LETTER TO THE EDITOR

Circulating tumor cells as a biomarker for response to therapy in multiple myeloma patients treated within the GMMG-MM5 trial

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During the last 15 years, the outcome of patients with multiple myeloma (MM) has improved significantly as a result of therapy with novel drugs.1 Up to 75–90% of fit patients reach CR or very good partial response according to the IMWG criteria.2 Nevertheless, most of the patients suffer from relapse, indicating the presence of minimal residual disease (MRD).2 Indeed, highly sensitive methods for detection of MRD, such as multicolor flow cytometry (MFC), allele-specific oligonucleotide PCR (ASO-PCR) and next-generation sequencing (NGS)-based assays, enable detection of residual tumor cells even in patients achieving clinical CR.3–5 Presence of MRD in these patients is associated with a worse PFS and overall survival.2–5 Recently, the IMWG has acknowledged these results in the new consensus criteria for response assessment in MM, which now includes MRD diagnostics when patients have reached CR and MRD negativity as the deepest response.2 Along with the new consensus criteria, the IMWG pointed out that circulating tumor cells (CTCs) should be investigated for their value as a biomarker for response and prognosis since CTCs have been found in the PB of most patients at the time of diagnosis, and their level was identified as an independent prognostic factor.2

In this study, we performed a longitudinal quantitative analysis of CTCs and malignant plasma cells in the bone marrow (BM) in MM patients treated with novel agents and autologous stem cell transplantation (ASCT) using a highly sensitive ASO-PCR (≤10−6). We aimed to examine if CTCs could be used as a minimal invasive biomarker for response to therapy beyond MRD diagnostics that are usually performed when patients reach CR. Samples were collected from patients who were treated within the open-label, randomized, multicenter phase III clinical trial MM5 for newly diagnosed MM patients of the German-speaking Myeloma Multicenter Group (GMMG, EudraCT no. 2010-019173-16),6 and who reached CR or suspected CR until spring 2014 (Table 1; N = 41; 104 PB; 29 BM). BM samples were collected at diagnosis and at the time of CR or suspected CR (CR N = 18/29), and PB samples were collected at diagnosis and after the induction therapy (IT: PAd or VCD), ASCT and consolidation therapy (Cons.) (CR N = 33/104; Table 1). Additional 20 PB samples (at diagnoses and/or after IT, eight pairs) of 11 patients treated within the HOVON-65/GMMG-HD4 sample set set.

Table 1. GMMG-MM5 and HOVON-65/GMMG-HD4 sample set

| Patients | N Samples—therapy regime at time point |
|----------|---------------------------------------|
|          | N Patients | IT | ASCT | Cons. | Sum |
|          | Diagnosis | PAd | VCD | PAD | PAd | VCD | PAD | PAd | VCD | PAD | VCD |
| GMMG-MM5 BM | 23 | 0 | 0 | — | 2 | 3 | — | 8 | 8 | 4 | 4 | 29 |
| GMMG-MM5 PB | 41 | 11 | 15 | — | 11 | 10 | — | 13 | 16 | 15 | 13 | 104 |
| GMMG-HD4 PB | 11 | 10 | — | — | 10 | — | — | — | — | — | — | — |
| GMMG-MM5 BM/PB pairs | 18 | — | — | — | 2 | 3 | — | 5 | 8 | 2 | 4 | 24 |

| Patients | N Samples—therapy regime per time point |
|----------|---------------------------------------|
|          | N Patients | PR | VGPR | CR |
|          | Diagnosis | PAd | VCD | PAd | VCD | PAd | VCD | PAd | VCD |
| GMMG-MM5 BM | 23 | — | — | 2 | 1 | 3 | 5 | 9 | 9 | 29 |
| GMMG-MM5 PB | 41 | 11 | 15 | 6 | 9 | 16 | 14 | 17 | 16 | 104 |
| GMMG-MM5 BM/PB pairs | 18 | — | — | 2 | 1 | 2 | 5 | 5 | 9 | 24 |

Abbreviations: ASCT = autologous stem cell transplantation; BM = bone marrow; Cons. = consolidation therapy; HR = gain 1q21 more than three copies, deletion 17p13 and t(4;14); ISS = International Staging System; IT = induction therapy; PAd = bortezomib/doxorubicin/reduced dose dexamethasone (240 mL per cycle); PAD = bortezomib/doxorubicin/dexamethasone (480 mg per cycle); SR = low risk (all others); VCD = bortezomib/cyclophosphamide/dexamethasone; VGPR = very good partial response.

