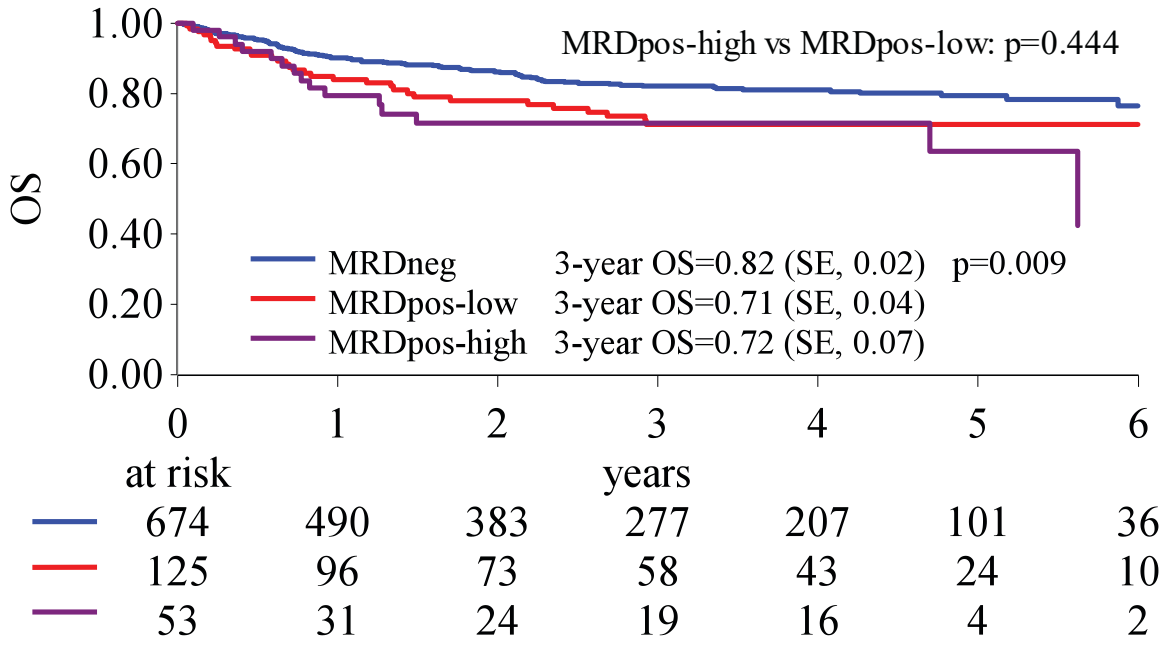
G Panel, Figure S3



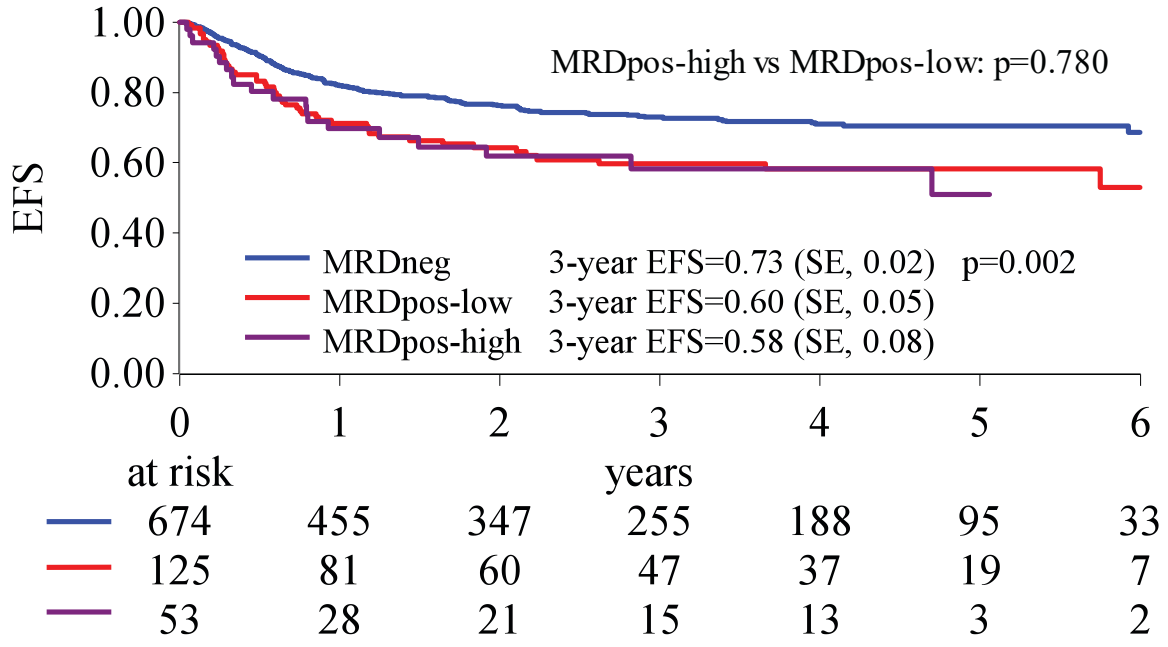
H Panel, Figure S3



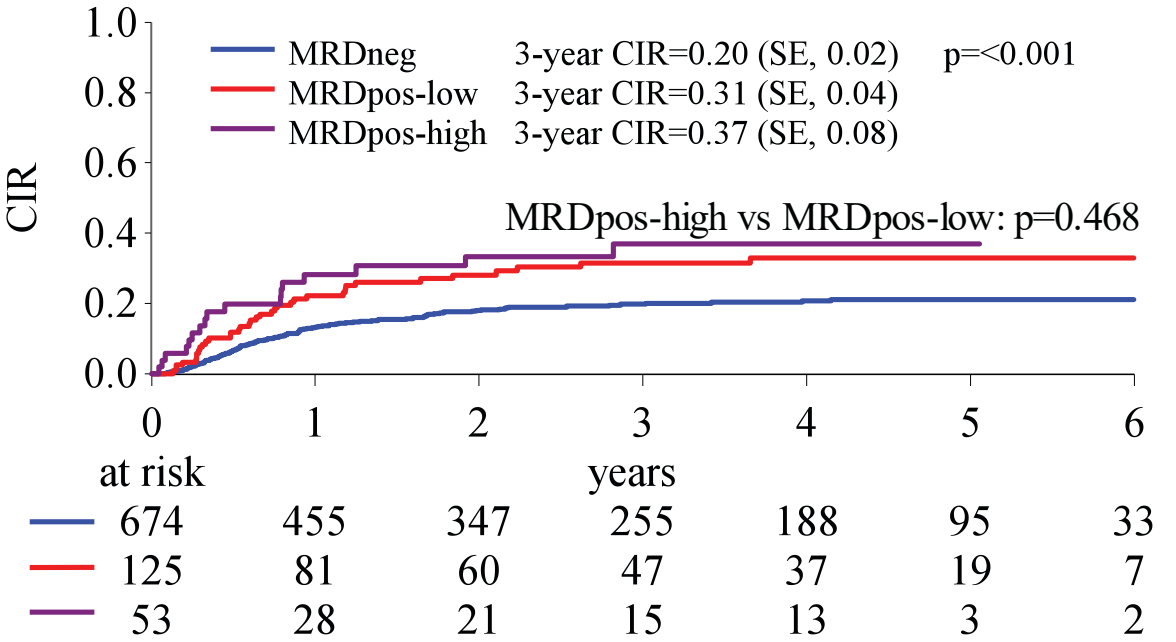
A Panel, Figure S4



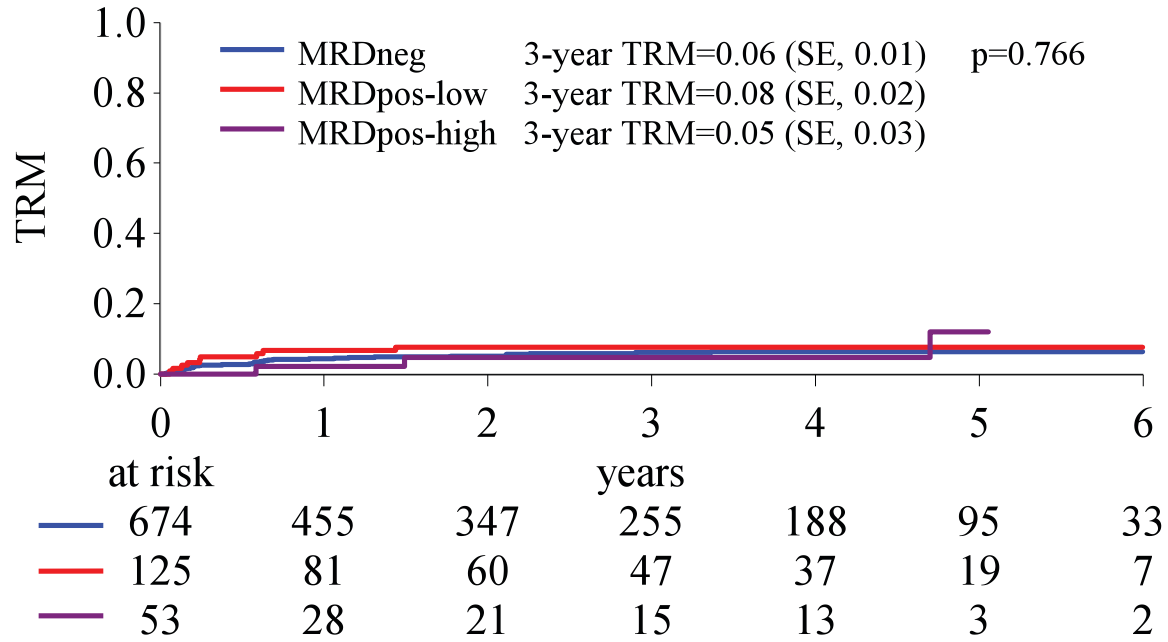
