

Molecular MRD status and outcome after transplantation in *NPM1* mutated AML: results from the UK NCRI AML17 study

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Abstract:

Relapse remains the most common cause of treatment failure for patients with acute myeloid leukaemia (AML) who undergo allogeneic stem cell transplantation (alloSCT) and carries a grave prognosis. Multiple studies have identified the presence of minimal residual disease (MRD) assessed by flow cytometry (FCM) prior to alloSCT as a strong predictor of relapse, but it is not clear how these findings apply to patients who test positive in molecular MRD assays which have far greater sensitivity. We analysed pre-transplant blood and bone marrow samples by reverse-transcription polymerase chain reaction (RT-qPCR) in 107 patients with *NPM1* mutant AML enrolled in the UK National Cancer Research Institute (NCRI) AML17 study. After a median follow-up of 4.9 years, patients with negative, low (<200 copies / 10⁵ ABL in the PB and <1000 copies in the BM) and high levels of MRD had an estimated 2y overall survival (OS) of 83%, 63% and 13% respectively (p<0.0001). Focussing on patients with low level MRD prior to alloSCT, those with *FLT3* ITD had significantly poorer outcome (hazard ratio, HR, 6.14, p=0.01). Combining these variables was highly prognostic, dividing patients into two groups with 2y OS of 17% and 82% (HR 13.2, p<0.0001). T-depletion was associated with significantly reduced survival both in the entire cohort (2y OS 56% vs 96%, HR 3.24, p=0.0005) and in MRD positive patients (2y OS 34% vs 100%, HR 3.78, p=0.003) but there was no significant effect of either conditioning regimen or donor source on outcome. Registered at ISRCTN (<http://www.isrctn.com/ISRCTN55675535>).

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Key points

Pre-transplant MRD level is highly predictive of outcome, thresholds of 200 copies / 10^5 *ABL* in PB and 1000 copies in BM are discriminatory.

Relapse in patients with pre-transplant MRD positivity below these levels is largely restricted to those with *FLT3* ITD.

Abstract

Relapse remains the most common cause of treatment failure for patients with acute myeloid leukaemia (AML) who undergo allogeneic stem cell transplantation (alloSCT) and carries a grave prognosis. Multiple studies have identified the presence of minimal residual disease (MRD) assessed by flow cytometry (FCM) prior to alloSCT as a strong predictor of relapse, but it is not clear how these findings apply to patients who test positive in molecular MRD assays which have far greater sensitivity.

We analysed pre-transplant blood and bone marrow samples by reverse-transcription polymerase chain reaction (RT-qPCR) in 107 patients with NPM1 mutant AML enrolled in the UK National Cancer Research Institute (NCRI) AML17 study. After a median follow-up of 4.9 years, patients with negative, low (<200 copies / 10^5 *ABL* in the PB and <1000 copies in the BM) and high levels of MRD had an estimated 2y overall survival (OS) of 83%, 63% and 13% respectively ($p < 0.0001$). Focussing on patients with low level MRD prior to alloSCT, those with *FLT3* ITD had significantly poorer outcome (hazard ratio, HR, 6.14, $p = 0.01$). Combining these variables was highly prognostic, dividing patients into two groups with 2y OS of 17% and 82% (HR 13.2, $p < 0.0001$).

T-depletion was associated with significantly reduced survival both in the entire cohort (2y OS 56% vs 96%, HR 3.24, $p = 0.0005$) and in MRD positive patients (2y OS 34% vs 100%, HR 3.78, $p = 0.003$) but there was no significant effect of either conditioning regimen or donor source on outcome.

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Introduction

Optimal therapy for patients with cytogenetically normal acute myeloid leukaemia (AML) remains controversial, particularly regarding the use of allogeneic stem cell transplantation (alloSCT)¹⁻³. Many recent studies have identified the presence of minimal residual disease detected by polymerase chain reaction (PCR)⁴⁻¹⁰, flow cytometry (FCM)¹¹⁻¹⁶ or next-generation sequencing (NGS)¹⁷⁻¹⁹ as a powerful predictor of outcome and MRD status is increasingly used to allocate patients for transplantation²⁰⁻²³, however peri-transplant management of MRD positive patients remains highly challenging.

Multiple studies have identified the presence of minimal residual disease (MRD) assessed by FCM²⁴⁻³², abnormal gene expression^{33,34} and NGS^{35,36} immediately prior to alloSCT as a strong predictor of adverse outcome; patients who test positive using these methods have a relapse risk of up to 69%³². As relapse after alloSCT carries a grave prognosis³⁷ there is growing interest in peri-transplant interventions to reduce or eliminate MRD, which might diminish relapse risk^{31,38}. In this regard, the effect of different conditioning regimens on the outcome of patients who are MRD positive remains uncertain^{28,39,40}.

Although the great majority of studies of pre-transplant MRD in AML have utilised FCM, over half of patients with cytogenetically normal AML harbour mutations in the gene encoding nucleophosmin (*NPM1*)^{41,42}. The recommended method for MRD assessment in these patients is reverse-transcription quantitative PCR (RT-qPCR)⁴³ which affords a sensitivity of up to 1:10⁷, around 1000-fold greater than that achieved by FCM or NGS⁴⁻¹⁰. Thus, the strongly adverse outcome reported in patients who are MRD positive using FCM and NGS may not be applicable to *NPM1* mutated patients with low level positivity by PCR. Despite this, only a few small studies have examined the impact of pre-transplant *NPM1* MRD status^{10,44,45}. Absence of robust outcome data for these patients is a barrier both to rational clinical decision making and to planning interventional studies in this setting.

In this study, we report the outcomes of a large cohort of patients with *NPM1* mutated AML treated on the NCRI AML17 protocol who had pre-transplant molecular MRD assessment.

Methods

Patients. Between 2009-2014 the NCRI AML17 study (ISRCTN 55675535) enrolled 3215 patients with non-M3 AML aged 16-77 eligible for intensive chemotherapy. The treatment protocol has been described previously⁴⁶. Central screening for *NPM1* mutations was positive in 861/2949 (29%) and 530 of these provided serial samples for MRD monitoring. Paired blood (PB) and bone marrow aspirates (BM) were requested on regeneration after each cycle of chemotherapy and then every three months. Post-remission treatment was determined according to the validated NCRI risk score, with poor-risk patients recommended for allogeneic stem-cell transplantation (alloSCT) during first complete remission (CR1). For patients receiving a transplant, additional samples were requested prior to alloSCT, at D+30 and D+100 and then at three-monthly intervals for at least two years. For this study, pre-SCT results were included if the sample was taken within 60 days before transplant and the patient had not received any further therapy between sampling and the start of conditioning. Results were issued to treating clinicians from June 2012 only and after this time patients could be treated for confirmed re-emergent or persistent molecular positivity.

Amplification of *NPM1* mutated transcripts. Samples were analysed by RT-qPCR as previously described⁴. Briefly, RNA was isolated using Trizol reagent (Life Technologies, Carlsbad, CA) and reverse transcribed using ThermoScript (Life Technologies). Primer and probe sets described by Gorello et al⁷ were used to amplify *NPM1* type A, B and D mutant transcripts and patient specific primers were designed to detect rare mutations. Samples were run in triplicate using an ABI 7900 thermocycler (Life Technologies) with parallel amplification of a control gene (*ABL*). Samples with *ABL* cycle threshold of ≥ 30 were excluded. Criteria proposed by the Europe Against Cancer programme⁴⁷ were used to define MRD positivity (i.e. amplification in at least two of three replicates with cycle-threshold values of 40 or less using a threshold setting of 0.1). Molecular relapse was diagnosed if there were two consecutive positive samples showing increasing transcript levels in a patient who had previously tested MRD negative in a technically adequate sample, consistent with ELN guidelines⁴³.

