Blood First Edition Paper, prepublished online September 25, 2018; DOI 10.1182/blood-2018-06-858613

Minimal Residual Disease Negativity using Deep Sequencing is a major Prognostic Factor in Multiple Myeloma

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Key Points

1) MRD using NGS identified patients with an excellent outcome in multiple myeloma.
2) MRD should be assessed in every prospective trial, and is a candidate to become a primary endpoint.

ABSTRACT

The introduction of novel agents has led to major improvements in clinical outcomes for patients with multiple myeloma. In order to shorten evaluation times for new treatments, health agencies are currently examining minimal residual disease (MRD) as a surrogate endpoint in clinical trials. We assessed the prognostic value of MRD, measured during maintenance therapy by next-generation sequencing. MRD negativity was defined as the absence of tumor plasma cell within 1,000,000 bone marrow cells ($<10^{-6}$). Data were analyzed from a recent clinical trial that evaluated the role of transplantation in newly diagnosed myeloma patients treated with lenalidomide, bortezomib, and dexamethasone (RVD). MRD negativity was achieved at least once during maintenance in 127 patients (25%). At the start of maintenance therapy, MRD was a strong prognostic factor for both progression-free survival (adjusted hazard ratio, 0.22; 95% confidence interval, 0.15 to 0.34; P<0.001) and overall survival (adjusted hazard ratio, 0.24; 95%confidence interval, 0.11 to 0.54; P=0.001). Patients who were MRD negative had a higher probability of prolonged progression-free survival than patients with detectable residual disease, regardless of

treatment group (RVD versus transplant), cytogenetic risk profile or international staging system disease stage at diagnosis. These results were similar after completion of maintenance therapy. Our findings confirm the value of MRD status, as determined by next-generation sequencing, as a prognostic biomarker in multiple myeloma, and suggest that this approach could be used to adapt treatment strategies in future clinical trials.

INTRODUCTION

Multiple myeloma is a hematologic malignancy characterized by the accumulation of malignant plasma cells, usually within the bone marrow.[1] This disease has long been considered incurable, supporting prolongation of overall survival as the major objective in both clinical practice and prospective trials. The introduction of several novel drugs over the past decade has dramatically improved patient outcomes, extending survival from 3 years to more than 10 years in young, transplant-eligible patients.[2-5] As a result, overall survival has become an unrealistic objective for clinical trials, and most investigators, as well as health agencies, now focus on alternative end points, such as progression-free survival or complete remission rates. While these two end points may be more realistic, in terms of the time required to answer the question, they are far from perfect. Furthermore, with the current highly effective drug combinations, complete response (CR) rates have increased dramatically from a few percent to up to 80%,[6] making this particular end point less useful. A solution to improve upon CR is to use more sensitive response assessment techniques, enabling quantification of the so-called 'minimal residual disease (MRD)'. This approach has been successfully used in several hematological malignancies such as acute lymphoblastic leukemia,[7] acute promyelocytic leukemia,[8] or chronic lymphocytic leukemia.[9] Number of pilot studies have shown benefit of achieving MRD negativity using currently available methods in myeloma: next-generation flow,[10-12] and next-generation sequencing.[13] Each of these techniques is capable of detecting a single residual malignant plasma cell within 1,000,000 bone marrow cells.

Using data from the recent phase 3, Intergroupe Francophone du Myélome (IFM) 2009 clinical trial, which evaluated the role of transplantation in patients with newly diagnosed myeloma treated with lenalidomide, bortezomib, and dexamethasone (RVD),[11] we assessed the prognostic value of MRD measured during maintenance by next-generation sequencing.

PATIENTS AND METHODS

PATIENTS

The protocol and clinical results of the IFM 2009 trial, including MRD results obtained by multiparametric flow cytometry, have been previously described in detail.[14] Briefly, 700 patients were enrolled in France, Belgium, and Switzerland between 2010 and 2012. The trial was approved by local and national health authorities, and all patients signed an informed consent form for MRD analyses. Participants were transplant-eligible patients younger than 66 years of age, with newly diagnosed, symptomatic multiple myeloma. Patients were randomized to receive either a conventional-dose strategy (i.e., eight courses of the RVD regimen) or an intensive approach comprising three courses of RVD followed by high-dose melphalan (200 mg/m²) with autologous stem cell transplantation, and consolidation with a further two cycles of RVD. All patients received lenalidomide maintenance therapy for 12 months. Randomization was stratified according to International Staging System disease stage[15] and cytogenetic risk profile, with high-risk patients defined at diagnosis by the presence of 17p deletion or either t(4;14) or t(14;16) translocation. When this trial was designed in 2008, the next-generation sequencing technique was not available; therefore, MRD was assessed by low-sensitivity (10^{-4}; i.e., one malignant plasma cell within 10,000 bone marrow cells) multiparametric flow cytometry in all patients who achieved at least a very good partial response following consolidation. Bone marrow samples were collected from all such patients at both the start and end of maintenance therapy (the minimal residual disease time points), for the measurement of MRD.

MINIMAL RESIDUAL DISEASE ASSESSMENT

Details of the multiparametric flow cytometry technique have already been published.[16] Collected bone marrow samples were frozen as dry pellets and stored at -80°C until analysis.

