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Measurable Residual Disease at Induction Redefines Partial Response in Acute Myeloid Leukemia and Stratifies Outcomes in Standard-Risk Patients Without NPM1 Mutations

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Value of MFC-MRD with Response Criteria in AML Risk Groups

Abstract

Purpose

We investigated the effect on outcome of measurable/minimal residual disease (MRD) status after each induction course in order to evaluate the extent of its predictive value for acute myeloid leukemia (AML) risk groups including *NPM1*wild-type standard-risk when incorporated with other induction response criteria.

Patients and Methods

As part of the NCRI AML17 trial, 2450 younger adult patients with AML or high risk MDS had prospective flow cytometric MRD (MFC-MRD) assessment. Post course-1 (C1) responses were categorised as resistant disease (RD), partial remission (PR) and CR/CRi by clinicians, with CR/CRi subdivided by MFC-MRD assay into MRD+ and MRD-. Patients without high-risk factors, including *Flt3*ITDwild-type/-*NPM1*wild-type subgroup, received a second daunorubicin/ara-C induction; course-2 (C2) was intensified for high-risk.

Results

Survival outcomes from PR and MRD+ responses post-C1 were similar, particularly for good/standard-risk subgroups (5 year OS: 33%RD vs 49%PR vs 51%MRD+ vs 70%MRD-, p<.0001). Adjusted analyses confirmed significant OS differences between C1 RD vs PR/MRD+ but not PR vs MRD+. CRi post-C1 reduced OS in MRD+ (19%CRi vs 45%CR, p=.001) patients with a smaller effect post-C2.

The prognostic effect of C2 MFC-MRD status (relapse, HR 1.88(1.50- 2.36), p<0.001; survival, HR 1.77(1.41-2.22) p<0.001) remained significant when adjusting for C1 response. MRD-positivity appeared less discriminatory in poor-risk patients by stratified analyses. For *NPM1*wild-type standard-risk subgroup, C2 MRD+ was significantly associated with poorer outcomes (OS, 32% vs 64%MRD-, p=0.003; relapse incidence 89% when MRD+ ≥0.1%); transplant benefit was more apparent in MRD+(HR 0.72(0.31-1.69) than MRD-(HR 1.68(0.75-3.85); (p=0.16 for interaction).

Conclusion MFC-MRD can improve outcome stratification by extending definition of partial response post first induction and may help predict *NPM1*wild-type standard-risk patients with poor outcome who benefit from transplant in CR1.

Introduction

In acute myeloid leukemia (AML) failure to achieve morphological complete remission (CR) after a first cycle of induction in previously untreated patients is an established independent prognostic factor from earlier studies[1-3]. Thus morphological response at this time-point is often incorporated with genetic and pre-treatment clinical parameters to guide further therapy[4], including second induction courses, choice of consolidation and whether intensification from allogeneic stem cell transplantation (SCT) may be appropriate in otherwise intermediate risk patients. Despite morphological response criteria being standard, a different approach for measuring response has been proposed[5,6] due to the independent prognostic value from measurable/minimal residual disease (MRD) assays when discrepant with morphology[7-9] or in CR[10-12] and the equivalent poor outcomes between MRD positivity and active disease pre-myeloablative SCT[13,14]

Previous studies have shown the prognostic value of MRD monitoring by PCR for patients with validated molecular targets, usually after 2 courses of chemotherapy[11,12,15]. Flow cytometric MRD (MFC-MRD) may identify as early as post course 1, patients with a poorer response despite achieving CR and is an assay that can be applied across AML genetic subgroups[12,16-20]. There is however insufficient data to ascertain the relative prognostic impact of MFC-MRD positivity in CR post course 1 compared to morphological active disease; it is feasible that the outcomes of patients with detectable MRD resemble those of refractory patients who achieve the cytoreduction criteria for a morphological partial remission[21,22]. Evaluating this will help refine which response categories are the most useful prognostic surrogate endpoints to assess effectiveness of the first induction course.

It is also uncertain for patients who complete a second chemotherapy course whether the quality of response after course 1, with inclusion of MFC- MRD assessment, adds prognostic information to CR-MRD status post course 2. The value of MFC-MRD status to differentiate outcome at either time-point is likely to be heterogeneous between established risk subgroups due to disease, treatment and assay factors but the extent of this has not been established.

Treatment decisions, including predicting the benefit of SCT, are particularly challenging for the standard-risk subgroup. MFC-MRD assays are most likely to influence therapeutic choices for *NPM1*-wild type standard-risk patients following recent data that post induction RT-qPCR quantitation of blood mutated transcripts reliably predicts outcome for *NPM1*-mutated patients[23,24]. There is thus a specific need to define the usefulness of MFC-MRD for risk stratification in this subgroup.

In this study we aimed to determine the prognostic effect of MFC-MRD measurement incorporated into response assessment post induction courses for the different risk subgroups, including *NPM1* wild type standard-risk patients, in a large cohort of younger AML patients who had undergone intensive treatment in the National Cancer Research Institute (NCRI) AML17 trial.

Methods

Patients

Patients were enrolled in the NCRI AML17 trial (ISRCTN: 55675535) from April 6, 2009, to December 31, 2014. (A list of treatments is provided in Appendix, Fig. A1).

The AML17 protocol was designed primarily for younger patients, generally age < 60 years. Patients with high risk myelodysplastic syndrome (MDS), which was defined as >10% marrow blasts at diagnosis, and secondary AML were eligible. Acute promyelocytic leukemia (APML) patients were not included in this MRD study. After first induction, patients were defined by risk of relapse, using a validated score comprising cytogenetics, WBC, age, secondary disease, morphological response to course-1[25,26] and *FLT-3* ITD/ *NPM1* mutation status.

Morphological-based response criteria were 1) complete response (CR), < 5% blasts in a cellular bone marrow, CRi when best response was with neutropenia <1000/μL or thrombocytopenia <100,000/μL; 2) partial remission (PR), decrease of pre-treatment bone marrow blast percentage by at least 50% to 5 -15% in a cellular marrow (hematological recovery not required)[1], 3) resistant disease (RD), >15% marrow blasts (patients surviving at least 7 days post completion of treatment). Responses were classified by centers.

Patients designated as favorable or standard-risk received the second daunorubicin/cytosine arabinoside course and were then randomized to receive either 1 or 2 courses of high-dose cytosine arabinoside. High-risk patients were offered a randomization between FLAG-Ida or daunorubicin/clofarabine with the intention of eventually proceeding to allogeneic stem cell transplantation (SCT). *FLT3*-ITD mutant patients were directed to the Lestaurtinib randomisation until 2012.

The trial was sponsored by Cardiff University, approved by Wales-REC3 and conducted in accordance with the Declaration of Helsinki.

Multiparameter Flow Cytometry (MFC) detection of MRD

Samples for MFC-MRD were requested at baseline (bone marrow and/or blood) and following each course (bone marrow). A summary of sample logistics and processing is provided in the Appendix. MFC-MRD analysis was performed centrally, using standardised gating strategy that screened for 'different-from-normal' LAIPs (Leukemia-Associated-Immunophenotypes) on blasts pre-treatment and tracked these (~0.02 -0.05% sensitivity thresholds) but also applied the 'different-to-normal' approach in follow-up (FU) samples to detect changes in blast LAIPs (~0.05 - 0.1% sensitivity threshold). In this study only samples for which there were pre-treatment LAIPs to monitor could be reported as MFC-MRD-negative whilst samples with any level of MRD detected above a diagnostic-LAIP or different-from-normal FU-LAIP threshold were reported as MFC-MRD-positive.

Clinicians were not informed of MFC-MRD results.

Statistical Analysis

All end points were based on the revised criteria of the International Working Group for Diagnosis[21]. Survival percentages were calculated using the Kaplan-Meier method with cumulative incidence of relapse calculated using competing risks methodology. Baseline characteristics were compared using chi-squared or Mantel-Haenszel tests, with continuous variables compared using the Wilcoxon rank sum test. Time-to-event outcomes were compared using logrank tests and Cox regression. Outcomes are reported as effect sizes with 95% confidence intervals; significance was set at $p<.05$. Stratified analyses use stratified logrank tests and are displayed as Forest plots with tests for interaction using standard methodology[27]. Comparison of transplantation versus not was analysed using the method of Mantel & Byar to mitigate immortal time bias.

Median follow-up for survival was 39.0 months (range 1.0-80.5 months).

Results

Induction Response by Morphology and MFC-MRD: Patient Characteristics

Between 2009 and 2014, 6539 samples (BM or PB at diagnosis, BM post treatment courses) from 2450 non-APML patients recruited to AML17 were prospectively analysed for flow cytometric detection of MRD (MFC-MRD) (Appendix-Fig A2). Among patients in complete remission post course-1 (C1), the presence of MRD data was associated with secondary AML, and the absence of an *NPM1* mutation (reflecting the prioritising of BM for RT-qPCR monitoring of *NPM1* mutations[23] during the second phase of the trial); survival at 5 years was 52% (with MRD data) vs 50% (without MRD data). In adjusted analyses, the presence of MRD data was not associated with survival (HR 0.99 (0.84-1.16) *P* =0.9).

Post-C1 1443 patients contributed data, 420 were refractory by morphology (197 resistant disease/ RD, 223 partial remission/ PR) and 1023 (70.9%) achieved morphological complete remission (CR/CRi) with MFC-MRD data (446 MFC-MRD negative [MRDneg], 577 MFC-MRD positive [MRDpos]). After the second course of induction (C2), 806 patients were in CR/CRi with MFC-MRD data (503 MRDneg, 303 MRDpos).

The clinical characteristics of patients according to response post-C1 and MRD status for patients in CR/CRi post-C1 or C2 are listed in Table 1. There was a significant association between responses post course-1 or course-2 and cytogenetic group; however, count recovery post course-1 was not significantly associated with MRD post either course.

