VOLUME 31 · NUMBER 20 · JULY 10 2013

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Minimal Residual Disease Assessed by Multiparameter Flow Cytometry in Multiple Myeloma: Impact on Outcome in the Medical Research Council Myeloma IX Study

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See accompanying editorial on page 2523; listen to the podcast by Dr San Miguel at www.jco.org/podcasts

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Published online ahead of print at www.jco.org on June 3, 2013.

Presented in part at the 13th International Myeloma Workshop, Paris, France, May 3-6, 2011, and 52nd Annual Scientific Meeting of the British Society for Haematology, Glasgow, United Kingdom, April 16-18, 2012.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical trial information: ISRCTN68454111.

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0732-183X/13/3120w-2540w/$20.00

DOI: 10.1200/JCO.2012.46.2119

ABSTRACT

Purpose
To investigate the prognostic value of minimal residual disease (MRD) assessment in patients with multiple myeloma treated in the MRC (Medical Research Council) Myeloma IX trial.

Patients and Methods
Multiparameter flow cytometry (MFC) was used to assess MRD after induction therapy (n = 378) and at day 100 after autologous stem-cell transplantation (ASCT; n = 397) in intensive-pathway patients and at the end of induction therapy in non–intensive-pathway patients (n = 245).

Results
In intensive-pathway patients, absence of MRD at day 100 after ASCT was highly predictive of a favorable outcome (PFS, $P < .001$; OS, $P = .0183$). This outcome advantage was demonstrable in patients with favorable and adverse cytogenetics (PFS, $P = .014$ and $P < .001$, respectively) and in patients achieving immunofixation-negative complete response (CR; PFS, $P = .0068$). The effect of maintenance thalidomide was assessed, with the shortest PFS demonstrable in those MRD-positive patients who did not receive maintenance and longest in those who were MRD negative and did receive thalidomide ($P < .001$). Further analysis demonstrated that 28% of MRD-positive patients who received maintenance thalidomide became MRD negative. MRD assessment after induction therapy in the non–intensive-pathway patients did not seem to be predictive of outcome (PFS, $P = .1$).

Conclusion
MRD assessment by MFC was predictive of overall outcome in patients with myeloma undergoing ASCT. This predictive value was seen in patients achieving conventional CR as well as patients with favorable and adverse cytogenetics. The effects of maintenance strategies can also be evaluated, and our data suggest that maintenance thalidomide can eradicate MRD in some patients.

J Clin Oncol 31:2540-2547. © 2013 by American Society of Clinical Oncology

INTRODUCTION

Historically, achievement of a stable plateau state rather than the magnitude of serologic response seemed to be the most important determinant of outcome in patients with myeloma.[1,2] However, with the introduction of high-dose melphalan (HDM) and supporting autologous stem-cell transplantation (ASCT), it became evident that the achievement of complete response (CR), defined by absence of monoclonal (M) protein in the serum and urine by immunofixation (IF), along with < 5% bone marrow (BM) plasma cells, predicted outcome.[3-7] With the advent of novel induction regimens, it has become apparent that the achievement of CR also predicts outcome in patients ineligible for ASCT.[8,9]

The sequential monitoring of M protein concentration has some limitations, despite its widespread availability and ease of use. For instance, categorical response assessment at specific time points is influenced by M protein type because clearance half lives vary considerably, typically 2 to 4 hours for free light chains and approximately 25 days for immunoglobulin G.[10] These data, along with clinical outcome data, have led to a

re-evaluation of high-quality categorical responses, and an additional category of stringent CR has been introduced, which incorporates serum free light-chain assessment and BM immunohistology or immunofluorescence.[11,12]

Low-level BM disease, so-called minimal residual disease (MRD), can be demonstrated by multiparameter flow cytometry (MFC) and allele-specific polymerase chain reaction (ASO-PCR) methods.[13,14] MFC, although less sensitive than ASO-PCR methods, seems to be applicable to a greater proportion of patients and is potentially more suited to routine practice.[15,16] MFC has been widely applied in the diagnostic and prognostic evaluations of patients with myeloma and related disorders.[17-22] Some reports have also suggested its value in response assessment, particularly in the context of patients undergoing ASCT.[23-29] Here we report the prospective evaluation of MRD using MFC in both intensively and nonintensively treated patients in the MRC (Medical Research Council) Myeloma IX trial.

PATIENTS AND METHODS

The MRC Myeloma IX trial was a multicenter, randomized phase III trial. The protocol was approved by the relevant institutional review boards, and all patients provided written informed consent.

Study Protocol

The study protocol and clinical results have been described in detail previously[30-33] and are summarized in the consort diagram (Fig 1). Patients were assigned to either intensive- or non–intensive-pathway treatment based on performance status, clinician judgment, and patient preference. In the intensive pathway, patients were randomly assigned to CTD (cyclophosphamide, thalidomide, and dexamethasone) or CVAD (cyclophosphamide, vincristine, doxorubicin, and dexamethasone). Both regimens were administered for four to six cycles until maximum response, after which patients proceeded to HDM (200 mg/m^2) and ASCT.

