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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Received: January 25, 2025.
Accepted: July 30, 2025.
Early view: August 7, 2025.

https://doi.org/10.3324/haematol.2025.287456

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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.

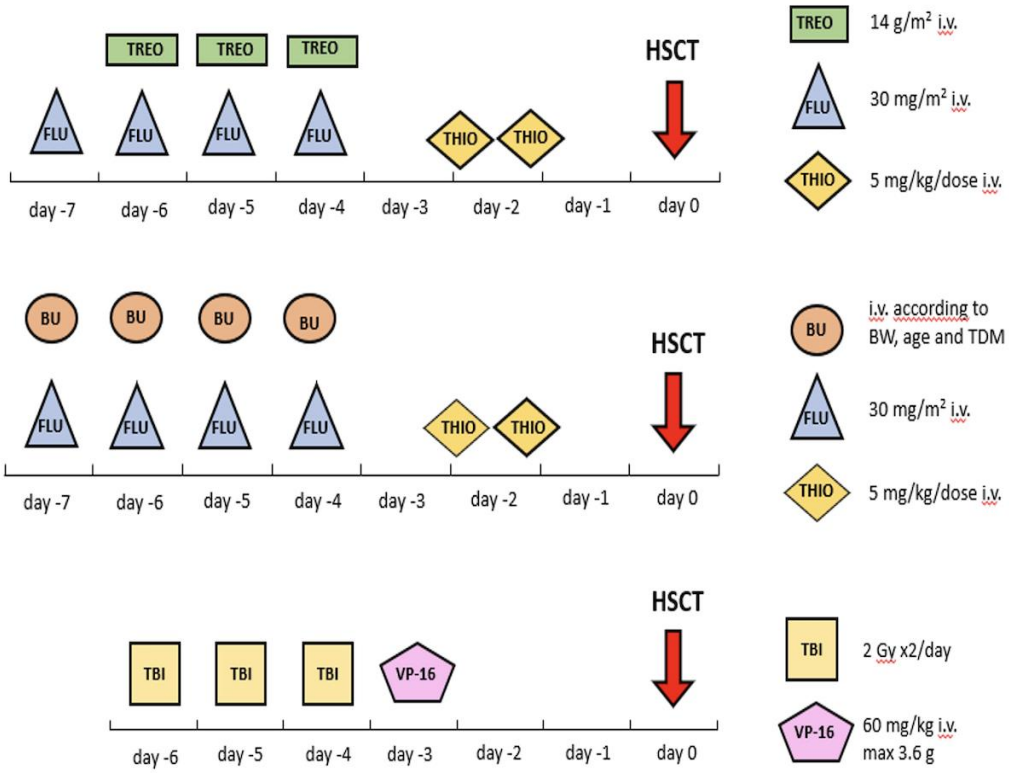
5. Kaplan, E.L. and Meier, P. Nonparametric Estimation from Incomplete Observations. Journal of the American Statistical Association, 1958; 53, 457-481.

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

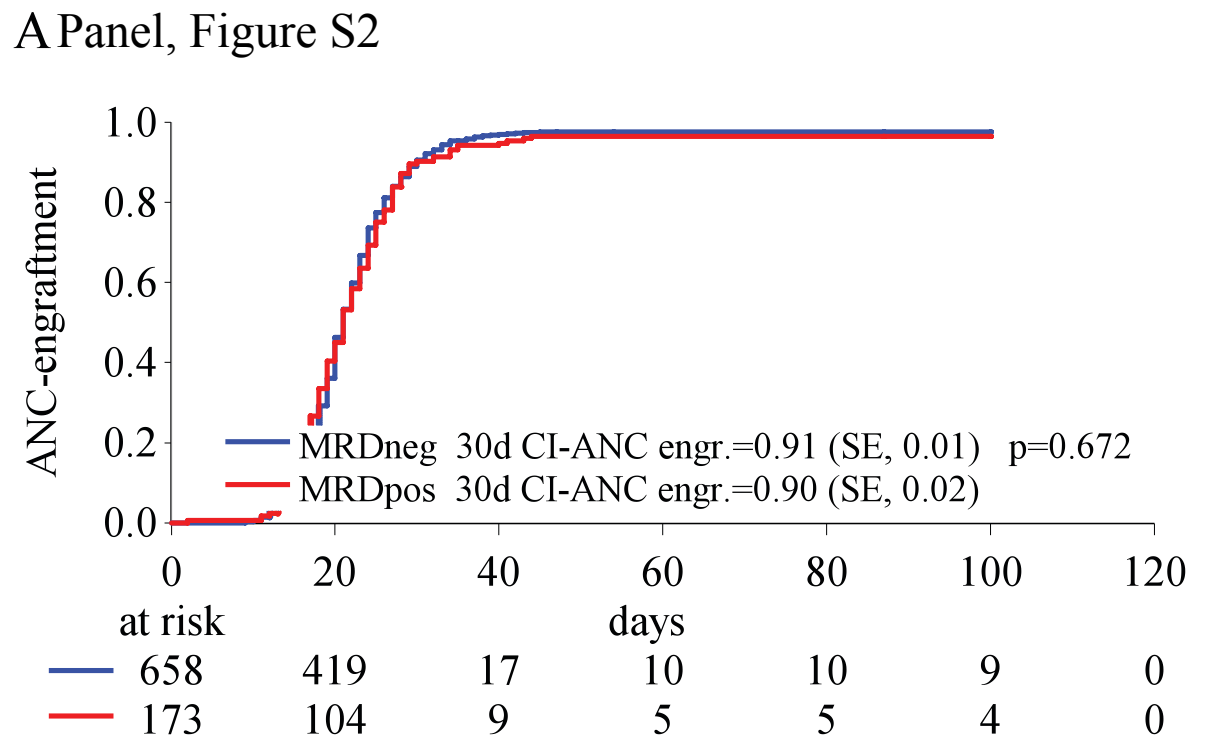
Risk factor	Subgroup	OS								EFS							
		MRD_{neg} (neg, NQ, $<10^{-4}$)			MRD_{pos} ($\geq 10^{-4}$)					MRD_{neg} (neg, NQ, $<10^{-4}$)			MRD_{pos} ($\geq 10^{-4}$)				
		N	events	3-year pOS	N	events	3-year pOS	*p-value*	3-year pOS	*p-value*	N	events	3-year pEFS	N	events	3-year pEFS	*p-value*	3-year 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

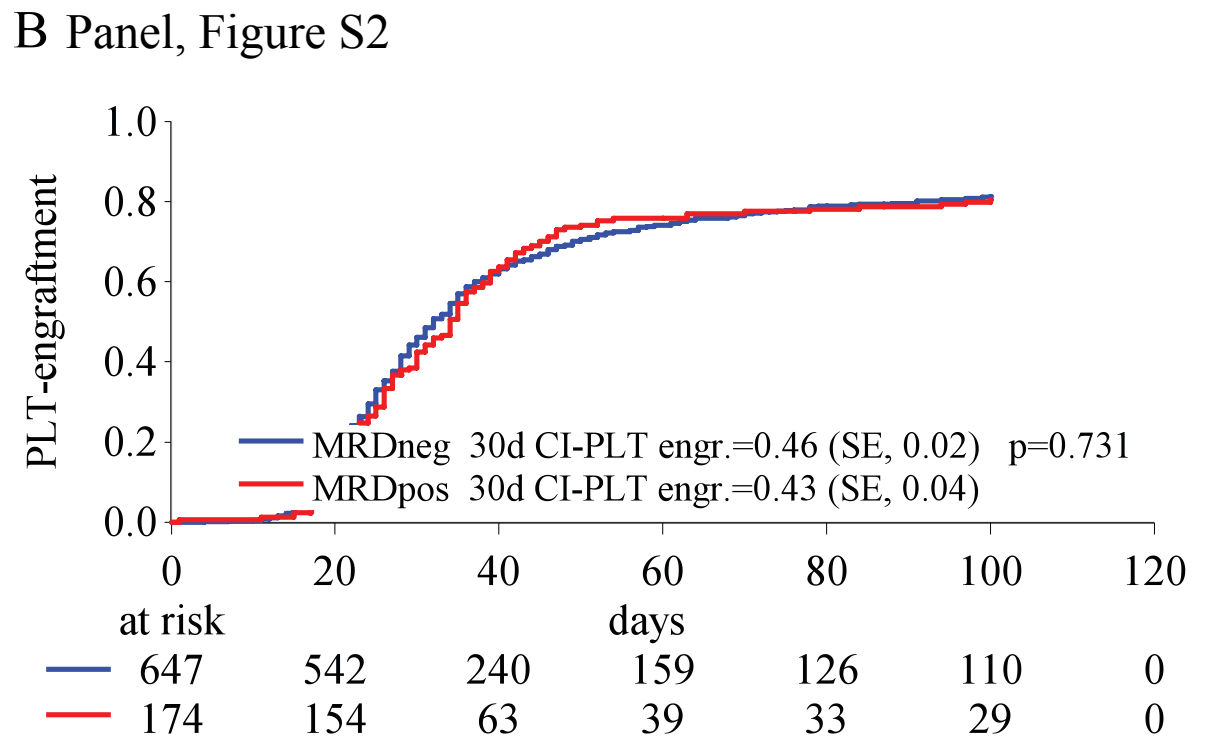
Risk factor	Subgroup	CI of relapse									CI of TRM							
		MRD_{neg} (neg, NQ, $<10^{-4}$)			MRD_{pos} ($\geq 10^{-4}$)						MRD_{neg} (neg, NQ, $<10^{-4}$)			MRD_{pos} ($\geq 10^{-4}$)				
		N	events	3-year CI of relapse	N	events	3-year CI of relapse	*p-value*	3-year CI of relapse	*p-value*	N	events	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.