Outcome Comparison for Morphologic Response and MFC-MRD Status after Course 1

We evaluated overall survival (OS) by C1 response status. Five year overall survival (OS) for all enrolled in AML17 excluding early deaths was 52% for those achieving CR/CRi vs 31% for refractory patients (*P* <.001). MRD status in CR/CRi versus PR or RD further differentiated 5 year survival outcomes (Fig 1A). A PR or MRDpos response gave intermediate survival at 5 years. Survival rates appeared equivalent between these two responses for the good / standard-risk patients; 5 year OS for MRDneg vs MRDpos vs PR vs RD were 63% vs 44% vs 35% vs 24% for all patients; 70% vs 51% vs 48% vs 27% when poor-risk patients excluded (Fig 1B); 66% vs 49% vs 46% vs 30% for standard-risk alone (Fig 1C) (*P* <.001 for all analyses). Similar results were observed for survival censored at SCT (Appendix-FigA3A, Fig 1D) and also for *NPM1*wild type (wt) standard-risk patients (Appendix-FigA3B-C).

Adjusted analyses confirmed significant survival differences between RD and PR / MRDpos but not between PR and MRDpos for good /standard-risk patients (RD vs PR /MRDpos, OS HR 2.28(1.38-3.75) P=.0009; PR vs MRDpos, HR=1.32,P=0.4) and for *NPM1*wt standard-risk patients (RD vs PR /MRDpos , OS HR 2.13(1.21-3.75) P=.008; PR vs MRDpos, HR=1.18,P=0.6) . Results were similar when censored at SCT (Table 2).

Thus the prognostic effect from morphological response criteria post first induction is restricted to RD in in the good/ standard-risk subgroups when MFC-MRD status is incorporated into response assessment.

Only 25 patients were refractory by morphology post-C1 but MRDneg (22 PR, 3 RD) with 61% 3-year and 49% 5-year OS. Seven of 577 MRDpos patients were in morphological CR but had ≥5% aberrant blasts by MFC (range 5.4-38%), Six died within 2 years, with one patient alive at 58.6 months.

Relative Prognostic Effect of MFC-MRD after Course 1 & Course 2 by Genetic / Risk Score Subgroup

In AML17 patients received 2 courses of induction regardless of remission status after course 1 but course 2 differed for patients designated as poor risk by trial risk score. Analyses of survival and relapse by MFC-MRD status of CR/CRi patients for C1 (n=1010) and C2 (n=803) were performed stratified by cytogenetic[28] and trial risk subgroups (Fig 2, Appendix-FigA4) to investigate the relative prognostic impact from clearance of blasts below MFC-MRD detection threshold at either of these response assessment time–points. There was some evidence that the benefit from MFC-MRD negativity on OS was lower in poor-risk patients compared to other subgroups with the NCRI AML17 treatment schedule (test for trend; p=0.01 for C1, p=0.05 for C2). Overall MFC-MRD status appeared more prognostic for relapse and OS at C2 (relapse, OR 1.88(1.50- 2.36), p<0.001; survival, HR 1.77(1.41-2.22) p<0.001) than C1 (relapse, HR 1.70(1.40-2.06), p<0.001; survival, HR 1.50(1.23-1.84) p<0.001) although this difference diminished when C1 analysis was restricted to patients who received course 2 and survived at least 30 days post-C2 (relapse, HR 1.80(1.49-2.18) p<.0001; survival, HR 1.87 (1.52-2.29) p<.0001).

Outcome from Combined Course 1 Response status and Course 2 MFC-MRD status

In patients with response / MFC-MRD data for both C1 and C2 time points (n=693), C2 MFC-MRD positivity remained significant on OS and relapse when adjusting for C1 response (5 year survival, HR 1.79(1.38-2.32) P<.0001; relapse, HR 1.52(1.18-1.96) P=.001) (Fig 3). A small number of patients (n=24) patients converted from C1 MRDneg to C2 MRDpos, with a particularly poor prognosis (15 relapses, 13 deaths); one had adverse risk cytogenetics and 5 were *Flt3*ITD mutated (Appendix-Table A1). Patients who were MRDneg at both C1 and C2 had the best outcome (n=217, 67 relapses /57 deaths); of these 80.8% were good /standard-risk and 26.3% *NPM1*wt standard-risk (Appendix-Table A2).

MRD status combined with peripheral count recovery

We examined the additional prognostic effect from combining MRD status with response by peripheral count recovery post-C1 and C2 (Appendix-Table A3). The frequencies of CRi as best response in the total cohort were similar in MRDpos vs MRDneg patients post-C1 (9.3%vs 9.6%) and C2 (13.1%vs12.0%); CRi frequencies were not relatively increased in the *NPM1*wt standard-risk subgroup. C1 CRi was associated with significantly decreased 5 year OS for total (39% vs 53%, p=.002) and in MRDpos (19% vs 45%, p=.001) but not for MRDneg patients. MRDpos *NPM1*wt standard-risk-patients in CRi also had a lower OS at 5 years (25%vs 48%, p=0.4) although difference was not significant. The effect of CRi vs CR was smaller post-C2 although outcomes were still worst in CRi/MRDpos patients. The reduced survival associated with CRi was not due to increased relapse.

Outcome by MFC-MRD status for *NPM1*-wild type Standard-Risk Patients

Since it is possible that the most appropriate MFC-MRD cut-off level for discriminating outcome may differ between AML genetic subgroups, we compared the 5 year cumulative incidence of relapse for C1 MRDneg vs MRDpos <0.1% vs MRDpos ≥0.1% by our assay in CBF-AML and *NPM1*-mutated as well as *NPM1*wt standard-risk patients. For CBF-AML and *NPM1*-mutated post C1 MRDpos at any level (<0.1% or ≥0.1%) significantly increased relapse (Appendix-FigA5). However in the *NPM1*wt standard-risk subgroup, low level MRDpos (<0.1%) post-C1 did not alter relapse risk compared to MRDneg but was associated with a higher CIR when detected post-C2 (Fig 4A). MRDpos levels of ≥0.1% detected in 35% and 13% *NPM1*wt standard-risk patients post-C1 and post-C2 respectively predicted a high probability of relapse (C1 3 year CIR 68%, C2 CIR 89%). MRD status post second induction was also significantly prognostic for survival; 33% for any level of MRD positivity vs 63% for MRDneg at 5 years (3 years 47% vs 69%, P=.003) (Fig 4B).

Of the 204 *NPM1*wt standard-risk patients with C2 MRD data, 83 had an allograft (44 in CR1: 29 MRDneg, 15 MRDpos). When survival was censored at any SCT, rates of 5 year OS were 35% vs 88% (3 years 47% vs 88%, P=.0005) (Appendix-FigA6). We next investigated the effect of SCT in CR1 according to C2 MRD status in Mantel-Byar analyses; although numbers were small, results suggested that transplant might be considered in MRDpos (HR 0.72(0.31-1.69) but not MRDneg patients (HR 1.68(0.75-3.85); p-value for interaction p=0.16) (Fig 4C).

Discussion

Response to induction therapy is a powerful prognostic indicator in AML. There are however differing practices for the implementation of technologies that measure residual leukemia to assess response. Flow cytometry is often used to support the definition of CR by morphology; those centres with access to experienced laboratories including some trial groups have extended its use to define CR without MRD[5]. It has recently been reported that outcomes post

myeloablative SCT for patients with pre-transplant flow cytometric MRD below 5% resemble those with at least 5% blasts by morphology[13]. This and the similar event-free survival observed in ~80 pediatric patients with MRD positivity after first induction whether <5% or ≥ 5% blasts by morphology[7] suggest that dichotomising patients by a 5% blast CR cut-off fails to capture some prognostic information. Our results confirm this. By incorporating flow cytometric MRD with established response criteria of partial remission and resistant disease distinct prognostic groups emerge post first course of standard induction for 5 year survival. Importantly the response subgroup with intermediate outcome comprises patients on either side of the current CR blast threshold, those with MRD positivity in CR and those who are refractory but clinically classified as a PR; both responses are associated with very similar 5 year survival, particularly in patients otherwise allocated as good /standard-risk subgroups. This is also the case when PR is defined by ELN criteria[5,21](Appendix-FigA7). From this, 3 response categories post first induction could be proposed: resistant disease, partial responders (flow cytometric MRD-positive whether below or above 5% blast threshold) and CR/CRi without MRD. CRi was an independent risk factor to MRD in a study that included relapsed/refractory AML and differing induction intensities[29,30]. From our data, outcomes for newly diagnosed AML patients achieving negative MRD are equivalent between CRi and CR after a single standard induction. However, the relatively few in our cohort (4.8%) with both CRi and MRD-positivity after first induction had as poor survival (OS 19% for all, 25% NPM1wt standard-risk) as RD patients.