When next-generation sequencing commercial kits became available (Sequenta, San Francisco, CA, USA),[13] DNA was extracted from the stored bone marrow samples and sent for sequencing. Briefly, the technique is based on the sequencing of immunoglobulin genes, which are clonally rearranged in all myeloma patients and thus represent unique, patient-specific biomarkers. As the technique required prior identification of the clonal rearrangements, tumor DNA obtained from CD138-positive cells at enrolment were sent to Sequenta for assessment. The clonal rearrangements were identified using the LymphoSight kit (Sequenta, San Francisco, CA, USA). For minimal residual disease quantification, DNA was extracted from the bone marrow samples and amplified by polymerase chain reaction using immunoglobulin gene-specific primers; the amplified products were then sequenced. The sensitivity was 10^{-6}, i.e, one malignant plasma cell within 1,000,000 bone marrow cells. MRD levels were reported as follows: less than 10^{-6}; from 10^{-6} to less than 10^{-5}; from 10^{-5} to less than 10^{-4}; and 10^{-4} or greater. Patients who failed to achieve at least a very good partial response, or who did not enter the maintenance phase of the trial, were considered minimal residual disease positive. Based on the results described below (Table S1 and Figs. S1A and S1B in the Supplementary Appendix), patients were considered minimal residual disease negative when the level was below 10^{-6}.

STATISTICAL ANALYSES

We assessed the association between minimal residual disease status during maintenance therapy and survival end points using two populations defined at the start, and after the completion, of 12 months of maintenance therapy (landmark times): including only patients in whom MRD status was known at this time; and **a modified intent-to-treat population that only excluded patients who had achieved VGPR, CR or SCR at both the start and end of maintenance therapy, but in whom minimal residual disease was not measured at either time**

point Progression-free survival was defined as the time from randomization or from the landmark points until either the first documentation of progressive disease or death from any cause. Overall survival was defined as the time from randomization or from the landmark points until death. The data were updated in February 2017, and follow-up was estimated using the reverse Kaplan-Meier method.[4] Survival functions were compared using the log-rank test or the Mantel-Byar test,[17] and graphed using the Kaplan-Meier method or the Kaplan-Meier method modified by the Simon−Makuch method[18] in the landmark analyses and the modified intent-to-treat analyses, respectively. The prognostic value of MRD was evaluated using a multivariate Cox proportional hazards model including MRD as a fixed covariate or as a time-dependent covariate. These models were adjusted for stratification factors and treatment groups. Analyses of progression-free survival in specific subgroups were performed entering an interaction term between subgroup and minimal residual disease status in the Cox models. The proportionality assumptions were checked with Cox-Snell residuals. All models were repeated using imputed values for missing assessments. Minimal residual disease negativity rates were compared between groups using Chi-square or Fisher's exact test. A logistic model adjusted for stratification factors was used to determine the association between treatment group and minimal residual disease status. Tests were two sided, and P values lower than 0.05 were considered significant. Given the exploratory nature of this post-hoc analysis of the IFM 2009 study, no adjustment was made for multiple testing. All analyses were conducted using Stata® Version 14.2 (StataCorp LP, College Station, TX, USA).

RESULTS

PATIENTS AND NEXT-GENERATION SEQUENCING MINIMAL RESIDUAL DISEASE ASSESSMENTS

Minimal residual disease status was assessed in 224 of 366 patients at the start and 183 of 239 patients after completing maintenance therapy, as they achieved at least a very good partial response and were included in the analyses. In all, 138 patients had MRD status assessed twice (Figure S2 in the Supplementary Appendix). The reasons for missing assessments in patients who achieved at least a very good partial response are summarized in Table S2 in the Supplementary Appendix. We also performed a modified intent-to-treat analysis which excluded 191 patients who achieved at least a very good partial response at both the start and end of maintenance therapy, but in whom MRD was not assessed at either time point. Among the 509 remaining patients, 269 in very good partial response were assessed at least once during maintenance therapy. Baseline characteristics were comparable between patients with known and unknown MRD status, and between treatment arms both at the landmark time points and overall (Tables S3 and S4 in the Supplementary Appendix). Initially evaluating the depth of MRD, we found progression-free survival to be related to the level of minimal residual disease at both time points, with the best outcome observed when MRD level was below 10^{-6} (Table S1 and Figs. S1A and S1B in the Supplementary Appendix). This level was hence considered as minimal residual disease negative.

MRD STATUS BY TREATMENT ARM, STRATIFICATION FACTORS, AND RESPONSE RATES IN THE MODIFIED INTENT-TO-TREAT POPULATION

Table 1 summarizes the MRD results by treatment arm, stratification factors, and treatment response. In total, 127 patients (25%) achieved MRD negative status at least once during maintenance: 54 out of 264 patients (20%) in the RVD-alone arm, and 73 out of 245 patients

(30%) in the transplantation arm (adjusted odds ratio for undetectable minimal residual disease 1.65; 95% confidence interval, 1.10 to 2.49; P=0.02). The MRD negativity rate did not differ according to International Staging System disease stage or cytogenetic risk profile (high versus standard risk). Among patients with high-risk cytogenetics, minimal residual disease negativity was achieved in 17 out of 42 patients with t(4;14) (40%), but in only three out of 28 patients with del(17p) (11%). Among patients who started maintenance therapy, 31% and 49% of patients who achieved a very good partial response and a complete response, respectively, were MRD negative (P=0.006). Of those 233 patients who had previously been found to be MRD negative by multiparametric flow cytometry, 120 (52%) were confirmed as MRD negative by next-generation sequencing. Change in MRD status before and after maintenance therapy is reported in the supplementary appendix (Table S5).