Analysis of *FLT3* ITD status and allelic ratio. PCR amplification of exons 14 and 15 of *FLT3* was performed using fluorescently labelled primers and analysed using capillary electrophoresis as previously described⁴⁸. The allelic ratio was determined by comparing the areas under the curves from the mutated and wild-type products.

Statistical Analyses. Kaplan-Meier estimates were used to calculate survival percentages. Time to event analysis was performed using the log rank test. Thresholds were selected by identifying cut-offs providing maximum additional discrimination. We used Cox regression with forward selection to identify independent prognostic factors. Categorical variables were analysed using Fisher's exact test. All reported P values are two-sided.

Results

In total 107/ 530 patients received alloSCT: 56 (52%) in CR1, 30 (28%) after molecular relapse (MR) and 21 (20%) in second remission after haematological relapse (CR2) (figure 1). Clinical and molecular details are shown in table 1. Median follow-up was 4.9 years from transplant (range 1.0 – 8.4y). Forty-two (39%) patients died, the cause of death was disease relapse in 21 patients, was not attributed to relapse in 19 and could not be determined in 2 patients. Overall survival 5 years from the date of transplant (5y-OS) was 65% for patients transplanted in CR1 and 54% for those transplanted after a molecular or haematological relapse (p=0.3).

Evaluable pre-SCT PB and BM samples taken in the 60 days preceding SCT were available for 103 and 78 patients, both were available for 74 patients. In total, 58 (54%) patients were MRD negative prior to SCT; 48 patients received additional chemotherapy prior to SCT for molecular (n=27) or haematological relapse (n=21) and 27/48 (56%) achieved MRD negativity (figure 1).

Survival according to pre-transplant molecular MRD status

Overall survival 2 years from allograft was 83% for MRD negative patients versus 45% for patients with any detectable MRD by RT-qPCR in the pre-transplant samples; median OS (mOS) was not reached (NR) and 10.5 months respectively (hazard ratio, HR, 3.60 95% confidence interval, CI, 1.92-6.77, p<0.0001, figure 2a). For patients with negative pre-SCT PB samples (n=73) 2y-OS was 81%, compared with 30% for patients with any PB positivity (n=30) (HR 8.30, CI 3.77-18.20, p<0.0001, fig 2b); mOS was NR and 7.4 months. Patients with a negative pre-SCT BM (n=37) had a 5y-OS of 84% compared with 49% if the BM was MRD positive (n=41); mOS was NR and 13.1 months (HR 3.17, CI 1.54-6.54, p=0.002, figure 2c).

A threshold of 200 mutant *NPM1* transcripts / 10^5 ABL copies in the pre-SCT PB sample provided maximum additional discrimination and split patients into three groups with 2y-OS of 81% (negative, n=73, mOS NR), 54% (low, 0.1-200 copies, n=13, mOS NR) and 12% (high, >200 copies, n=17, mOS 6.5 months, HR by group 2.81, CI 1.96-4.02, p<0.0001, figure 2d).

In the BM, a threshold of 1000 copies provided maximum additional discrimination and defined 3 groups with 2y-OS of 84% (negative, n=37, mOS NR), 56% (low, 0.1-1000 copies, n=32, mOS NR) and 22% (high, >1000 copies, n=9, mOS 5.8 months, HR by group 2.87, CI 1.69-4.86, p<0.0001, figure 2e).

Overall (applying the higher level where there was a discrepancy between PB and BM results), 2y-OS was 83% (n=58, mOS NR) 63% (n=30, mOS NR) and 13% (n=19, mOS 6.5 months) for patients with negative, low and high levels of MRD (HR by group 2.83, CI 1.92-4.19, p<0.0001, figure 2f).

Impact of FLT3 status on post-transplant outcome

We next stratified patients according *FLT3* ITD status. Thirty-four patients were positive for *FLT3*-ITD at diagnosis and 73 were negative; 2y-OS was 62% and 67% respectively (HR 1.14, CI 0.59-2.19, p=0.7). *FLT3* ITD status was not associated with outcome in patients who were MRD negative before transplant (2y-OS ITD negative 77%, n=40, ITD positive 94%, n=18, HR 0.42, CI 0.14-1.28, p=0.13) or those who had high levels of MRD (2y-OS ITD negative 0%, n=11, ITD positive 25%, n=8, mOS 5.8 vs 6.8 months, HR 0.71 CI 0.26-1.92, p=0.5). In contrast for patients with low levels of MRD, *FLT3* status was strongly associated with outcome: 2y-OS was 77% for ITD negative (n=22, mOS NR) and 25% for ITD positive patients (n=8, mOS 7.1 months, HR 6.14 CI 1.50-25.13, p=0.01, figure 3).

Owing to small numbers, we were unable to reliably assess the effect of *FLT3* ITD allelic ratio.

Impact of first line post-induction MRD status on post-transplant outcome

Peripheral blood MRD status after the second induction cycle of first line therapy (PBPC2) has previously been shown to be highly prognostic⁴ and retained power in this cohort (2y OS 76% vs 33% for PBPC2 negative and positive patients, mOS NR vs 9.6 months, HR 4.93, CI 2.05-11.90, p=0.0004). There was an association between PBPC2 and pre-SCT MRD negativity (p=0.002, table 1). Of those patients who were PBPC2 negative and experienced molecular or haematological relapse, 60% (21/35) achieved MRD negativity following salvage therapy and a further 14% (5/35) were MRD positive at levels below the thresholds defined above and were *FLT3* WT; 2y OS for these patients was 88%.

Multivariable model for prediction of post-transplant outcome

We performed a multivariate analysis taking into account remission status at time of transplant (CR1 vs other), age at time of transplant, *FLT3* ITD status, PBPC2 status and pre-transplant MRD level (negative, low or high). The only factors which retained independent prognostic power were age (HR per decade 1.54, CI 1.08-2.19 $p=0.02$) and pre-transplant MRD level (HR 3.02, CI 1.97-4.62, $p<0.0001$).

We developed a two-group prognostic model incorporating MRD status (negative, low or high) and *FLT3* ITD (positive or negative). Patients who had high levels of MRD were allocated to the high-risk group together with patients with low levels of MRD who had *FLT3* ITD at diagnosis. The remaining patients were allocated to the low risk group. Patients with a negative PB and absent BM sample could not be reliably allocated to a risk group and were excluded from this analysis. There was sufficient information to assign a risk group in 83 patients. In the low-risk group ($n=56$) 2y-OS was 82% compared to 17% in the high-risk group ($n=27$, mOS NR vs 6.5 months, HR 13.2, CI 5.80-30.2, $p<0.0001$, figure 4).

When risk group (low or high) was introduced as a candidate variable into the multivariable model described above, the only factors to retain prognostic significance were age at time of transplant (HR per decade 1.60, CI 1.08-2.37, $p=0.02$) and risk group (HR 9.5, CI 4.24-21.42, $p<0.0001$).

Effect of transplant-related factors on outcome according to MRD status

Donor source was a matched sibling in 43 patients, a volunteer unrelated donor (VUD) in 63 and umbilical cord blood in 1. Although a trend for greater overall survival in patients whose donor was a sibling compared to a VUD was noted, this was not statistically significant (2y-OS 72% vs 62%, HR 1.81, CI 0.97-3.35, $p=0.06$, figure 5a).