For those completing a second induction with a CR/CRi, post course-2 MRD status increases prognostic discrimination. Although sample attrition bias may limit analyses comparing time-points, MRD negativity post course-2 improves outcome overall even when adjusting for slower blast clearance by course-1 response. This differs from our previous results in older adults[17] and might reflect the better treatment tolerance and mutation profiles of younger adults. However following the second daunorubicin/cytosine arabinoside induction, ~33% of standard-risk /~34% of NPM1wt standard-risk patients in CR/CRi had persistent bone marrow MRD by our assay. Whether detectable MFC-MRD after completion of conventional induction is a sufficiently specific prognostic surrogate to guide therapy has been debated. The post-consolidation time-point was more informative in the GIMEMA study for a cohort that included ~70% with intermediate cytogenetics [31,32]. This suggests that in a proportion of those with post-induction MRD positivity, consolidation may confer a favourable outcome by further MFC-MRD clearance (although it is of note that for some younger adults in the GIMEMA trials the induction/consolidation regime comprised 2 courses in total). Genetic profile, treatment intensity and the later effects of any transplant may also modify interpretation and utility of MFC-MRD to inform post-remission therapy. Our data is consistent with this since the prognostic impact as well as best MFC-MRD cut-off level differed between AML risk groups; MRD status appeared less discriminatory in the poor-risk

patients. Importantly however, in the NPM1wt standard-risk subgroup, detectable MFC-MRD at ≥0.1% early in treatment is associated with significantly higher relapse rates (89% after second course). The 'false negative' 50% CIR observed for post induction MRD-negative NPM1wt standard-risk patients could reflect MFC-MRD sensitivity limitations although a similar CIR was observed for DNTM3A-mutated /NPM1-mutated patients who were MRD-negative by NPM1-mutated transcript RT-qPCR[23]. Exploratory analyses could not identify any significant clinical parameters that predicted MRD-negative relapses. Longitudinal broad molecular studies may disclose whether increased pre-leukemic instability re-initiating AML[33,34] or persistence of pre-treatment minor or major leukemic clones[35,36] contributes to these 'false-negative' relapse risks. Notwithstanding, NPM1wt standard-risk patients achieving MRD negativity post second induction had a significantly better survival. As their survival increased to 88% when censored for transplant, this raises the possibility that transplant in first remission could be avoided in this subset. The Mantel-Byar analysis support this with some evidence of interaction, although this should be interpreted cautiously as small numbers and the interaction was not significant.

Transplant decisions have mainly been arbitrary in this subgroup with no accepted approach to distinguish those patients likely to be cured with chemotherapy alone (or those likely to be successfully salvaged if they do relapse) from those who benefit from transplantation in first remission or potentially experimental therapy. Our results suggest that allogeneic transplant in first remission could be directed to those who are MRD-positive rather than MRD-negative. This is the first indication that MRD status might have utility directing therapy for NPM1wt standard-risk patients despite their molecular heterogeneity. Large patient data sets likely requiring collaborative efforts will determine whether integrating MFC-MRD status with genomic profiles[37,38] further informs outcome prediction.

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Author Disclosures

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Figure Legends

Figure 1. Overall Survival according to post course 1 response status.
(A) all patients (B) good and standard risk patients (known poor risk excluded) C) standard-risk patients D) OS for standard-risk patients censored at allogeneic stem cell transplantation

RD, refractory disease; PR, partial remission; CR, complete remission; MRD, measurable residual disease; MRD-, MRD negative; MRD+, MRD positive; ASCT, allogeneic stem cell transplantation

Figure 2. Forest Plots for Overall Survival by MFC - MRD status for patients in CR (A) post course 1, (B) post course 2 stratified by cytogenetic risk group and NCRI AML17 risk score group

CR, complete remission; MRD, measurable residual disease; MRD -, MRD negative; MRD+, MRD positive

Figure 3. Forest Plots for (A) Overall Survival and (B) Relapse by combined post course 1 and 2 response data.
Effect of MFC-MRD status in CR post course 2 stratified by post course 1 response status

CR, complete remission; RD, refractory disease; PR, partial remission; MRD, measurable residual disease; MRD-, MRD negative; MRD+, MRD positive

Figure 4. Standard-risk *NPM1*-wild type: (A) Cumulative Incidence of Relapse (CIR) by MRD level
(MRD– vs MRD+ <0.1% vs MRD+ ≥ 0.1%) post course 1 and post course 2,
(B) Overall Survival according to post course 2 MRD status, MRD- vs MRD+.
(Not shown: MRD+ ≥ 0.1%, overall survival of 24%; MRD+ <0.1%, overall survival of 39%)
(C) Mantel Byar for survival according to CR1 SCT by MRD status post course 2
MRD, measurable residual disease; MRD-, MRD negative; MRD+, MRD positive

SCT, allogenic stem cell transplant; CR1, first CR; MRD-, MRD negative; MRD+, MRD positive

In Appendix

Appendix Figure A1. Flow chart for treatments given to patients in NCRI AML17

Appendix Figure A2. CONSORT: Outline of patient sample flow for MRD study

Appendix Figure A3. OS according to post course 1 response status. (A) All patients, OS censored at allogeneic stem cell transplantation, (B) *NPM1* wild type standard risk patients, (C) *NPM1* wild type standard risk patients, censored at allogeneic stem cell transplantation

OS, overall survival; RD, resistant disease; PR, partial remission; CR, complete remission; MRD, measurable residual disease; MRD-, MRD negative; MRD+, MRD positive; SCT, allogeneic stem cell transplantation

Appendix Figure A4. Forest Plots for Relapse by FCM - MRD status for patients in CR (A) post course 1, (B) post course 2 stratified by cytogenetic risk group and NCRI AML 17 risk score group

CR, complete remission; MRD, measurable residual disease; MRD-, MRD negative; MRD+, MRD positive

Appendix Figure A5. Cumulative Incidence of Relapse (CIR) by MFC-MRD level (MRD- vs MRD+ <0.1% vs MRD+ ≥ 0.1%) post course 1
(A) CBF AML (B) Standard Risk NPM1 Mutant
MRD, measurable residual disease; MRD-, MRD negative; MRD <0.1%, MRD positive <0.1%; MRD 0.1%+, MRD positive ≥ 0.1%

Appendix Figure A6. Standard Risk NPM1wild type: Overall Survival according to post course 2 FCM-MRD status, censored at any allogeneic stem cell transplantation

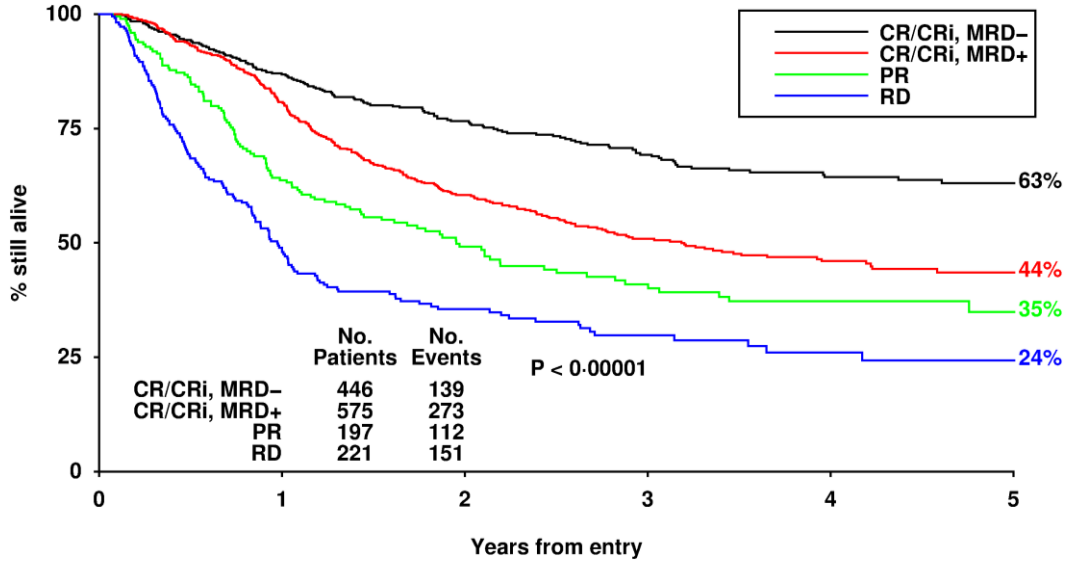
CR, complete remission; MRD, measurable residual disease; MRD-, MRD negative; MRD+, MRD positive

Appendix Figure A7. OS according to post course 1 response status applying ELN /Cheson criteria for PR and RD instead of MRC criteria. **(A)** All patients. **(B)** good and standard risk patients (known poor risk excluded) **(C)** standard risk patients, **(D)** standard risk patients, OS censored at allogeneic stem cell transplantation.

OS, overall survival; RD, resistant disease by ELN defintion; PR, partial remission by ELN definition; CR, complete remission; MRD, measurable residual disease; MRD-, MRD negative; MRD+, MRD positive; SCT, allogeneic stem cell transplantation
ELN criteria for PR: all hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25% with decrease of pretreatment bone marrow blast percentage by at least 50%

FIGURE 1A - D

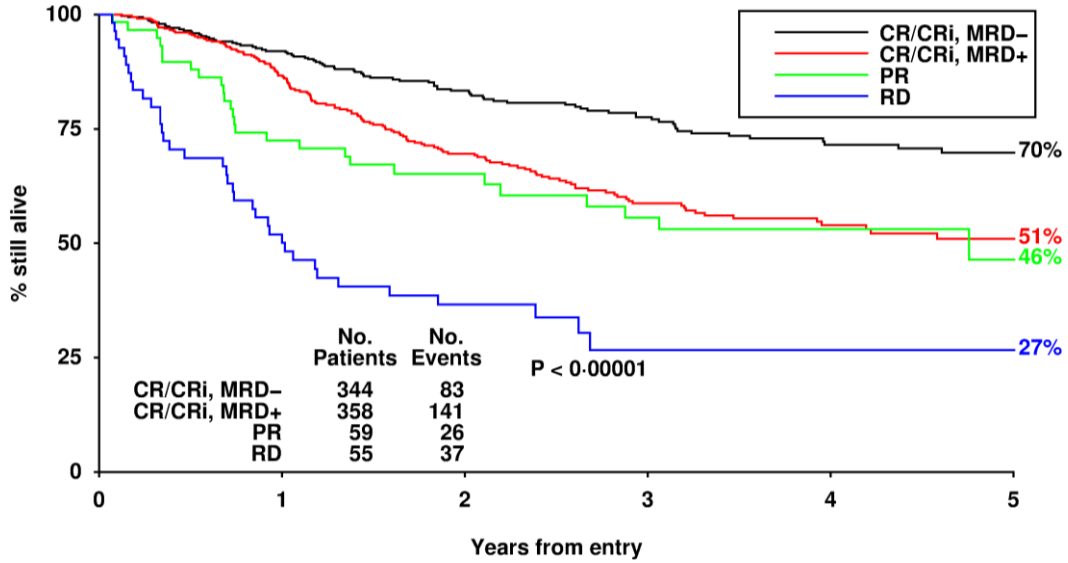
A Overall Survival All patients



At risk:

