

## Post-transplant events

# Extramedullary vs medullary relapse after autologous or allogeneic hematopoietic stem cell transplantation (HSCT) in multiple myeloma (MM) and its correlation to clinical outcome

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### Summary:

**Risk-adapted treatment of multiple myeloma (MM) includes autologous (auto-) and allogeneic (allo-) hematopoietic stem cell transplantation (HSCT). Case reports on extramedullary (EM) compared to bone marrow (BM) relapse after HSCT suggest a dismal prognosis. We compared the outcome of 78 MM patients relapsing after auto- (group A:  $n = 53$ ) or allo- (group B:  $n = 25$ ) HSCT, stratified into BM (64 patients) vs EM (14 patients) relapse. The relapse-specific groups were also compared with respect to risk factors, including age,  $\beta 2$ -microglobulin, pretreatment, cytogenetics and stage. EM relapse sites were lungs (5), soft tissue (4), pericardium (2), bone (1), skin (1) and CNS (1). As of May 2004, the overall (OS) and progression-free (PFS) survival after HSCT in patients relapsing from EM sites was not significantly different from BM relapse patients, both after auto- and allo-HSCT. Although MM patients relapsing from EM sites after allo-HSCT used to be regarded as having few therapeutic options, we observed encouraging responses to donor lymphocyte infusions (DLI). Treatment responses to DLIs were observed in 5/9 (56%) BM relapse patients, and in 3/4 (75%) EM relapse patients. These observations suggest that EM relapse after HSCT is common and needs an individualized diagnostic and therapeutic approach in MM during clinical follow-up after HSCT.** *Bone Marrow Transplantation* (2004) **34**, 1057–1065. doi:10.1038/sj.bmt.1704713

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(HSCT) is widely pursued in multiple myeloma (MM) patients, which has shown to induce long periods of disease control and to improve long-term survival.<sup>1–5</sup> However, despite treatment intensification, MM relapse is still a major concern after auto- and allo-HSCT.<sup>3,6</sup> Relapse after HSCT seems to result from malignant plasma cells, which may have acquired a remarkable ability to survive various conditioning regimens. The transformation from primarily medullary (BM) to secondary extramedullary (EM) MM after HSCT raises the question of whether transplantation can induce selection of plasma-cell clones with an altered biology. Preliminary reports provide evidence supporting this hypothesis.<sup>7–9</sup>

Patients responding badly or relapsing after auto-HSCT may profit from a second auto-HSCT,<sup>2</sup> an allo-HSCT<sup>3</sup> or novel therapeutics, such as thalidomide, bortezomib and combinations of those with standard anti-MM drugs.<sup>10</sup> Relapse after allo-HSCT can be successfully treated with cell- or cytokine-mediated immunotherapy, including donor lymphocyte infusions (DLI), or novel agents.<sup>11–13</sup>

Location of relapse after HSCT can occur in BM, EM sites or both, and to date few studies have analyzed patients' outcome with stratification according to these distinct relapse patterns. Therefore, we analyzed the progression-free (PFS) and overall (OS) survival of MM patients having undergone auto- or allo-HSCT, with respect to BM vs EM relapse sites.

### Patients and methods

#### Patients

Consecutive MM patients treated in the Department of Hematology and Oncology of the Freiburg University and receiving an auto- or allo-HSCT between January 1992 and May 31, 2004, were included in this study. Patients were treated after informed consent from both patients and donors, according to the guidelines of the Declaration of Helsinki and good clinical practice. The relapse rates were 53/84 (63%) after auto-HSCT, while 25/37 (68%) patients relapsed after allo-HSCT. The median time of follow-up of the survivors was 42 months after auto- and 28 months after allo-HSCT, respectively. The median time to progression was 21 and 13 months after auto- and allo-transplant,

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High-dose chemotherapy with reinfusion of either autologous (auto)- or allogeneic (allo)-hematopoietic stem cells

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respectively. The higher relapse rate after allo-HSCT correlated with the more unfavorable risk factors of allo-patients, such as advanced age, more intensive prior treatment and more advanced disease prior to allo-HSCT (SD/PD in 53% (auto-patients) vs SD/PD in 80% (allo-patients)), as detailed in Tables 1 and 2.