B Panel, Figure S4



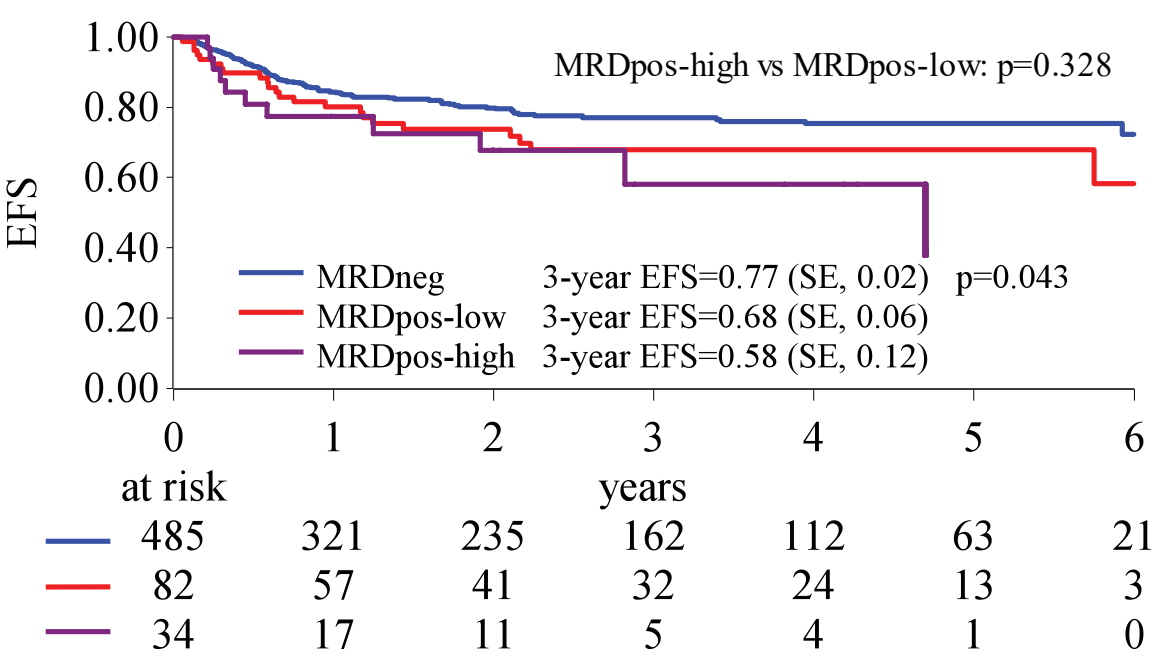
C Panel, Figure S4



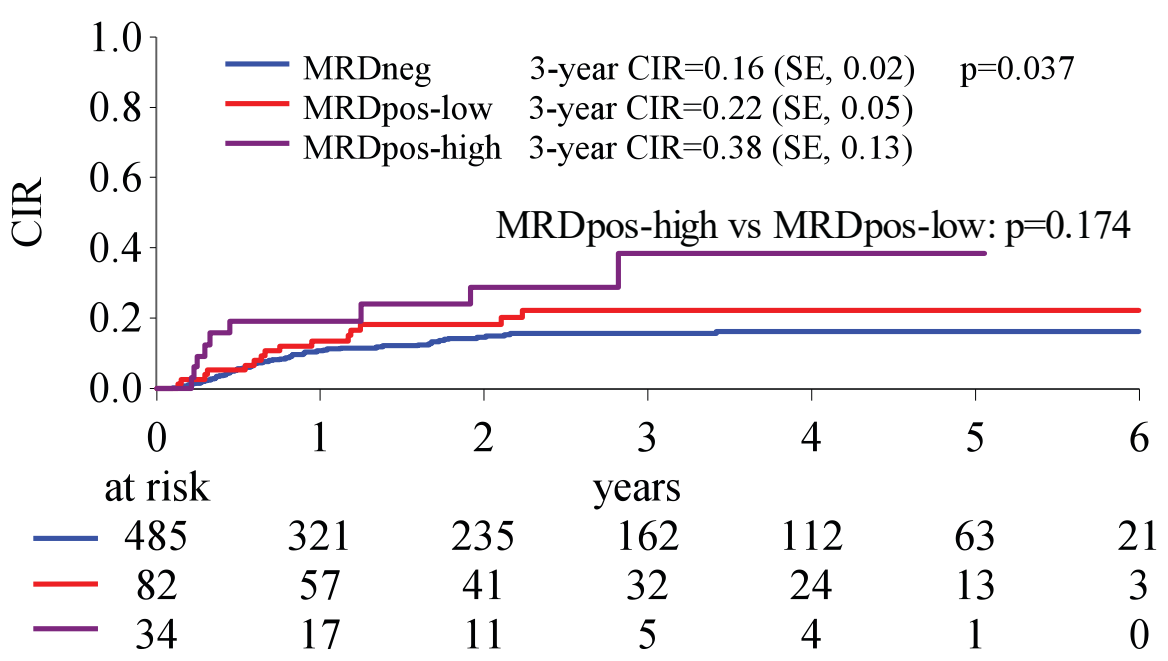
D Panel, Figure S4



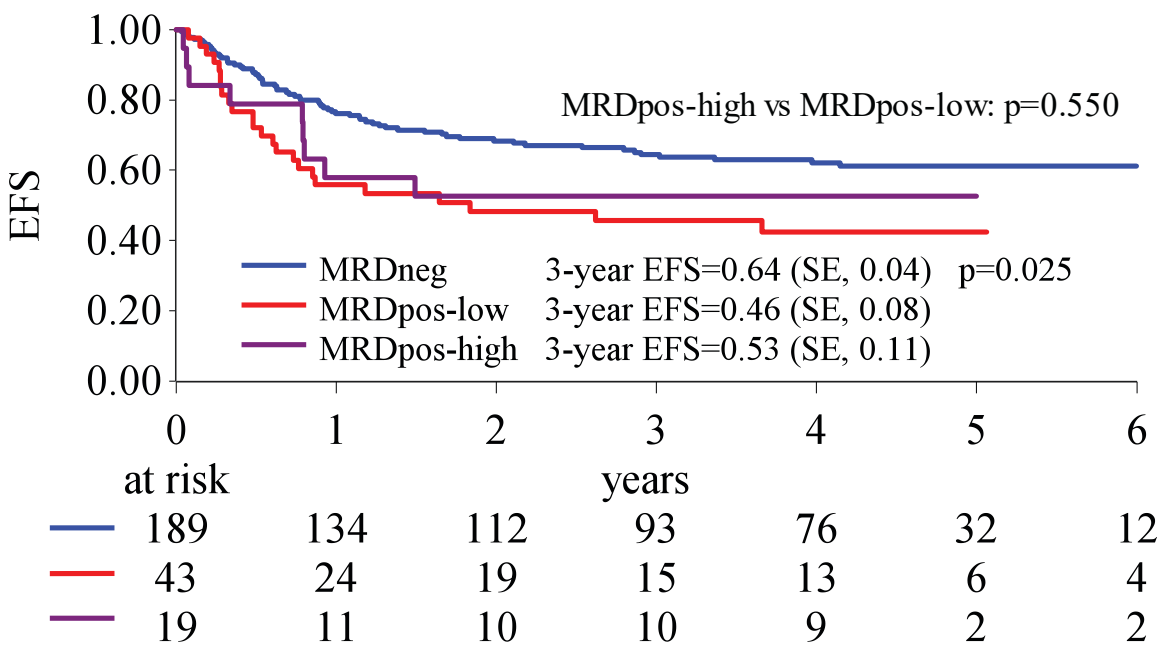