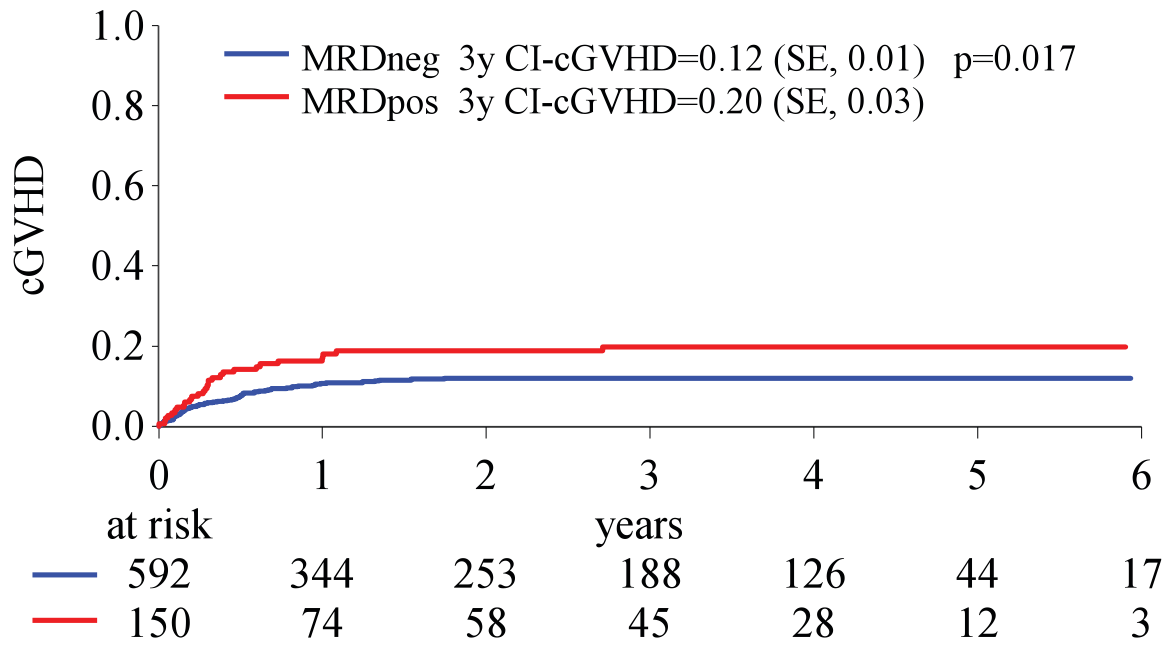
A Panel, Figure S2



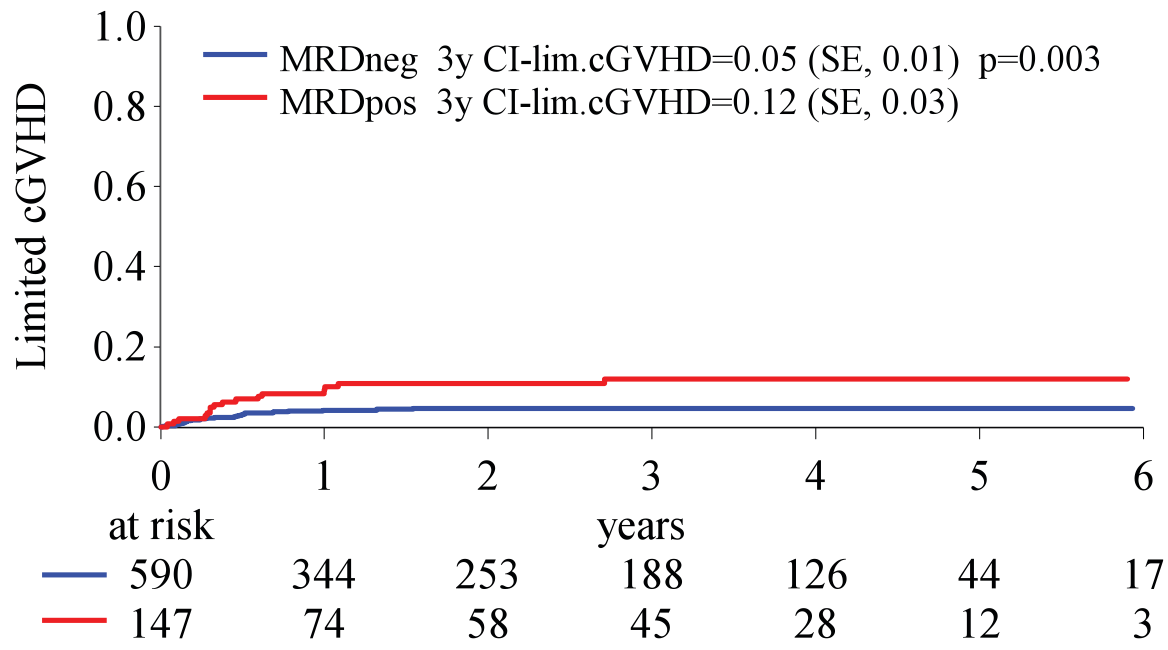
B Panel, Figure S2



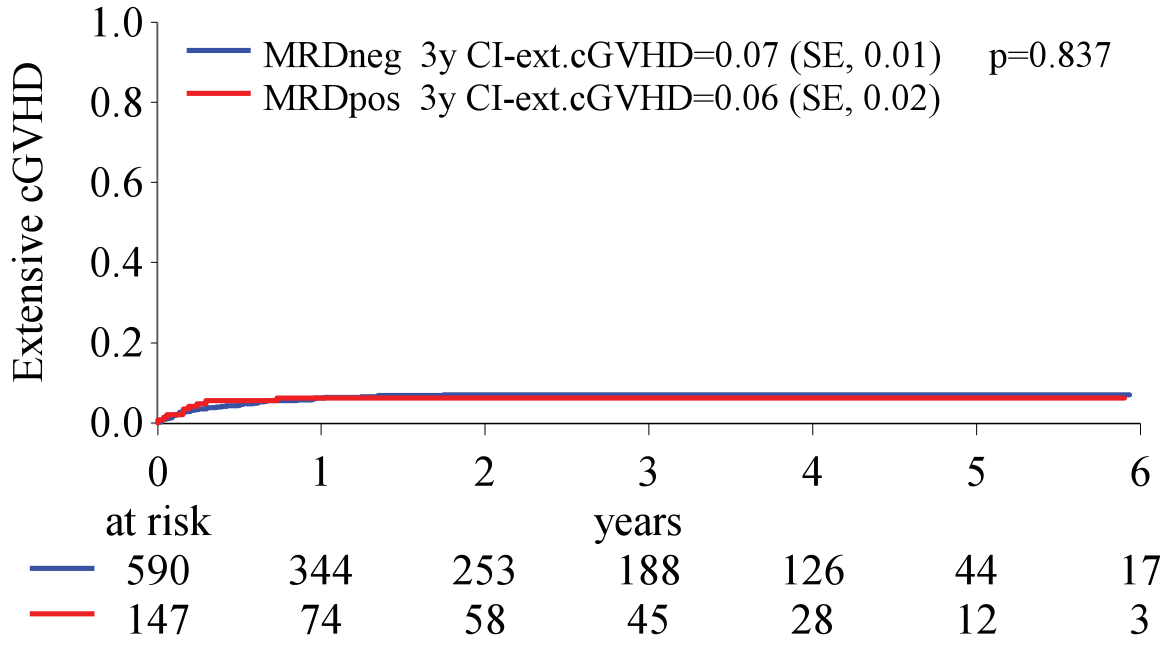
A Panel, Figure S3



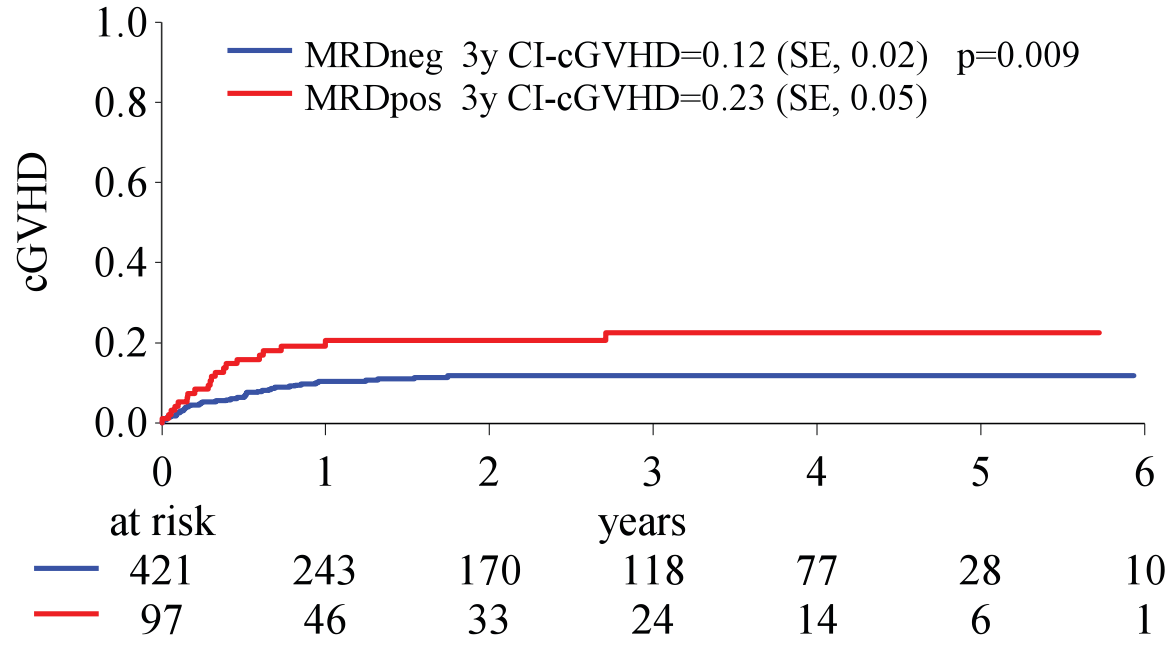
B Panel, Figure S3



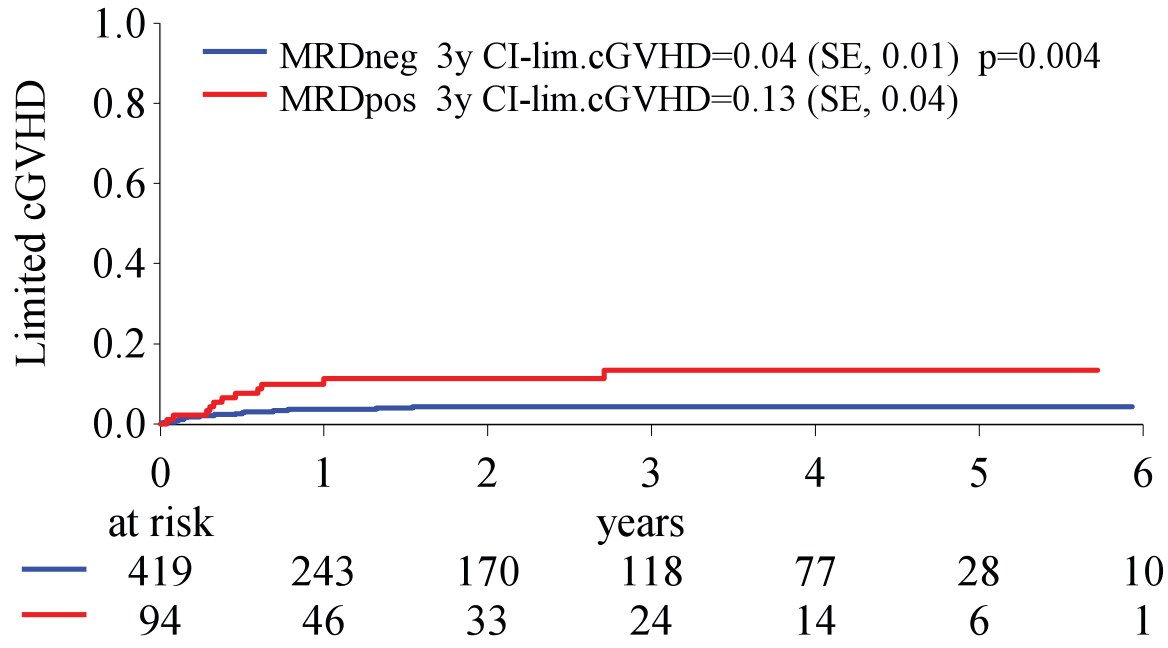
C Panel, Figure S3