The clinical course of MM patients relapsing after auto- (group A:  $n=53$ ) or allo- (group B:  $n=25$ ) HSCT was stratified in those with BM vs those with EM relapse: in group A, BM relapse was observed in 47 patients (A1) vs EM relapse in six patients (A2). EM relapse was defined as at least one extramedullary manifestation of MM. The

**Table 1** Group A: Characteristics of patients relapsing after auto-HSCT ( $n=53$ )

Relapse	A1: Medullary (BM)		A2: Extramedullary (EM)	
	<i>n</i>	%	<i>n</i>	%
Total patients	47	100	6	100
Sex				
M	28	60	4	67
F	19	40	2	33
Age (years) (median/range)	52 (37–63)		53 (45–59)	
Myeloma type				
IgG	23	48	2	33
IgA	13	28	2	33
kappa	4	9	0	0
lambda	6	13	1	17
Nonsecretory	1	2	1	17
Stage at transplantation <sup>a</sup>				
I	1	2	0	0
II	1	2	0	0
III	45	96	6	100
Cytogenetics				
del 13 q positive	10	21	1	17
del 13 q negative	17	36	2	33
Not evaluated	20	43	3	67
$\beta$ 2-MG at presentation				
> UNL <sup>b</sup>	17	36	2	33
$\leq$ UNL	25	53	4	67
Not evaluated	5	11	0	0
LDH levels at presentation				
> UNL <sup>b</sup>	27	5	4	67
$\leq$ UNL	18	38	2	33
Not evaluated	2	4	0	0
No. of treatments prior transplantation (median/range)	2.2 (1–5)		2.6 (2–6)	
Time from diagnosis to transplantation in months (median/range)	19.8 (2.9–65.2)		14.7 (3.0–57.1)	
Staging at transplantation				
PD	5	11	1	17
SD	20	42	2	33
PR	15	32	2	33
CR	7	15	1	17
Transplant conditioning				
TBI/Melphalan or Melphalan	46	98	5	83
BEAM	1	2	0	0
Busulfan/Cyclophosphamid	0	0	1	17
Graft				
Unselected PBSC <sup>c</sup>	21	45	5	83
CD34 <sup>+</sup> selected PBSC	26	55	1	17
Best response after auto-HSCT				
SD	11	23	1	17
PR	17	36	2	33
CR	19	40	3	50

**Table 1** Continued

Relapse	A1: Medullary (BM)		A2: Extramedullary (EM)	
	n	%	n	%
<i>Site of relapse</i>				
Lungs			2	33
Soft tissue			2	33
Pericardial			1	17
Bone			1	17
<i>Treatment after relapse</i>				
Allogeneic HSCT	19	40	4	67
Thalidomide	10	21	0	0
Radiation	9	19	2	33
Dexamethasone	5	11	0	0
Best supportive care	4	9	0	0
<i>Response to relapse treatment</i>				
Responders <sup>d</sup>	28/43	60	4/6	67

<sup>a</sup>Stage according to classification of Durie and Salmon.

<sup>b</sup>UNL: upper normal limit.

<sup>c</sup>PBSC: peripheral blood stem cells.

<sup>d</sup>Response was defined as stable disease, partial remission or complete remission. Only patients treated after relapse were evaluated (43 patients with BM relapse and 6 patients with EM relapse).

**Table 2** Group B: Characteristics of patients relapsing after allo-HSCT (n = 25)

Relapse	B1: Medullary (BM)		B2: Extramedullary (EM)	
	n	%	n	%
Total patients	17	100	8	100
<i>Sex</i>				
M	10	59	6	67
F	7	41	2	33
Age (years) (median/range)	52 (44–61)		54 (43–63)	
<i>Myeloma type</i>				
IgG	8	47	4	40
IgA	6	35	3	38
kappa	1	6	0	0
lambda	2	12	1	12
<i>Stage at transplantation<sup>a</sup></i>				
II	4	24	2	25
III	13	76	6	75
<i>Cytogenetics</i>				
del 13 q positive	6	35	2	25
del 13 q negative	6	35	3	37
Not evaluated	5	29	3	37
<i>β2-MG at presentation</i>				
> UNL <sup>b</sup>	9	53	3	37
≤ UNL	5	29	3	37
Not evaluated	3	18	2	25
<i>LDH levels at presentation</i>				
> UNL <sup>b</sup>	8	47	2	33
≤ UNL	6	35	4	67
Not evaluated	3	18	0	0
No. of treatments prior transplantation (median/range)	3.4 (2–7)		3.4 (2–8)	
Prior auto-HSCT	13	76	6	75