E Panel, Figure S4



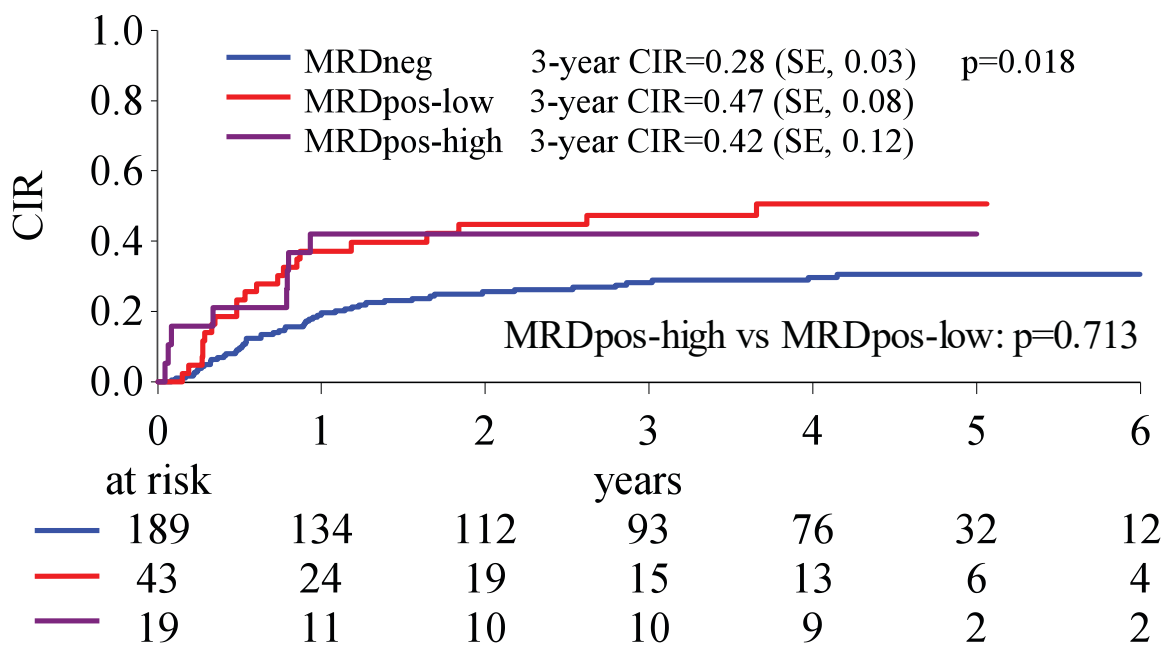
F Panel, Figure S4



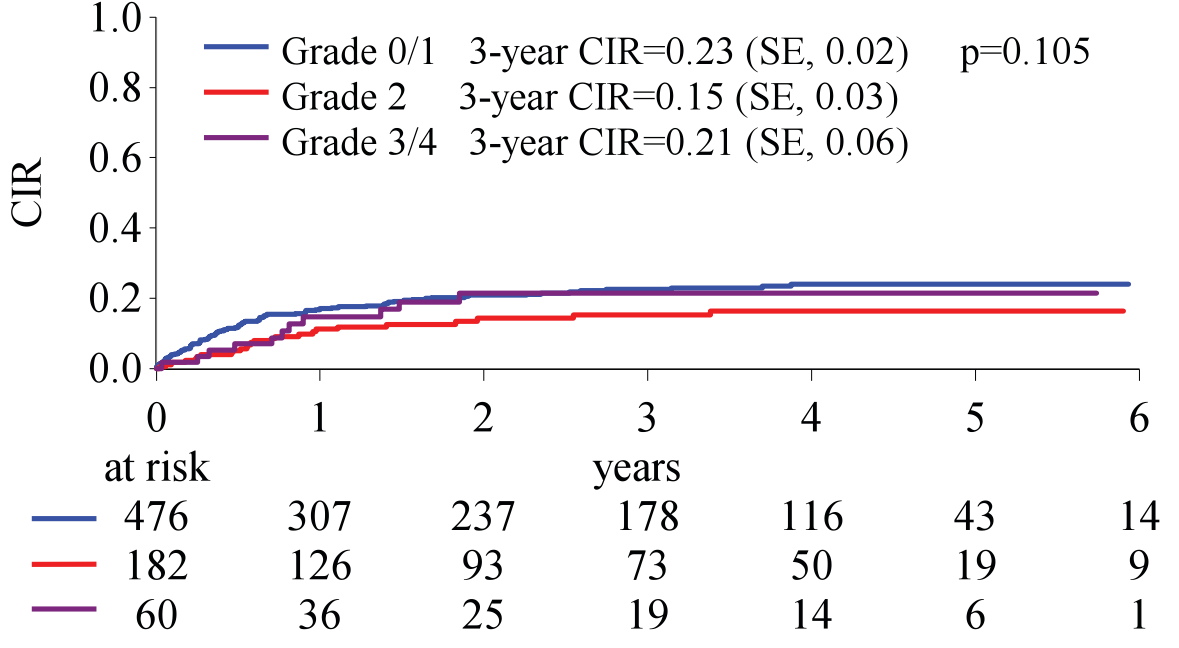
G Panel, Figure S4



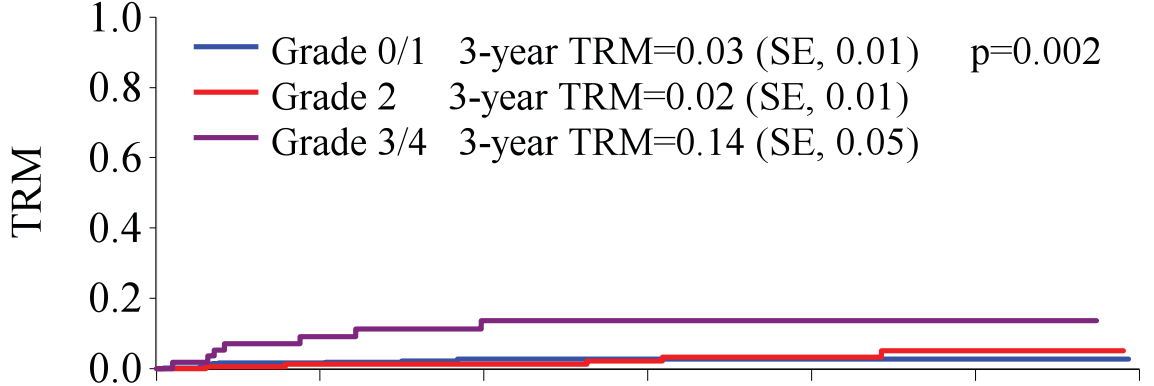
H Panel, Figure S4



A Panel, Figure S5