CR/CRi, MRD–	446	373	272	192	122	71
CR/CRi, MRD+	575	451	272	173	97	41
PR	197	121	72	47	31	11
RD	221	104	56	29	16	10

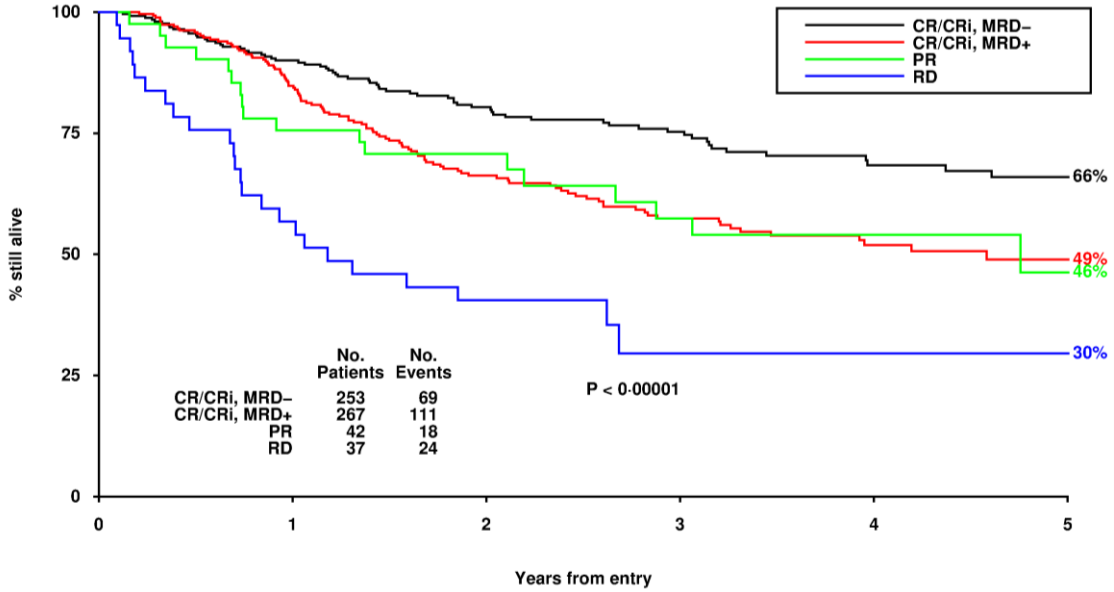
B Overall Survival Excluding Poor Risk



At risk:

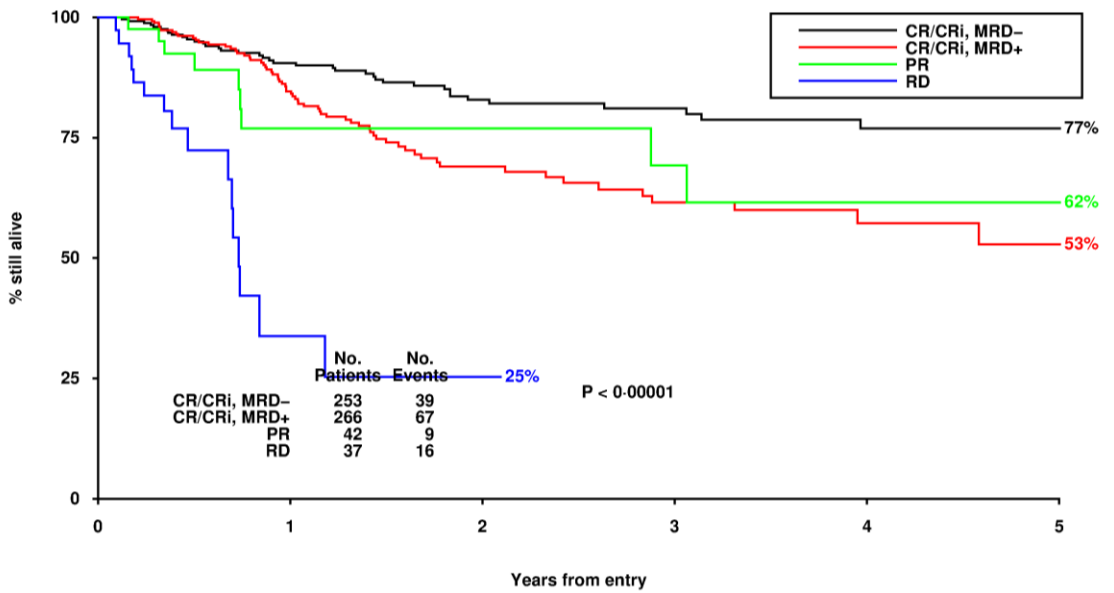
CR/CRi, MRD–	344	306	225	161	104	62
CR/CRi, MRD+	358	300	190	120	69	32
PR	59	42	28	22	15	5
RD	55	28	16	5	3	2

C Overall Survival Standard Risk



At risk:						
CR/CRi, MRD–	253	221	160	114	69	41
CR/CRi, MRD+	267	219	133	91	49	22
PR	42	31	22	17	12	4
RD	37	21	13	3	1	1

D Overall Survival Standard Risk censored at SCT



At risk:						
CR/CRi, MRD–	253	170	110	70	43	24
CR/CRi, MRD+	266	165	69	45	20	9
PR	42	17	12	9	7	4
RD	37	4	2	0	0	0

Figure 2A: Effect of FCM-MRD status post course 1 stratified by cytogenetic and risk score group
Overall Survival

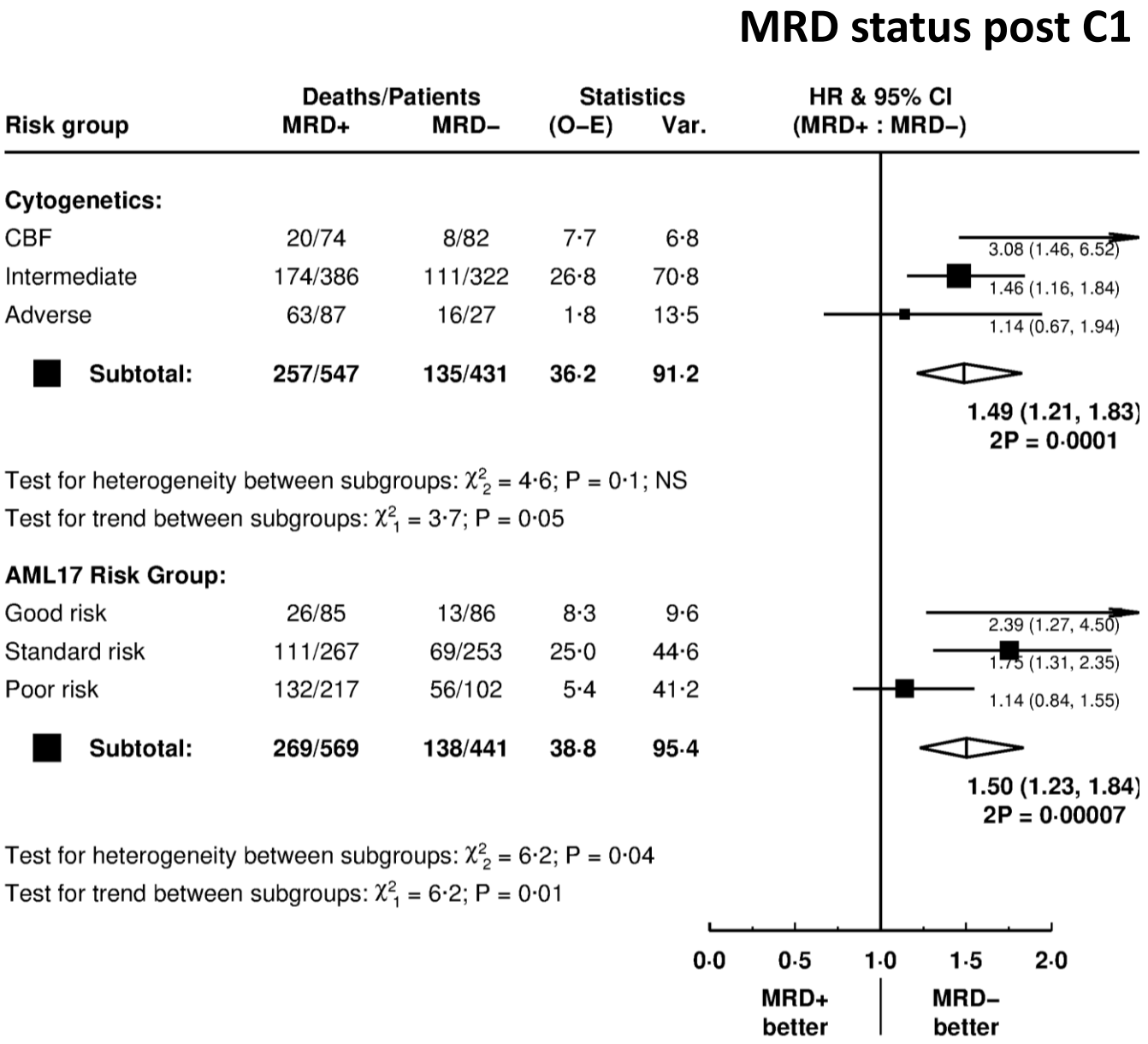


Figure 2B: Effect of FCM-MRD status post course 2 stratified by cytogenetic and risk score group
Overall Survival