PROGRESSION-FREE SURVIVAL

The median duration of follow-up was 55, 50, and 38 months from randomization, start and completion of maintenance therapy, respectively. Progression-free survival was significantly prolonged in minimal residual disease-negative versus minimal residual disease-positive patients (Fig. 1). A similar significant difference is also observed using landmark and modified intent to treat population (Supplementary Figure S6). Among the 37 minimal residual disease-negative patients who progressed or died during follow-up, 34 had a serological relapse. The progression-free survival benefit associated with MRD negativity was similar among the different patient subgroups (supplementary Fig. S3). In multivariate Cox models adjusted for stratification factors and treatment group, MRD negativity was the strongest prognostic factor for progression-free survival (Table2). Similar results were observed after having imputed values for missing minimal residual disease assessments (supplementary Table S6). As the summary of the results of the Cox models, adjusted

probabilities of progression-free survival presented in Figure 2 and in supplementary figures S4 and S5 shows improvement in progression-free survival in those achieving MRD negativity irrespective of the treatment received (Transplantation or RVD only arms); or standard versus high-risk group or ISS stage I versus stage II or III.

OVERALL SURVIVAL

Nine deaths occurred among the 127 minimal residual disease-negative patients (7%): four in the RVD-alone group, and five in the transplantation group. Five of these deaths were myeloma related. Overall survival was significantly prolonged in minimal residual disease-negative patients versus minimal residual disease-positive patients (Fig. 3 and Supplementary Figure S7). Median overall survival was not reached in either group. Owing to the small number of deaths among patients who were minimal residual disease negative, subgroup analyses were not performed.

Survival analyses, limited to the population of patients assessed by next-generation sequencing and according to the change in MRD status before and after maintenance therapy, shows a similar progression-free and overall-survival for patients who maintained MRD negativity at both measurements or those who became MRD negative after 12 months of maintenance. Their survival was significantly superior to those who were either MRD positive at both measurements or became positive at the later measurement. (Supplementary Figures S8-S9-S10).

DISCUSSION

As a result of major improvements in clinical outcomes in recent years, the search for surrogate markers that enable early prediction of survival end points and reduce the time

required to evaluate new treatments has become a major objective for the myeloma community. Minimal residual disease is one of the most promising such biomarkers identified to date.[19-21] In a recent meta-analysis of studies published between January 1990 and January 2016, MRD negativity was found to confer an approximate 50% relative reduction in the risk of both progression and mortality.[22] Although this risk reduction appears to be clinically important, a significant proportion of patients classified as MRD negative still relapsed and died from disease. An explanation for this observation is the lower level of sensitivity of the older multiparametric flow cytometry techniques used in the studies included in the meta-analysis. With the development of more advanced technologies and the analysis of larger numbers of cells (10 to 20 million), higher sensitivity is now observed.[10]

Using next-generation sequencing,[13] a technique that offers sensitivity at the 10^{-6} level, we are able to demonstrate that an ability to measure deeper response provides superior outcome; e.g. MRD level below 10^{-6} is predictive of superior PFS compared to 10^{-5} or 10^{-4}. With 10^{-6} sensitivity, we found MRD negativity to be a strong prognostic biomarker of progression-free survival and overall survival. This approach demonstrated a higher level of discrimination than had previously been achieved with the less sensitive multiparametric flow cytometry technique.[14] However, recent data from the Spanish group showed that 10^{-6} sensitivity can be achieved by next-generation flow (NGF) as well. Thus it is important to note that the methodology used to measure MRD is not as important, as long as we are able to measure deeper levels of MRD. For clinical practice and based on availability, any platform which achieves adequate sensitivity and reproducibility can be used. Our findings support and enhance the recently published International Myeloma Working Group criteria[23].

Survival was prolonged in both high- and standard-risk myeloma patients who were MRD negative versus standard-risk MRD-positive patients. This finding raises the possibility that achieving MRD negativity may overcome certain adverse risk factors identified at

diagnosis. These results, coupled with the lack of impact of disease stage or cytogenetic risk profile on MRD negativity rates, should be kept in mind when discussing prognoses with patients not only at diagnosis, but also during the different phases of treatment, as their prognosis could be substantially altered by their achieving MRD negative status.

We found lower rates of MRD negativity among patients who received RVD alone than among those who underwent transplantation. However, due to the 80% relative risk reduction conferred by MRD negativity, regardless of treatment assignment, progression-free survival in MRD-negative patients in the RVD-alone group was similar to that in patients in the transplantation group with the same MRD status. Although the risk of progression was reduced in the transplantation group versus the RVD-alone group as a whole, it seems possible that the role of transplantation may be less clear in patients who achieve MRD negativity after induction therapy. As MRD assessments were only available at the start and end of maintenance therapy in our study, we were not able to test this hypothesis. Our study now provides the rationale to address these questions in future prospective trials to determine whether MRD status can be used to inform induction, consolidation, and/or maintenance treatments.