Non–intensive-pathway patients were randomly assigned to either MP (melphalan and prednisolone) or attenuated CTD (CTDa). Each was administered for a minimum of six cycles to a maximum of nine. Full details of induction regimens available in the Appendix (online only).

Patients were then randomly assigned to maintenance thalidomide (50 to 100 mg daily) or no further therapy at day 100 after ASCT. This was done at time of maximum response in the non–intensive-pathway patients.

Cytogenetic Characterization

BM aspirates were obtained at study entry to determine cytogenetic profiles by fluorescence in situ hybridization on CD138 purified plasma cells (Miltenyi Biotec, Bisley, United Kingdom). This analysis was performed in a central laboratory (Wessex Regional Genetics Laboratory, Salisbury, United Kingdom). In intensive-pathway patients, adverse interphase fluorescence in situ hybridization cytogenetic profiles were defined as gain(1q), del(1p32), t(4;14), t(14;20), t(14;16), and del(17p). Favorable profiles comprised the remainder and included hyperdiploidy, t(11;14), and t(6;14).

Assessment of MRD

BM aspirates were obtained at presentation and after induction therapy and at day 100 after ASCT in the intensive-pathway and at completion of

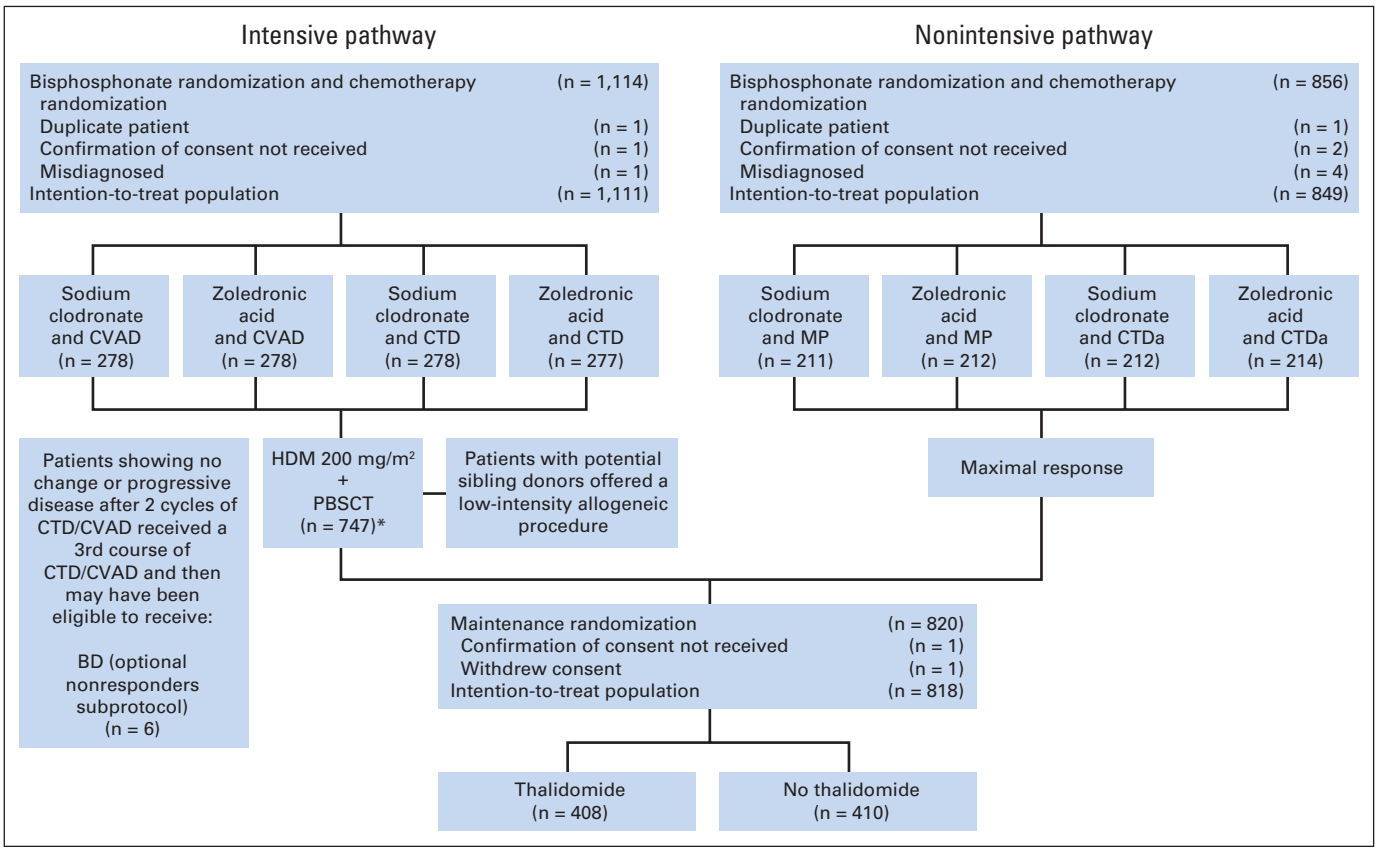

Fig 1. MRC (Medical Research Council) Myeloma IX trial CONSORT diagram. BD, bortezomib and dexamethasone; CTD, cyclophosphamide, thalidomide, and dexamethasone; CTDa, attenuated CTD; CVAD, cyclophosphamide, vincristine, doxorubicin, and dexamethasone; HDM, high-dose melphalan; MP, melphalan and prednisolone; PBSCT, peripheral blood stem-cell transplantation.

induction therapy in the non–intensive-pathway patients. These marrow assessments were planned in all patients irrespective of clinical or categorical response. Additional marrow samples were obtained in a subset of patients.