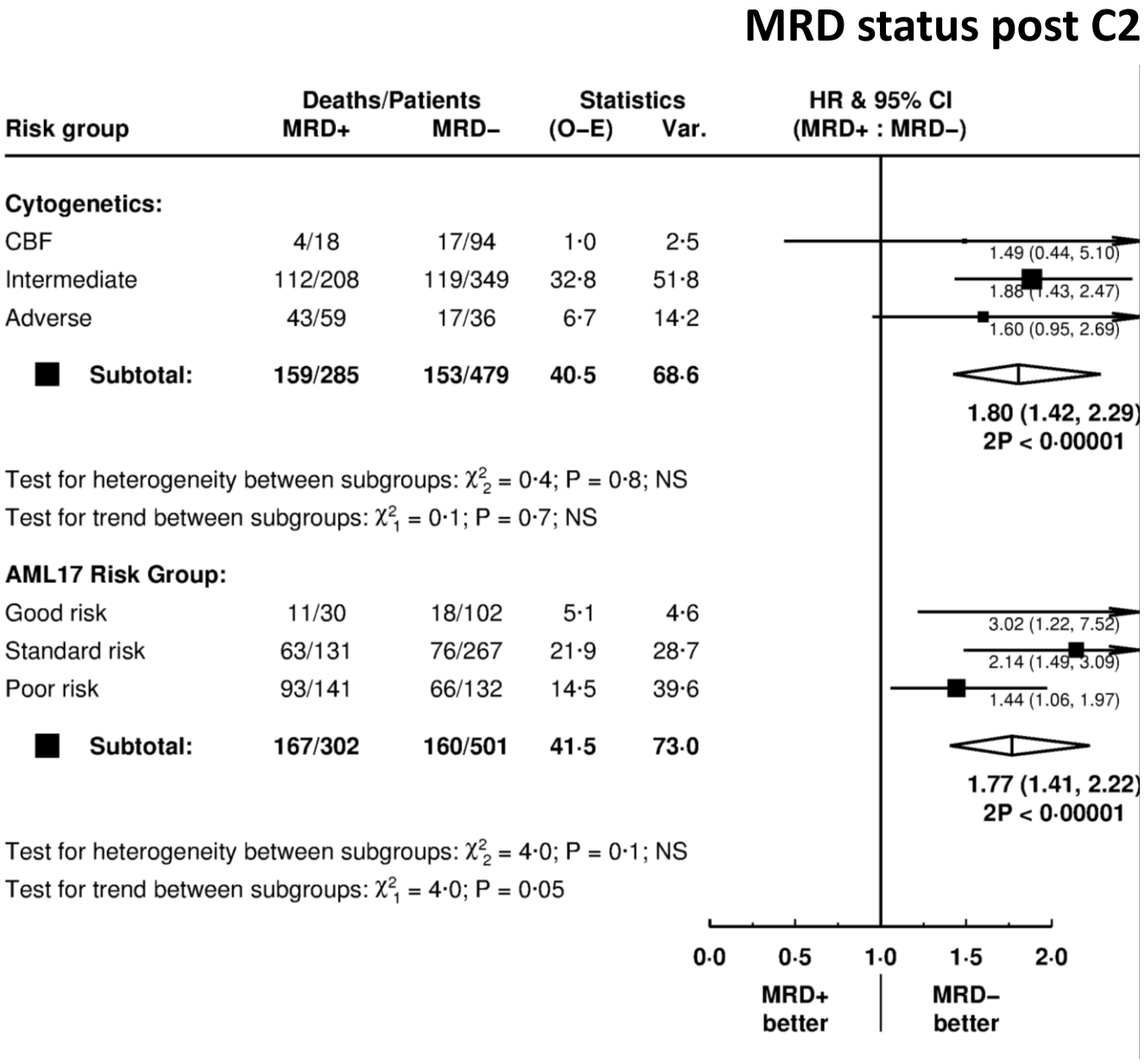


Figure 3A: Combined post course 1 and 2 response status
Overall Survival

MRD status in CR post C2

Status post C1	Deaths/Patients MRD+	MRD–	Statistics (O–E)	Var.	HR & 95% CI (MRD+ : MRD–)
Response Status post C1:					
CR MRD–	13/24	58/224	7·5	5·1	4.34 (1.82, 10.34)
CR MRD+	93/169	60/166	20·0	37·9	1.69 (1.23, 2.33)
PR	23/33	11/26	4·6	8·3	1.74 (0.88, 3.44)
RD	14/28	9/22	1·0	5·6	1.19 (0.52, 2.73)
■ **Subtotal:**	**143/254**	**138/438**	**33·0**	**56·9**	**1.79 (1.38, 2.32)** **2P = 0·00001**

Test for heterogeneity between subgroups: $\chi^2_3 = 5{\cdot}0$; P = 0·2; NS

Test for trend between subgroups: $\chi^2_1 = 2{\cdot}7$; P = 0·1; NS

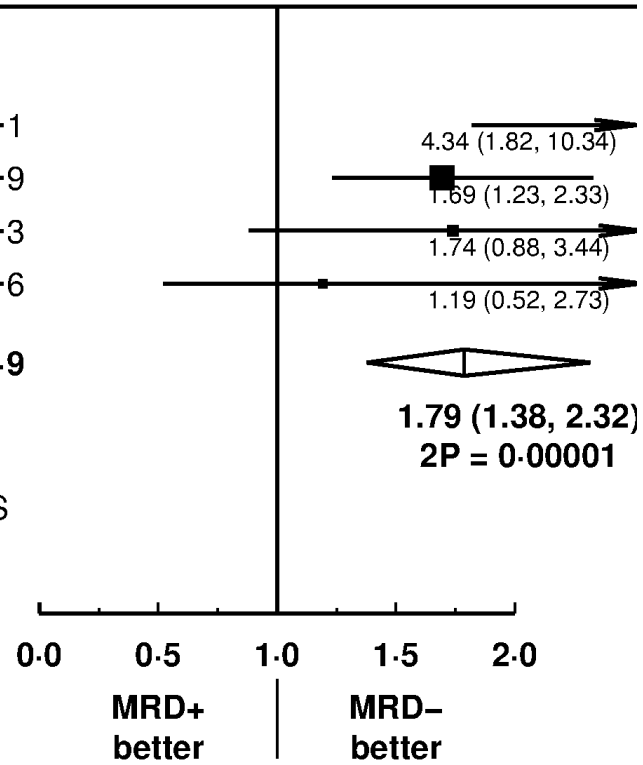


Figure 3B: Combined post course 1 and 2 response status Relapse

MRD status in CR post C2

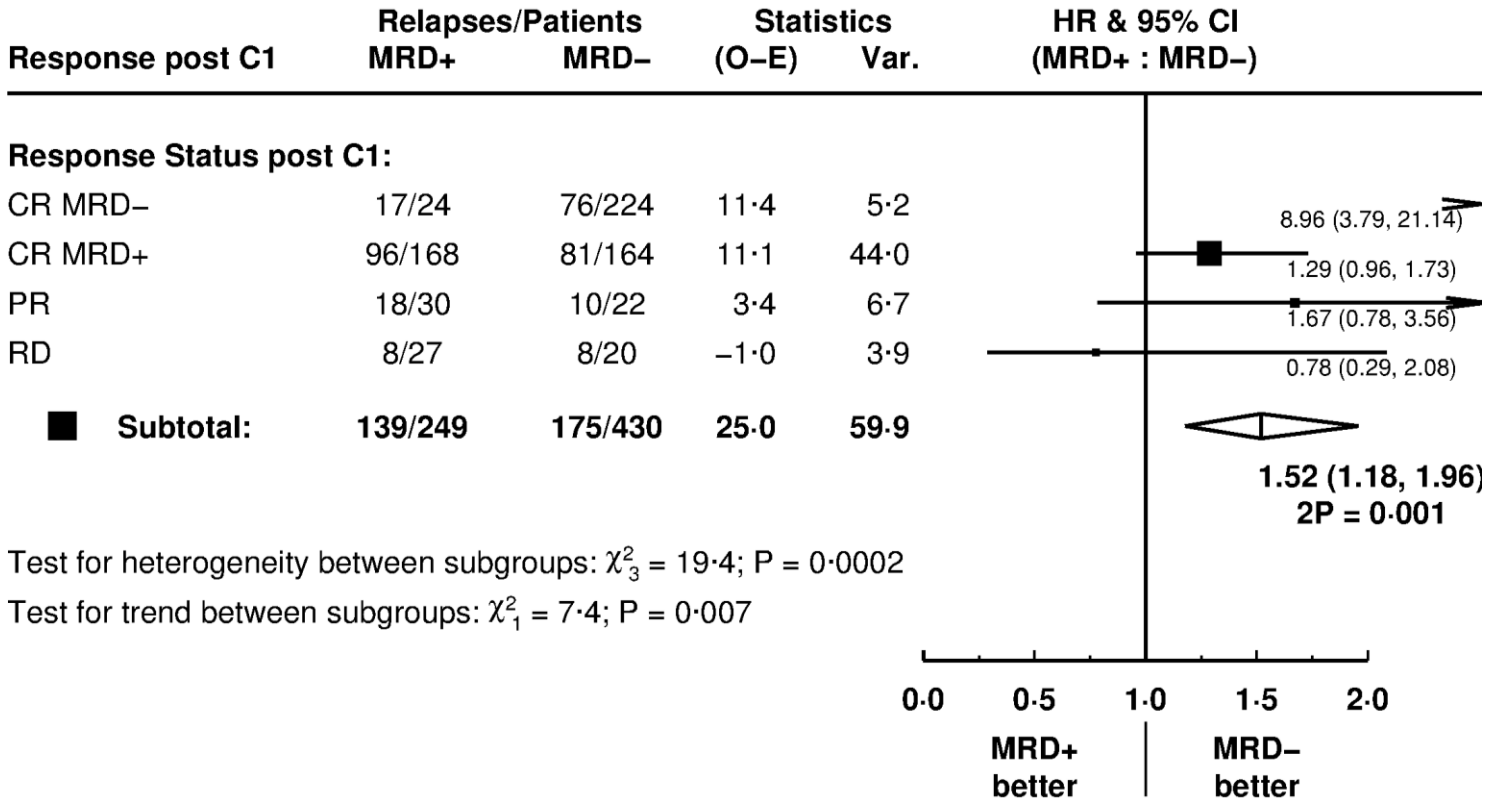
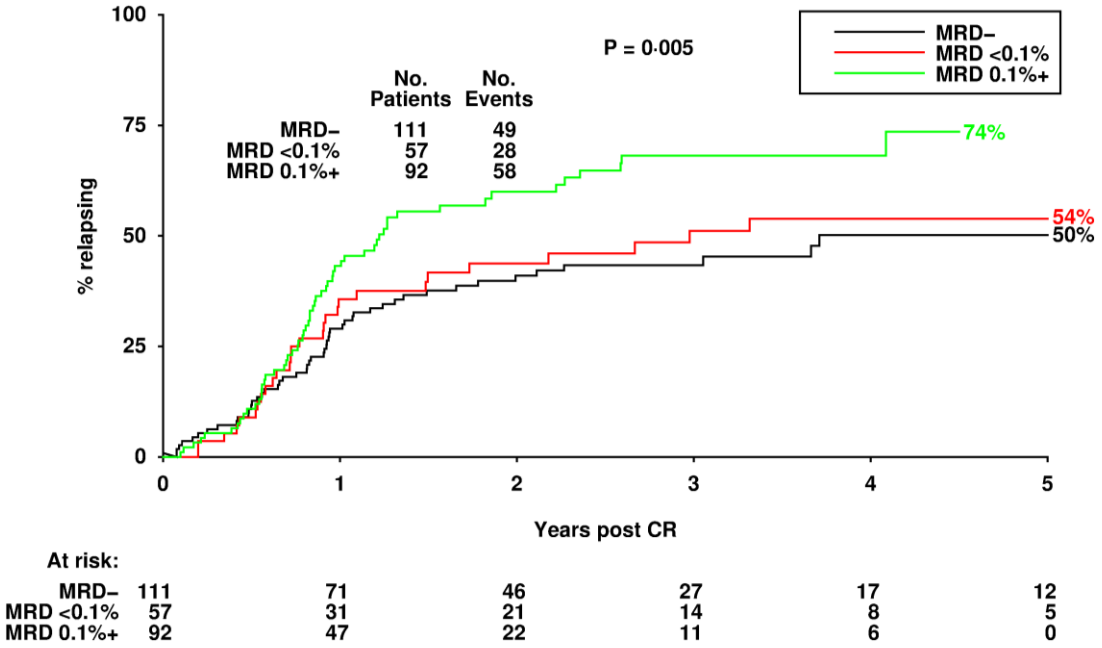