Despite higher sensitivity, biological relapses still occur in MRD negative patients. This finding, along with the observed changes in MRD status over one year of maintenance therapy, highlight the requirement for serial minimal residual disease assessments to better assess the risk of progression. At present, the feasibility of monitoring MRD over time is limited by the invasive bone marrow biopsy procedures, but serum based tests to facilitate this monitoring are currently undergoing clinical evaluation.

Although the characteristics of patients excluded from the analyses were comparable to those of patients who were included, we should emphasize that potential bias cannot be totally excluded since MRD data were unavailable for 50% of patients at the specified time

points. The main reason that MRD was not assessed was the absence of a sample stored at the time of diagnosis to identify the clonal rearrangements.

In a significant number of patients, MRD evaluation was not feasible mainly because of lack of diagnostic sample for calibration. Since MRD evaluation by NGS was not planned in 2008 (because not existing), we did not make significant efforts to spare plasma cells for calibration. This point could be a limitation of NGS vs NGF, but, currently, we are able to calibrate > 98% of the patients (personal data, HAL). The main advantage of NGS vs NGF is the required number of cells to achieve the 10^{-6} threshold. With NGS, only 2 millions of cells are needed, vs at least 20 millions for NGF. In this study we used the Sequenta/Adaptive platform. Other platforms (commercial and academic) are available, but to our knowledge, the Adaptive kit is the only one which is (almost) FDA-approved.

In conclusion, next-generation sequencing-determined MRD status enables the identification of patient subpopulations with highly different prognoses. Consequently, in the future, this end point could be used to inform treatment decisions and provide significant reassurance for myeloma patients who achieve MRD negativity, regardless of their cytogenetic risk profile or disease stage. It is also clear that MRD opens the door to evaluation of stratified therapy for multiple myeloma patients in future randomized clinical trials.

COI disclosure: Authors have no conflict of interest to declare for this paper

Author contribution: AP, VLC, JC, MA, HAL, and NM collected the data, performed analyses, and wrote the paper, VC, MM, TW, and MF performed the MRD experiments. All other authors provided samples and clinical data.

Support: This study has been supported by grants from Celgene and Janssen, the CAPTOR program,

and a NIH PO1 grant (PO1-155258). The UGM laboratory is labelled by the Association Recherche Cancer.

Acknowledgments: This study was supported by grants from Celgene and Janssen, a National Institutes of Health PO1 grant (CA100707-12), and the Cancer Pharmacology of Toulouse-Oncopole and Region (CAPTOR) program. The UGM laboratory is sponsored by the Association Recherche Cancer.

REFERENCES

1. Palumbo A, Anderson K. Multiple myeloma. The New England journal of medicine 2011;364:1046-60.

2. Richardson PG, Weller E, Lonial S, et al. Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. Blood 2010;116:679-86.

3. Moreau P, Masszi T, Grzasko N, et al. Oral Ixazomib, Lenalidomide, and Dexamethasone for Multiple Myeloma. The New England journal of medicine 2016;374:1621-34.

4. Dimopoulos MA, Oriol A, Nahi H, et al. Daratumumab, Lenalidomide, and Dexamethasone for Multiple Myeloma. The New England journal of medicine 2016;375:1319-31.

5. Stewart AK, Rajkumar SV, Dimopoulos MA, et al. Carfilzomib, lenalidomide, and dexamethasone for relapsed multiple myeloma. The New England journal of medicine 2015;372:142-52.

6. Jakubowiak AJ, Dytfeld D, Griffith KA, et al. A phase 1/2 study of carfilzomib in combination with lenalidomide and low-dose dexamethasone as a frontline treatment for multiple myeloma. Blood 2012;120:1801-9.

7. Cave H, van der Werff ten Bosch J, Suciu S, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer--Childhood Leukemia Cooperative Group. The New England journal of medicine 1998;339:591-8.

8. Grimwade D, Jovanovic JV, Hills RK, et al. Prospective minimal residual disease monitoring to predict relapse of acute promyelocytic leukemia and to direct pre-emptive arsenic trioxide therapy. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2009;27:3650-8.

9. Kovacs G, Robrecht S, Fink AM, et al. Minimal Residual Disease Assessment Improves Prediction of Outcome in Patients With Chronic Lymphocytic Leukemia (CLL) Who Achieve Partial Response: Comprehensive Analysis of Two Phase III Studies of the German CLL Study Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2016;34:3758-65.

10. Flores-Montero J, Flores LS, Paiva B, et al. Next generation flow (NGF) for highly

sensitive and standardized detection of minimal residual disease in multiple myeloma. Leukemia 2017.

11. Paiva B, van Dongen JJ, Orfao A. New criteria for response assessment: role of minimal residual disease in multiple myeloma. Blood 2015;125:3059-68.