**Table 2** Continued

Relapse	B1: Medullary (BM)		B2: Extramedullary (EM)	
	n	%	n	%
Time from diagnosis to transplantation in months (median/range)	29.4 (4.8–79.2)		26.3 (3.9–100.6)	
<i>Staging at transplantation</i>				
PD	6	35	2	25
SD	8	47	4	50
PR	3	18	2	25
<i>Transplant conditioning</i>				
Fludarabin/BCNU/Melphalan	9	53	3	38
Busulfan/Cyclophosphamid	5	29	3	38
TBI/Cyclophosphamid	2	12	1	12
Fludarabin/Thiotepa	1	6	0	0
Fludarabin/Melphalan	0	0	1	12
<i>Graft</i>				
Unselected PBSC <sup>c</sup>	10	59	5	63
CD34 <sup>+</sup> selected PBSC	5	29	2	25
Bone marrow	2	12	1	12
<i>Best response</i>				
SD	4	24	2	25
PR	8	47	2	25
CR	5	29	4	50
<i>Site of relapse</i>				
Lungs			3	38
Soft tissue			2	25
Pericardial			1	12
Skin			1	12
CNS			1	12
<i>Treatment after relapse</i>				
DLI <sup>d</sup>	5	29	3	38
DLI plus Rx or Cx	4	24	1	12
Chemotherapy	4	24	2	25
Best supportive care	4	24	2	25
<i>Response to relapse treatment<sup>e</sup></i>				
Responders	7/13	54	3/6	50
DLI responders	5/9	56	3/4	75
<i>Graft-versus-host disease</i>				
Acute GvHD ( $\geq$ I <sup>o</sup> )	7	41	4	50
Chronic GvHD ( $\leq$ II <sup>o</sup> )	5	29	3	38

<sup>a</sup>Stage according to classification of Durie and Salmon.

<sup>b</sup>UNL: upper normal limit.

<sup>c</sup>PBSC: peripheral blood stem cells.

<sup>d</sup>DLI: donor lymphocyte infusions.

<sup>e</sup>Response was defined as stable disease, partial remission or complete remission. Only patients treated after relapse were evaluated (13 patients with BM relapse and six patients with EM relapse).

number of patients with both EM and BM involvement was 2/53 in group A and 3/25 in group B. In both groups, most patients had stage III disease at transplantation (A1: 96%, A2: 100%; Table 1). In group B, BM relapse was observed in 17 patients (B1) vs EM relapse in eight patients (B2) (Table 2). The median time between diagnosis and transplantation was 19.8 months in group A1 and 14.7 months in group A2 as compared to 29.4 in group B1 and 26.3 months in group B2, again demonstrating the later performance of allo- as compared to auto-HSCT. As summarized in Tables 1 and 2, adverse prognostic factors,

including disease stage at transplantation, cytogenetics (del 13q),  $\beta$ 2-microglobulin ( $\beta$ 2-MG) serum levels, number of pretreatments and response status prior HSCT were comparable for both groups. There were no patients with plasma-cell leukemia receiving transplantation in the study period.

In order to detect patient- or transplant-related factors which could predicted EM relapse, we investigated the following criteria: gender, age, myeloma type, number of pretreatments, preparative regimens, CD34 selection of the graft, cytogenetics and  $\beta$ 2 MG.

### Staging procedures

The standard MM staging procedures included physical examination, ECOG-performance status, routine blood analysis with serum electrophoresis, Bence Jones proteinuria, chest X-rays, abdominal ultrasound and BM smear and biopsy. Partial remission (PR) was defined as at least 50% or more tumor reduction, as measured by decrease in myeloma proteins in serum and/or urine measured at least twice.<sup>14,15</sup> In responding patients, BM evaluation had to be in agreement with myeloma protein measurement and had to be accompanied by improvement of clinical symptoms. A complete remission (CR) was defined as the complete disappearance of myeloma proteins and absence of BM monoclonal plasma cells.<sup>14,15</sup> Relapse was defined as increase in 25% of myeloma protein levels, measured at least twice or as >10% plasma-cell infiltration of the BM.<sup>15</sup> Response and relapse were defined using immunofixation. In order to exclude progressive osteolytic lesions in patients with solitary EM relapse, skeletal magnetic resonance imaging (MRI) scans were performed.