Flow cytometric analysis was performed in a single laboratory (Haematological Malignancy Diagnostic Service, Leeds, United Kingdom) according to the principles outlined by the European Myeloma Network.[13] Leukocytes were prepared by incubation of a volume of BM aspirate containing 10^6 leukocytes with 5 mL of ammonium chloride (8.6 g/L in distilled water) for 10 minutes at 37°C, washed twice, and resuspended in 5 mL of FACSFlow (BD Biosciences, Oxford, United Kingdom) containing 0.3% bovine serum albumin. The cell pellet was resuspended in pretitered antibody mixtures and incubated for 30 minutes at 4°C in the dark, washed twice, and resuspended in FACSFlow. For pretreatment samples, 100,000 events were acquired, and for post-treatment samples, a minimum of 500,000 events were acquired and analyzed for each antibody combination using a Canto II flow cytometer with FACSDiva software (BD Biosciences).

A six-color panel of antibodies was used: CD138 APC (B-B4; Miltenyi Biotec), CD45 APC-Cy7 (2D1; BD Pharmingen, Oxford, United Kingdom), CD38 PE-Cy7 (HIT2; BD Pharmingen), and CD19 PerCP-Cy5.5 (HIB19; BD Pharmingen). In all cases, expression of CD56 PE (MY31; BD Biosciences) and CD27 FITC (M-T271; BD Pharmingen) on gated plasma cells was assessed. Patient cases were classified as having residual disease if a discrete population of phenotypically aberrant plasma cells comprising ≥ 50 events was identified in the 500,000-event file (0.01% limit of detection). An aberrant phenotype was classified as a lack of CD19 expression, strong CD56 expression, weak CD27 expression, and/or weak CD45 expression. Patient cases with < 20 aberrant-phenotype plasma cells were considered to have no evidence of residual disease. Occasional (< 1%) patient cases, with 20 to 50 events or with a potentially aberrant phenotype but without a discrete population, were analyzed further either by acquiring more events or by additional analysis of CD81 FITC (JS-81; BD Pharmingen), CD117 PE (YB5.B8; BD Pharmingen), CD200 PE (MRC OX-104; BD Pharmingen), and/or CD52 FITC (HI186; AbD Serotec, Kidlington, United Kingdom).

Efficacy End Points and Statistical Analysis

Categorical responses were defined according to the modified European Group for Blood and Marrow Transplantation/International Bone Marrow Transplant Registry criteria.[34] All statistical analyses were landmarked from date of MRD assessment. Progression-free survival (PFS) was defined as time from MRD assessment to documented progression or death and overall survival (OS) as time from MRD assessment to death resulting from any cause. Analyses were based on the intention-to-treat population and included all patients with MRD data. Between-group comparisons were performed using Fisher's exact test, and OS and PFS were assessed with Kaplan-Meier analyses and the log-rank test. Statistical analysis was performed using SAS software (version 9.2; SAS Institute, Cary, NC) or Fortran software (Digital Equipment, Maynard, MA). All hypothesis tests were two sided, with a 5% significance level.

RESULTS

A total of 1,970 patients were enrolled from 121 centers; 1,114 received intensive-pathway treatment, and 856 received non–intensive-pathway treatment. Median follow-up was 71 months.

Response Rates: Superiority of Thalidomide-Containing Regimens

MRD was assessed in intensive-pathway patients at the end of induction chemotherapy (n = 378; 190 received CVAD; 188 received CTD) and at day 100 after ASCT (n = 397; 208 received CVAD; 189 received CTD). Overall, 72 (19.0%) of 378 patients had no detectable aberrant-phenotype plasma cells at the end of induction and were considered MRD negative. When present, residual aberrant plasma cells comprised a median of 0.7% of BM leukocytes (range, 0.01% to 49.9%). After ASCT 247 (62.2%) of 397 assessed patients were MRD negative, and in those considered MRD positive, residual aberrant plasma cells comprised 0.2% of BM leukocytes (range, 0.01% to 90.0%). However, there was a significant difference when the two induction regimens were compared; 25% of CTD-treated patients became MRD negative, compared with 13% of CVAD-treated patients ($P = .0039$). This superiority was maintained after ASCT; 71% of CTD-treated patients were MRD negative, compared with 54% of CVAD-treated patients ($P < .001$).

Similarly, MRD was assessed at the end of induction therapy in the non–intensive-pathway patients (n = 245; 119 received MP; 126 received CTDa). Overall, 36 (14.7%) of 245 of patients became MRD negative, whereas residual aberrant plasma cells comprised 1.6% of BM leukocytes in those remaining MRD positive. Again, there was a highly significant difference between the two regimens; 26% of CTDa-treated patients became MRD negative, compared with 3% of MP-treated patients ($P < .001$).