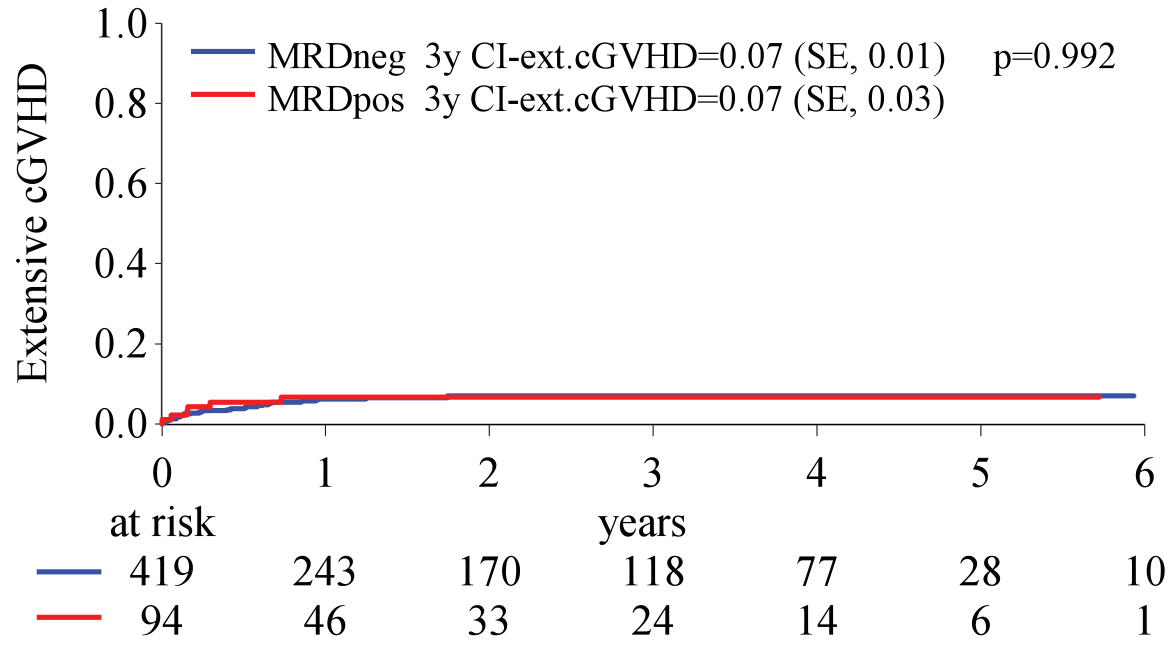
D Panel, Figure S3



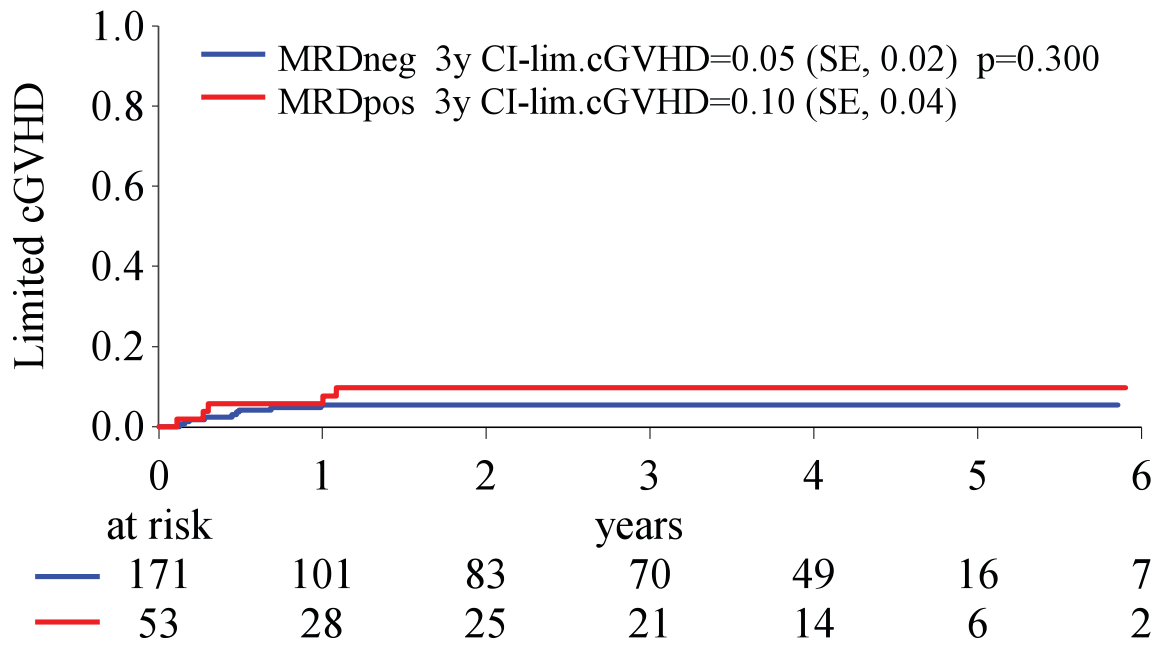
E Panel, Figure S3



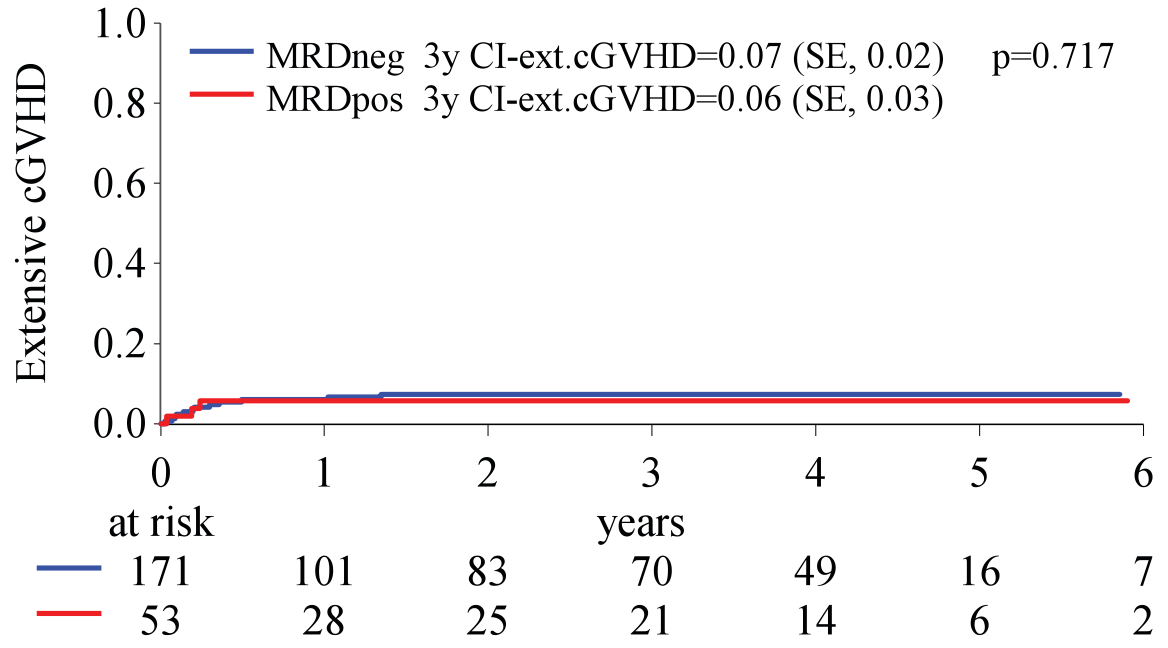
F Panel, Figure S3



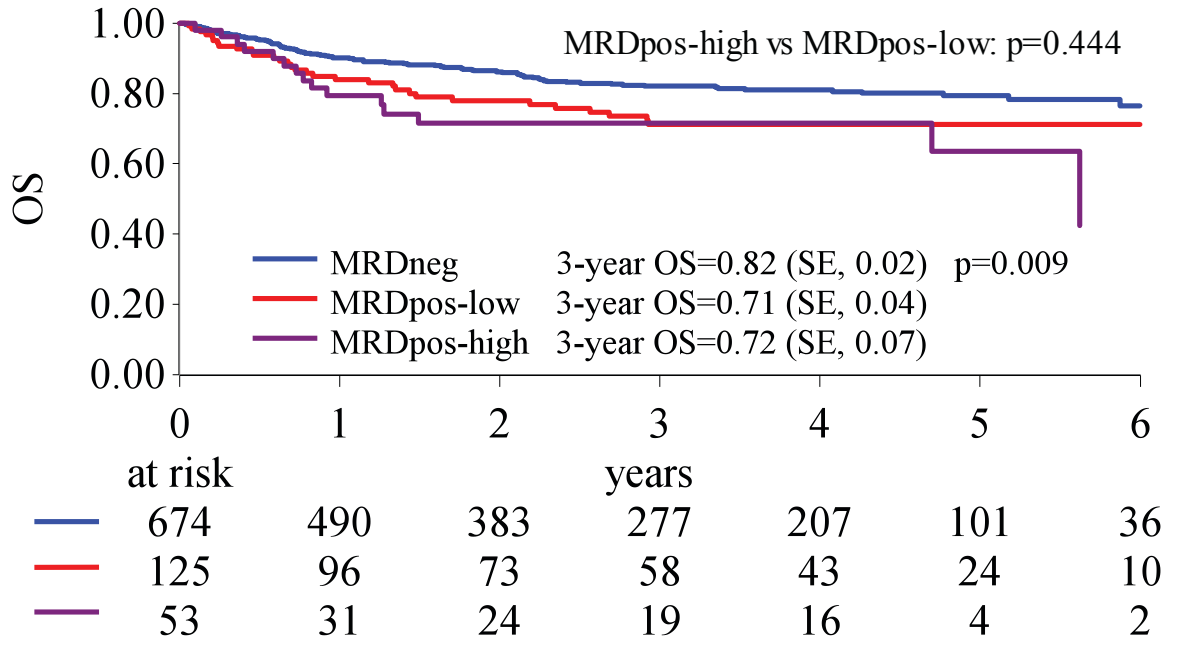
G Panel, Figure S3



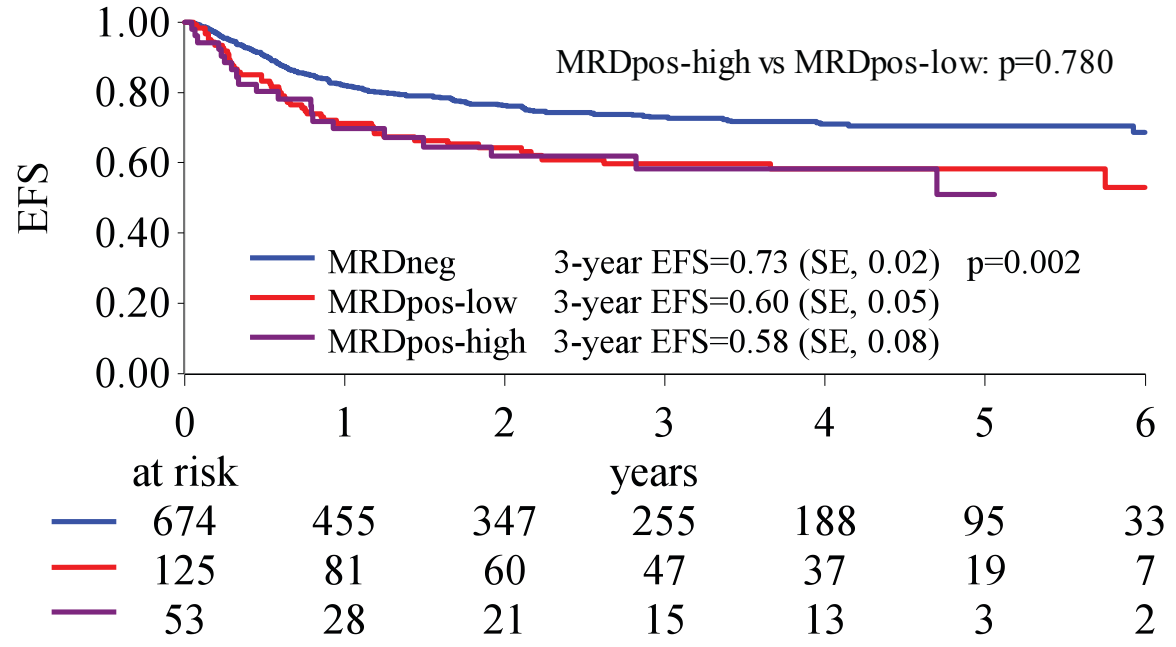
H Panel, Figure S3



