The prognostic value of the depth of response in multiple myeloma depends on the time of assessment, risk status and molecular subtype

Complete remission (CR) rates for multiple myeloma (MM) have increased to 60% with current treatment approaches, including high dose melphalan-based autologous stem cell transplant (ASCT) and novel agents, and are associated with improved survival.[1-3] Despite this improvement, highly sensitive methods to detect minimal residual disease (MRD), including multiparameter flow-cytometry (MFC), allele-specific oligonucleotide polymerase chain reaction (ASO-PCR), and next generation sequencing (NGS) have shown that residual tumor cells persist in these states.[4,5] Achieving MRD negativity at a level of 10^{-5} to 10^{-6} has been associated with improved progression free (PFS) and overall survival (OS) suggesting that being negative at this level could constitute a therapeutic endpoint.[6-8]

Applying clinical tools for CR assessment, we have shown that there are profound differences in the time to maximum response and level of response dependent upon risk status and molecular subgroup, Figure 1A.[1,9] To understand the varying impact of the achievement of clinical CR and MRD negativity on patient outcome based on the time point at which they are assessed, their molecular subgroup and risk status, we analyzed patients enrolled into the total therapy (TT) protocols and assessed clinical CR and MRD level at different time points after ASCT, during maintenance, and in long-term survivors.

The TT3b-TT5a studies comprised 883 patients with newly diagnosed MM,[10,11] who were treated with protocols using induction therapy consisting of bortezomib, thalidomide and dexamethasone (VTD) in conjunction with chemotherapy (cisplatin, doxorubicin, cyclophosphamide, etoposide [PACE]) followed by intended tandem transplantation, consolidation therapy and 3 years' maintenance with bortezomib, lenalidomide and dexamethasone (VRD), *Online Supplementary Tables S1* and *S2*. The median follow-up time for the study population was 89 months. The Kaplan-Meier method was used to estimate the distributions of OS, PFS, and CR duration. Group comparisons for survival endpoints and cumulative incidence were performed using the log-rank test. Chi-square test or Fisher's exact test were used to compare MRD positive and negative. *P*-values <0.05 were considered statistically significant.

For gene expression profiling (GEP), plasma cells were enriched by CD138 immunomagnetic bead selection of bone marrow (BM) aspirates and analyzed by the Affymetrix U133Plus2.0 microarray platform (Santa Clara, CA, USA) using methods previously described.[12] Risk status was determined according to the GEP70 model and molecular subtypes were determined by the University of Arkansas for Medical Sciences (UAMS) classification [Hyperdiploidy (HY), Cyclin D1 (CD1), Cyclin D2 (CD2), MAF (MF), Low Bone (LB), Proliferation (PR), MMSET/FGFR3 (MS)].[12]

For MRD assessment by NGS, we used the Adaptive Biotechnologies NGS technology.[13] In brief, genomic DNA was amplified using locus-specific primer sets for immunoglobulin heavy-chain complete (IGH-VDJH) and incomplete (IGH-VDH) as well as for immunoglobulin κ locus (IGκ). The amplified products underwent sequencing and a clonal immunoglobulin gene rearrangement was identified when at least 2 identical sequencing reads were obtained. The frequency of each clonotype in a sample was determined by calculating the sequencing reads per clonotype divided by the total number of reads in the sample. MRD negativity was defined as the absence of clonal plasma cells seen in the BM aspirate to a sensitivity of 1 clonal plasma cell in 10^5 nucleated BM cells.

For the flow-cytometric assessment of response, BM samples were immuno-phenotyped using an 8-color technique [CD138 (V-500), CD38 (FITC), CD19 (PE-Cy7), CD45 (V-450), CD27 (PercpCy5.5), CD81 (APC-H-