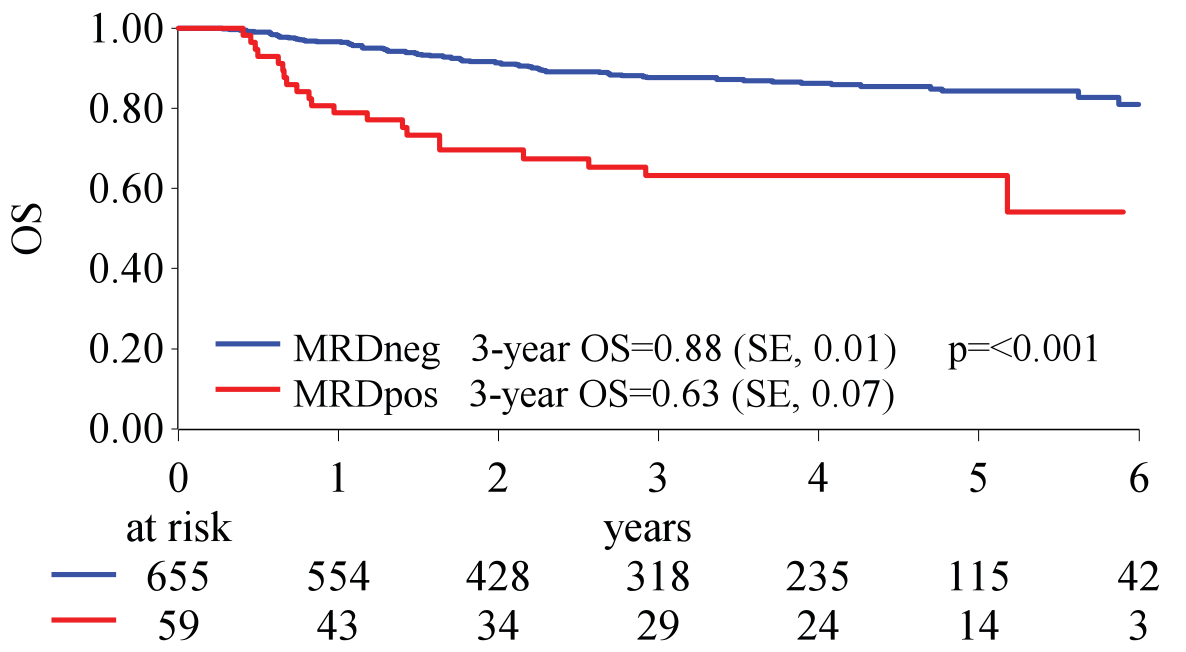


B Panel, Figure S5

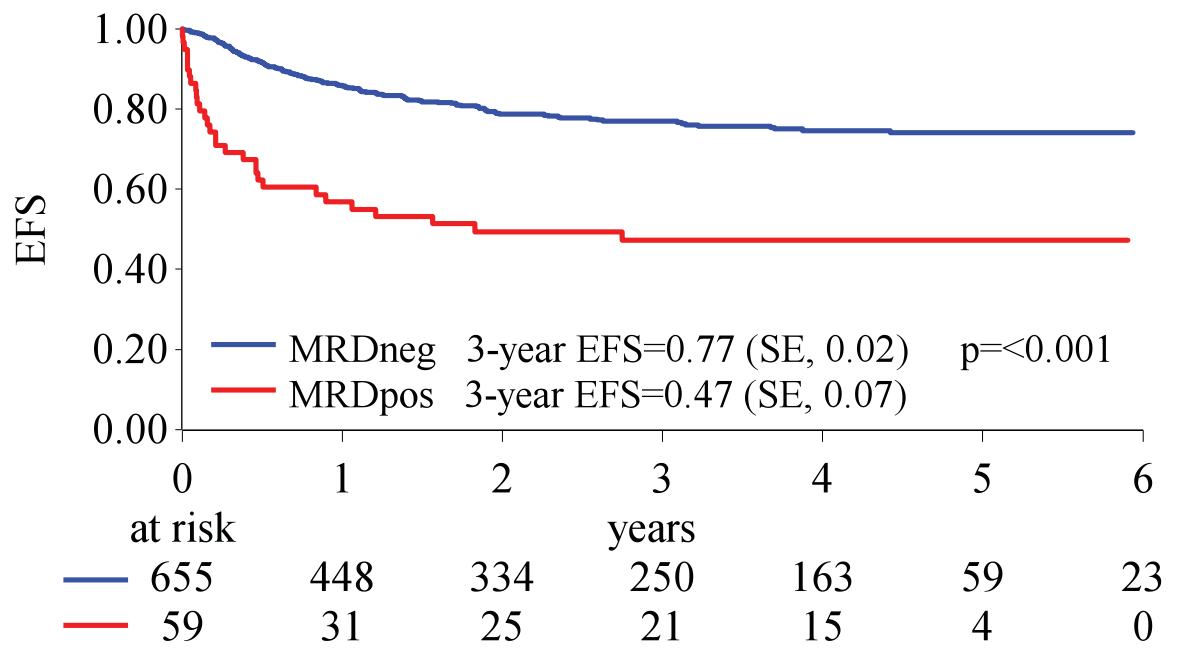


at risk						
476	307	237	178	116	43	14
182	126	93	73	50	19	9
60	36	25	19	14	6	1

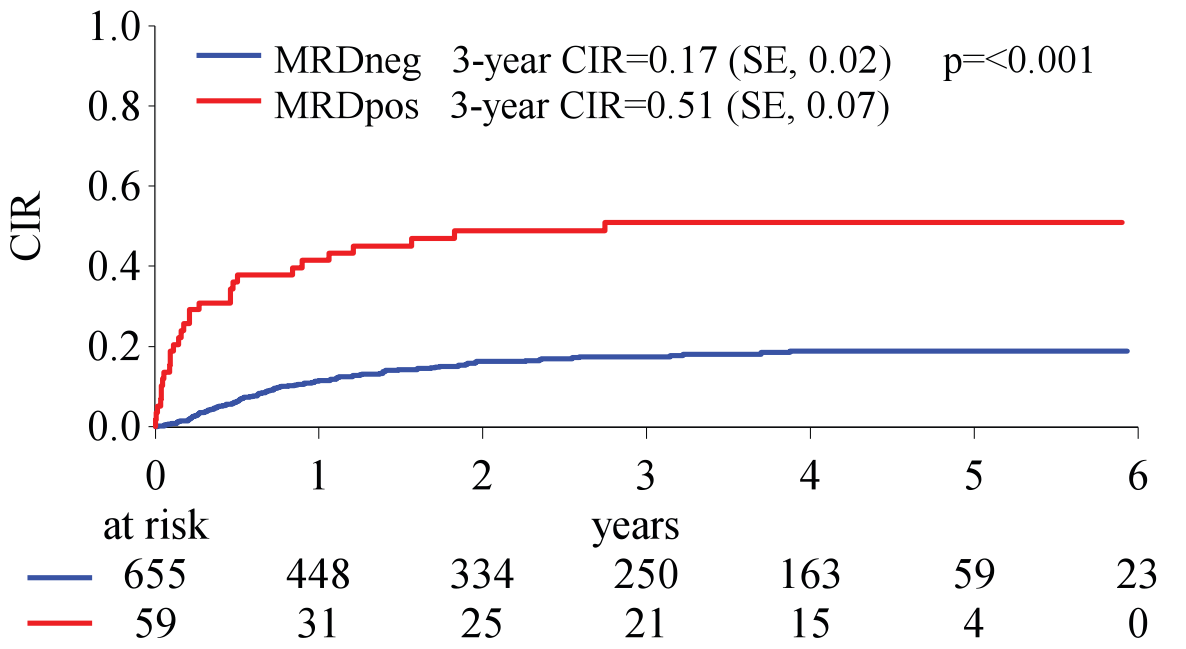