Conditioning regimens were considered myeloablative (MAC) in 30 patients (28%, BuCy 4, CyTBI 20, FB4C 6) and reduced-intensity (RIC) in 77 (72%, FluMel 48, FluBu 11, FLAMSA-Bu 8, FluTBI 6, FluCy 2, FluCyTBI 2). Patients who received MAC were significantly younger (mean 43 vs 56 years $p<0.0001$). There was no difference in overall survival according to conditioning regimen type (2y OS MAC 71%, RIC 63%, HR 1.18, CI 0.61-2.29, $p=0.6$, figure 5b).

Alemtuzumab was given to 70 (65%) and anti-thymocyte globulin (ATG) to 12 (11%) patients for T-depletion; 2y-OS was 56% for these patients with no difference by T-depletion agent, compared to 96% in patients who did not receive T-depletion (n=25, HR 3.24, CI 1.69-6.42, p=0.0005, figure 5c). Patients who received T-depletion were older (mean 54 vs 47y, p=0.0028) and were more likely to have been transplanted using a VUD (67% vs 33% for non-T-depleted, p=0.004) and with RIC (80% vs 44% for non-T depleted, p=0.0008). Cumulative incidence of relapse at 5 years was 24% in patients who underwent T-depletion compared with 4% in those who did not (p=0.0149). Cumulative incidence of non-relapse mortality at 5 years was 23% in patients who underwent T-depletion compared with 4% in patients who did not (p=0.0148).

Considering patients who were MRD positive prior to allograft, there was a trend for lower OS in patients who had received a VUD transplant (2y-OS 38% vs 55% for SIB, HR 1.94, CI 0.92-4.08, p=0.08, figure 5d). There was no association between OS and type of conditioning (2y OS 50% for MAC vs 43% for RIC, HR 1.22, CI 0.54-2.76, p=0.6, figure 5e). Specifically, MRD positive patients treated with the sequential FLAMSA protocol had no difference in overall survival. Patients who were MRD positive and who received T-depletion showed inferior overall survival than those who did not (2y-OS 34% vs 100%, mOS 7.8m vs NR, HR 3.78, CI 1.57-19.2, p=0.003, figure 5f).

Discussion

Patients with *NPM1* mutated AML who test MRD negative by RT-qPCR prior to transplant have an excellent chance of long-term survival regardless of other risk factors including *FLT3* status and independent of the intensity of the transplant conditioning regimen.

As expected, *NPM1* MRD positivity had an overall adverse effect on transplant outcome, but in contrast to patients who are MRD positive by FCM or NGS, patients who test positive for *NPM1* mutant transcripts prior to alloSCT do not have a universally poor outcome, indeed many become long-term survivors. Factors associated with adverse outcome are high levels of MRD (above 200 copies / 10^5 *ABL* in the PB or 1000 copies in the BM) and the presence of a *FLT3* ITD mutation at diagnosis. Patients who are MRD positive before transplant and have one or both of these features have a poor prognosis due to a high risk of disease relapse.

In this study 27/48 (56%) of patients with a haematological or confirmed molecular relapse achieved MRD negativity with salvage chemotherapy and a further 8/48 (17%) became low risk as defined in our risk score. These 35 patients had an excellent outcome with an overall survival of 80% at 2 years. Additionally, 74% of patients who tested negative for MRD in the PB after second induction (PBPC2) and who subsequently relapsed achieved MRD negativity or low-risk status after salvage and had a 2y OS of 88%. This supports the approach adopted in the current NCRI AML19 protocol where such patients are not recommended for transplantation in CR1 and are offered sequential MRD monitoring to allow early detection and treatment of relapse.

Selection of transplant protocol remains controversial, particularly for patients who are MRD positive, and a key question is whether augmented conditioning can eliminate MRD and thereby improve outcome. Studies to date have provided conflicting results and have not examined the effect of transplant related factors specifically in *NPM1* mutated patients^{28,39,40,49}. Surprisingly, we observed no effect on survival according to conditioning type, either overall or in patients who were MRD positive. In contrast, we observed a strong association between use of T-depletion and adverse outcome. Relatively few patients who were MRD positive received T-replete transplants (n=8) and this retrospective non-randomised analysis clearly has significant limitations, however these results highlight T-depletion as a potentially critical factor which has not been consistently reported in other studies to date.

These findings require independent validation, however patients at highest risk of relapse identified here may benefit from an alternative approach prior to transplant such as the use of *FLT3* inhibition to reduce the level of MRD below the thresholds identified. Alternatively, augmentation of the graft-versus-leukaemia effect (e.g. through avoidance or minimisation of T-depletion, early withdrawal of immunosuppression and / or donor lymphocyte infusion) may be considered. Randomised studies to investigate these approaches are urgently required.

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Authorship contributions

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Statistical analysis: RD, RH. Clinical data collection: AK, JC, MS, HBO, UMO, MD, SK, HK, DT, PM, KR, INB, MN, RD, PK, KH, DF, SA, EH, PJ, AK, RSa, RSp, AB, NR, CC. Trial co-ordination: NR, AB, SK, MD. Manuscript preparation: RD, SF, NR, RH

Disclosure of conflicts of interest

The authors have no relevant conflicts of interest to declare.

Appendix

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References

1. Burnett AK, Goldstone A, Hills RK, et al. Curability of patients with acute myeloid leukemia who did not undergo transplantation in first remission. *J Clin Oncol* 2013;31(10):1293-301.
2. Cornelissen JJ, Blaise D. Hematopoietic stem cell transplantation for patients with AML in first complete remission. *Blood* 2016;127(1):62-70.
3. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* 2017;129(4):424-47.
4. Ivey A, Hills RK, Simpson MA, et al. Assessment of Minimal Residual Disease in Standard-Risk AML. *N Engl J Med* 2016(5);374:422-33.
5. Balsat M, Renneville A, Thomas X, et al. Postinduction Minimal Residual Disease Predicts Outcome and Benefit From Allogeneic Stem Cell Transplantation in Acute Myeloid Leukemia With NPM1 Mutation: A Study by the Acute Leukemia French Association Group. *J Clin Oncol* 2017;35(2):185-93.
6. Kronke J, Schlenk RF, Jensen KO, et al. Monitoring of minimal residual disease in NPM1-mutated acute myeloid leukemia: a study from the German-Austrian acute myeloid leukemia study group. *J Clin Oncol* 2011;29(19):2709-16.
7. Gorello P, Cazzaniga G, Alberti F, et al. Quantitative assessment of minimal residual disease in acute myeloid leukemia carrying nucleophosmin (NPM1) gene mutations. *Leukemia* 2006;20(6):1103-8.
8. Chou WC, Tang JL, Wu SJ, et al. Clinical implications of minimal residual disease monitoring by quantitative polymerase chain reaction in acute myeloid leukemia patients bearing nucleophosmin (NPM1) mutations. *Leukemia* 2007;21(5):998-1004.
9. Schnittger S, Kern W, Tschulik C, et al. Minimal residual disease levels assessed by NPM1 mutation-specific RQ-PCR provide important prognostic information in AML. *Blood* 2009;114(11):2220-31.
10. Shayegi N, Kramer M, Bornhauser M, et al. The level of residual disease based on mutant NPM1 is an independent prognostic factor for relapse and survival in AML. *Blood* 2013;122(1):83-92.
11. Freeman SD, Hills RK, Virgo P, et al. Measurable Residual Disease at Induction Redefines Partial Response in Acute Myeloid Leukemia and Stratifies Outcomes in Patients at Standard Risk Without NPM1 Mutations. *J Clin Oncol* 2018;36(15):1486-97.
12. Freeman SD, Virgo P, Couzens S, et al. Prognostic relevance of treatment response measured by flow cytometric residual disease detection in older patients with acute myeloid leukemia. *J Clin Oncol* 2013;31(32):4123-31.
13. Terwijn M, van Putten WL, Kelder A, et al. High prognostic impact of flow cytometric minimal residual disease detection in acute myeloid leukemia: data from the HOVON/SAKK AML 42A study. *J Clin Oncol* 2013;31(31):3889-97.
14. Ravandi F, Jorgensen J, Borthakur G, et al. Persistence of minimal residual disease assessed by multiparameter flow cytometry is highly prognostic in younger patients with acute myeloid leukemia. *Cancer* 2017;123(3):426-35.
15. Othus M, Wood BL, Stirewalt DL, et al. Effect of measurable ('minimal') residual disease (MRD) information on prediction of relapse and survival in adult acute myeloid leukemia. *Leukemia* 2016;30(10):2080-3.
16. Buccisano F, Maurillo L, Spagnoli A, et al. Cytogenetic and molecular diagnostic characterization combined to postconsolidation minimal residual disease assessment by

flow cytometry improves risk stratification in adult acute myeloid leukemia. *Blood* 2010;116(13):2295-303.