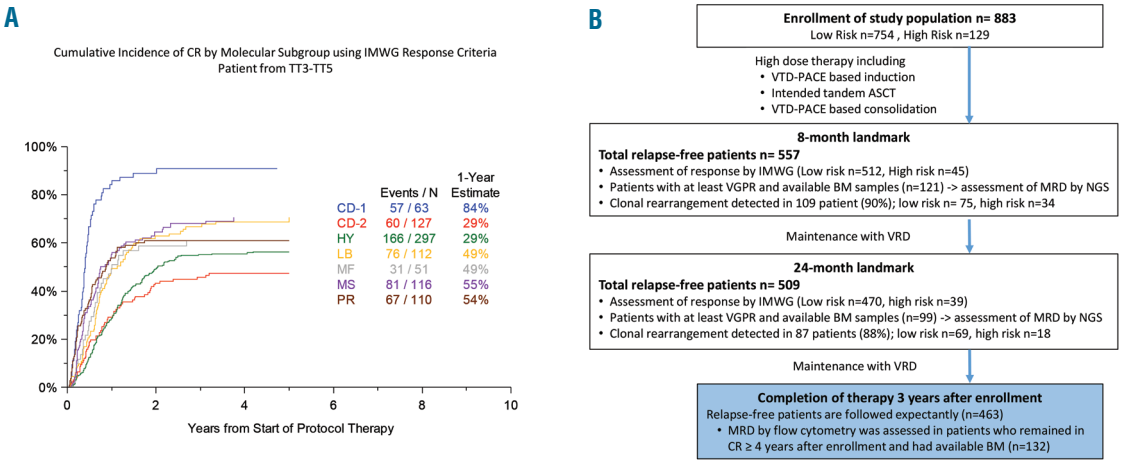

Figure 1. Cumulative incidence of CR by molecular subgroup and study population with time points of assessment. The curves show the cumulative incidence of CR by molecular subgroup [Hyperdiploidy (HY), Cyclin D1 (CD1), Cyclin D2 (CD2), MAF (MF), Low Bone (LB), Proliferation (PR), MMSET/FGFR3 (MS)] and depict different response dynamics by molecular subgroup with the CD2 subgroup having the lowest and slowest cumulative incidence of response, yet their outcome is very favorable, A). Study population and treatment schema [VTD: Velcade, thalidomide,dexamethasone; PACE: cisplatin, doxorubicin, cyclophosphamide, etoposide; SCT: stem cell transplantation with melphalan; VRD: Velcade, Revlimid, dexamethasone], B).

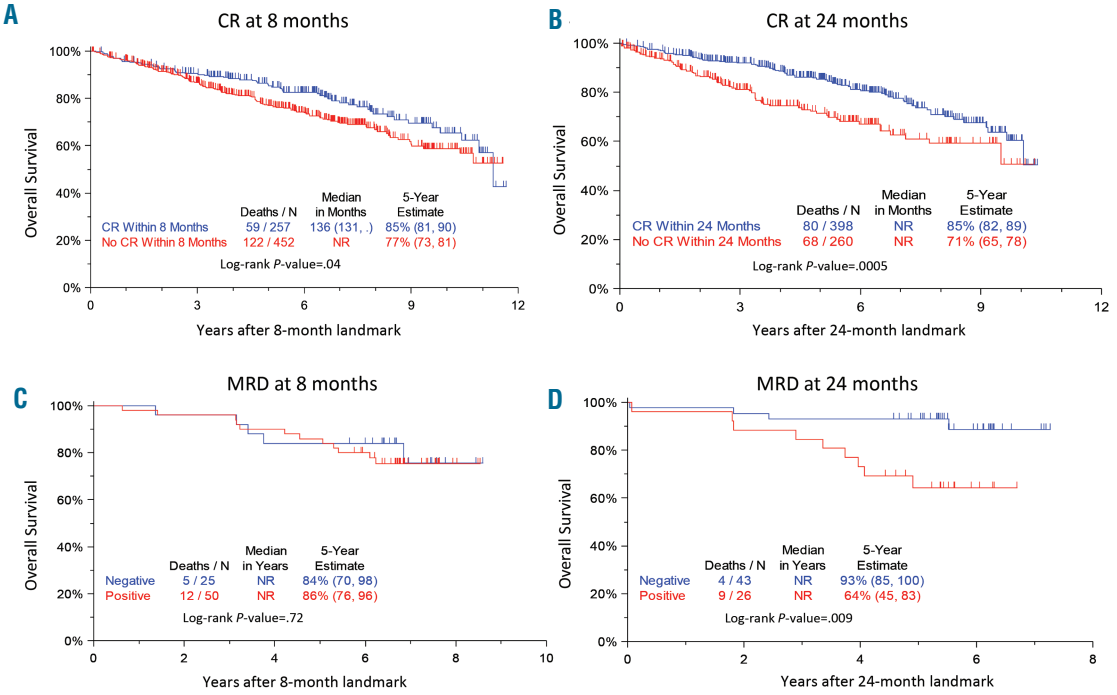

Figure 2. Overall survival from 8 and 24-month Landmarks by CR and MRD for GEP70 Low Risk patients. CR status at 8 months, A) and 24 months, B). Landmark analysis shows that optimum clinical benefit and most significant impact on OS is achieved only at the later time point (*P*=0.0005). Similarly, assessment of MRD status by NGS, C) post ASCT (at 8-month landmark), and D) during maintenance (24 months) depicts that MRD negativity had only prognostic value during maintenance (*P*=0.009).