12. Paiva B, Puig N, Garcia-Sanz R, San Miguel JF, Grupo Espanol de Mieloma /Programa para el Estudio de la Terapeutica en Hemopatias Malignas cooperative study g. Is this the time to introduce minimal residual disease in multiple myeloma clinical practice? Clinical cancer research : an official journal of the American Association for Cancer Research 2015;21:2001-8.

13. Martinez-Lopez J, Lahuerta JJ, Pepin F, et al. Prognostic value of deep sequencing method for minimal residual disease detection in multiple myeloma. Blood 2014;123:3073-9.

14. Attal M, Lauwers-Cances V, Hulin C, et al. Lenalidomide, Bortezomib, and Dexamethasone with Transplantation for Myeloma. The New England journal of medicine 2017;376:1311-20.

15. Greipp PR, San Miguel J, Durie BG, et al. International staging system for multiple myeloma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2005;23:3412-20.

16. Roussel M, Lauwers-Cances V, Robillard N, et al. Front-line transplantation program with lenalidomide, bortezomib, and dexamethasone combination as induction and consolidation followed by lenalidomide maintenance in patients with multiple myeloma: a phase II study by the Intergroupe Francophone du Myelome. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2014;32:2712-7.

17. Mantel N, Bvar DP. Evaluation of response time data involving transient states: An illustration using heart transplant data. J Am Stat Assoc 1974;69:81-6.

18. Simon R, Makuch RW. A non-parametric graphical representation of the relationship between survival and the occurrence of an event: application to responder versus non-responder bias. Statistics in medicine 1984;3:35-44.

19. Lahuerta JJ, Paiva B, Vidriales MB, et al. Depth of Response in Multiple Myeloma: A Pooled Analysis of Three PETHEMA/GEM Clinical Trials. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2017;35:2900-10.

20. Gormley NJ, Turley DM, Dickey JS, et al. Regulatory perspective on minimal residual disease flow cytometry testing in multiple myeloma. Cytometry Part B, Clinical cytometry 2016;90:73-80.

21. Rawstron AC, Child JA, de Tute RM, et al. Minimal residual disease assessed by multiparameter flow cytometry in multiple myeloma: impact on outcome in the Medical Research Council Myeloma IX Study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2013;31:2540-7.

22. Munshi NC, Avet-Loiseau H, Rawstron AC, et al. Association of Minimal Residual Disease With Superior Survival Outcomes in Patients With Multiple Myeloma: A Meta-analysis. JAMA oncology 2017;3:28-35.

23. Kumar S, Paiva B, Anderson KC, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. The Lancet Oncology 2016;17:e328-46.

TABLES

Table 1. Minimal Residual Disease Status During Maintenance Therapy, According to Patients' Baseline Characteristics and Treatment Response at the Start and after 12 months of Maintenance Therapy.

	Minimal Residual Disease Status		
	Negative	**Positive**	**P Value**
	N = 127	**N = 382**	
Age — no. (%)			0.14
<60 years	62 (22.4)	215 (77.6)	
≥60 years	65 (28.0)	167 (72.0)	
Gender — no. (%)			0.19
Male	68 (22.8)	230 (77.2)	
Female	59 (28.0)	152 (72.0)	
International Staging System disease stage — no. (%)			0.61
Stage I	38 (23.0)	127 (77.0)	
Stage II	67 (26.9)	182 (73.1)	
Stage III	22 (23.2)	73 (76.8)	
Cytogenetic risk profile — no. (%)			0.40*
Standard risk	85 (26.2)	239 (73.8)	
High risk	23 (31.1)	51 (68.9)	
FISH failure	19 (17.1)	92 (82.9)	
Treatment arm — no. (%)			0.01
RVD-alone	54 (20.5)	210 (79.5)	
Transplantation	73 (29.8)	172 (70.2)	
Response at the beginning of maintenance therapy — no. (%)†			0.006‡

Complete response stringent or not	54 (49.5)	55 (50.5)	
Very good partial response	36 (31.3)	79 (68.7)	
Partial response	0 (0.0)	134 (100.0)	
Stable disease	0 (0.0)	8 (100.0)	
Response after 12 months of maintenance therapy — no. (%)†			<.001‡
Complete response stringent or not	83 (59.7)	56 (40.3)	
Very good partial response	9 (20.5)	35 (79.5)	
Partial response	0 (0.0)	56 (100.0)	

* The FISH failure category was excluded.
† Among the 127 patients who were minimal residual disease negative during maintenance therapy, 90 achieved negativity at the beginning of maintenance therapy, and 92 after completion of maintenance therapy.
‡ Patients who achieved less than a very good partial response were excluded.
Abbreviations: FISH, fluorescence in-situ hybridization; RVD, lenalidomide, bortezomib, dexamethasone.

Table 2. Multivariate Cox Proportional Hazards Regressions for Progression-Free Survival.