A Panel, Figure S6



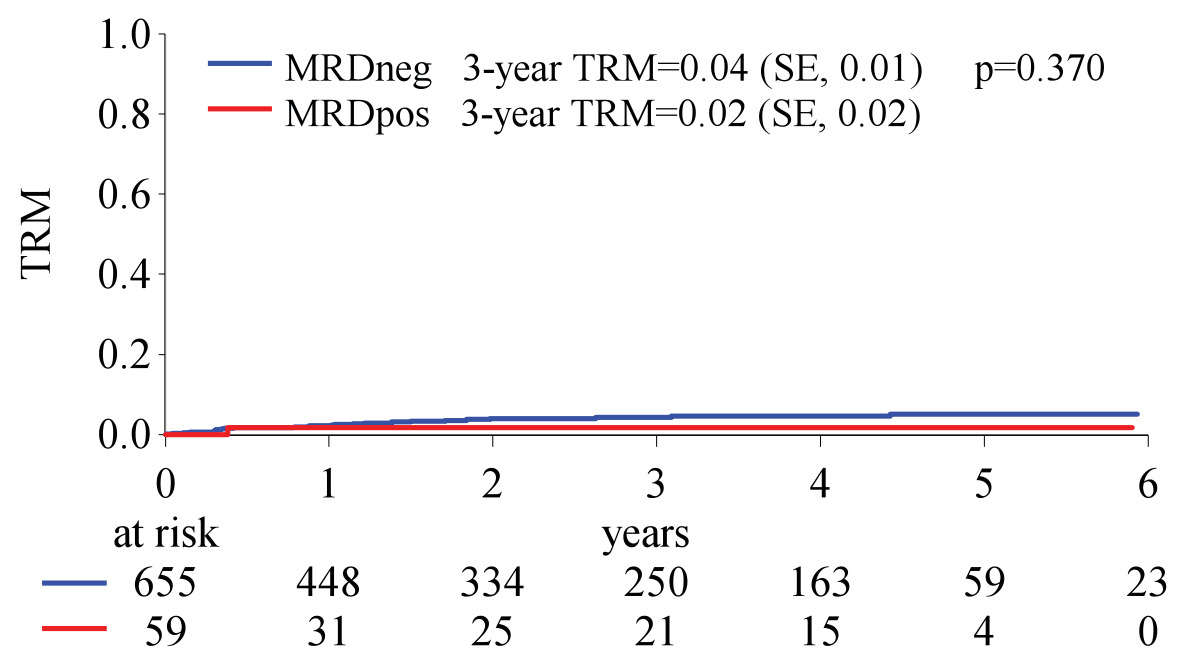
B Panel, Figure S6



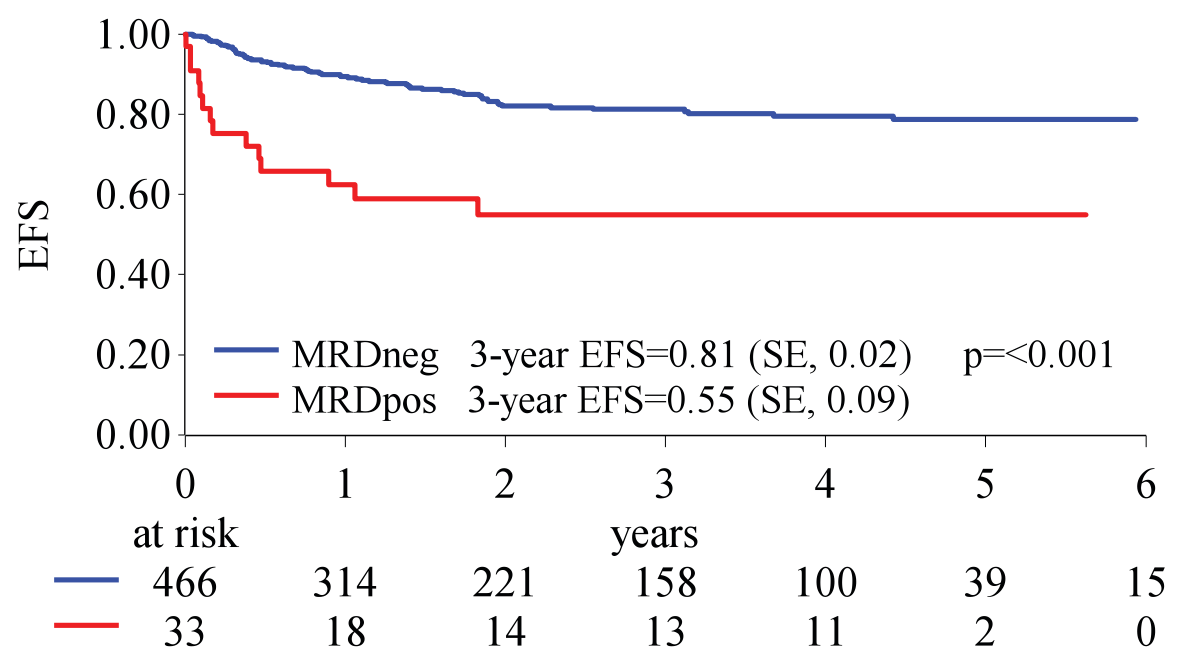
C Panel, Figure S6



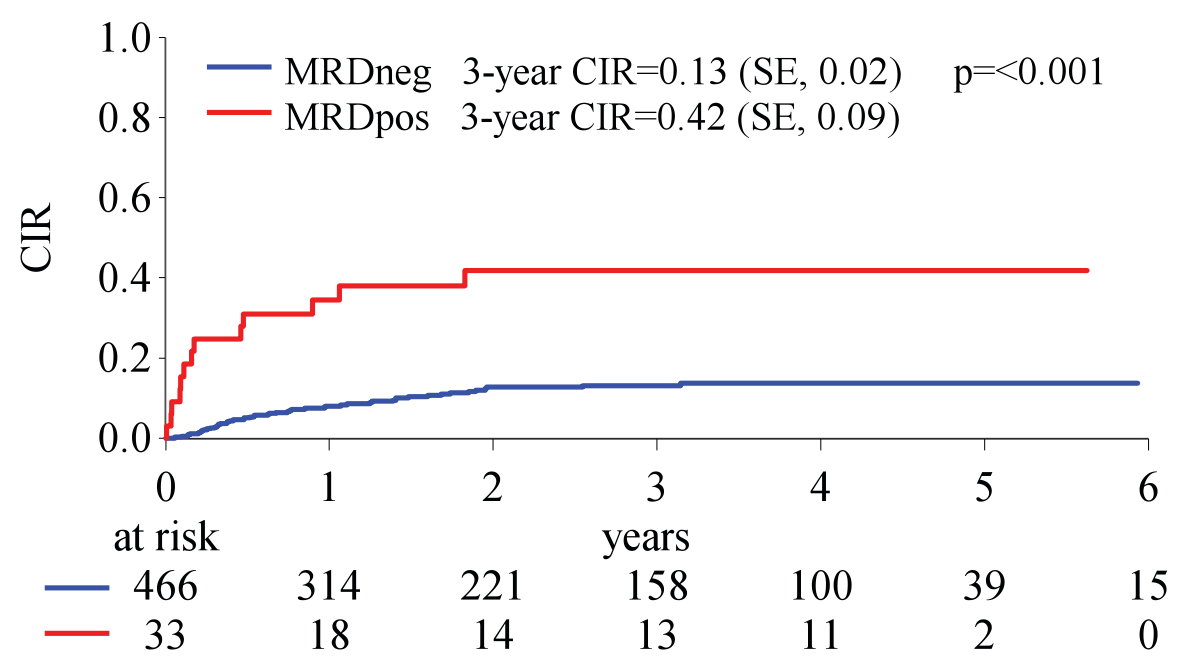