17. Jongen-Lavrencic M, Grob T, Hanekamp D, et al. Molecular Minimal Residual Disease in Acute Myeloid Leukemia. *N Engl J Med* 2018;378(13):1189-99.
18. Morita K, Kantarjian HM, Wang F, et al. Clearance of Somatic Mutations at Remission and the Risk of Relapse in Acute Myeloid Leukemia. *J Clin Oncol* 2018;36(18):1788-97.
19. Klco JM, Miller CA, Griffith M, et al. Association Between Mutation Clearance After Induction Therapy and Outcomes in Acute Myeloid Leukemia. *JAMA* 2015;314(8):811-22.
20. Craddock C, Raghavan M. Which patients with acute myeloid leukemia in CR1 can be spared an allogeneic transplant? *Curr Opin Hematol* 2019; 26(2):58-64.
21. Ossenkoppele G, Schuurhuis GJ. MRD in AML: does it already guide therapy decision-making? *Hematology Am Soc Hematol Educ Program* 2016;2016(1):356-65.
22. Rowe JM. AML in 2017: Advances in clinical practice. *Best Pract Res Clin Haematol* 2017;30(4):283-6.
23. Buccisano F, Dillon R, Freeman SD, Venditti A. Role of Minimal (Measurable) Residual Disease Assessment in Older Patients with Acute Myeloid Leukemia. *Cancers (Basel)* 2018;10(7) 215.
24. Anthias C, Dignan FL, Morilla R, et al. Pre-transplant MRD predicts outcome following reduced-intensity and myeloablative allogeneic hemopoietic SCT in AML. *Bone Marrow Transplant* 2014;49(5):679-83.
25. Araki D, Wood BL, Othus M, et al. Allogeneic Hematopoietic Cell Transplantation for Acute Myeloid Leukemia: Time to Move Toward a Minimal Residual Disease-Based Definition of Complete Remission? *J Clin Oncol* 2016;34(4):329-36.
26. Walter RB, Gooley TA, Wood BL, et al. Impact of pretransplantation minimal residual disease, as detected by multiparametric flow cytometry, on outcome of myeloablative hematopoietic cell transplantation for acute myeloid leukemia. *J Clin Oncol* 2011;29(9):1190-7.
27. Walter RB, Buckley SA, Pagel JM, et al. Significance of minimal residual disease before myeloablative allogeneic hematopoietic cell transplantation for AML in first and second complete remission. *Blood* 2013;122(10):1813-21.
28. Walter RB, Gyurkocza B, Storer BE, et al. Comparison of minimal residual disease as outcome predictor for AML patients in first complete remission undergoing myeloablative or nonmyeloablative allogeneic hematopoietic cell transplantation. *Leukemia* 2015;29(1):137-44.
29. Bastos-Oreiro M, Perez-Corral A, Martinez-Laperche C, et al. Prognostic impact of minimal residual disease analysis by flow cytometry in patients with acute myeloid leukemia before and after allogeneic hemopoietic stem cell transplantation. *Eur J Haematol* 2014;93(3):239-46.
30. Buckley SA, Wood BL, Othus M, et al. Minimal residual disease prior to allogeneic hematopoietic cell transplantation in acute myeloid leukemia: a meta-analysis. *Haematologica* 2017;102(5):865-73.
31. Buckley SA, Appelbaum FR, Walter RB. Prognostic and therapeutic implications of minimal residual disease at the time of transplantation in acute leukemia. *Bone Marrow Transplant* 2013;48(5):630-41.

32. Zhou Y, Othus M, Araki D, et al. Pre- and post-transplant quantification of measurable ('minimal') residual disease via multiparameter flow cytometry in adult acute myeloid leukemia. *Leukemia* 2016;30(7):1456-64.
33. Goswami M, McGowan KS, Lu K, et al. A multigene array for measurable residual disease detection in AML patients undergoing SCT. *Bone Marrow Transplant* 2015;50(5):642-51.
34. Jentzsch M, Bill M, Grimm J, et al. High BAALC copy numbers in peripheral blood prior to allogeneic transplantation predict early relapse in acute myeloid leukemia patients. *Oncotarget* 2017;8(50):87944-54.
35. Getta BM, Devlin SM, Levine RL, et al. Multicolor Flow Cytometry and Multigene Next-Generation Sequencing Are Complementary and Highly Predictive for Relapse in Acute Myeloid Leukemia after Allogeneic Transplantation. *Biol Blood Marrow Transplant* 2017;23(7):1064-71.
36. Thol F, Gabdoulline R, Liebich A, et al. Measurable residual disease monitoring by NGS before allogeneic hematopoietic cell transplantation in AML. *Blood* 2018;132(16):1703-13.
37. Schmid C, Labopin M, Nagler A, et al. Treatment, risk factors, and outcome of adults with relapsed AML after reduced intensity conditioning for allogeneic stem cell transplantation. *Blood* 2012;119(6):1599-606.
38. Craddock C, Hoelzer D, Komanduri KV. Current status and future clinical directions in the prevention and treatment of relapse following hematopoietic transplantation for acute myeloid and lymphoblastic leukemia. *Bone Marrow Transplant* 2019;54(1):6-16.
39. Milano F, Gooley T, Wood B, et al. Cord-Blood Transplantation in Patients with Minimal Residual Disease. *N Engl J Med* 2016;375(10):944-53.
40. Hourigan CS, Haferlach T, Hokland P. Cord-Blood Transplantation in Patients with Minimal Residual Disease. *N Engl J Med* 2016;375(22):2204.
41. Falini B, Mecucci C, Tiacci E, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med* 2005;352(3):254-66.
42. Schlenk RF, Dohner K, Krauter J, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. *N Engl J Med* 2008;358(18):1909-18.
43. Schuurhuis GJ, Heuser M, Freeman S, et al. Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. *Blood* 2018;131(12):1275-91.
44. Bill M, Grimm J, Jentzsch M, et al. Digital droplet PCR-based absolute quantification of pre-transplant NPM1 mutation burden predicts relapse in acute myeloid leukemia patients. *Ann Hematol* 2018;97(10):1757-65.
45. Kayser S, Benner A, Thiede C, et al. Pretransplant NPM1 MRD levels predict outcome after allogeneic hematopoietic stem cell transplantation in patients with acute myeloid leukemia. *Blood Cancer J* 2016;6(7):449.
46. Knapper S, Russell N, Gilkes A, et al. A randomized assessment of adding the kinase inhibitor lestaurtinib to first-line chemotherapy for FLT3-mutated AML. *Blood* 2017;129(9):1143-54.
47. Gabert J, Beillard E, van der Velden VH, et al. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia - a Europe Against Cancer program. *Leukemia* 2003;17(12):2318-57.