	Adjusted Hazard Ratio*	95% Confidence Interval	P Value
First landmark analysis at start of maintenance therapy (N=366)			
Minimal residual disease			
Negative / positive	0.22	0.15-0.34	<0.001
Treatment arm			
Transplantation / RVD alone	0.68	0.52-0.88	0.004
Second landmark analysis after 12 months of maintenance therapy (N=239)			
Minimal residual disease			
Negative / positive	0.18	0.12-0.29	<0.001
Treatment arm			
Transplantation / RVD alone	0.76	0.54-1.07	0.115
Modified intent-to-treat population (N=509)			
Minimal residual disease			
Negative / positive	0.19	0.13–0.26	<0.001
Treatment arm			
Transplantation / RVD alone	0.76	0.62–0.94	0.01

Abbreviations: RVD, lenalidomide, bortezomib, dexamethasone.
*Hazard ratio were adjusted on International Staging System disease stage and cytogenetic risk profile

Figure Legends

Figure 1 A. Kaplan-Meier Survival Curves for Progression-Free Survival According to Minimal Residual Disease Status at the Start of Maintenance Therapy.

The median progression-free survival from the start of maintenance therapy was not reached among MRD-negative patients, and was 29 months among MRD-positive patients

Figure 1 B. Kaplan-Meier Survival Curves for Progression-Free Survival According to Minimal Residual Disease Status after 12 months of maintenance therapy.

The median progression-free survival from the completion of maintenance therapy was not reached among MRD-negative patients, and was 20 months among MRD-positive patients.

Figure 1C. Kaplan-Meier Survival Curves Modified by the Simon−Makuch Method for Progression-Free Survival According to Minimal Residual Disease Status.

The number of patients at risk in the MRD-negative group was 0 at the beginning of follow-up, because of the time-dependent nature of MRD. Prior to the start of maintenance therapy, all patients were included in the MRD-positive group; patients who were found to be MRD-negative on assessment during maintenance were switched to the MRD-negative group. The median progression-free survival from randomization was not reached among MRD-negative patients, and was 29 months among MRD-positive patients.

Abbreviation: MRD, minimal residual disease.

Figure 2. Probability of progression-free survival adjusted for minimal residual disease status and treatment group (A), cytogenetic risk status (B), and International Staging System disease stage with stages II and III grouped together, as hazard ratios in these subgroups were numerically identical (C).

These curves were constructed according to the results of the Cox model performed on the

366 patients with known minimal residual disease status at start of maintenance therapy. Abbreviations: ISS, International Staging System; MRD, minimal residual disease; RVD, lenalidomide, bortezomib, dexamethasone.

Figure 3A. Kaplan-Meier Survival Curves for Overall Survival According to Minimal Residual Disease Status at the Start of Maintenance Therapy.

The overall survival at 4 years after the start of maintenance therapy was 94% among MRD-negative patients, and 79% among MRD-positive patients (adjusted hazard ratio for death, 0.24; 95% confidence interval, 0.11 to 0.54).

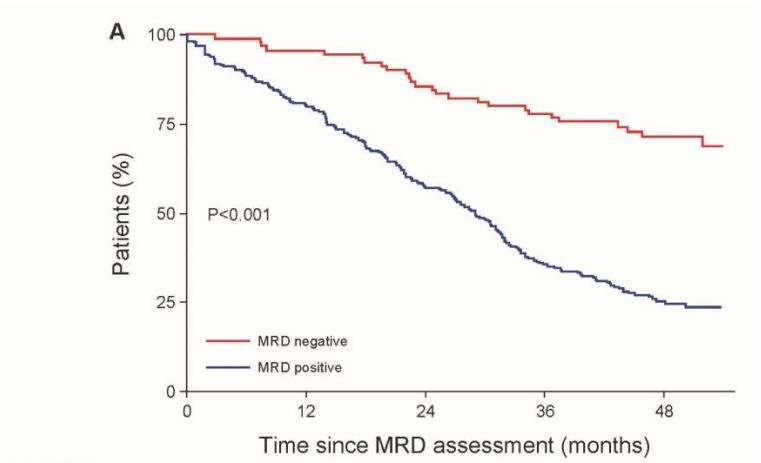
Figure 3B. Kaplan-Meier Survival Curves for Overall Survival According to Minimal Residual Disease Status After 12 months of Maintenance Therapy.

The overall survival at 3 years after the completion of maintenance therapy was 96% among MRD-negative patients, and 86% among MRD-positive patients (adjusted hazard ratio for death, 0.26; 95% confidence interval, 0.10 to 0.68).

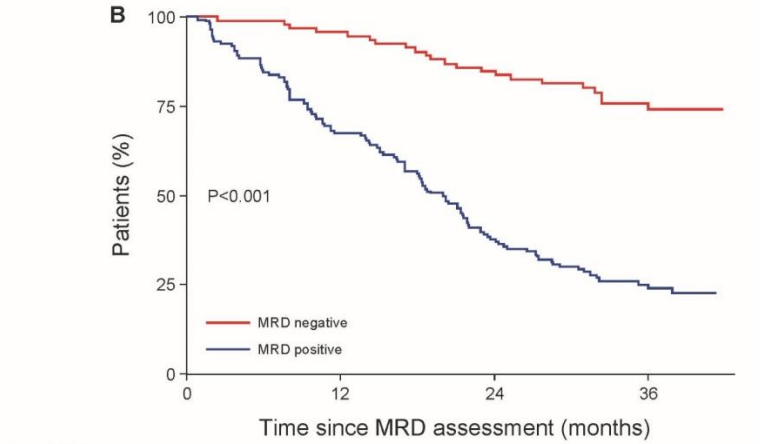
Figure 3C. Kaplan-Meier Survival Curves Modified by the Simon−Makuch Method for Overall Survival According to Minimal Residual Disease Status.