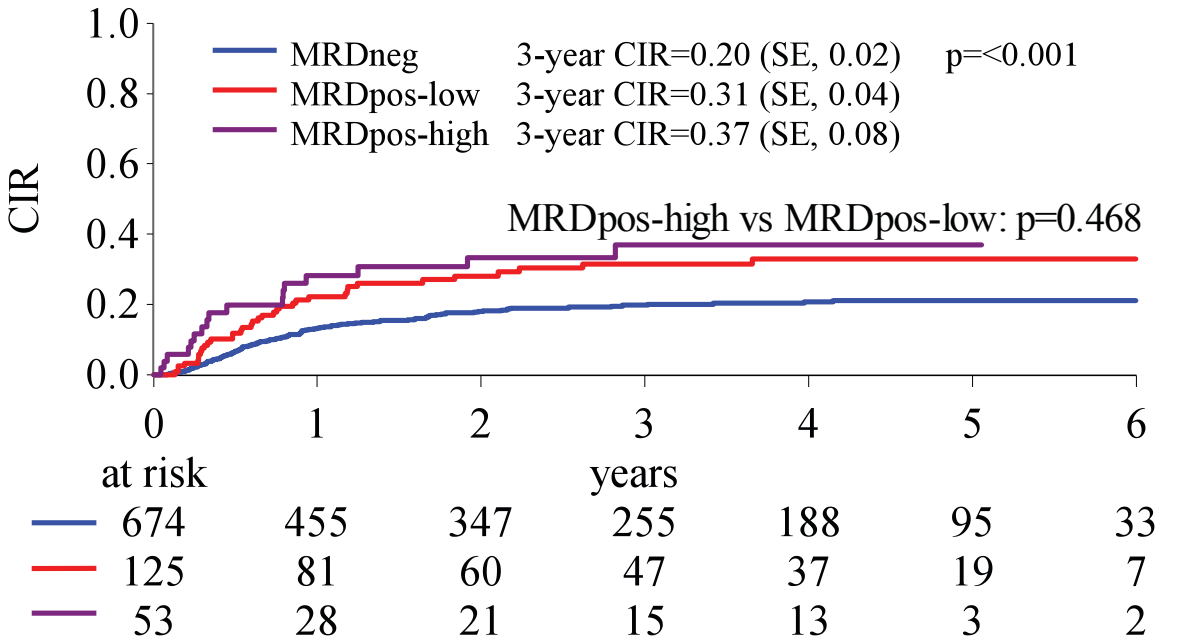
A Panel, Figure S4



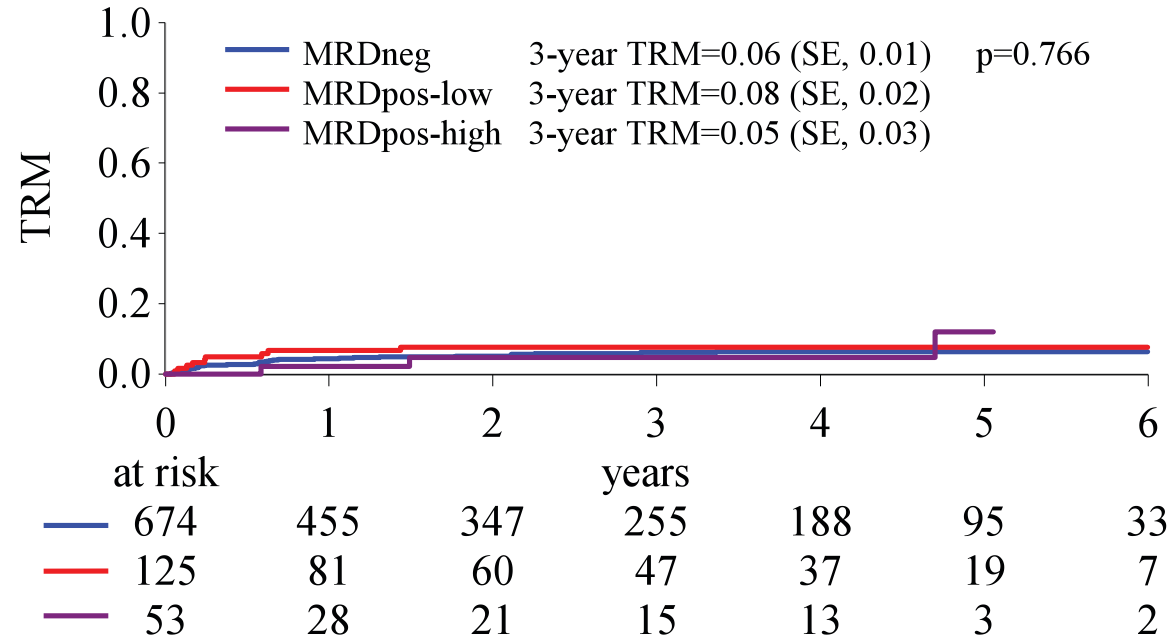
B Panel, Figure S4



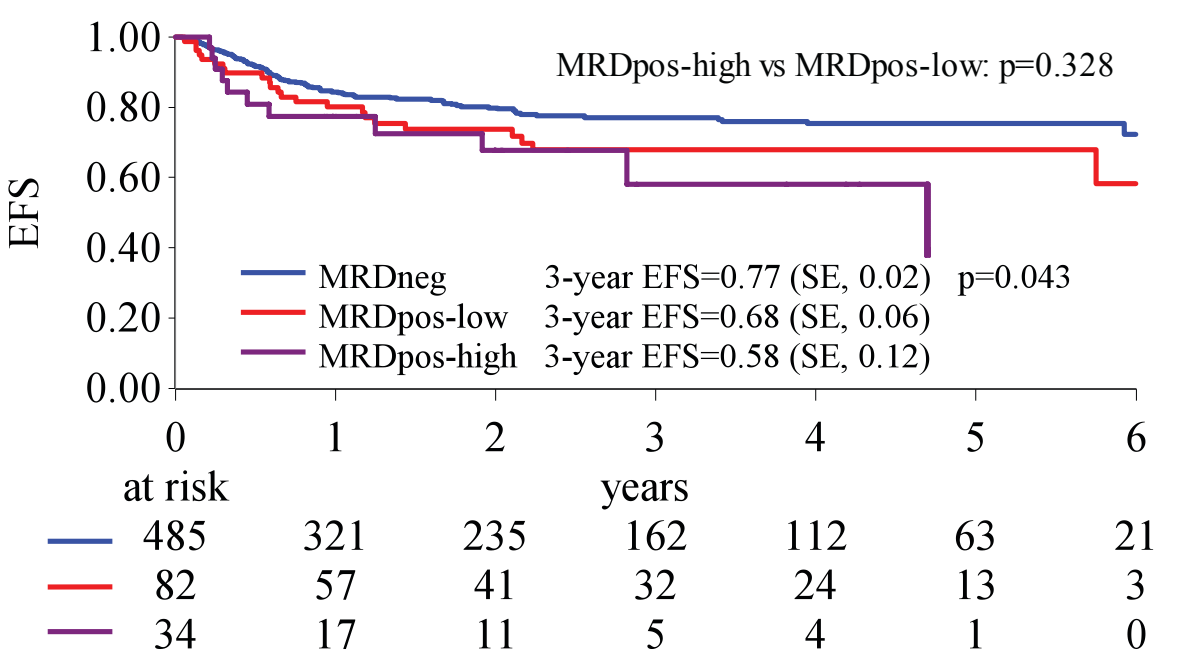
C Panel, Figure S4



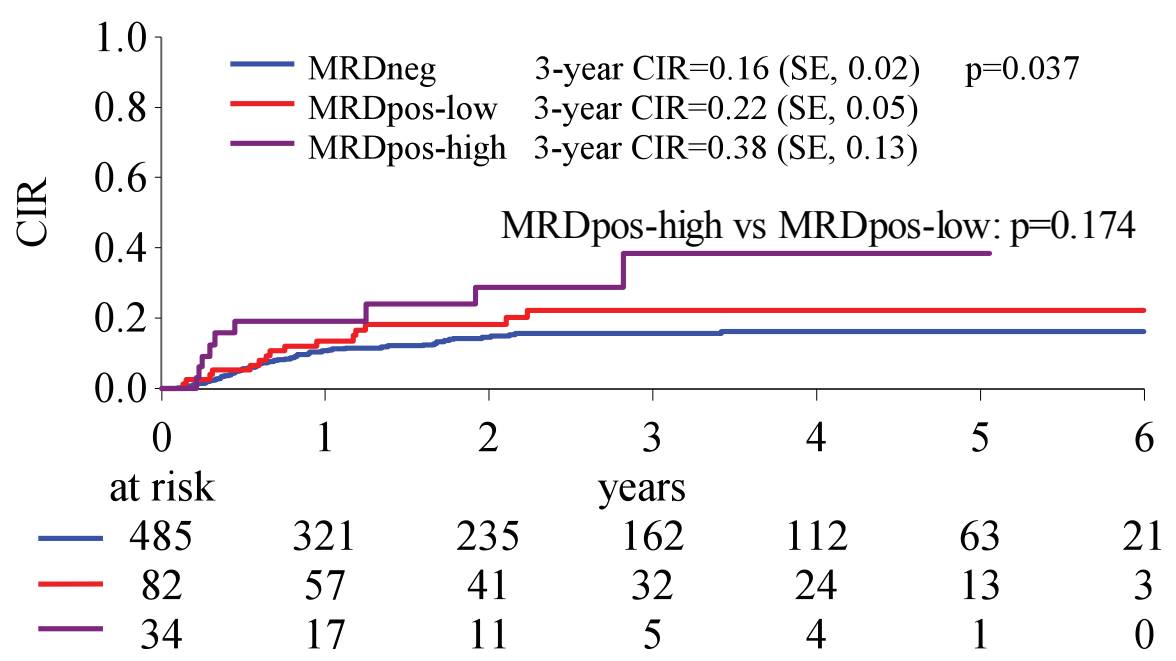
D Panel, Figure S4



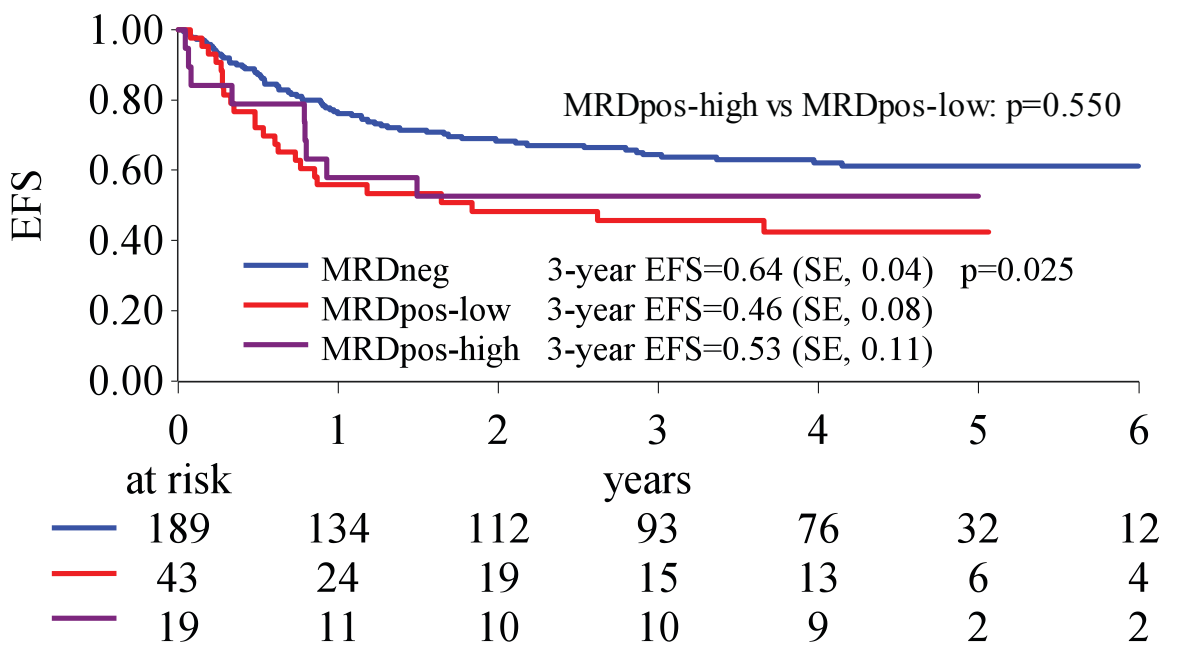
E Panel, Figure S4