48. Gale RE, Green C, Allen C, et al. The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. *Blood* 2008;111(5):2776-84.
49. Ustun C, Courville EL, DeFor T, et al. Myeloablative, but not Reduced-Intensity, Conditioning Overcomes the Negative Effect of Flow-Cytometric Evidence of Leukemia in Acute Myeloid Leukemia. *Biol Blood Marrow Transplant* 2016;22(4):669-75.

Table Legends

Table 1.

Clinical, molecular and transplant-related variables in each MRD-defined group. Spearman correlation *p* value is provided for the age comparison and Mantel-Haenszel *p* value is provided for all other variables. CR1 first complete remission. PB peripheral blood

Table 1

Pre-transplant MRD status	High n=19	Low n=30	Negative n=58	p
Median age, years Range	53 40-69	53 17-65	54 24-66	1.0
<i>FLT3</i> ITD positive	8 (42%)	8 (27%)	18 (31%)	0.5
<i>FLT3</i> ITD allelic ratio >0.5	4 (21%)	3 (10%)	7 (12%)	0.4
PB Post #2 MRD positive	6/17 (35%)	11/26 (42%)	4/53 (8%)	0.002
Transplanted in CR1	5 (26%)	20 (67%)	31 (53%)	0.16
Myeloablative conditioning	8 (42%)	6 (20%)	17 (29%)	0.5
Sibling donor	6 (32%)	14 (47%)	23 (40%)	0.7
T cell depletion	16 (84%)	25 (83%)	41 (71%)	0.15

Figure Legends

Figure 1.

CONSORT diagram showing the number of patients in each part of the trial, therapy given prior to transplant and outcomes in each group. CT chemotherapy, MRD minimal residual disease, NRM non-relapse mortality, REL relapse, UNK unknown cause of death.

Figure 2.

Overall survival from date of transplant according to pre-transplant molecular MRD status.

Panels A-C show the difference in survival between patients with positive and negative MRD (A) overall (B) in the peripheral blood, (C) in the bone marrow. Panels D-F show the difference in survival between patients with negative, low and high levels of MRD (D) in the peripheral blood using a cut-off at 200 copies per 10^5 *ABL* (E) in the bone marrow with level of >1000 copies and (E) with either, defining “high level” MRD. Percentages indicate estimated 2 year OS.

Figure 3.

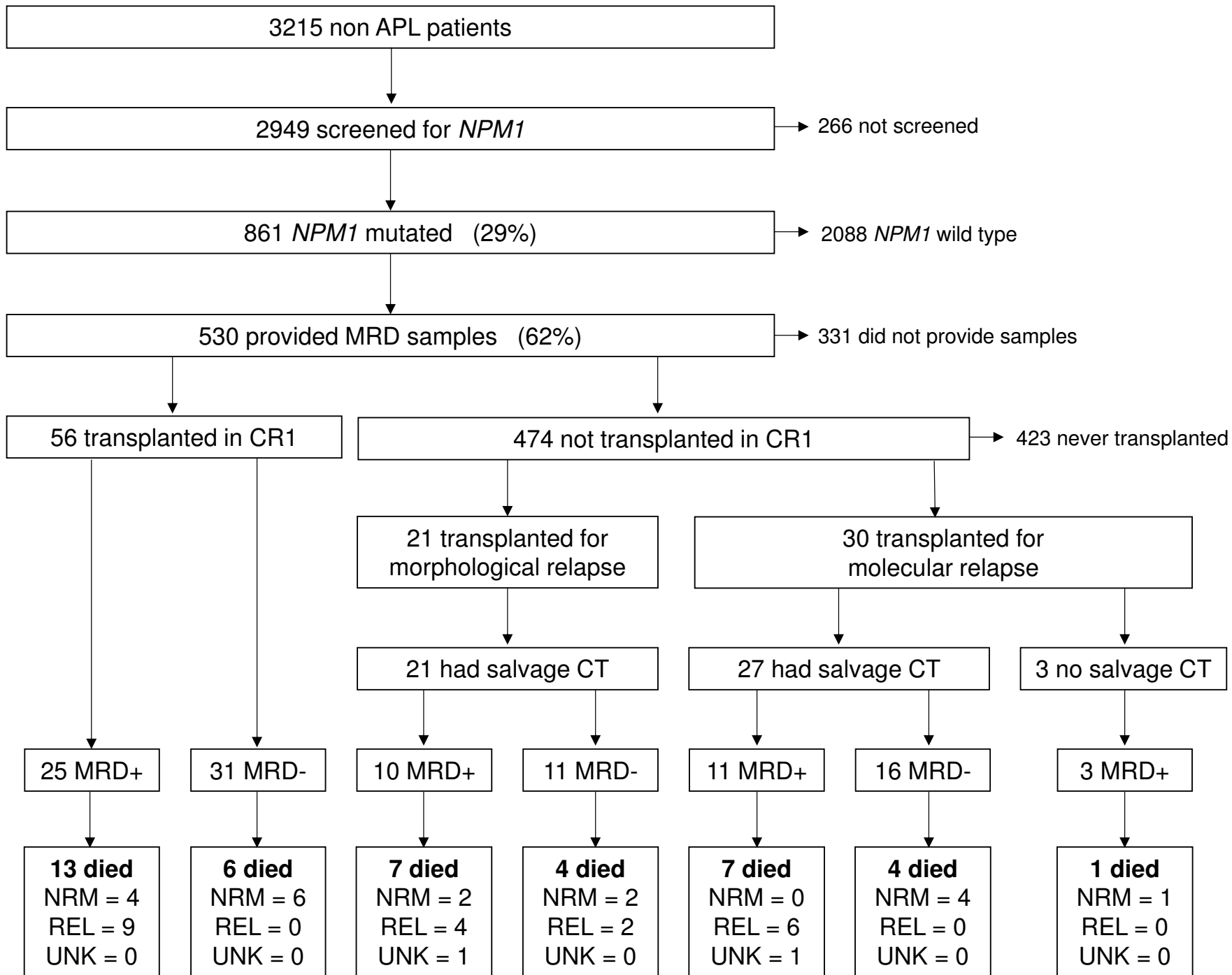
Effect of *FLT3* ITD on outcome according to pre-transplant MRD status. (A) Hazard ratio and 95% confidence intervals for *FLT3* ITD mutation in each group. (B-D) Overall survival from transplant for patients with high (B), low (C) and negative (D) pre-transplant MRD. Percentages indicate estimated 2 year overall survival.

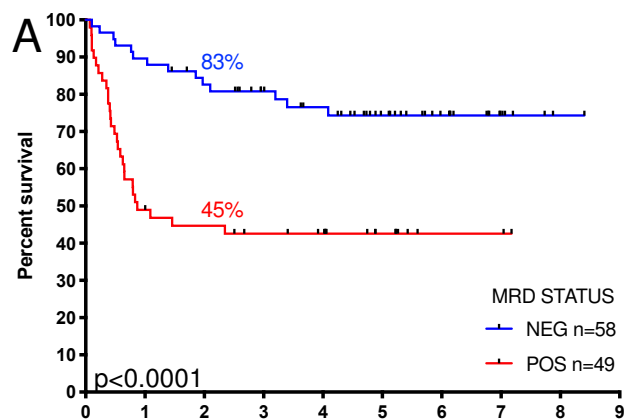
Figure 4.

Overall survival from transplant according to the risk group. The risk group was derived from *FLT3* ITD status and pre-transplant MRD level. Patients with high levels of MRD, and those with low levels who had the *FLT3* ITD mutation were allocated to the high-risk group. All other patients were allocated to the low-risk group. Percentages indicate estimated 2 year overall survival.