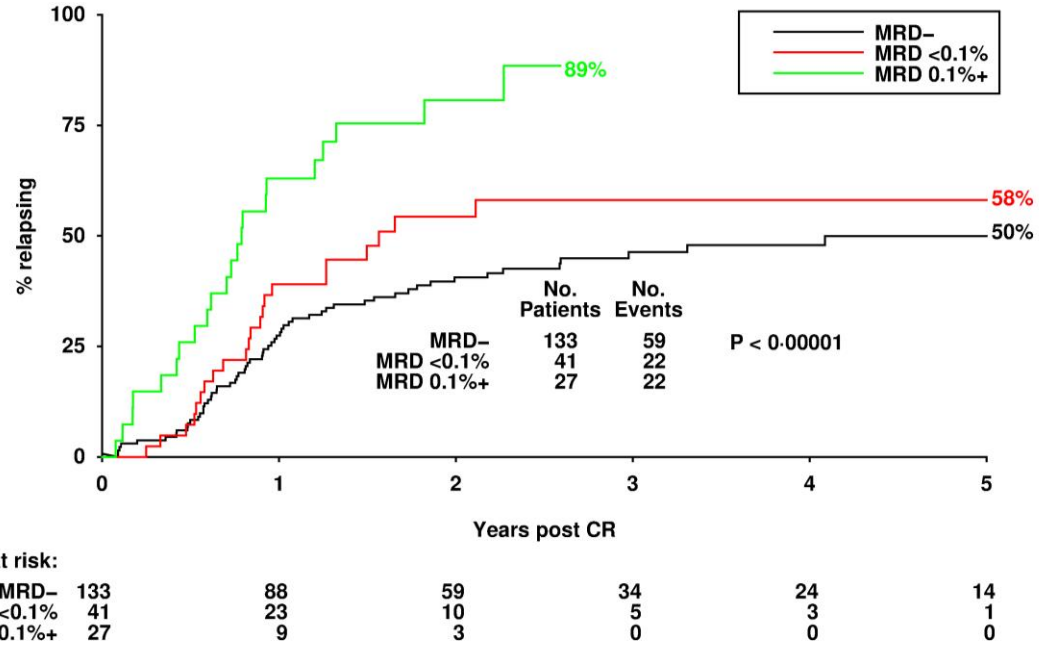
Figure 4

Standard Risk NPM1wt

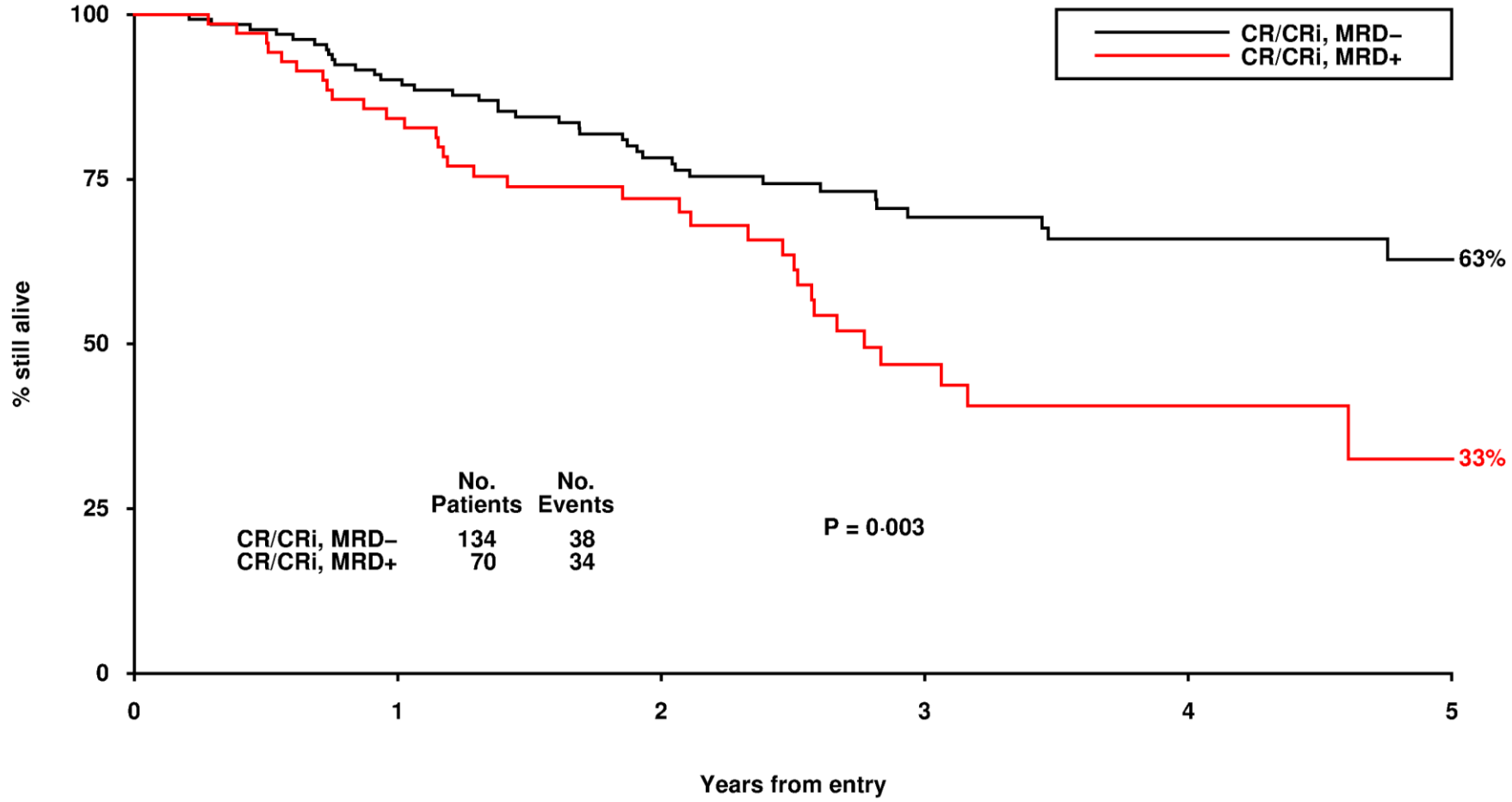
A CIR by post course 1 FCM-MRD level



CIR by post course 2 FCM-MRD level



B Overall Survival by post course 2 FCM-MRD



At risk:

CR/CRi, MRD–	134	116	84	50	32	18
CR/CRi, MRD+	70	58	36	15	9	4

C Mantel-Byar analysis of CR1 allograft in Standard Risk NPM1wt by post course 2 MRD