| Patients | N Patients—clinical parameter at the time of diagnosis |
|----------|-------------------------------------------------------|
|          | N       | PAd | VCD | PAD | HR | SR | ISS I | ISS II | ISS III |
| GMMG-MM5 PB | 41 | 20 | 21 | — | 16 | 25 | 16 | 15 | 10 |
| GMMG-HD4 PB | 11 | — | — | 11 | 5 | 3 | 4 | 2 |
Figure 1. (a) GMMG-MM5—correlation between tumor load in PB and therapy cycle; N = 106. (b) GMMG-MM5—correlation between tumor load in PB and response to therapy. (c) GMMG-MM5 and GMMG-HD4—tumor load in PB at diagnosis and after different bortezomib-based induction therapy regimes. PAD = bortezomib/doxorubicin/reduced dose dexamethasone (240 mL per cycle); VCD = bortezomib/cyclophosphamide/dexamethasone; PAD = bortezomib/doxorubicin/dexamethasone (480 mg per cycle). (d) GMMG-MM5—tumor load in BM and PB at the time point at which the patients had reached CR (after IT, after ASCT and after Cons.). (e) GMMG-MM5—correlation between tumor load in PB and therapy cycle, stratified for the presence or absence of high-risk cytogenetics (HR = amp(1q) more than three copies, deletion 17p13, t(4;14) and t(14;16); SR = low risk (all others)); HR = 1; SR = 0. (f) GMMG-MM5—correlation between tumor load in PB and therapy cycle, stratified for ISS Stage. Ordinary boxplots ignoring censoring. (g) GMMG-MM5—correlation between tumor load in BM and PB if PB is positive; N = 14 pairs. (h) Tumor load in BM and PB if PB is positive; N = 14 pairs.
MRD-negative results (MRD $= 2.98 \times 10^{-5}$) study wide weakest sensitivity of $9.08 \times 10^{-6}$ were de
NADA for left-censored data (Kendall material was available to reach a sensitivity for MRD
contained the number of tested cells as individual censoring value for each
was reduced signi
diadagnosis and after ASCT (99.6% reduction; mean $= 7.1 \times 10^{-5}$; Figures 1a and
b; Supplementary Table 1). Of note, in 8/19 patients in CR (42%), we detected CTCs (Figure 1d).
Stratifying the data for risk according to cytogenetics, we found a significantly higher number of CTCs at the time of diagnosis in HR patients than in SR patients (median: $1.6 \times 10^{-3}$ vs $1.1 \times 10^{-4}$, $P = 0.005$; Figure 1e). After IT, the number of CTCs was significantly reduced in HR patients (99.8% reduction) and SR patients (89% reduction) (HR median IT: $1.1 \times 10^{-5}$, S$R$ median IT: $5.35 \times 10^{-6}$, $P = 0.04$), and no significant difference after IT could be detected between the two risk groups ($P = 0.95$) (Figure 1e; Supplementary Table 1).
Between the different ISS stages, no significant differences in the number of CTCs at the time of diagnosis and after ASCT were detected (Figure 1f; Supplementary Table 1). However, while patients with ISS I and II already showed a significant reduction of CTCs from diagnosis to IT (ISS I 88.9% reduction, $P = 0.01$; and ISS II 99.5% reduction, $P = 0.004$, respectively) in ISS III patients, CTCs were only reduced by ASCT (99.97% reduction, $P = 0.005$) (Figure 1f; Supplementary Table 1).
Comparing the tumor load in BM and PB, we found that in only 3/24 pairs, were both entities MRD$^-$ (12.5%; median sensitivity: BM $= 8.95 \times 10^{-7}$, PB $= 9.36 \times 10^{-7}$). In 16/24 pairs, BM was MRD$^+$, while PB was MRD$^-$ (66.6%; median tumor load BM$^+$ $= 1.56 \times 10^{-5}$; median sensitivity PB$^-$ $= 6.36 \times 10^{-6}$). In only 5/24 pairs, was PB MRD$^+$, but most interestingly, all but one corresponding BM sample was MRD$^+$. Adding an additional eight BM/PB pairs collected after stem cell mobilization or during maintenance therapy, we could confirm that as long as PB is MRD$^-$, BM is also MRD$^+$ (N = 14 PB$^-$ pairs; Figure 1h; median BM$^-$ $= 6.3 \times 10^{-5}$; median PB$^-$ $= 6.9 \times 10^{-5}$; Supplementary Table 1). Further analysis showed a strong correlation between tumor load in PB and BM if the paired PB sample was MRD$^+$ (tau = 0.604; $P = 0.0031$; Figure 1g). In the only PB$^+$/BM$^-$ case, tumor load in PB was $7.75 \times 10^{-4}$ and sensitivity of the BM measurement was $9.13 \times 10^{-7}$.
Taken together, our analysis showed both a significant correlation with the number of tumor cells in BM if PB was MRD$^+$ and a significant correlation of the number of CTCs with response to therapy. Accordingly, CTCs could as such be a promising minimal invasive biomarker for the general activity of the disease in the BM.
In comparison to other recently published studies about MRD diagnostics in BM at CR, our rates of MRD$^+$ patients are low (14.3%).
This might be due to the fact that our MRD assay reaches a sensitivity that is even below $10^{-6}$. When applying the so far best published sensitivity thresholds for MFC ($10^{-5}$) and NGS ($10^{-6}$) to our data for BM samples at CR, the numbers are well in line with published proportions of MRD$^-$ patients with 42–68% MRD$^-$ by MFC and 19–35% by NGS.\(^7\)\(^-\)\(^9\) Nevertheless, by increased sensitivity, we were able to identify 43% more BM MRD$^-$ patients and 12% more PB MRD$^-$ patients at CR compared to MFC. This highlights the fact that sensitivity is essential for MRD diagnostics in BM as well as for the analysis of CTCs. We conclude that CTCs could serve as a surrogate for BM evaluations until PB is MRD$^-$, but cannot stand alone for MRD detection. Larger studies of CTCs in MM patients and the analysis of their effect on PFS and overall survival are needed to confirm and evaluate our findings. Future developments in improvement of MRD assay sensitivity and applicability, potential automation, high-throughput applications and cost reduction will determine which assay serves best for the clinical application of MRD diagnostics and CTC evaluation.\(^1\)\(^4\)\(^,\)\(^1\(^5\)