D Panel, Figure S6



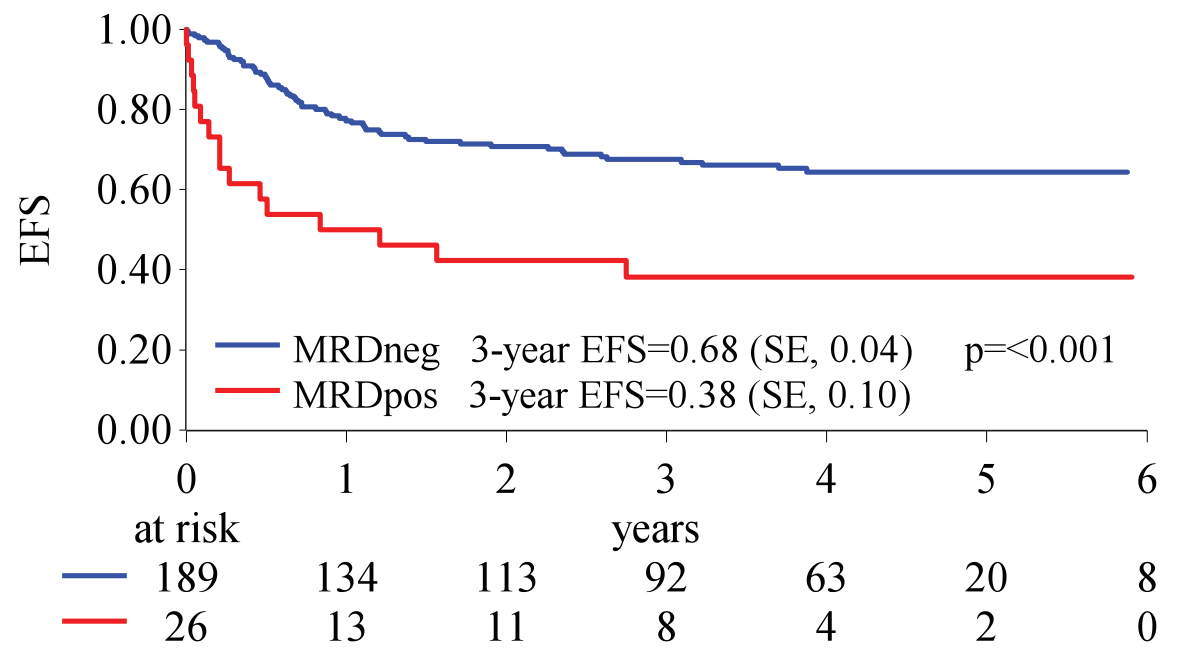
E Panel, Figure S6



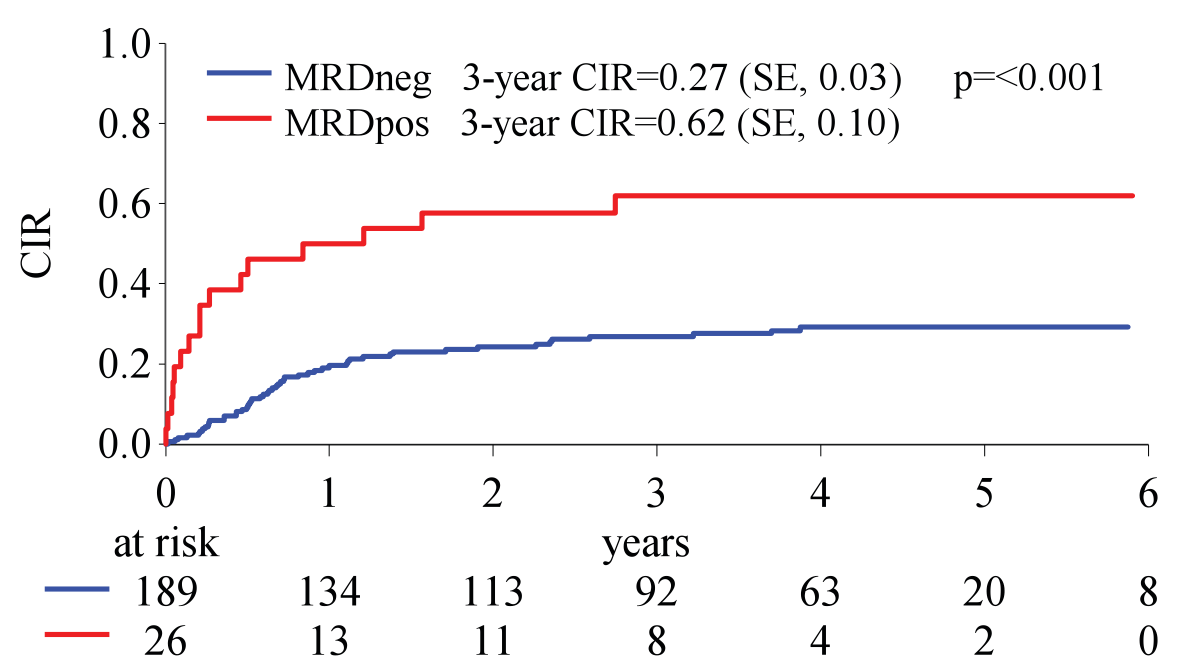
F Panel, Figure S6



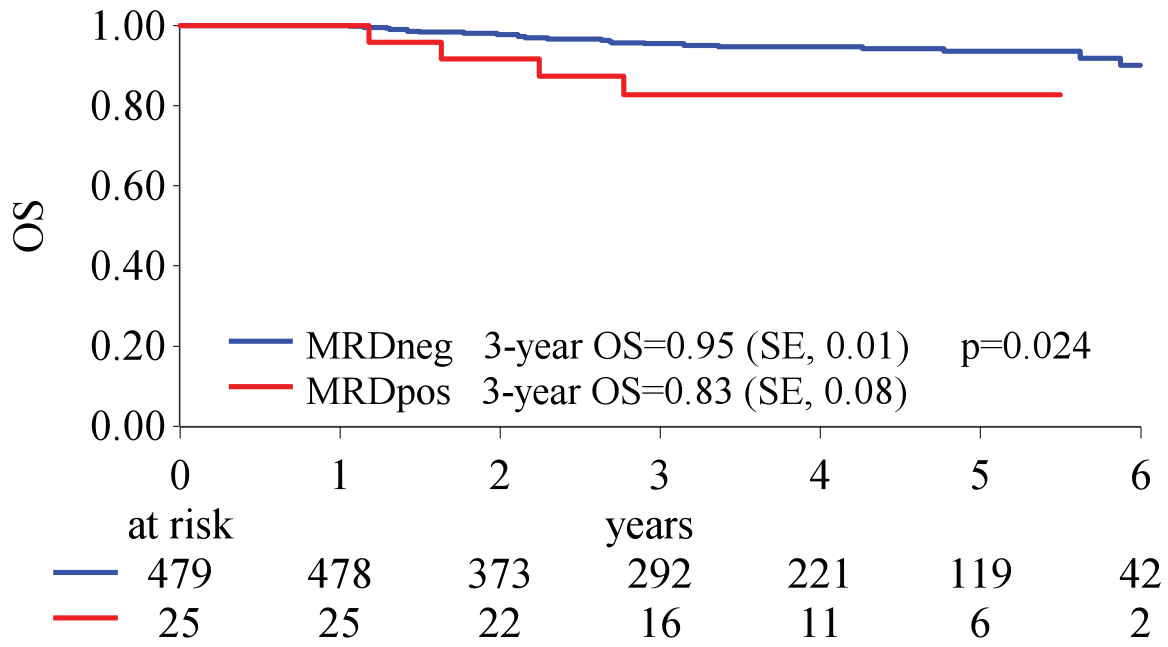
G Panel, Figure S6