The number of patients at risk in the MRD-negative group was 0 at the beginning of follow-up, because of the time-dependent nature of minimal residual disease. Prior to the start of maintenance therapy, all patients were included in the MRD-positive group; patients who were found to be MRD-negative on assessment during maintenance were switched to the MRD-negative group. MRD was a strong prognostic factor for overall survival (adjusted hazard ratio, 0.16; 95% confidence interval, 0.08 to 0.32).

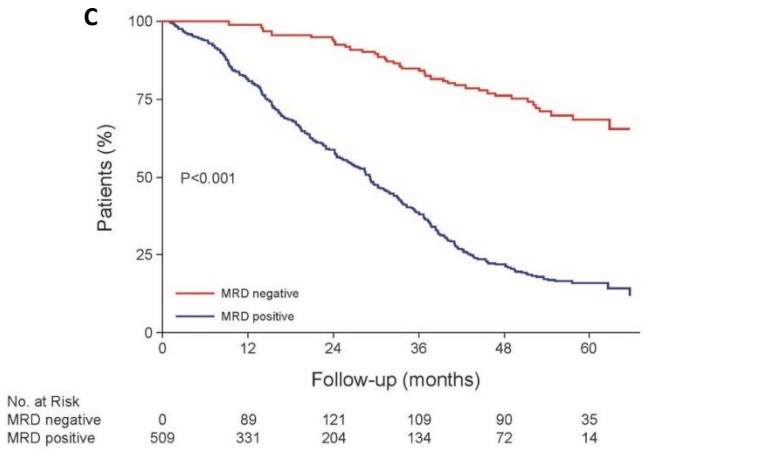
Figure 1.



No. at Risk					
MRD negative	90	86	77	69	40
MRD positive	276	221	157	96	40



No. at Risk				
MRD negative	92	88	77	42
MRD positive	147	99	55	23



No. at Risk						
MRD negative	0	89	121	109	90	35
MRD positive	509	331	204	134	72	14

Figure 2.