Figure 5.

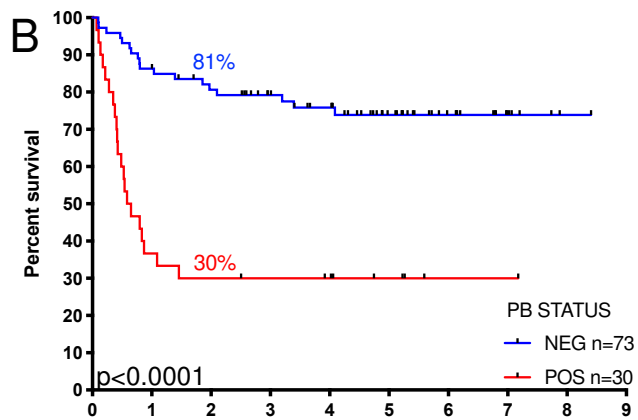
Effect of transplant-related factors on overall survival. Panels A-C show the effect of transplant related variables in the entire cohort, panels D-F show their effect in patients who were MRD-positive prior to transplantation. (A,D) Effect of donor source. (B,E) Effect of conditioning type. (C,F) Effect of T-cell depletion. SIB sibling donor, VUD volunteer unrelated donor, MAC myeloablative conditioning, RIC reduced intensity conditioning.



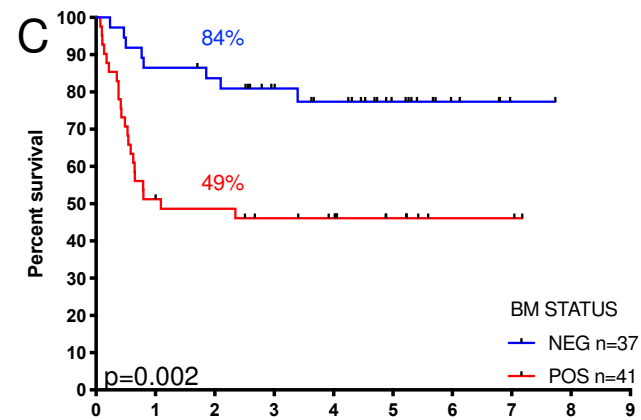


At risk:

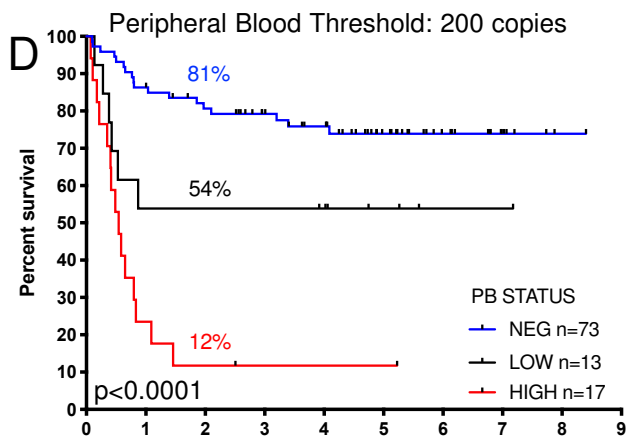
	Years from Transplant									
	0	1	2	3	4	5	6	7	8	9
Neg	58	52	46	39	34	24	15	6	1	0
Pos	49	24	21	18	16	8	2	2	0	0



	Years from Transplant									
	0	1	2	3	4	5	6	7	8	9
NEG	73	63	56	48	42	27	15	6	1	0
POS	30	11	9	8	7	4	1	1	0	0

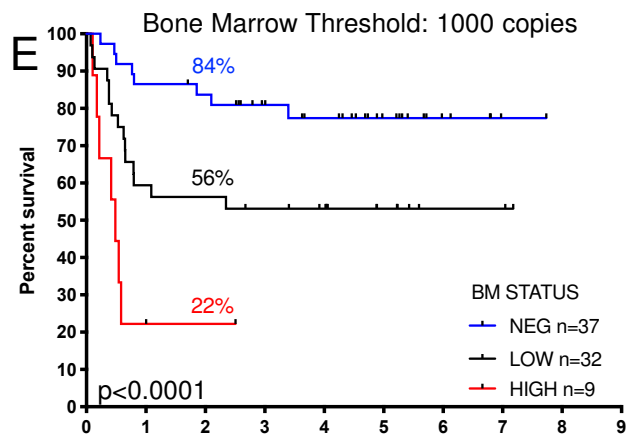


	Years from Transplant									
	0	1	2	3	4	5	6	7	8	9
NEG	37	32	30	24	20	12	5	1	0	0
POS	41	21	19	16	14	7	2	2	0	0

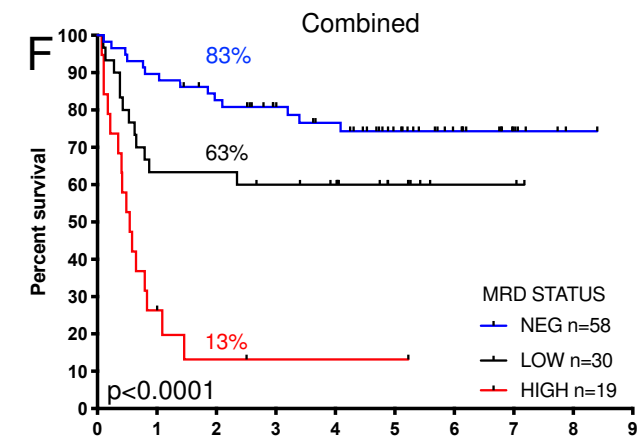


At risk:

	Years from Transplant									
	0	1	2	3	4	5	6	7	8	9
Neg	73	63	56	48	42	27	15	6	1	0
Low	13	7	7	7	6	3	1	1	0	0
High	17	4	2	1	1	1	0	0	0	0