7), CD56 (APC) and CD20 (PE)]. A minimum of 2 million cells were counted. MRD negativity by MFC was defined by the absence of phenotypically aberrant clonal plasma cells on BM aspirates with a minimum sensitivity of 1 in 10^5 nucleated cells.

The patient populations and the time points of assessment are shown, Figure 1B. Conventional response was assessed by the International Myeloma Working Group (IMWG) criteria at 8 months in 557 patients,[14] a time point at which patients had completed consolidation therapy. From these cases, 109 patients had achieved at least a very good partial response (VGPR), had a stored BM sample available for MRD analysis, and had a detectable clonal rearrangement by NGS. At the 24-month landmark, 509 patients remained relapse-free, and of these, 87 patients were in at least a VGPR and had a detectable clonal rearrangement to be assessed for MRD by NGS. Maintenance was completed at 3 years after enrollment, and 463 patients were observed expectantly following this time. From this population, 132 patients remained relapse-free ≥ 4 years after enrollment and underwent MRD assessment by 8 color flow cytometry.

A landmark analysis for GEP70 low risk (LR) patients achieving a clinical CR *versus* non-CR patients at 8 and 24 months showed that LR patients with clinical CR have significantly better PFS and OS at both time points compared to non-CR patients. The optimum clinical benefit for achieving clinical CR, in terms of improved PFS and OS, was seen at the 24-month landmark, where 85% of CR patients remained alive and 74% relapse-free 5 years after landmark compared to 71% of non CR patients still alive and 51% progression-free, Figure 2 and *Online Supplementary Figure S1*.

Looking specifically at the molecular response in LR patients defined by the GEP 70 who had achieved at least a VGPR at the 8-month timeline, MRD negativity was associated with a trend to a better PFS, with 80% of MRD negative patients remaining relapse-free at 5-year follow up compared to 72% of patients who did not achieve MRD negativity, *P*=0.19, *Online Supplementary Figure S1*. However, OS was not different between the groups, with approximately 85% of patients still alive after 5 years in both, Figure 2C. At the 24-month time point, the MRD negative patient group was associated with improved outcome, with 83% remaining relapse-free at the 5-year follow-up point compared to 57% in patients with detectable residual disease (*P*=0.04), *Online Supplementary Figure S1*. This survival benefit was also seen in the OS results at the 24-month landmark where MRD negativity was associated with a significantly better 5-year OS rate, with 93% of patients still alive compared to only 64% in patients who were positive (*P*=0.009), Figure 2D. Interestingly, in patients with the LR CD2 molecular subgroup who had achieved at least a VGPR, MRD status did not appear to significantly impact PFS and OS. CD2 patients that remained MRD positive at 8 or 24 months had similar PFS and OS compared to any other LR patients with MRD negativity, while patients of any other molecular subgroup that remained MRD positive after therapy had significant worse PFS and OS, *Online Supplementary Figure S2.*

Moving on to look at the impact of response in the GEP70 defined high risk (HR) patients, at either the 8 or 24-month landmark, there was no difference in PFS or