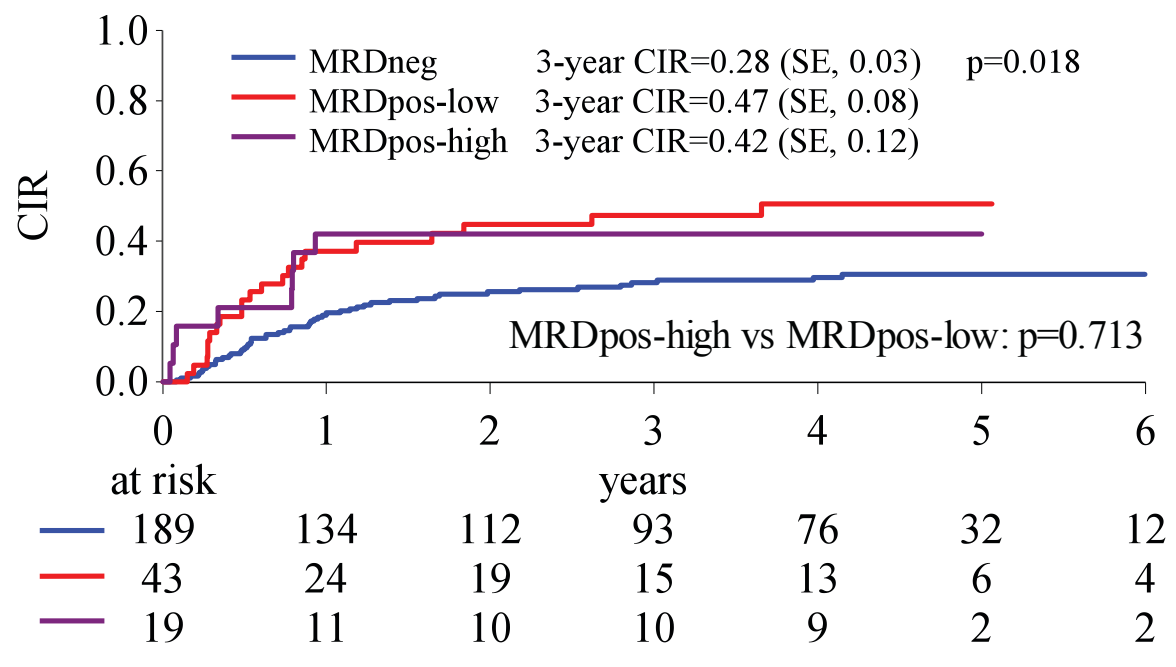
F Panel, Figure S4



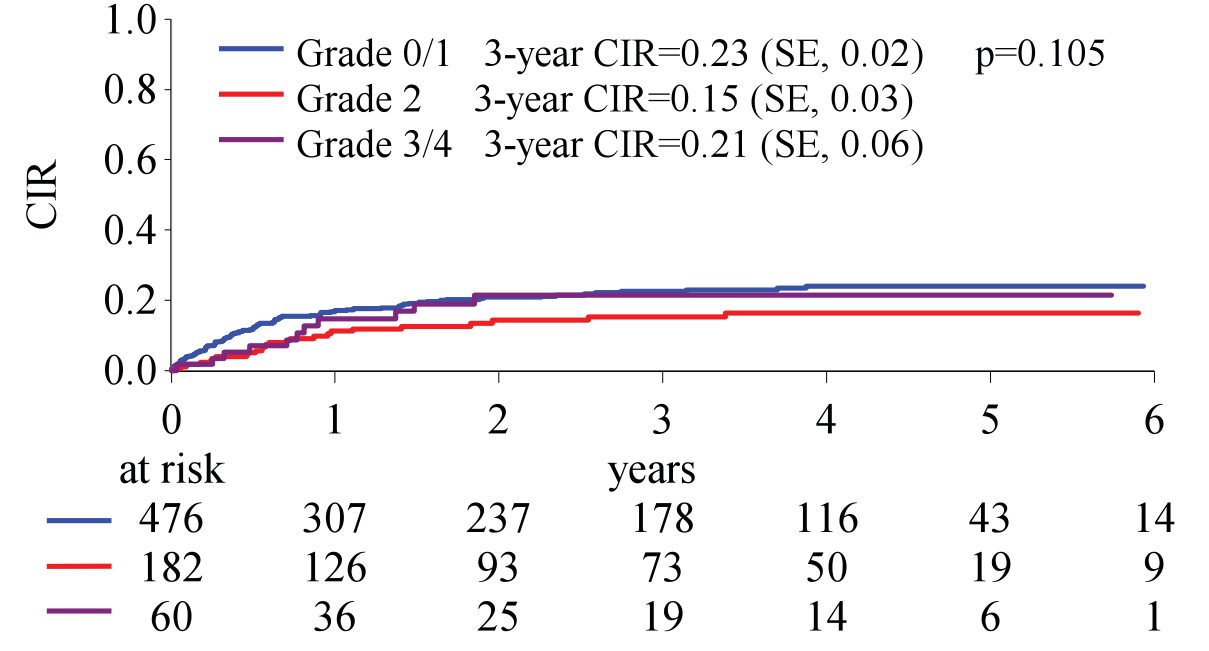
G Panel, Figure S4



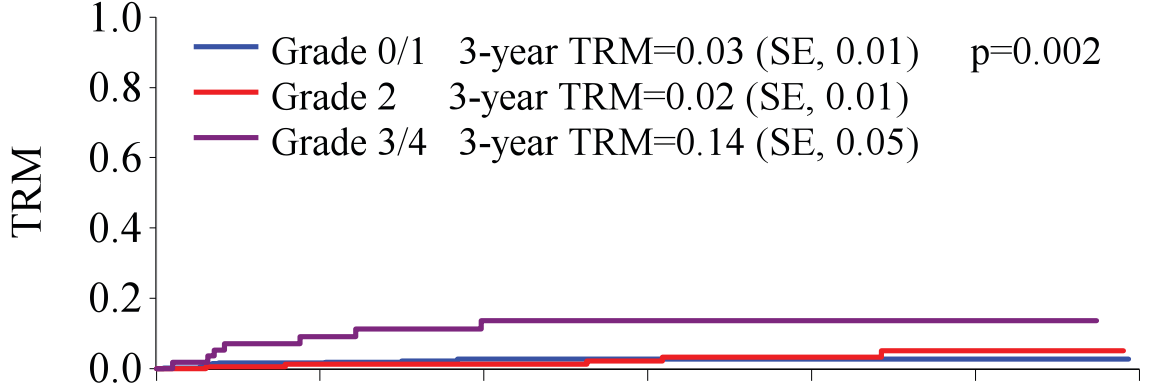
H Panel, Figure S4



A Panel, Figure S5

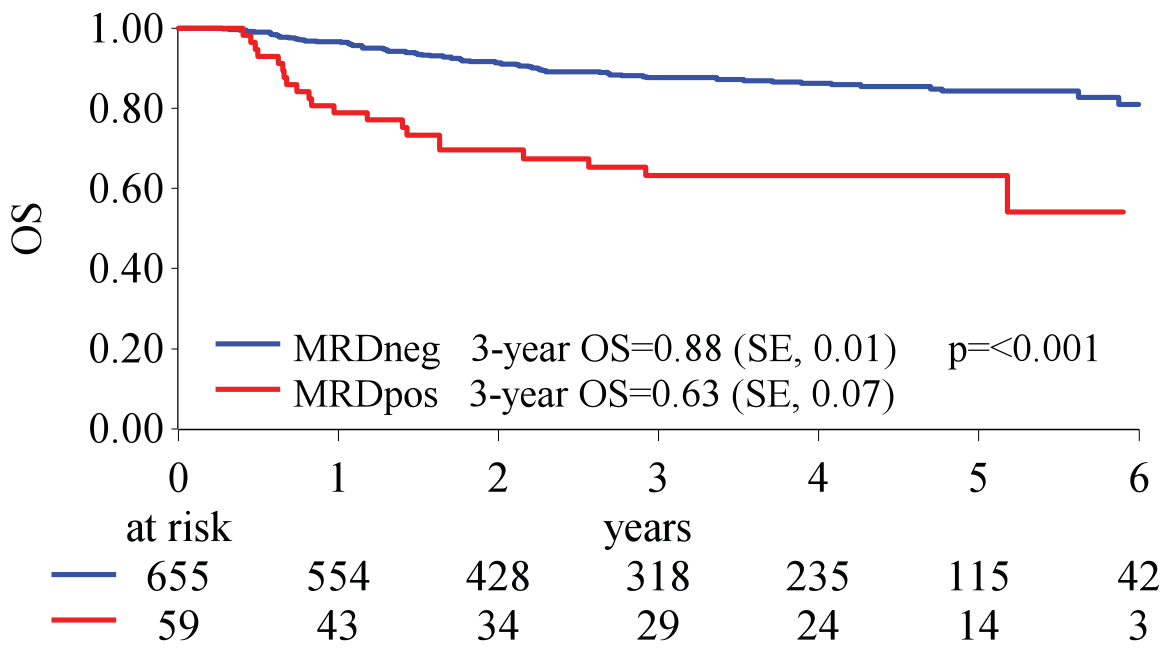


B Panel, Figure S5

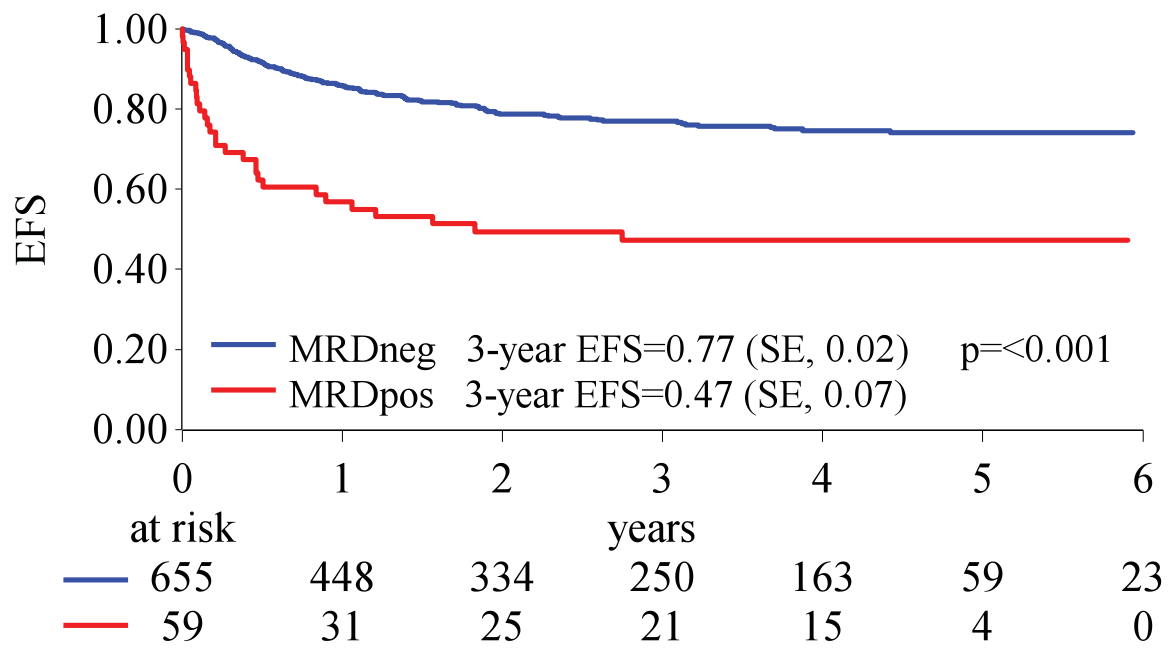


at risk						