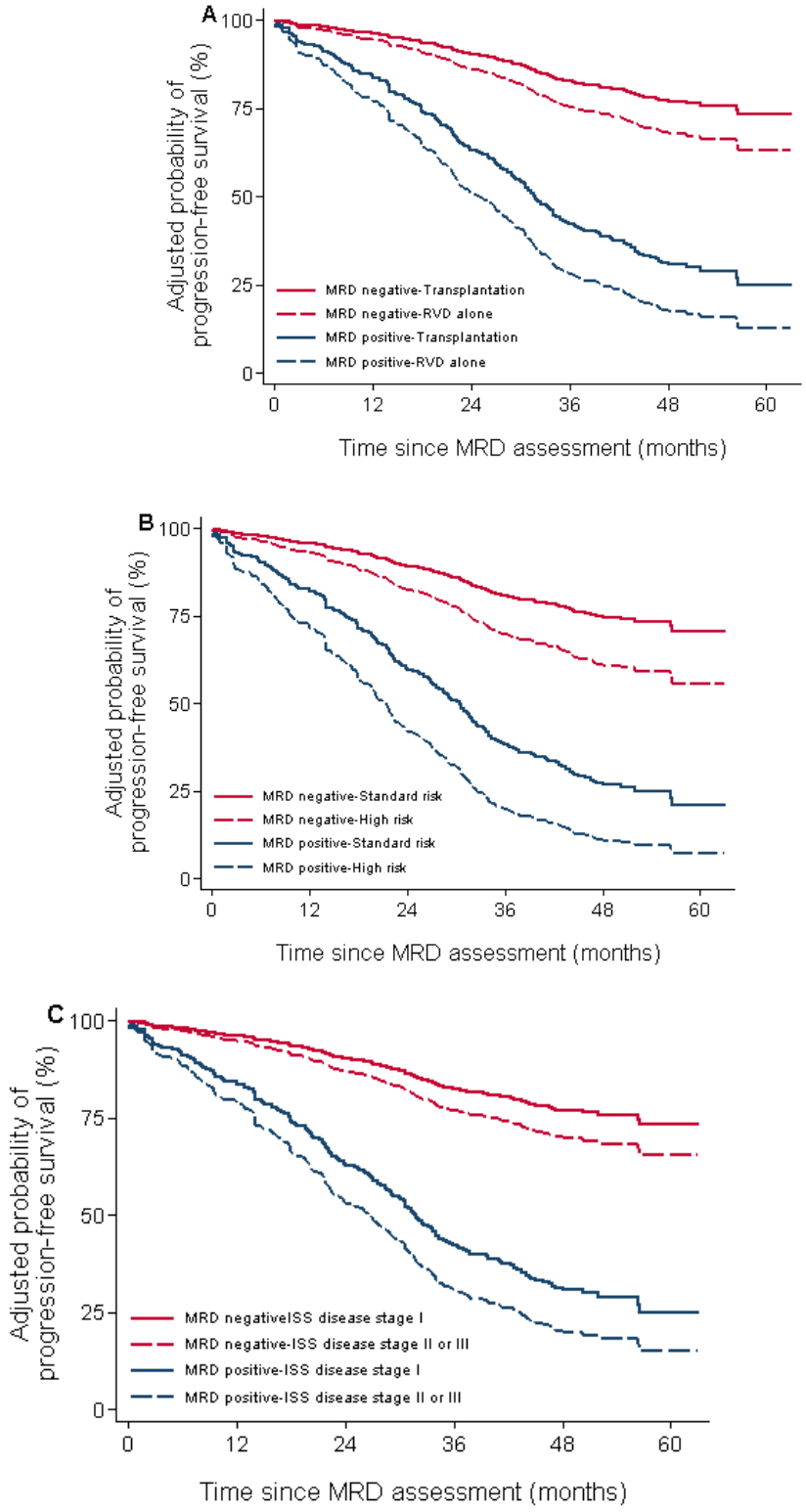
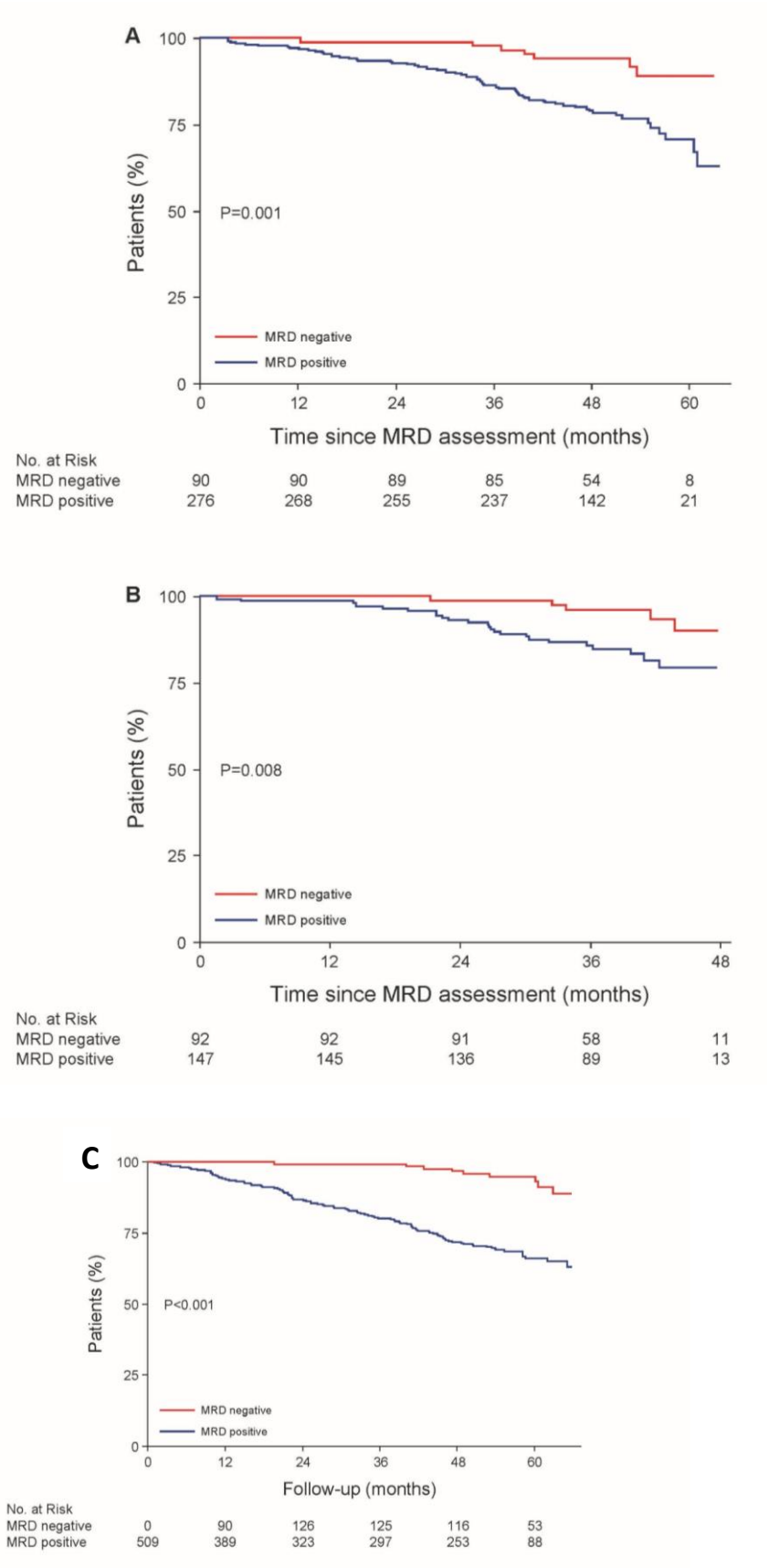


Figure 3



Prepublished online September 24, 2018;
doi:10.1182/blood-2018-06-858613 originally published online
September 24, 2018

Minimal residual disease negativity using deep sequencing is a major prognostic factor in multiple myeloma

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Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.