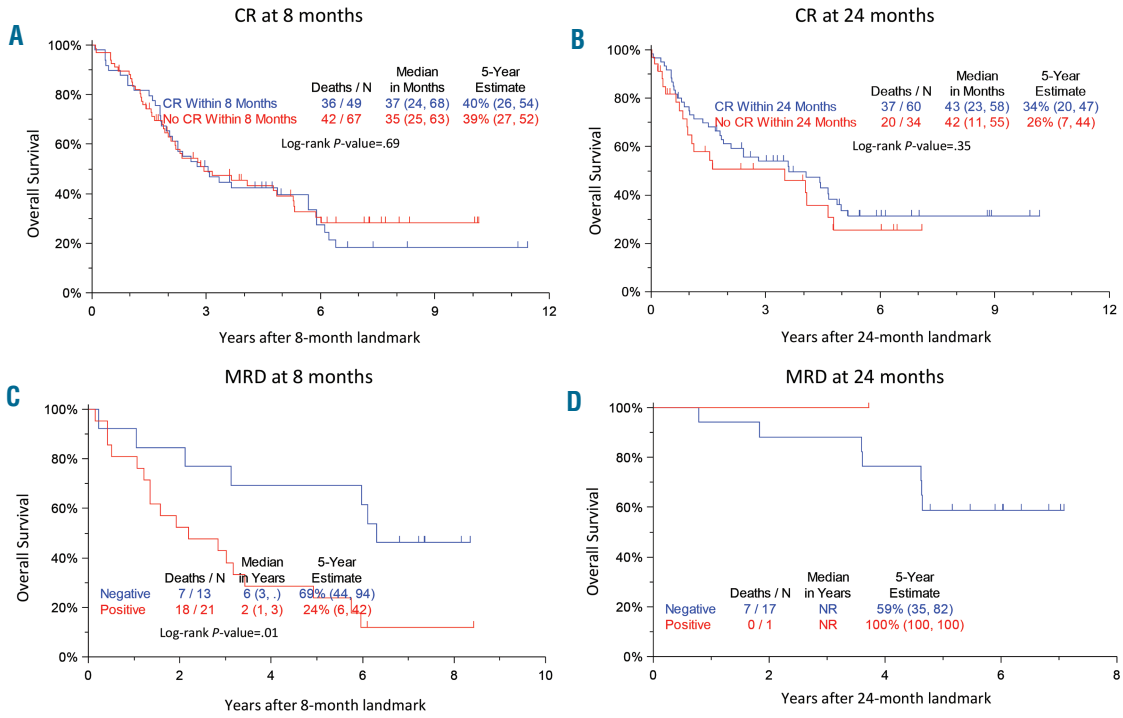

Figure 3. Overall survival of GEP70 High Risk patients according to CR and MRD status at different landmarks. CR status at 8 months, A) and 24 months, B). Landmark analysis shows no prognostic impact on OS at either time point. In contrast, MRD negativity, measured by NGS, post ASCT, C) is strongly associated with improved OS (*P*=0.01). D) Only 18 HR patients remained relapse-free at 24-month landmark and, of those, 17 were MRD negative.

OS depending on the achievement of clinical CR, Figure 3A and 3B. In contrast, MRD negativity in this population was highly predictive of a better outcome; at the 8-month landmark, MRD negative cases had a PFS of 42% and OS of 69% compared to patients with residual disease who had a dismal outcome with only 11% of patients progression-free and 24% alive after 5 years, Figure 3C and *Online Supplementary Figure S3*. The data further showed that HR patients with MRD negativity at a 2-year landmark continued to have a relatively high risk of relapse and death with only 41% patients remaining relapse-free and 59% patients alive at 5 years, Figure 3D and *Online Supplementary Figure S3*.

In an attempt to understand the MRD status of long-term survivors, we further measured MRD levels by flow cytometry in a group of 132 patients who were off therapy and progression-free between 4 and 7 years after enrollment. This analysis showed that the percentage of patients achieving MRD negativity at 10^{-5} increased each year, and that beyond 6 years follow up patients are overwhelmingly MRD negative (95%), *Online Supplementary Table S3*.

In conclusion, this study shows the importance of taking into account the risk group, molecular subtype and the time point at which response is assessed when interpreting the results of depth of response on clinical outcomes. Patients with LR MM enhance their response during maintenance therapy, and the prognostic implications of clinical CR and MRD negativity become most significant at the end of the treatment protocol. Interestingly, the CD2 molecular subgroup seems to be an exception to this observation as CD2 MRD positive patients had similar PFS an OS compared to patients with MRD negativity. In contrast, HR patients have significantly better outcomes when they achieve MRD negativity early at the 8-month time point. Importantly, even for HR patients who achieve MRD negativity at 8 or 24 months, they still have a very high risk of relapse, indicating that currently relying solely on molecular MRD methods to assess long-term outcome in HR populations is likely insufficient. Lastly, we show that MRD negativity in long-term survivors increases over time, and remains an important marker for most patients to be attained if long-term survival and cure is the treatment aim.

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