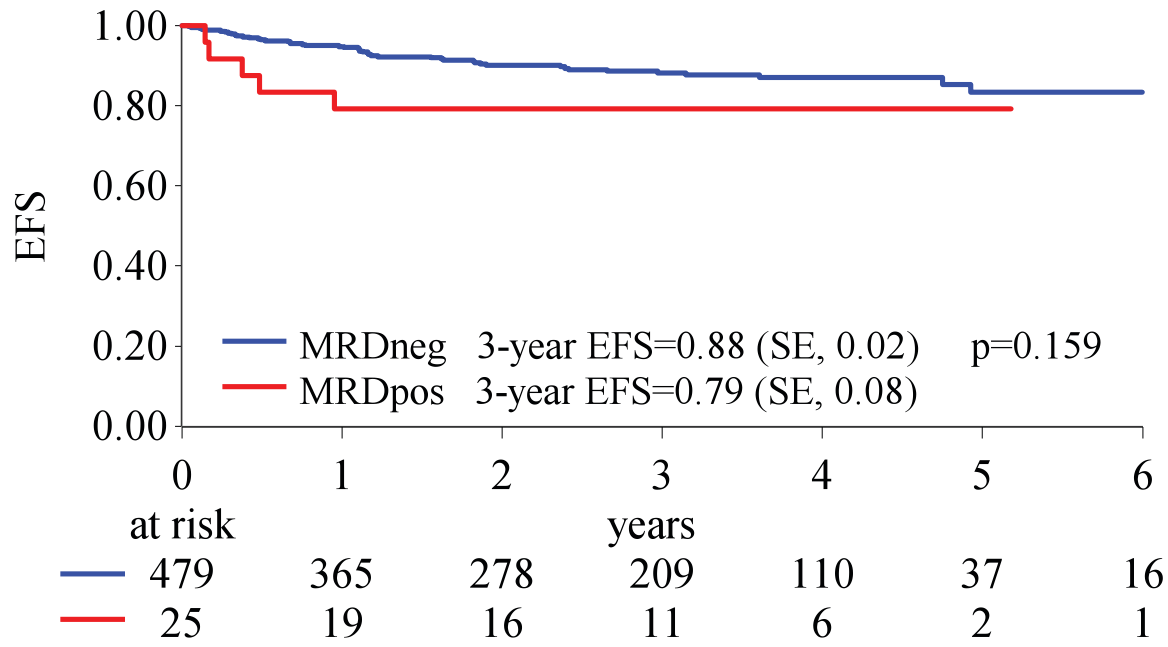
H Panel, Figure S6



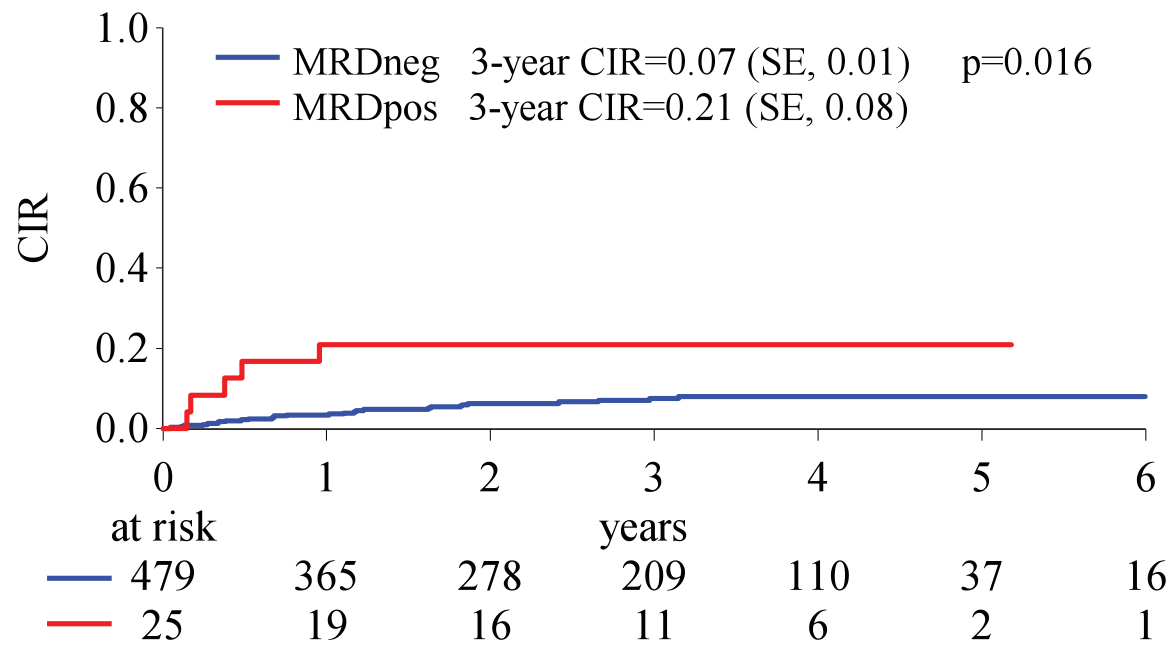
A Panel, Figure S7



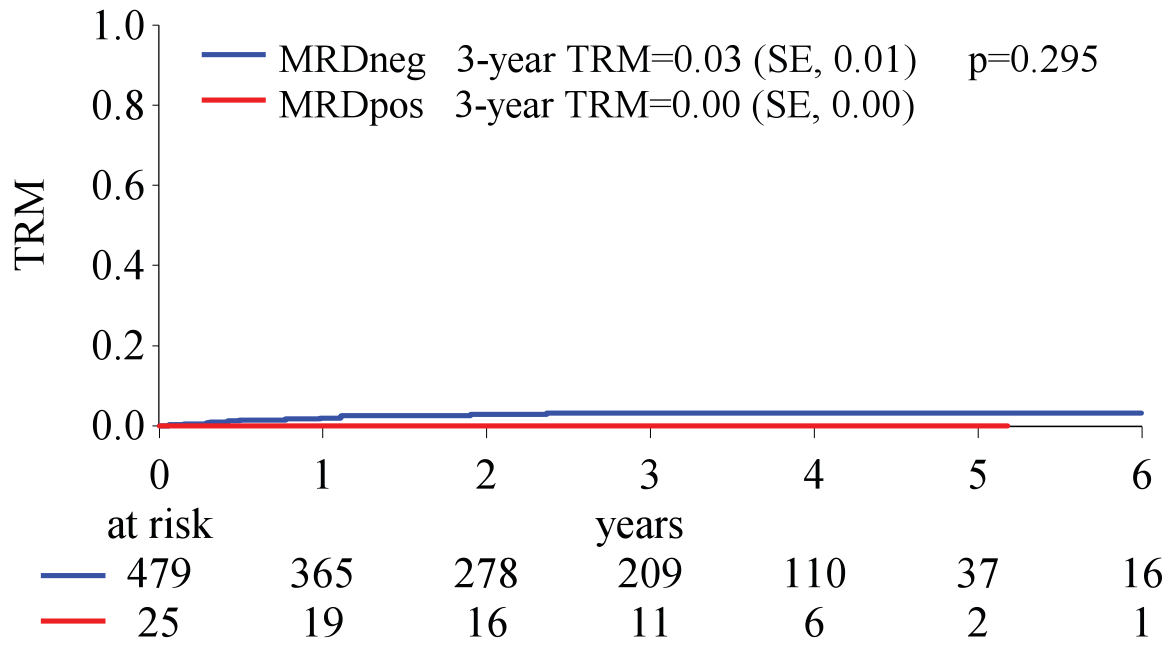
B Panel, Figure S7



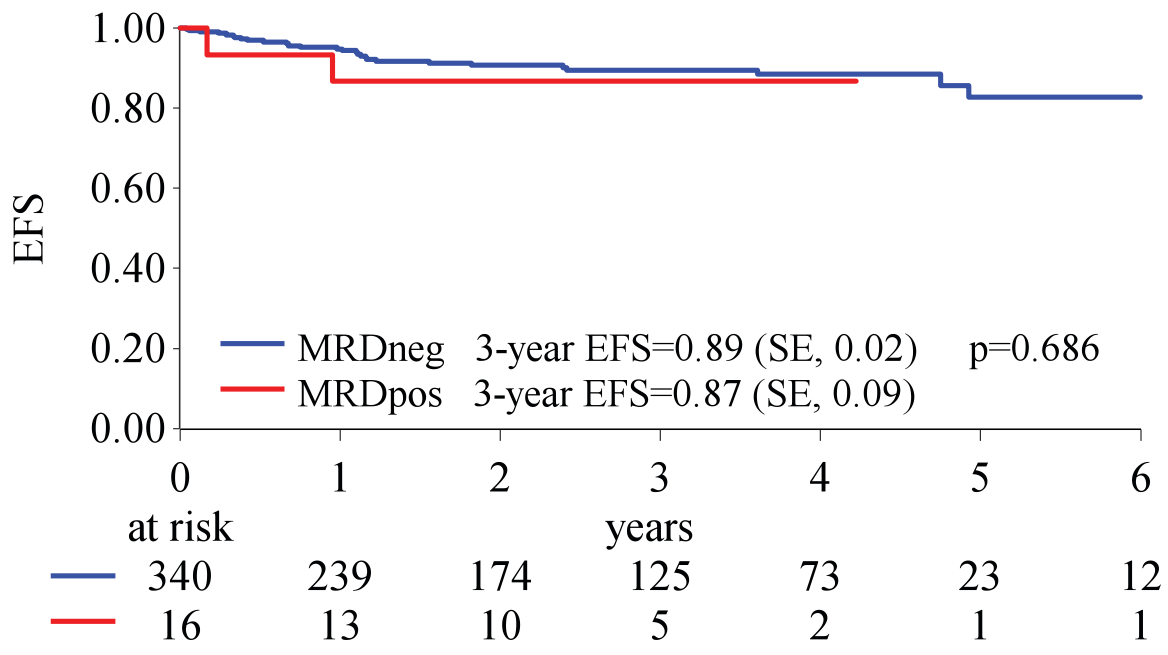