Impact of MRD on Outcome

Presence of MRD was associated with inferior outcome in the intensive-pathway cohort. Median PFS for those patients who were MRD positive after ASCT was 15.5 months versus 28.6 months in MRD-negative patients ($P < .001$; Fig 2A). A significant OS advantage was also demonstrable, with a median OS of 59.0 months in MRD-positive patients versus 80.6 months in MRD-negative patients ($P = .0183$; Fig 2B). When the impact of MRD status at the end of induction was also considered, those patients achieving MRD negativity at the end of induction had the most favorable outcome with regard to PFS (median PFS, 44.2 months; $P < .001$; Fig 2C), but their OS did not seem different from that of patients who became MRD negative after ASCT ($P = .16$; Fig 2D).

The effect of MRD after ASCT was then assessed according to cytogenetic risk group in a subset of 241 patients with available data. The proportion of patients achieving MRD negativity was not influenced by cytogenetic risk group; it was demonstrable in 79 (59.8%) of 132 patients with favorable cytogenetic profiles and 67 of (61.5%) 109 with adverse cytogenetics. Presence of MRD was predictive of outcome in patients with favorable cytogenetic profiles (median PFS, 44.2 *v* 33.7 months; $P = .014$) and adverse cytogenetic profiles (median PFS, 15.7 *v* 8.7 months; $P < .001$). The combination of MRD status at day 100 and cytogenetic risk group provided a powerful discriminator of outcome. The best outcome was demonstrable in those patients with favorable cytogenetics and MRD negativity; the worst was in those patients with adverse cytogenetics and persistent disease (PFS and OS, $P < .001$; Fig 3).

Impact of MRD was also assessed in the non–intensive-pathway cohort. Here, presence of MRD at the end of induction was associated with nonsignificantly inferior PFS (7.4 *v* 10.5 months; $P = .1$).

Outcome Prediction in Patients Achieving CR

When MRD was evaluated according to categorical response, it was noted that 31 (14.5%) of 214 patients achieving IF-negative CR had detectable disease by MFC. Similarly, it was noted that 63 (25.6%) of 246 MRD-negative patients failed to achieve CR. Formal responses documented in this latter group of patients included 34 very good partial responses (VGPRs), 24 PRs, and five other. That 29 (11.6%) of 246 MRD-negative patients failed to achieve at least VGPR suggests