**CONFLICT OF INTEREST**

SH, NW, JN, MP, TH, MHu, UB, BH-D, MHa, JD, MG, HK, UG, MHo, PR, AJ, NP and KD declare no conflict of interest. MV and RA are employees of Janssen and hold stock in Johnson & Johnson; HJS—Celgene: honoraria, travel grants and Amgen: honoraria; KW—Honoraria and Advisory Board of Amgen, BMS, Celgene, Janssen, Novartis, Takeda; FL—advisory role: BioNtech, Bristol-Myers-Squibb, Eli Lilly, GANYMED Pharmaceuticals, Merck Sharp & Dohme, Roche Pharma AG. Lecture honoraria: Amgen, Astra Zeneca, Eli Lilly, Merck Sharp & Dohme, Roche Pharma AG, Servier. Research grant: Boehringer Ingelheim, Fresenius Biotech. Travel grants: Amgen, Bayer, Merck Sharp & Dohme, Roche Pharma AG, Taiho Pharmaceutical; IWB—scientific grants Jabsen-Clag and Celgene. TM is an employee of inVentiv Health; PW—Honoraria and membership on Advisory Boards of Sanofi-Aventis. Membership on Advisory Boards and Travel Grants from Hexal AG; HG—research support (institutions): Celgene, Janssen, Chugai, Novartis, BMS; Advisory Boards (institutions): Janssen, Celgene, Novartis, Amgen Takeda, BMS; Honoraria: Celgene, Janssen, Novartis, Chugai, BMS.

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**AUTHOR CONTRIBUTIONS**

Conceived and designed the study: SH, NW and HG; Performed the experiments and analyzed the data: SH, NW, JW, JW, JW, MP, TH and MHu; Acquired study material and data: SH, JW, JW, JW, JW, MP, UB, BH-D, MHa, JW, JW, JW, JW, JW, JW, JW, MG, HK, UG, FL, MHo, PR, IWB, AJ, TM, PW and HG; Interpreted the results: SH and NW; Drafted the manuscript: SH and NW; Revised the manuscript: SH, JW, JW, MP, TH, MHu, UB, BH-D, MV, RA, MHa, JW, JW, JW, JW, JW, MG, HK, UG, FL, MHo, PR, IWB, ADH, AJ, KD, TM, PW and HG; Approved the final version: SH, JW, JW, MP, TH, MHu, UB, BH-D, MV, RA, MHa, JW, JW, JW, JW, MG, HK, UG, FL, MHo, PR, IWB, ADH, AJ, KD, TM, PW and HG.

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