476	307	237	178	116	43	14
182	126	93	73	50	19	9
60	36	25	19	14	6	1

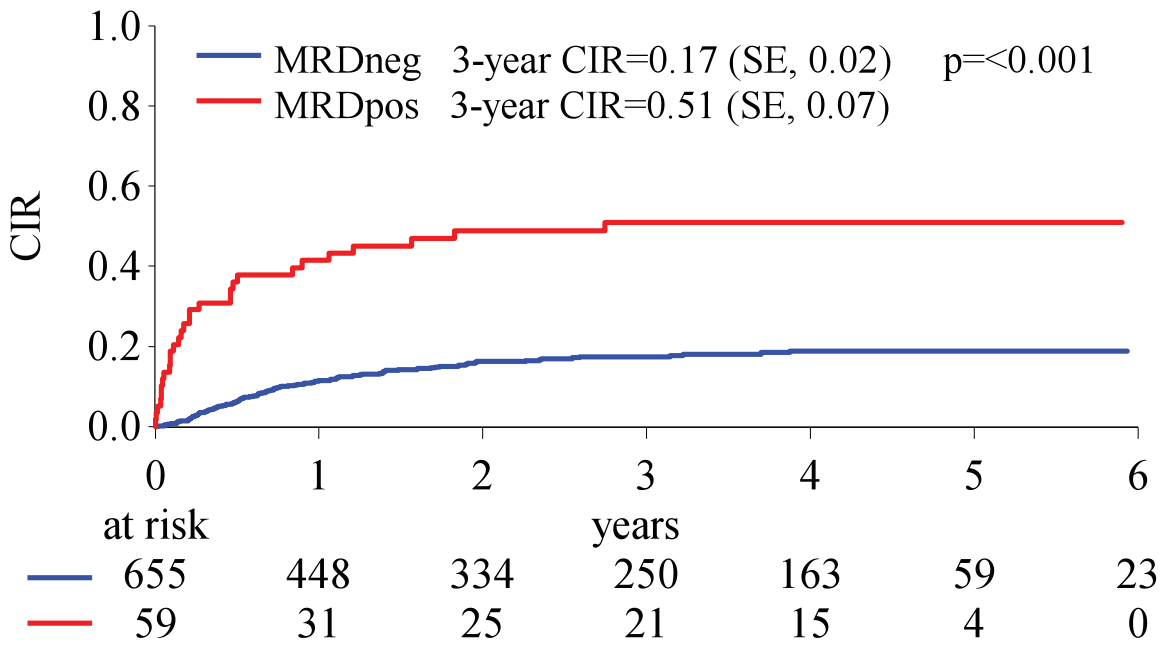
A Panel, Figure S6



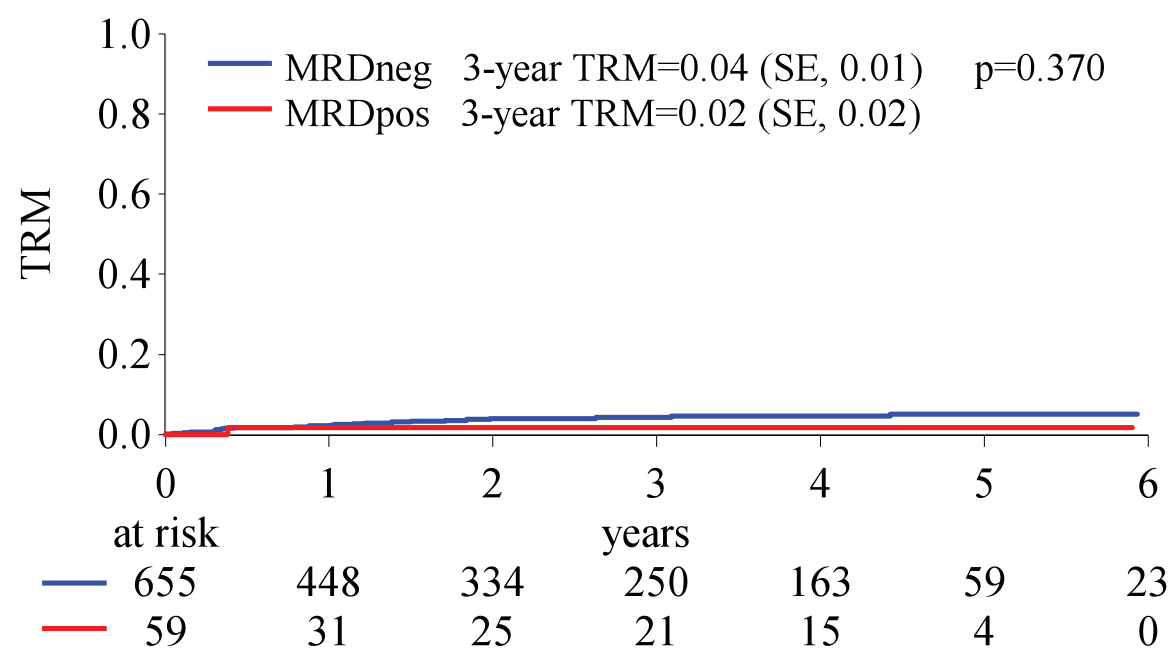
B Panel, Figure S6



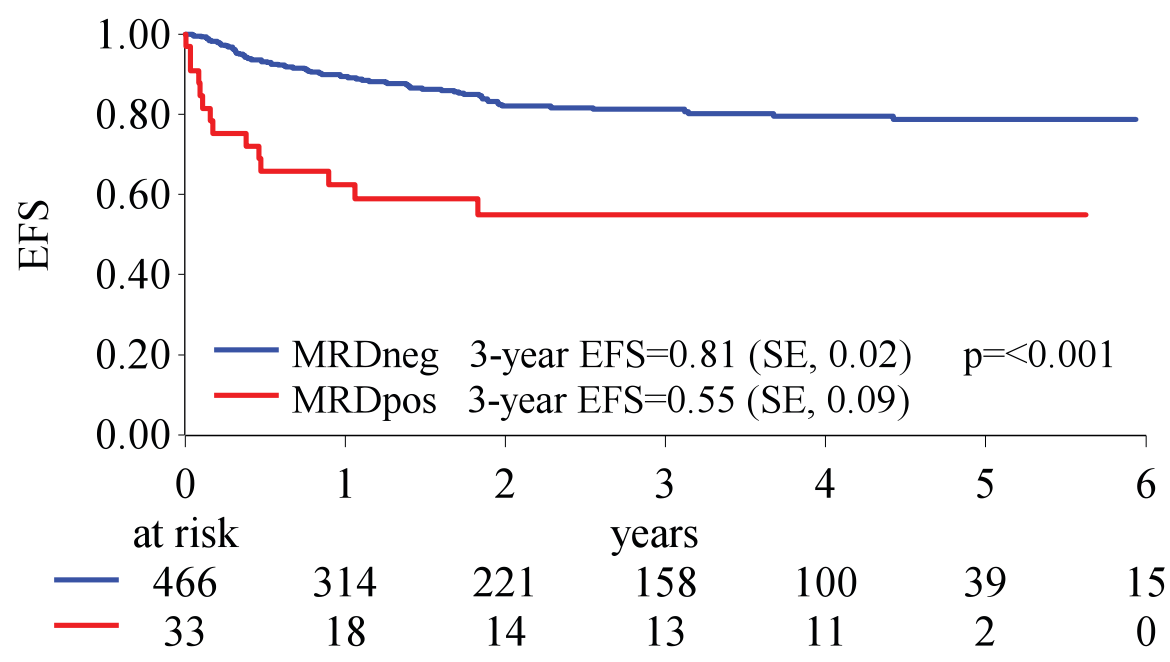
C Panel, Figure S6



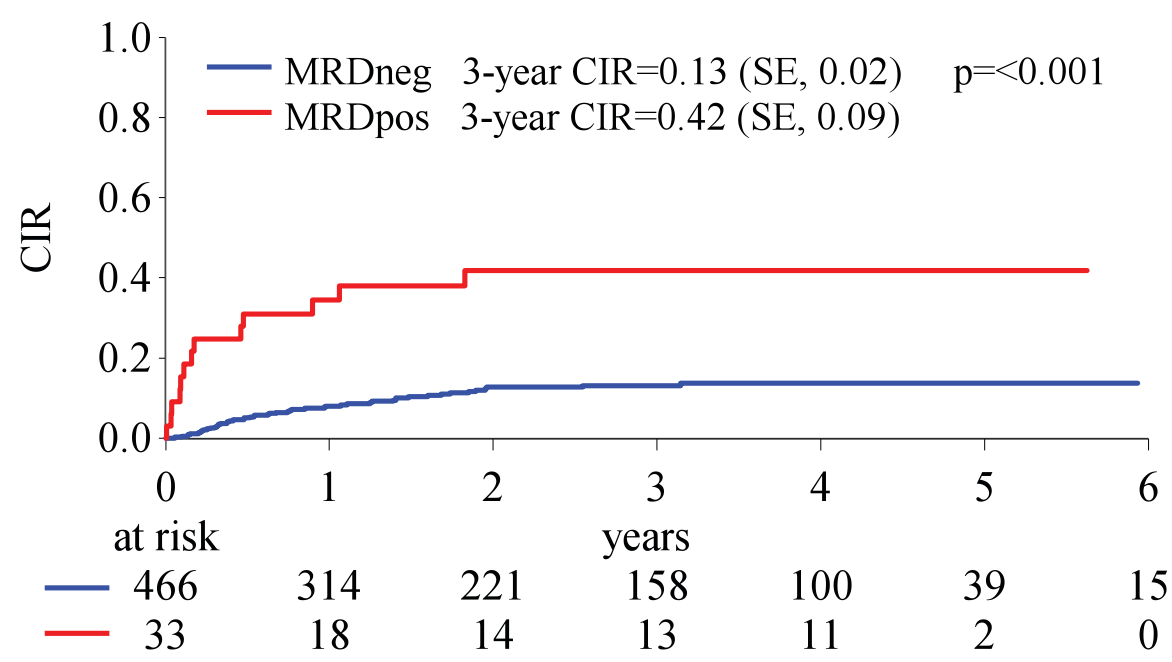
D Panel, Figure S6