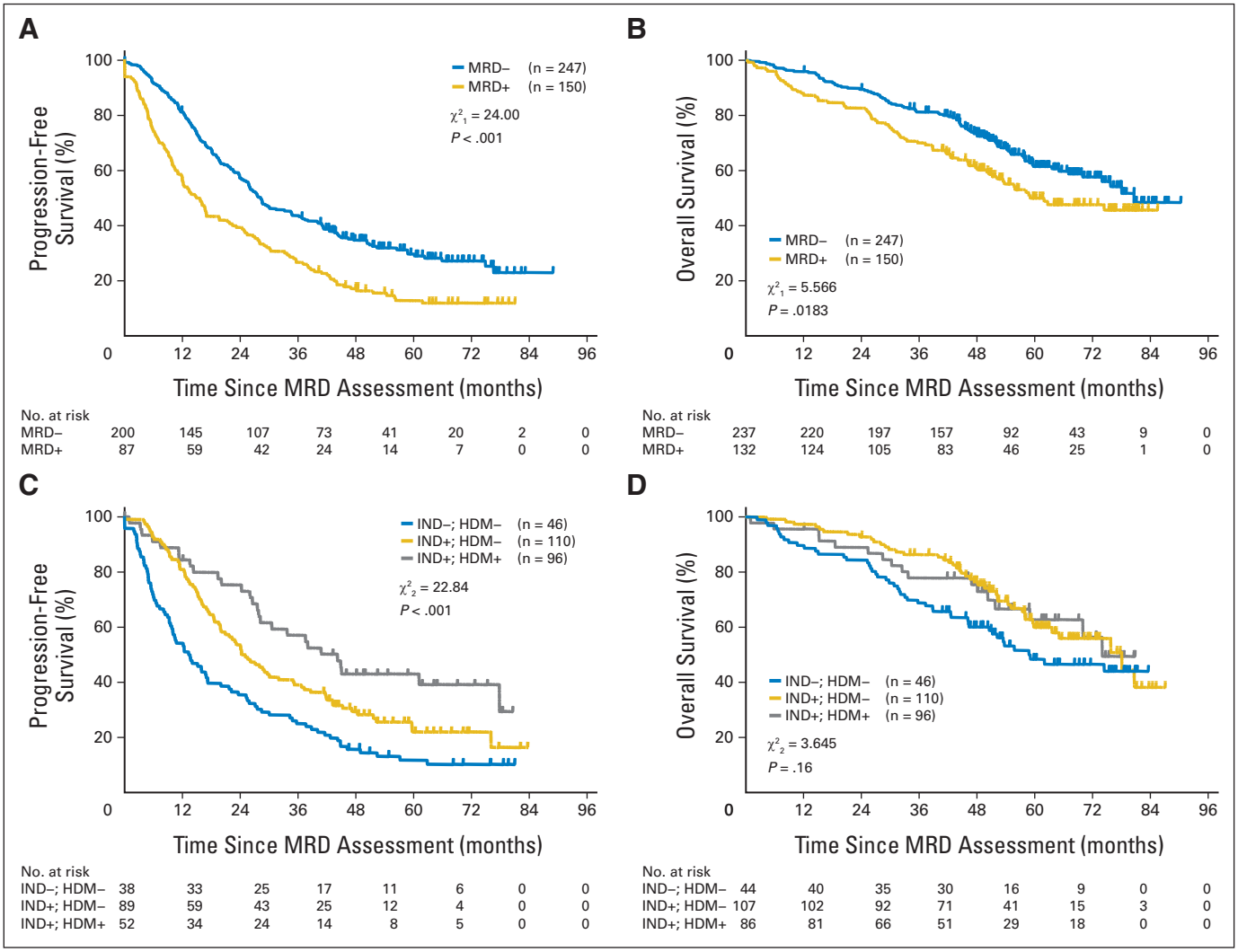

Fig 2. Outcome according to minimal residual disease (MRD) status before and after autologous stem-cell transplantation (ASCT). Presence of MRD at day 100 after ASCT was associated with inferior outcome; (A) progression-free survival (PFS; $P < .001$; (B) overall survival (OS; $P = .0183$). When MRD status at the end of induction was also considered, patients achieving MRD negativity at the end of induction had a favorable outcome with regard to PFS (C; $P < .001$), but their OS did not seem different from that of patients who subsequently became MRD negative after ASCT (D; $P = .16$). HDM, high-dose melphalan; IND, induction.

that MRD analysis should be performed in all responding patients regardless of M protein response.

With regard to outcome in CR patients, presence of MRD was associated with inferior PFS (14.1 *v* 34.3 months; $P = .0068$) and nonsignificantly inferior OS (median OS, 61.9 months *v* not reached; $P = .0928$). When outcome was assessed according to both conventional response criteria and MRD, it was clear that those patients who were both IF negative and MRD negative had the most favorable outcome, whereas those patients who were MRD negative but failed to achieve conventional CR had an outcome identical to that of those patients who were MRD positive (PFS, $P < .001$; OS, $P = .0385$; Fig 4).

Assessment of Maintenance Thalidomide

The relationship between MRD after ASCT and thalidomide maintenance was assessed in 292 patients who underwent maintenance random assignment. Outcome seemed best in those patients who achieved MRD negativity after ASCT and were receiving maintenance and worst in those MRD-positive patients who did not receive maintenance (PFS, $P < .001$; Fig 5). When the effect of maintenance was evaluated according to MRD status, an improvement in PFS was noted in the MRD-positive but not MRD-negative group ($P = .036$ and $P = .1$, respectively). Similarly, when the effect of MRD was assessed according to maintenance, the effect on PFS seemed greatest in those receiving no maintenance (no maintenace, $P = .0038$; maintenance, $P = .1$).

Further insight into the effects of thalidomide maintenance was gained when a subset of patients (n = 99) had additional BM assessments. These were performed at a median of 7 months after the day 100 post-ASCT assessment. Although there was some variability in the timing of this BM assessment, there was no difference between those receiving maintenance and those randomly assigned to no further therapy. Eight (27.6%) of 29 MRD-positive patients receiving thalidomide became MRD negative, compared with one (3.4%) of 29 patients randomly assigned to no further therapy ($P = .025$). Likewise, 24 (96%) of 25 MRD-negative patients receiving thalidomide