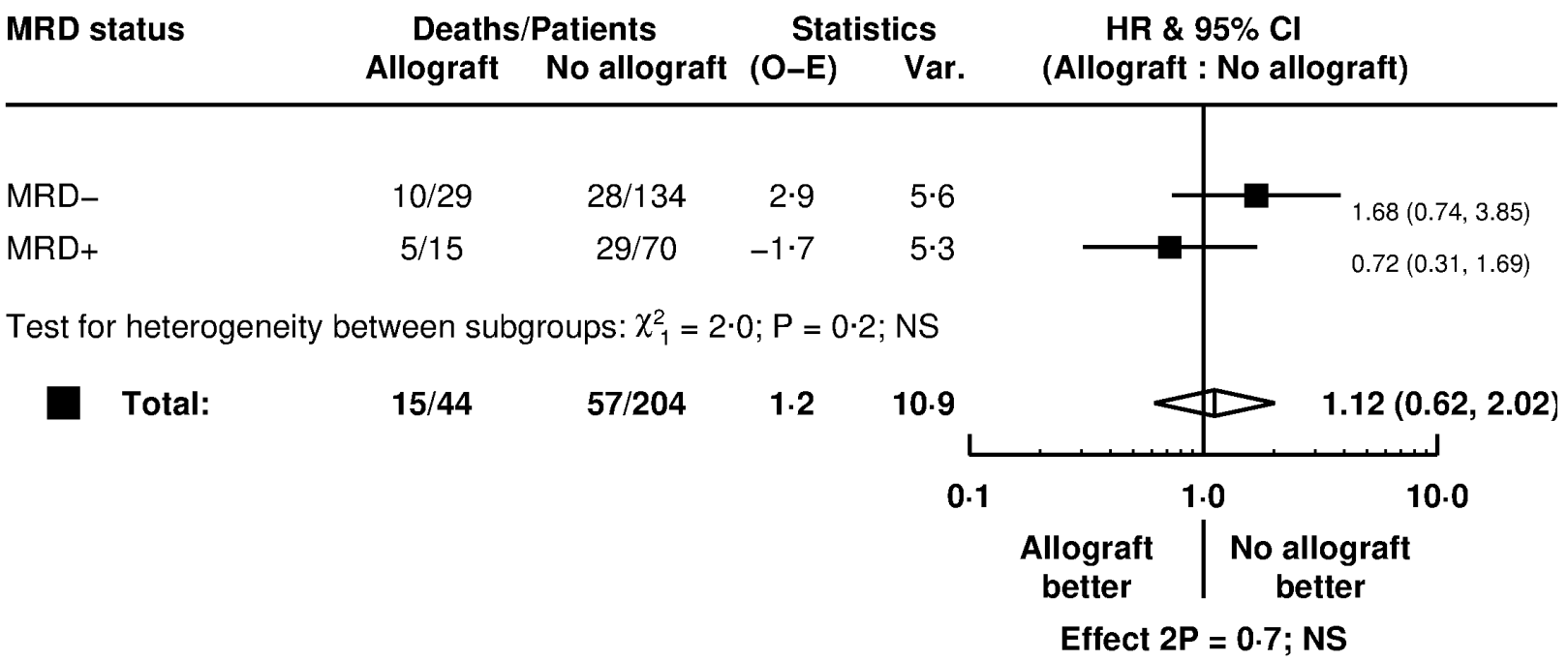


Table 1: Characteristics of Study Population by Response

	Post Course 1 Response (n= 1443)							Post Course 2 MRD status in CR (n= 806)		
	MRD- No. (%)	MRD+ No. (%)	PR No. (%)	RD No. (%)	P-value MRD- vs MRD+	P-value (4 categories)	P-value MRD+ vs PR	MRD- No. (%)	MRD+ No. (%)	P-value
All patients	446 (30.9%)	577 (40.0%)	197 (13.7%)	223 (15.5%)				503 (62.4%)	303 (37.6%)	
Age, years										
16-29	48 (10.8)	67 (11.6)	22 (11.2)	26 (11.7)				67 (13.3)	29 (9.6)	
30-39	59 (13.2)	55 (9.5)	15 (7.6)	24 (10.8)				64 (12.7)	33 (10.9.)	
40-49	104 (23.3)	135 (23.4)	36 (18.3)	39 (17.5)				122 (24.3)	62 (20.5)	
50-59	145 (32.5)	187 (32.4)	75 (38.1)	68 (30.5)				168 (33.4)	99 (32.7)	
60+	90 (20.2)	133 (23.1)	49 (24.9)	66 (29.6)				82 (16.3)	80 (26.4.)	
Median Age, years (range)	50 (16-71)	51 (16-72)	53 (16-72)	53 (17-73)	0.3**	0.02**	0.3**	49 (16-72)	53 (16-72)	0.002**
Sex					0.06	0.03*	1.0			.006
Female	220 (49.3)	250 (43.3.)	85 (43.1.)	91 (40.8.)				248 (49.3.)	119 (39.3.)	
Male	226 (50.7)	327 (56.7)	112 (56.9)	132 (59.2.)				255 (50.7.)	184 (60.7.)	
Diagnosis					0.007	0.0009	0.5			0.12
De Novo	410 (91.9.)	492 (85.3)	162 (82.2.)	193 (86.5.)				459 (91.3.)	265 (87.5.)	
Secondary	18 (4.0.)	52 (9.0.)	23 (11.7)	25 (11.2)				25 (5.0)	26 (8.6.)	
High risk MDS	18 (4.0)	33 (5.7)	12 (6.1)	0 (0)				19 (3.7)	12 (4.0)	
WHO Performance Status					1.0*	0.4*	0.8*			0.7*
0	314 (70.4)	413 (71.6)	138 (70.1)	148 (66.4)				359 (71.4)	219 (72.3)	
1	115 (25.8)	138 (23.9)	53 (26.9)	65 (29.1)				124 (24.7)	72 (23.8)	
2	11 (2.5)	14 (2.4)	4 (2.0)	6 (2.7)				13 (25.8)	9 (3.0)	
3	5 (1.1)	12 (2.1)	2 (1.0)	4 (1.8)				6 (1.2)	3 (1.0)	
4	1 (0.2)	0 (0)	0 (0)	0 (0)71.4				1 (0.1)	0 (0)	
WBC ($x10^9$/L)										
0-9.9	219 (49.1)	301 (52.2)	114 (57.9)	112 (50.2)				260 (51.7)	155 (51.2)	
10-49.9	156 (35.0)	186 (32.2)	47 (23.9)	55 (24.7)				166 (33.0)	88 (29.0)	
50-99.9	42 (9.4)	45 (7.8)	21 (10.7)	26 (11.7)				46 (9.1)	29 (9.6)	

100+	29 (6.5)	45 (7.8)	15 (7.6)	30 (13.5)				31 (61.6)	31 (10.2)	
Median WBC (x10^9/L) (range)	10.6 (0.3-319.6)	8.9 (0.4-456.0)	6.0 (0.4-430.0)	9.9 (0.6-430.0)	0.3**	0.3**	0.13**	9.3 (0.7-275.2)	9.5 (0.4-430.0)	0.5**
Cytogenetics					<.0001*	<.0001*	<.0001*			<.0001
Favorable	82 (18.4)	75 (13.0)	8 (4.1)	3 (1.3)				94 (18.7)	18 (5.9)	
Intermediate	322 (72.2)	386 (66.9)	126 (64.0)	130 (58.3)				349 (69.4)	208 (41.4)	
Adverse	27 (6.1)	87 (15.1)	53 (26.9)	79 (35.4)				36 (7.2)	59 (11.7)	
Unknown	15 (3.3)	28 (4.9)	10 (5.1)	11 (4.9)				24 (4.8)	17 (3.4)	
***FLT3-ITD / NPM1c* Status**					.001	<.0001	0.05			0.005
ITD WT, *NPM1c* WT	229 (51.3)	356 (61.7)	129 (65.5)	174 (78.0)				274 (54.5)	192 (63.4)	
ITD WT, *NPM1c* mutant	99 (22.2)	88 (15.3)	18 (9.1)	8 (3.6)				102 (20.3)	39 (12.9)	
ITD mutant, *NPM1c* WT	22 (5.0)	42 (7.3)	17 (8.6)	29 (13.0)				27 (5.4)	24 (7.9)	
ITD mutant, *NPM1c* mutant	63 (14.1)	63 (10.9)	12 (6.1)	5 (2.2)				66 (13.1)	28 (9.2)	
Unknown	33 (7.4)	28 (4.9)	21 (10.7)	7 (3.1)				34 (6.8)	20 (6.6)	
Post-course 1 Response					0.9					<.0001*; CR vs CRi (p=0.8)
CR	407 (91.3)	528 (91.5)						412 (81.9)	219 (72.3)	
CRi	39 (8.7)	49 (8.5)						37 (7.4)	18 (5.9)	
PR								26 (5.2)	33 (10.9)	
RD								22 (4.4)	28 (9.2)	
								6 (1.2)	5 (1.7)	
Post-course 1 Risk Score					<.0001*	<.0001*	<.0001*			<.0001*
Good	86 (19.3)	85 (14.7)	11 (5.6)	3 (1.3)				102 (20.3)	30 (9.9)	
Standard	253 (56.7)	267 (46.3)	42 (21.3)	37 (16.6)				267 (53.1)	131 (43.2)	
Poor Risk	102 (22.9)	218 (37.8)	138 (70.1)	167 (74.9)				132 (26.2)	141 (46.5)	
Not Assessable	6 (1.3)	6 (1.0)	6 (3.0)	16 (7.2)				2 (0.4)	1 (0.3)	
Number of Chemotherapy Cycles (poor risk excluded)					n/a	n/a	n/a			n/a
3	95 (21.3)	128 (22.2)	12 (6.1)	0 (0)				113 (22.5)	64 (21.1)	
4	117 (26.2)	116 (20.1)	10 (5.1)	1 (0.4)				139 (27.6	49 (16.2)	
Not Randomised	139 (31.2)	130 (22.5)	37 (18.8)	56 (25.1)				128 (25.4)	56 (18.5)	
Allogeneic SCT					0.07	0.0008*	0.01			0.04
Any	142 (31.8)	215 (37.3)	102 (51.8)	89 (39.9)				193 (38.4)	139 (45.9)	
CR1	82 (18.4)	128 (22.2	75 (38.1	71 (31.8)				114 (22.7)	91 (30.0)	

CR2	38 (8.5)	68 (11.8)	4 (2.0)	2 (0.9)				54 (10.7)	32 (10.6)	

Chi-square test unless specified otherwise. *: Mantel-Haenszel test for trend;**: Wilcoxon rank sum test/Spearman correlation; n/a not assessed as 3 v 4 courses randomised.