C Panel, Figure S7



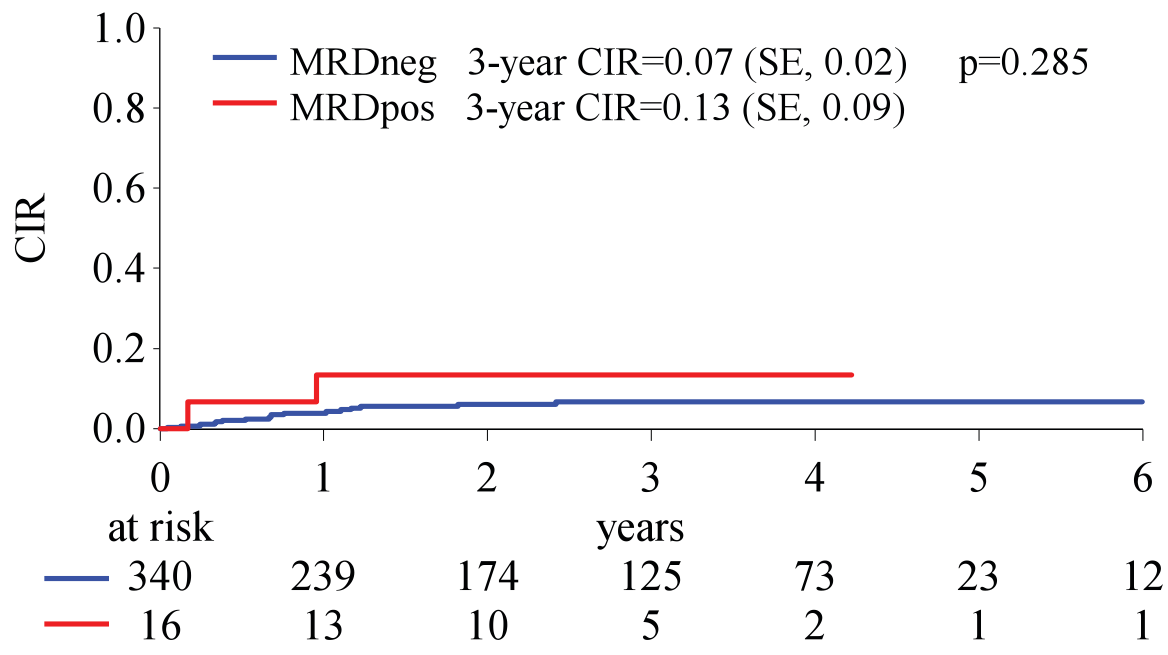
D Panel, Figure S7



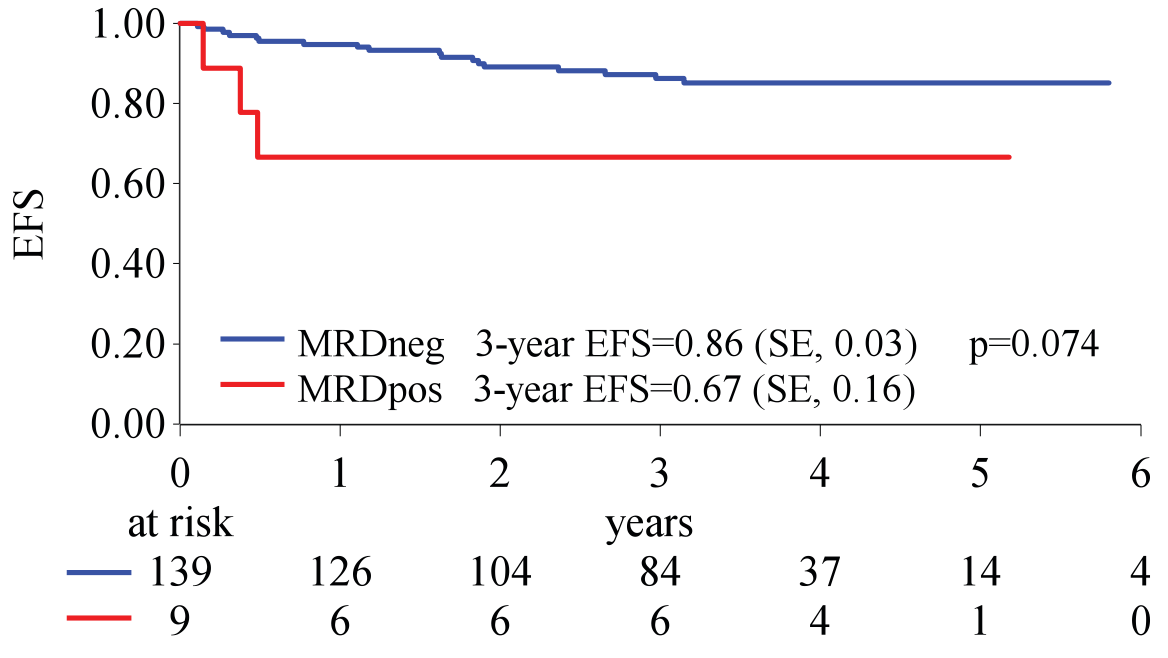
E Panel, Figure S7



F Panel, Figure S7



G Panel, Figure S7



H Panel, Figure S7