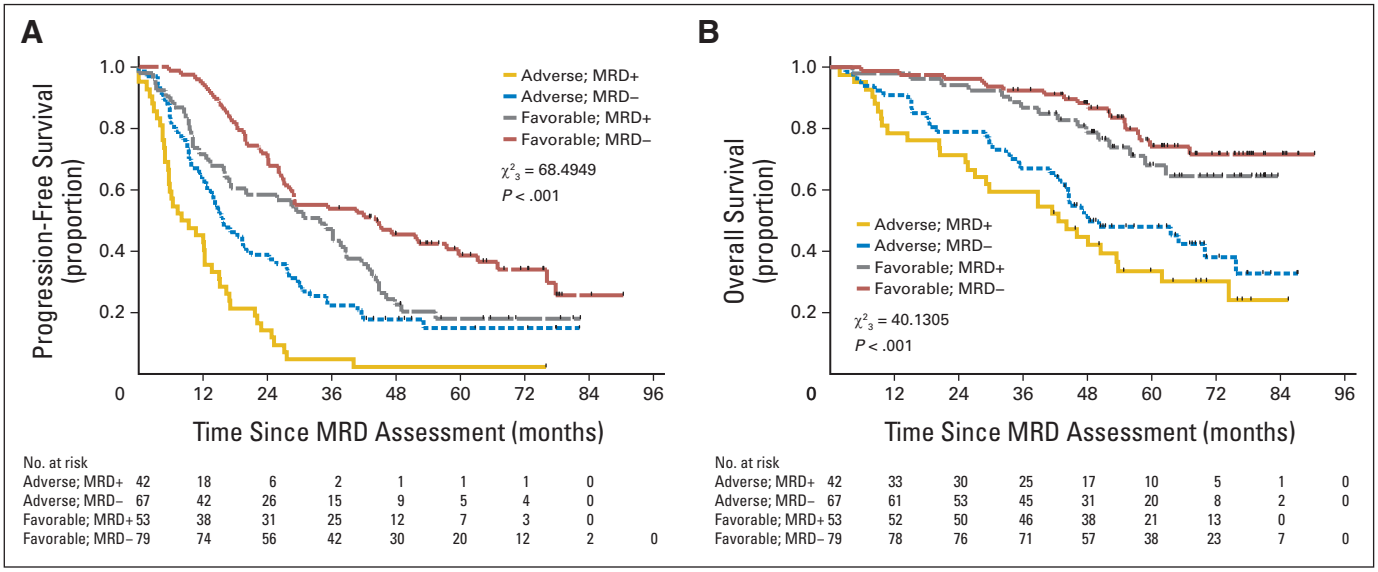

Fig 3. Outcome according to minimal residual disease (MRD) status after autologous stem-cell transplantation and cytogenetic risk profile. (A) Progression-free survival; (B) overall survival.

maintenance remained MRD negative, compared with 11 (68.8%) of 16 patients who received no further therapy ($P = .026$; Fig 6).

DISCUSSION

Recent therapeutic advances have improved OS in myeloma.[35] Response rates are improving, and the proportion of patients achieving high-quality categorical responses is also increasing. These factors have resulted in a need to continually re-evaluate response criteria, particularly the definition of CR. Similarly, treatment schedules are becoming more complex and may typically include induction, consolidation, and maintenance components. Assessment of the efficacy of individual components of such multicomponent therapy can be difficult because planned treatments are often introduced before maximum M protein responses are achieved. This is particularly relevant for patients with immunoglobulin G M proteins, in which the half-life is approximately 25 days.[10] Furthermore, response assessment after ASCT is not infrequently complicated by presence of oligoclonal bands.

There is a clear rationale for the direct evaluation of residual BM plasma cells in the assessment response. Assessment of kappa-to-lambda ratios by immunohistochemistry or immunofluorescence has limited sensitivity in the post-treatment setting because of the regeneration of normal polyclonal plasma cells.[36] MFC is currently an

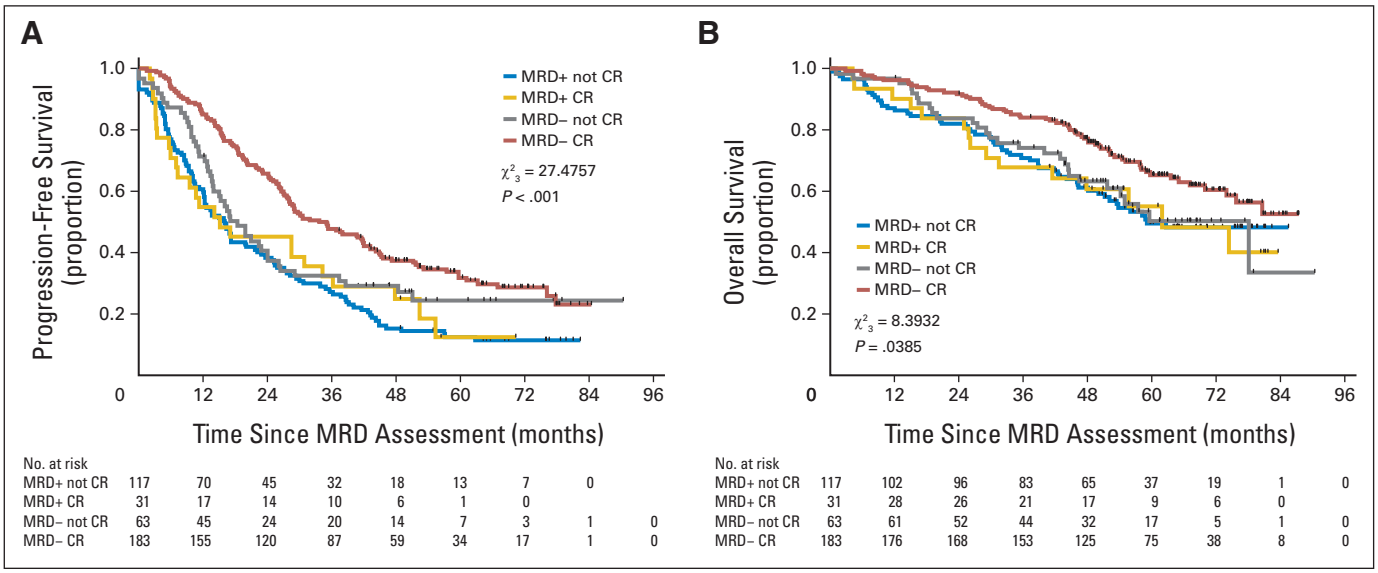

Fig 4. Outcome according to minimal residual disease (MRD) and immunofixation (IF) status after autologous stem-cell transplantation. Best outcome was demonstrated in those patients with IF-negative complete response (CR) and no demonstrable MRD. (A) Progression-free survival ($P < .001$); (B) overall survival ($P = .0385$).