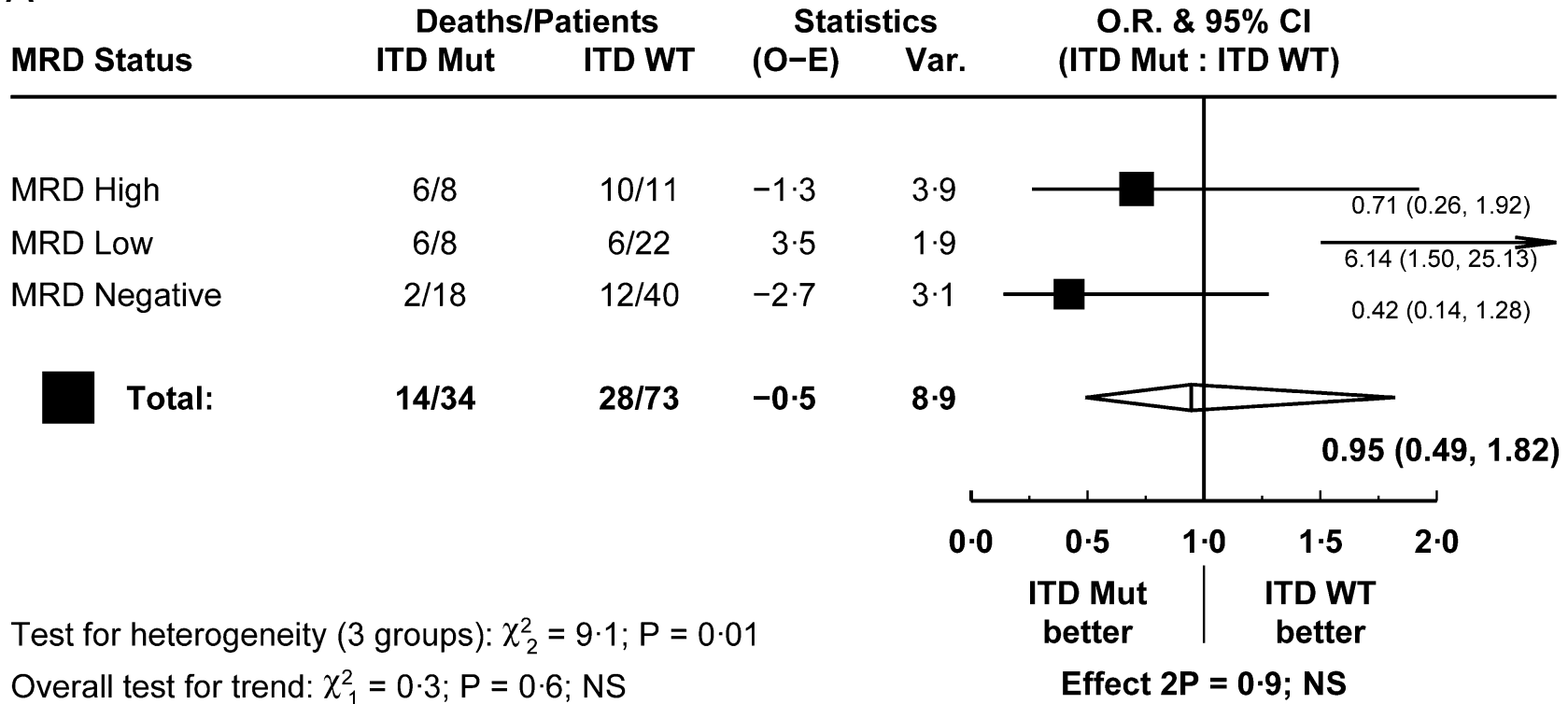


	Years from Transplant									
	0	1	2	3	4	5	6	7	8	9
NEG	37	32	30	24	20	12	5	1	0	0
LOW	32	19	18	16	14	7	2	2	0	0
HIGH	9	2	1	0	0	0	0	0	0	0



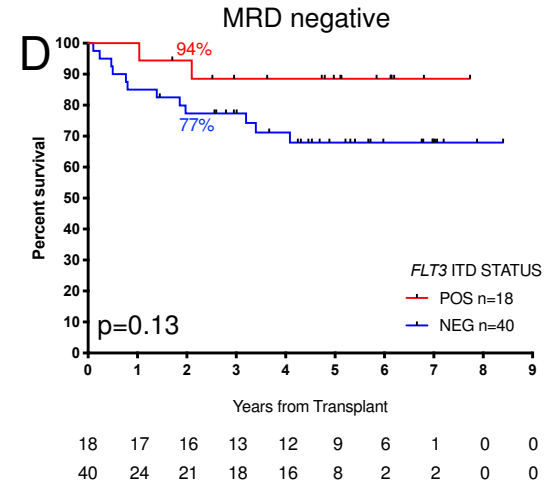
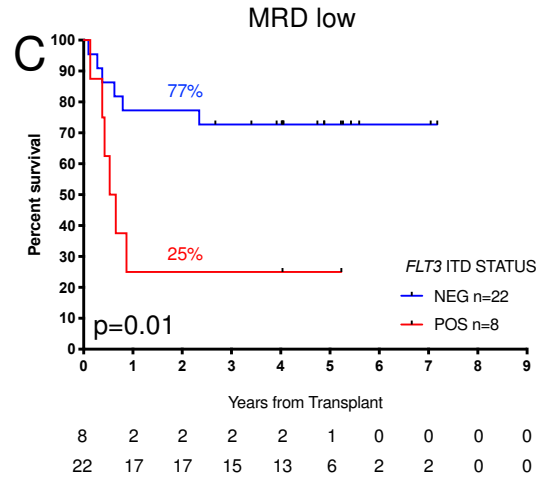
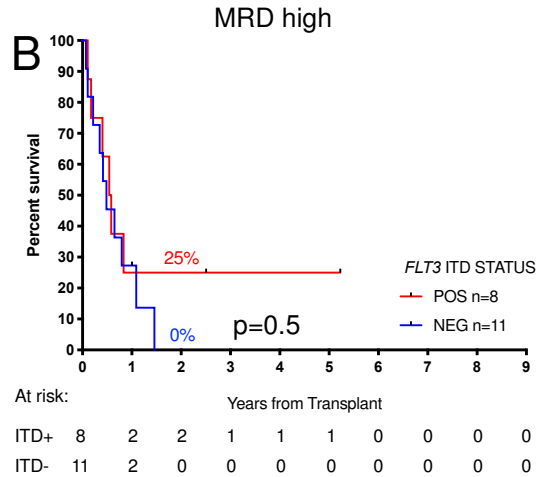
	Years from Transplant									
	0	1	2	3	4	5	6	7	8	9
NEG	58	52	46	39	34	24	15	6	1	0
LOW	30	19	19	17	15	7	2	2	0	0
HIGH	19	5	2	1	1	1	0	0	0	0