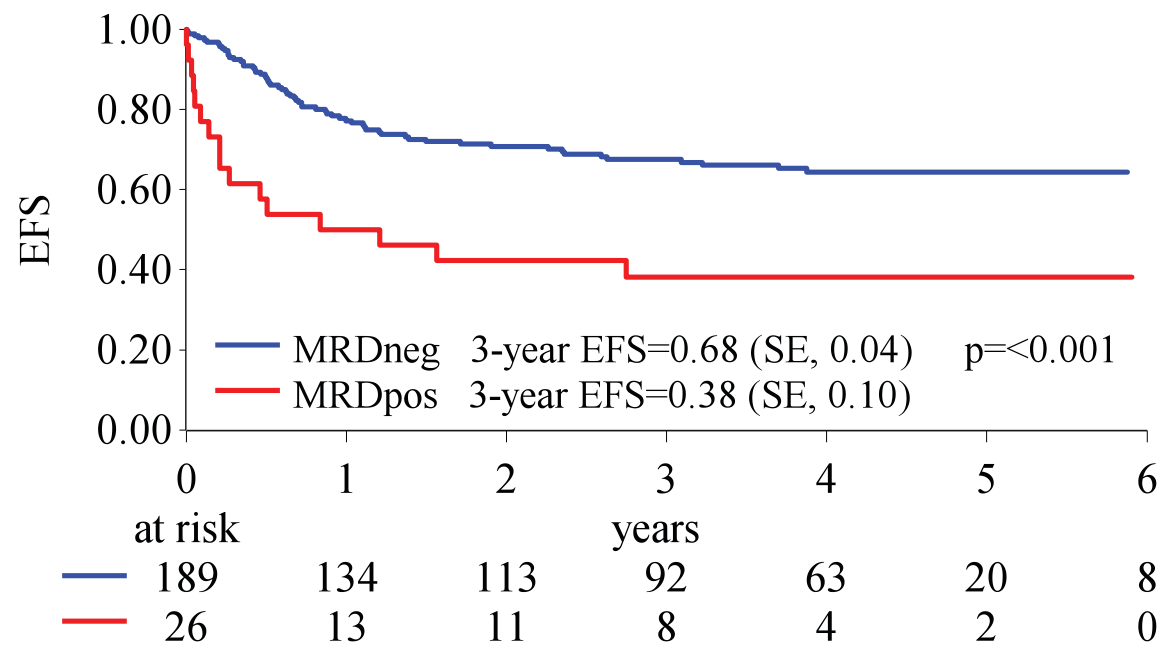
E Panel, Figure S6



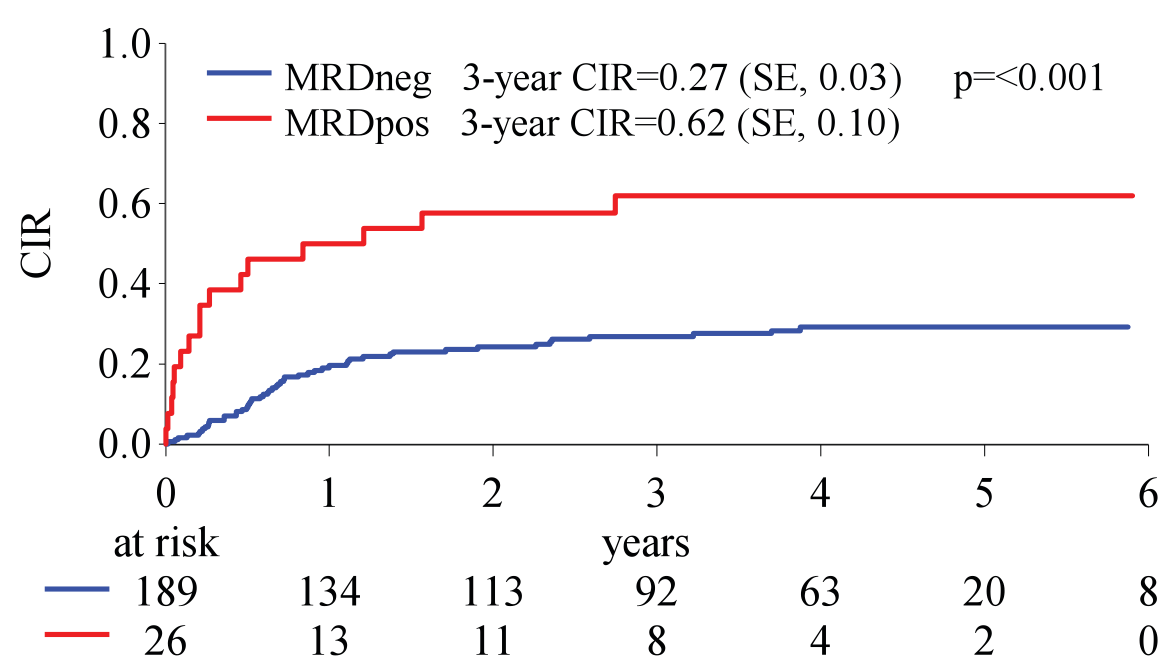
F Panel, Figure S6



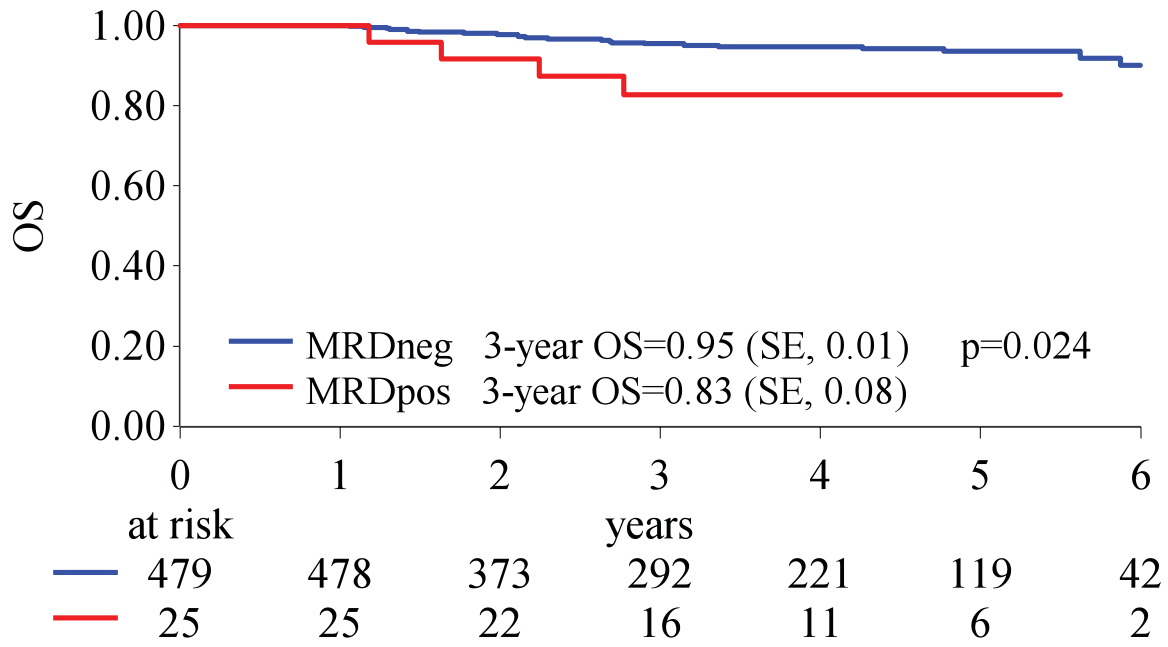
G Panel, Figure S6



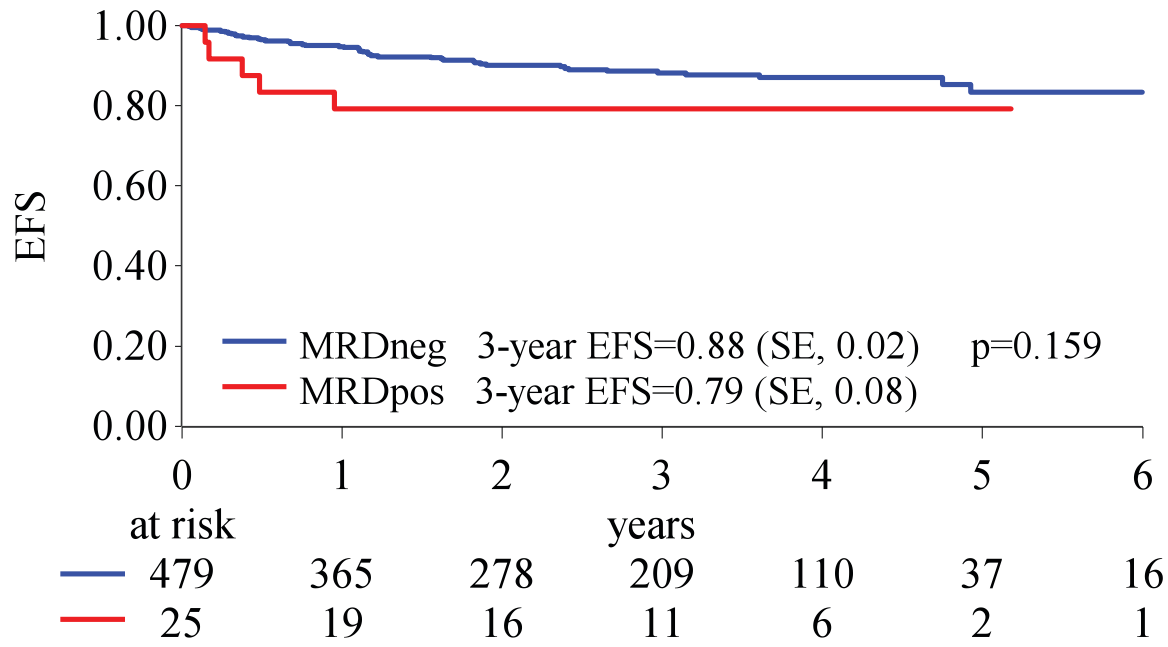
H Panel, Figure S6



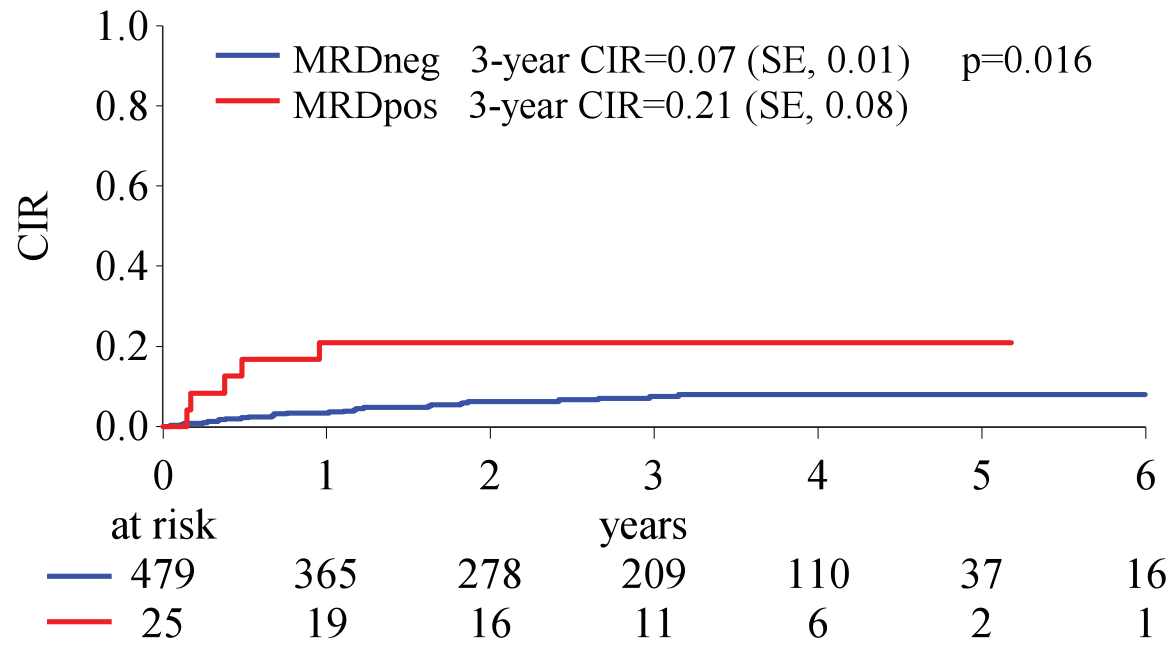
A Panel, Figure S7