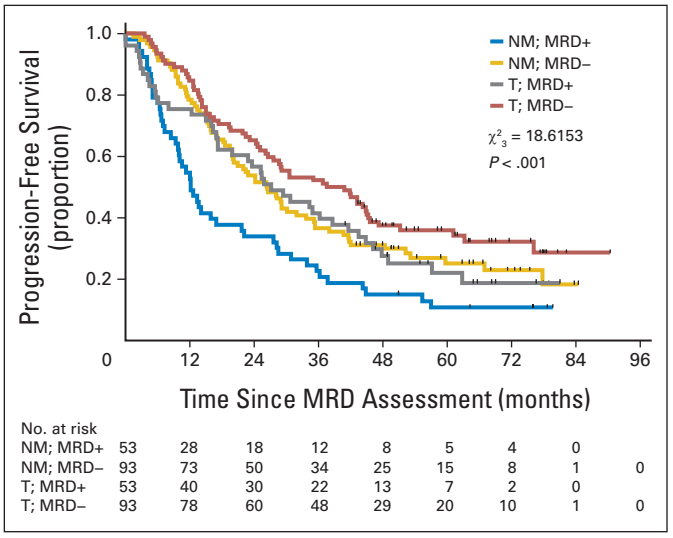

Fig 5. Progression-free survival (PFS) according to minimal residual disease (MRD) status after autologous stem-cell transplantation and maintenance random assignment. When effect of maintenance was assessed according to MRD status, improvement in PFS was seen in MRD-positive but not MRD-negative patients ($P = .036$ and $P = .1$, respectively). Similarly, when effect of MRD was assessed according to maintenance treatment, effect on PFS seemed greatest in those not receiving thalidomide (T; no maintenance, $P = .0038$; maintenance, $P = .1$). NM, no maintenance.

appropriate method for MRD assessment, because although its sensitivity is less than that of ASO-PCR at 10−4, it is applicable to a greater proportion of patients (approximately 97% in our study; data not shown), and it is rapid and inexpensive.[13,15,16]

In this study, we evaluated two induction regimens in the intensive pathway and found CTD to be superior, with 25% of patients becoming MRD negative, compared with 13% of those receiving CVAD ($P = .0039$). This superiority was maintained after ASCT, with 71% becoming MRD negative, compared with 54% ($P < .001$).

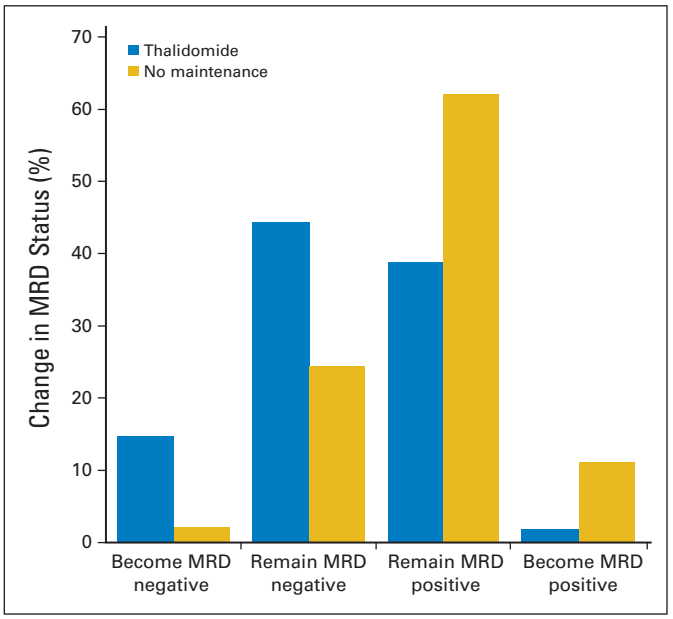

Fig 6. Change in minimal residual disease (MRD) status according to maintenance random assignment.

These results are broadly comparable to previous reports, in which we and others have shown that 42% to 58% of patients become MRD negative after ASCT.[23,25] These data argue strongly that ASCT remains a highly effective component of myeloma therapy. We demonstrated a 2.8-fold increase in MRD negativity after ASCT in CTD-treated patients and a 4.2-fold increase in CVAD-treated patients. Paiva et al[27] assessed MRD using MFC after a number of different induction regimens. The highest rates of MRD negativity were noted with bortezomib-containing regimens, but in all instances, a further improvement (minimum two-fold) was seen after ASCT.

In this study, presence of MRD after ASCT was associated with significantly inferior PFS and OS. These data are similar to those reported previously after conventional chemotherapy induction and ASCT.[23,25] When outcome was assessed in patients according to MRD status after induction as well as after ASCT, it was clear that PFS was best in those who achieved MRD negativity after induction (Fig 2C). These data are similar to those reported by Paiva et al,[25] although we did not demonstrate an OS advantage for those achieving MRD negativity after induction. However, these studies do suggest that the role of ASCT should only be questioned in patients who achieve MRD negativity after induction.