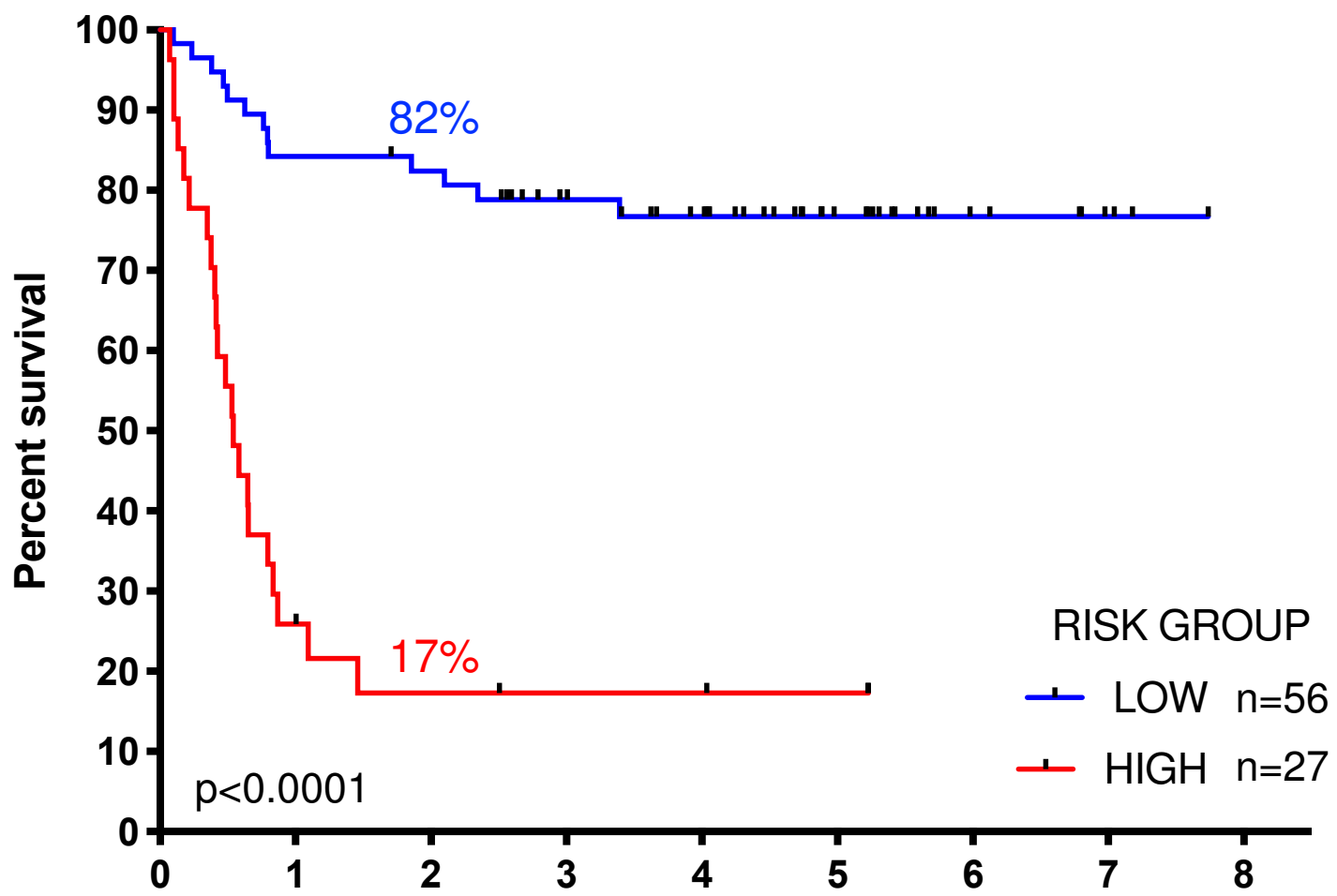
A



Test for heterogeneity (3 groups): $\chi^2_2 = 9.1$; P = 0.01

Overall test for trend: $\chi^2_1 = 0.3$; P = 0.6; NS

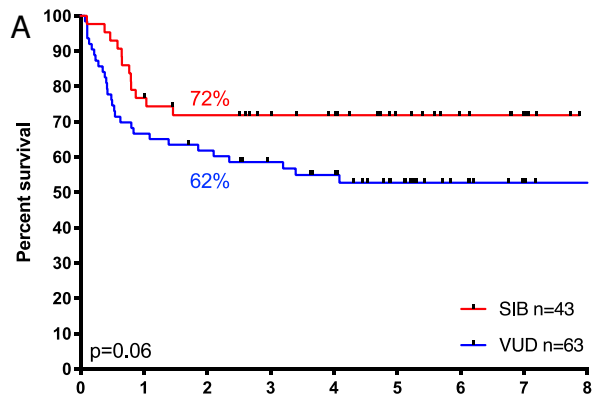




At risk:

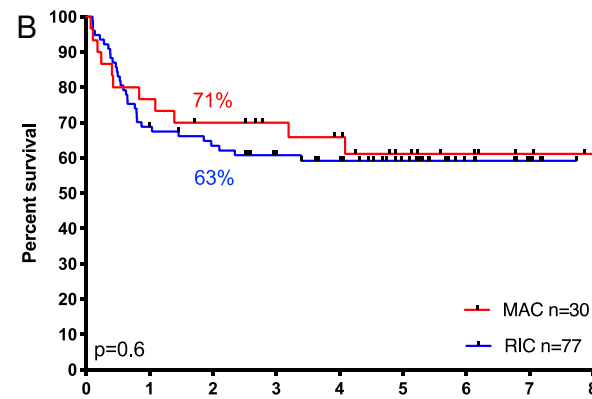
Years from Transplant

	0	1	2	3	4	5	6	7	8
Low	56	48	46	38	32	17	8	4	0
High	27	8	4	3	3	2	0	0	0



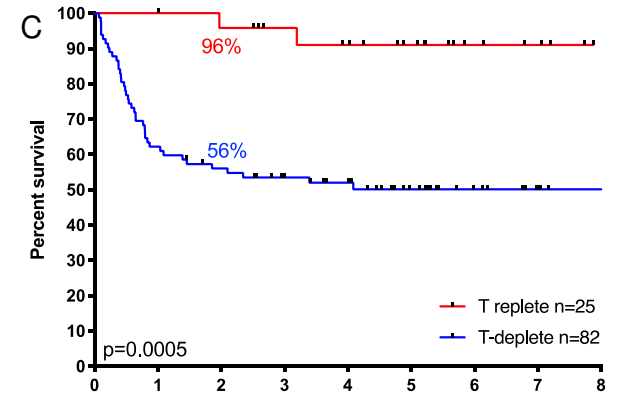
At risk:

	0	1	2	3	4	5	6	7	8
SIB	43	33	29	25	22	14	9	5	0
VUD	63	42	38	32	28	18	8	3	1



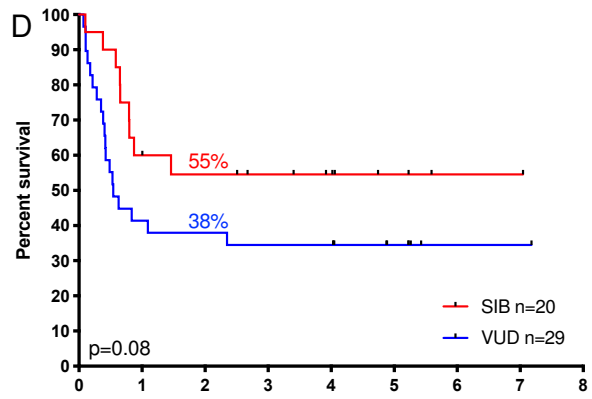
At risk:

	0	1	2	3	4	5	6	7	8
MAC	30	23	20	17	15	10	6	3	1
RIC	77	53	47	40	35	22	11	5	0



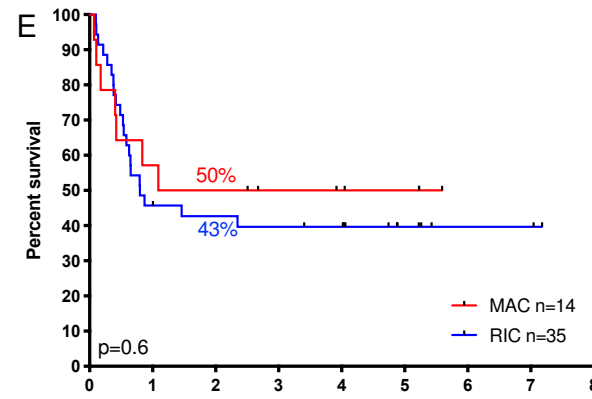
At risk:

	0	1	2	3	4	5	6	7	8
T-rep	25	25	23	20	18	14	7	4	1
T-dep	82	51	44	37	32	18	10	4	1



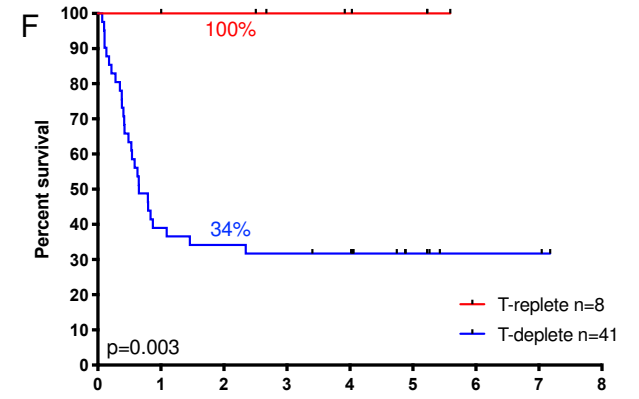
At risk:

	0	1	2	3	4	5	6	7	8
SIB	20	12	10	8	6	3	3	1	0
VUD	29	12	11	11	10	5	1	1	0



At risk:

	0	1	2	3	4	5	6	7	8
MAC	14	8	7	5	4	3	0	0	0
RIC	35	16	14	13	12	5	2	2	0



At risk:

	0	1	2	3	4	5	6	7	8
T-rep	8	8	7	5	4	3	0	0	0
T-dep	41	16	14	13	12	5	2	2	0