MRD, measurable residual disease by flow cytometry; MRD+, MRD positive; MRD- , MRD negative; CR, Complete Remission; CRi, complete remission with absolute neutrophil count <1,000/µl or thrombocytopenia <100,000/µ; RD, Resistant Disease (less than a 50% reduction in blast numbers with >15% residual blasts) PR, Partial Response (at least a 50% reduction in blast numbers with 5% -15% residual blasts

Table 2: Outcomes by post course 1 response status

	PR	MRD+ in CR	Unadjusted HR/OR, 95% CI; p-value	adjusted HR/OR, 95% CI; p-value	RD	PR or MRD+ in CR	Unadjusted HR/OR, 95% CI; p-value	adjusted HR/OR, 95% CI; p-value
All patients	N= 197	N= 577			N= 223	N= 774		
5yr (3yr) OS	35% (41%)	44% (51%)	1.50 (1.18-1.91) P=.001	1.32 (1.05-1.66) p=0.02	24% (30%)	41% (48%)	2.43 (1.93-3.06) p<.0001	1.61 (1.31-1.97) p<.0001
5yr (3yr) OS censored at SCT	41% (48%)	49% (54%)	1.81 (1.25-2.61) P=.002	1.62 (1.16-2.25) p=0.004	11% (11%)	48% (53%)	8.17 (5.76-11.6) p<.0001	2.64 (2.02-3.45) p<.0001
Good / Standard Risk patients	N= 53	N= 352			N= 40	N= 405		
5yr (3yr) OS	49% (58%)	51% (59%)	1.11 (0.69-1.76) P=0.7	1.320 (0.65-2.68) P=0.4	33% (33%)	51% (59%)	16.6 (9.24-30.0) P<.0001	2.28 (1.38-3.75) p=.0009
5yr (3yr) OS censored at SCT	67% (73%)	60% (66%)	1.05 (0.54-2.06) P=0.9	1.38 (0.68-2.80) P=0.4	37% (at 3, 4 yrs)	61% (67%)	22.4 (8.23-60.8) P<.0001	3.56 (1.78-7.12) p=.0002
Standard Risk NPM1 WT patients	N= 27	N= 149			N= 34	N= 176		
5yr (3yr) OS	38% (55%)	47% (55%)	1.10 (0.60-2.04) P=0.8	1.18 (0.61-2.28) P=0.6	28% (28%)	45% (55%)	3.09 (1.65-5.80) p=0.0004	2.13 (1.21-3.75) p=.008
5yr (3yr) OS censored at SCT	53% (71%)	52% (59%)	1.38 (0.52-3.63) P=0.5	1.51 (0.58-3.93) P=0.4	28% (at 3 yrs)	53% (60%)	18.2 (6.38-52.1) p<.0001	3.88 (1.68-8.94) p=0.001

PR , morphological partial response; CR, morphological complete remission; MRD+, MRD positive; CRi, complete remission with absolute neutrophil count <1,000/µl or thrombocytopenia <100,000/µl; OS – overall survival; SCT – allogeneic stem cell transplant; RFS– relapse free survival ; CIDCR – cumulative incidence of death in remission.
Adjusted analyses included age, wbc, sex, performance status, disease type (secondary or de novo) and cytogenetic group.

Appendix Table A1

karyotype	Cytogenetic risk group	NPM1 mutation	Flt3 ITD
46,XY,t(8;21)(q22;q22)[7]/47,idem,+8[3]	good	negative	negative
46,XY[20]	intermediate	NA	NA
46,XY[20]	intermediate	+	+
46,XY[20]	intermediate	+	+
46,XX[16]	intermediate	+	+
46,XX[20]	intermediate	negative	+
46,XY[20]	intermediate	negative	+
46,XX[20]	intermediate	+	negative
46,XY[20]	intermediate	negative	negative
46,XX[20]	intermediate	negative	negative
46,XX[20]	intermediate	negative	negative
46,XX[20]	intermediate	negative	negative
46,XX,t(11;19)(q23;p13.1)[10]	intermediate	negative	negative
46,XY,del(9)(q?2q?3)[9]/46,XY[2]	intermediate	negative	negative
46,XX,t(2;9)(p22;p21)[12]/46,XX[1]	intermediate	negative	negative
46,XY,t(6;9)(p23;q24)[9]/46,XY[1]	intermediate	negative	negative
46,XY[20]	intermediate	negative	negative
45,XX,dic(17;18)(p11.2;p11.2)[9]/46,XX[1]	intermediate	negative	negative
46,XX[20]	intermediate	negative	negative
46,XY[20]	intermediate	negative	negative
47,XY,+8[6]/ 46,XY[4]	intermediate	negative	negative
50,XY,+X,+4,t(10;11)(p12;q14),+15,+19[9]/ 46,XY[1]	adverse	negative	negative
Failed	NA	negative	negative
Failed	NA	negative	negative

Table A2: Correlation of Risk group and Clinical Response by combined C1 and C2 response status

	All No. (%)	C1 MRD- C2 MRD- No. (%)	C1 MRD- C2 MRD+ No. (%)	C1 MRD+ C2 MRD- No. (%)	C1 MRD+ C2 MRD+ No. (%)	C1 PR C2 MRD- No. (%)	C1 PR C2 MRD+ No. (%)	C1 RD C2 MRD- No. (%)	C1 RD C2 MRD+ No. (%)
All (patients with both C1 and C2 data)	693	224 (32.3)	24 (3.5)	166 (24.0)	170 (24.5)	26 (3.8)	33 (4.8)	22 (3.2)	28 (4.0)
Post-course 1 Risk Score Good Standard Poor Risk Not Assessable	 110 (15.9) 347 (50.1) 234 (33.4) 2 (0.3)	 48 (21.4) 133 (59.4) 41 (18.3) 2 (0.9)	 2 (8.3) 15 (62.5%) 7 (29.2) 0	 34 (20.5) 87 (52.4) 45 (27.1) 0	 19 (11.2) 87 (51.2) 64 (37.6) 0	 2 (7.7) 12 (46.2) 12 (46.2) 0	 3 (9.1) 4 (12.1) 26 (78.8) 0	 1 (4.5) 6 (27.3) 15 (68.2) 0	 1 (3.6) 3 (10.7) 24 (85.7) 0
*NPM1*wt standard risk	180 (26)	59 (26.3)	10 (41.2)	50 (30.1)	43 (25.3)	8 (30.8)	4 (12.1)	6 (27.3)	3 (10.7)
Post-course 1 Response CR (excluding CRi/CRp) CRi / CRp	 538 (77.6) 46 (6.6)	 202 (90.2) 22 (9.8)	 23 (95.8) 1 (4.2)	 157 (94.6) 9 (5.4)	 156 (91.8) 14 (14.4)				

C1, course 1 induction; C2, course 2 induction; MRD, measurable residual disease by flow cytometry; MRD+, MRD positive; MRD- , MRD negative; CR, Complete Remission; CRi, complete remission with absolute neutrophil count <1,000/µl or thrombocytopenia <100,000/µl; RD, Resistant Disease (less than a 50% reduction in blast numbers with >15% residual blasts; PR, Partial Response (at least a 50% reduction in blast numbers with 5% -15% residual blasts

Appendix Table A3 Outcomes for patients by peripheral count recovery response combined with MRD status

		No. (%CRi)	5 year (3 year) OS	p- value	5 year (3 year) CIR	p- value
All Patients	**Post Course 1**					
	CR vs CRi	933 / 88 (9.4%)	53% vs 39% (60% vs 46%)	0.002	50% vs 43% (46% vs 40%)	0.6
	MRD- CR vs MRD- CRi	407 / 39 (9.6%)	63% vs 63% (70% vs 63%)	0.2	40% vs 33% (35% vs 33%)	0.7
	MRD+ CR vs MRD+ CRi	526 /49 (9.3%)	45% vs 19% (52% vs 33%)	0.001	58% vs 53% (54% vs 47%)	0.6
	Post Course 2					
	CR vs CRi	716 / 89 (12.4%)	54% vs 38% (59% vs 46%)	0.02	51% vs 47% (48% vs 44%)	0.9
	MRD- CR vs MRD- CRi	449 / 54 (12.0%)	63% vs 52% (68% vs 52%)	0.05	61% vs 57% (59% vs 57%)	0.9
	MRD+ CR vs MRD+ CRi	267 /35 (13.1%)	37% vs 20% (46% vs 40%)	0.3	45% vs 40% (41% vs 36%)	0.9
Standard Risk NPM1 wt	**Post Course 1**					
	CR vs CRi	241 / 19 (7.9%)	52% vs 42% (64% vs 56%)	0.16	58% vs 66% (53% vs 66%)	0.2
	MRD- CR vs MRD- CRi	100 / 11 (11.0%)	60% vs 64% (77% vs 64%)	0.2	49% vs 66% (41% vs 66%)	0.07
	MRD+ CR vs MRD+ CRi	141 /8 (5.7%)	48% vs 25% (55% vs 50%)	0.4	65% vs 69% (61% vs 69%)	0.8

	Post Course 2					
	CR vs CRi	180 / 24 (13.3%)	54% vs 47% (63% vs 47%)	0.3	58% vs 43% (55% vs 43%)	0.6
	MRD- CR vs MRD- CRi	118/ 16 (13.6%)	63% vs 61% (70% vs 61%)	0.6	52% vs 34% (48% vs 34%)	0.5
	MRD+ CR vs MRD+ CRi	62 /8 (12.9%)	35% vs 23% (50% vs 23%)	0.10	70% vs 67% (70% vs 67%)	0.7

MRD, measurable residual disease by flow cytometry; MRD+, MRD positive; MRD- , MRD negative; CR, Complete Remission; CRi, complete remission with absolute neutrophil count <1,000/µl or thrombocytopenia <100,000/µl;