It is also clear from this study that MRD assessment is predictive of outcome in patients achieving IF-negative CR. We have demonstrated that 14.5% of patients achieving CR after ASCT had detectable MRD. We previously reported MRD in 27% of CR patients in a smaller study,[23] whereas Paiva et al[25] demonstrated MRD in 36% of CR patients treated with conventional chemotherapy and ASCT. In all three studies, presence of MRD in patients achieving CR predicted for inferior outcome. The outcome of those MRD-negative patients who did not achieve CR seemed to be identical to that of MRD-positive patients, suggesting that both MRD and conventional categorical response assessment should be used to define the highest quality categorical responses. Paiva et al[25] suggested an intermediate outcome after ASCT in this group, but in a more recent analysis of non-ASCT patients, they demonstrated that a favorable outcome was best defined by stringent CR and MRD negativity.[28] MRD-negative non-CR patients are likely to represent a heterogenous group. Some patients will ultimately achieve CR with follow-up, whereas in others, MRD assessment may represent a false-negative result as a consequence of patchy marrow disease.[37]

The prognostic significance of MRD after ASCT was seen in patients with adverse as well as favorable cytogenetic risk profiles. The combination of MRD and cytogenetics provided a particularly powerful prognostic model for both PFS and OS (Fig 3). Similarly, Paiva et al[38] recently showed that for patients achieving CR, presence of adverse cytogenetics and persistent MRD define a particularly poor-risk group and suggested that these patients be considered for experimental strategies.

Our study also provided useful insight into the effects of maintenance thalidomide after ASCT. It is interesting to note that thalidomide maintenance seemed to prolong PFS in MRD-positive patients only and that further tumor depletion was demonstrable in 28% of patients with detectable disease after ASCT. Similarly, the prognostic impact of MRD seemed most significant in those patients who did not receive maintenance. These data could have a potential impact on the

design of future maintenance studies. We would consider that sequential MRD assessments have a central role in the assessment of the efficacy of different maintenance/consolidation strategies within clinical trials.

The prognostic significance of CR on outcome in patients not undergoing transplantation has historically been less clear. However, with the introduction of novel induction regimens, it has become evident that CR attainment determines overall outcome.[8,9] In this study, we have demonstrated that MRD negativity is demonstrable in a minority of patients treated with less intensive schedules and that this is associated with a nonsignificant improvement in PFS. Paiva et al[28] assessed MRD in this patient group and demonstrated that 30% of a selected cohort treated with bortezomib containing regimens became MRD negative and that this predicted outcome. These data suggest that MRD assessment is likely to become more relevant in transplantation-ineligible patients as induction regimens improve.

We conclude that MRD assessment by MFC is a powerful prognostic tool in myeloma. It provides prognostic data complimentary to conventional categorical response assessment, can also provide insight into individual components of planned sequential therapy, and seems particularly suited to the monitoring and assessment of maintenance strategies after ASCT.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory Role:** Andy C. Rawstron, Celgene (C), BD Biosciences (C); Faith E. Davies, Celgene (C), Ortho Biotech (C), Novartis (C); Walter M. Gregory, Celgene (C); Gordon Cook, Celgene (C), Janssen-Cilag (C); Gareth J. Morgan, Celgene (C), Merck (C), Novartis (C), Johnson & Johnson (C) **Stock Ownership:** None **Honoraria:** Andy C. Rawstron, Genzyme, GlaxoSmithKline, Roche; J. Anthony Child, Science Agency and Network; Faith E. Davies, Ortho Biotech, Celgene; Gordon Cook, Celgene, Janssen-Cilag; Graham H. Jackson, Celgene, Janssen-Cilag; Gareth J. Morgan, Celgene, Merck, Novartis, Johnson & Johnson; Roger G. Owen, Celgene, Ortho Biotech **Research Funding:** Gordon Cook, Celgene **Expert Testimony:** None **Other Remuneration:** Andy C. Rawstron, BD Biosciences; J. Anthony Child, Science Agency and Network; Faith E. Davies, Ortho Biotech, Celgene; Walter M. Gregory, Celgene, Ortho Biotech; Sue E. Bell, Celgene, Ortho Biotech; Alexander J. Szubert, Celgene, Ortho Biotech; Gordon Cook, Celgene, Janssen-Cilag; Gareth J. Morgan, Celgene, Merck, Novartis, Johnson & Johnson; Roger G. Owen, Celgene, Ortho Biotech

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Final approval of manuscript: All authors

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Appendix

Induction Regimens

Intensive CTD. Oral cyclophosphamide 500 mg per week; oral thalidomide 100 mg per day, increasing to 200 mg per day if tolerated; and oral dexamethasone 40 mg per day on days 1 to 4 and 12 to 15 of 21-day cycle.

Intensive CVAD. Oral cyclophosphamide 500 mg per week; vincristine 0.4 mg per day and doxorubicin 9 mg/m^2 per day as continuous intravenous infusion for 4 days; and oral dexamethasone 40 mg per day on days 1 to 4 and 12 to 15 of 21-day cycle.

Nonintensive MP. Melphalan 7 mg/m^2 per day and prednisolone 40 mg per day, administered on days 1 to 4 of 28-day cycle.

Nonintensive CTDa. Cyclophosphamide 500 mg per week; thalidomide 50 mg for 4 weeks, increasing every 4 weeks in 50-mg increments to maximum of 200 mg per day; and dexamethasone 20 mg per day on days 1 to 4 and 15 to 18 of 28-day cycle.