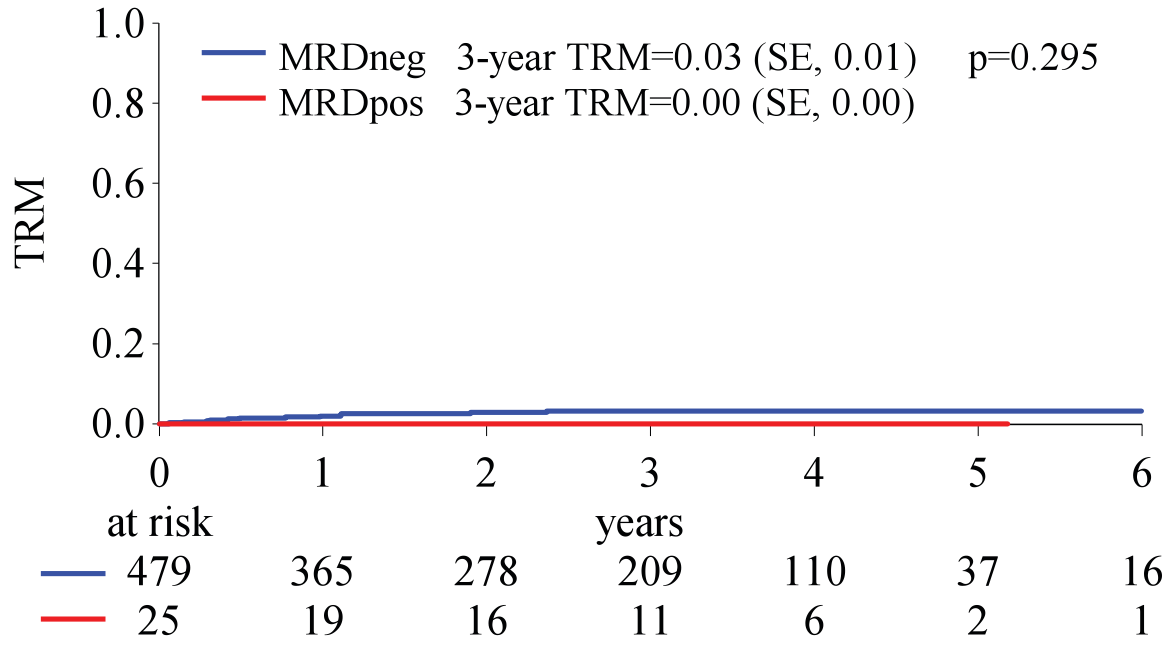
B Panel, Figure S7



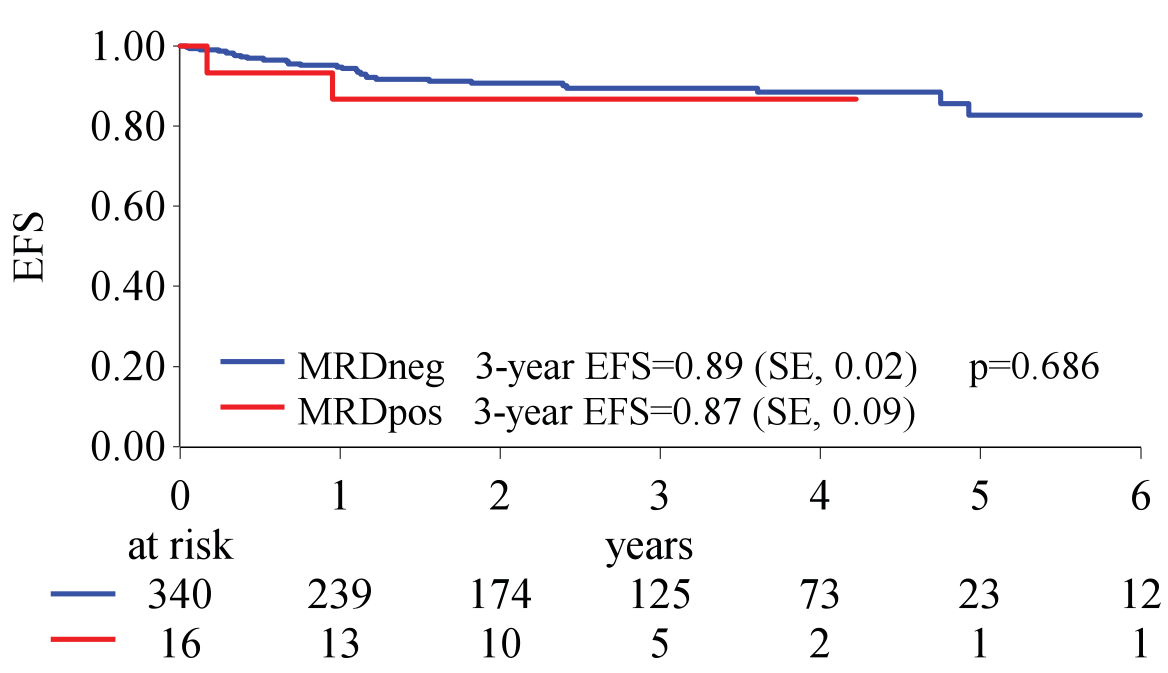
C Panel, Figure S7



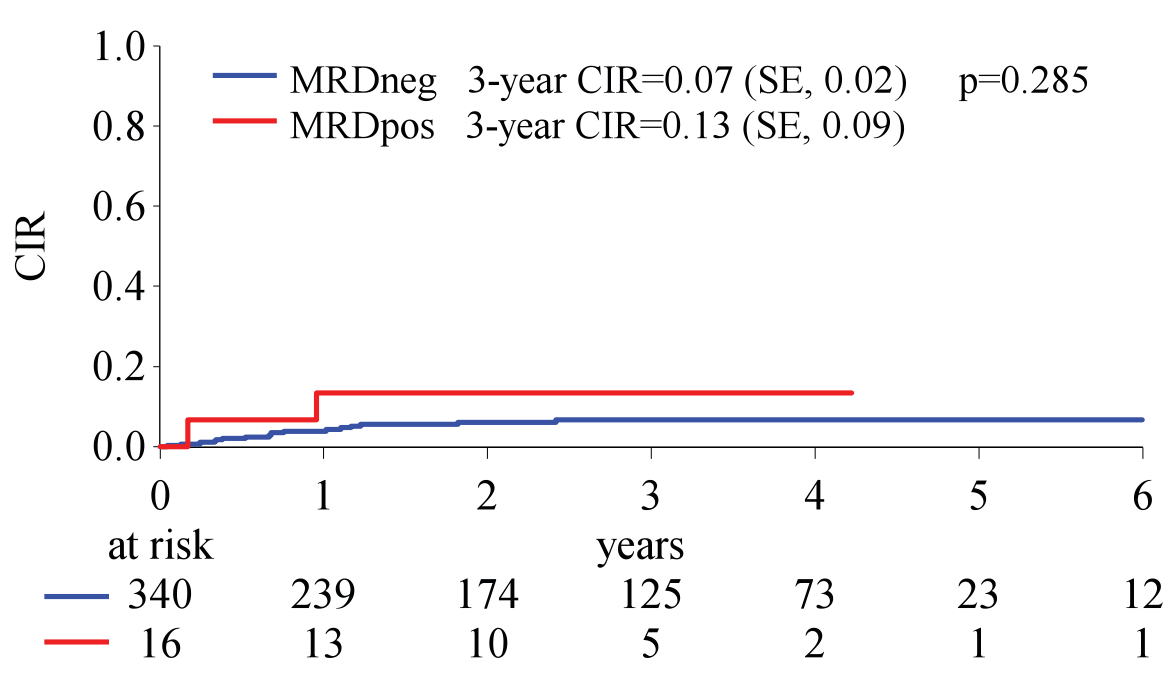
D Panel, Figure S7



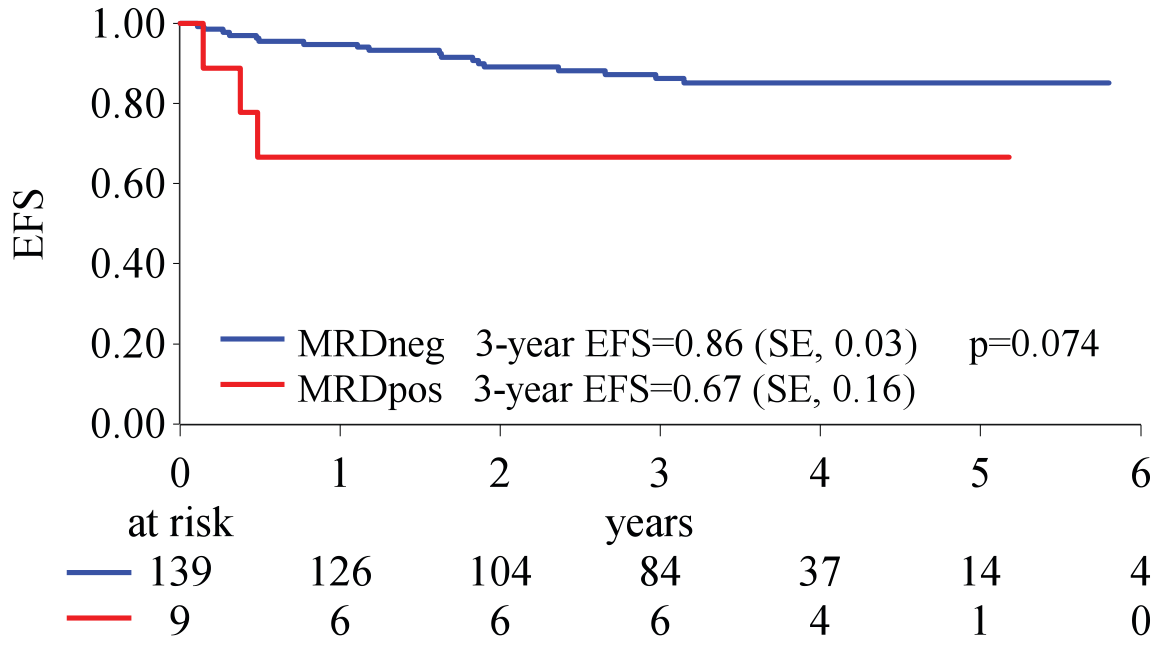
E Panel, Figure S7



F Panel, Figure S7



G Panel, Figure S7



H Panel, Figure S7