### Treatment schedule and HSCT

Stem cell mobilization therapy consisted of either VI (VP16 500 mg/m<sup>2</sup>, Ifosfamid 4000 mg/m<sup>2</sup>) or IEV (Epirubicin 100 mg/m<sup>2</sup>, VP16 150 mg/m<sup>2</sup>, Ifosfamid 2500 mg/m<sup>2</sup>) chemotherapy, followed by RhG-CSF (Filgrastim, Neupogen®; 5 µg/kg body weight (b.w.)) as reported previously.<sup>16,17</sup> Stem cells were collected by leukapheresis and cryopreserved with a targeted minimum cell dose of  $\geq 2 \times 10^6$  CD34<sup>+</sup> cells/kg b.w. According to a collaborative multicenter study (internal study protocol #277), a CD34<sup>+</sup> positive selection (Clinimacs®) was performed in 27/53 auto-patients as described.<sup>18,19</sup> In seven allo-patients, CD34<sup>+</sup> selection was applied for single major HLA mismatches.<sup>19</sup> In three allo-patients, unmanipulated BM was used.

Conditioning regimens for groups A1 and A2 included TBI/melphalan or melphalan in most patients.<sup>20</sup> Busulfan/cyclophosphamide (BU/CY) or BEAM were applied in one patient of each group (Table 1), employing previously described protocols.<sup>20,21</sup> Conditioning regimens for groups B1 and B2 included fludarabin/BCNU/melphalan (FBM) in nine and three patients, respectively, BU/CY in five and three patients, respectively, TBI/cyclophosphamide in two and one patients, respectively, fludarabin/melphalan (Flu/Mel) in one patient (B2) and fludarabin/thiothepa (FluTT) in one patient both (B1), also according to previous protocols (Table 2).<sup>22–24</sup>

Restaging was performed on days +30, +100, every 3 months post HSCT and any time of clinically suspected relapse. GvHD prophylaxis for patients undergoing allo-HSCT consisted of antithymocyte-globuline ATG-S® in matched unrelated donors (Fresenius, Graefeling; 20 mg/kg/day for 2 days), cyclosporine A (CsA) and/or mycophenolatmofetil (2 g/day) as described for the individual protocols.<sup>22–24</sup>

### Statistical analysis

Analyses were performed to estimate OS and PFS employing the Kaplan–Meier method.<sup>25</sup> Survival was calculated

until patients' death (OS), or disease progression or patients' death (PFS). To compare the survival distributions with respect to the relapse pattern, the log-rank test was applied at a 95% confidence interval (CI), resulting in a *P*-value and a standard error.<sup>26</sup> To screen for significant correlation to the relapse pattern, a  $\chi^2$  analysis was applied.

All statistical analysis was performed using SPSS version 11.0® and GraphPad Prism3® soft ware. Statistical significance was defined as *P* < 0.05.

## Results

### Comparability of the subgroups

Between January 1992 and May 31, 2004, 84 MM patients underwent auto-HSCT, and 37 allo-HSCT at our institution. Relapse or progression eventually occurred in 78 patients, who had undergone auto- (53 relapse patients) or allo-HSCT (25 relapse patients). Tables 1 and 2 summarize the remission prior to transplantation and the best response after HSCT. Patients with BM relapse (group A1) after auto-HSCT were on average 1 year younger than those with EM relapse (group A2). Apart from that, adverse prognostic factors were comparable for both subgroups (A1 vs A2; Table 1). Response rates (CR/PR) before, and after auto-HSCT, were also similar in both subgroups (A1 vs A2: 47 vs 50% before HSCT, and 85 vs 83% after HSCT). Patients relapsing after allo-HSCT were with a median of 2 years older in group B2 than in group B1. The median  $\beta$ 2-MG (mg/l) was 3.1 in B1 vs 4.4 in B2, indicating an additional unfavorable prognostic factor for the latter group. Since cytogenetics were in former times less commonly performed worldwide, these were not for all patients available. Cytogenetics were available in 30/57 (53%) auto-patients and in 17/25 (68%) allo-patients. Del 13q- was detected in 17% of all EM relapse patients and in 21% of all BM relapse patients after auto-HSCT (Table 1). In patients relapsing after allo-HSCT, 13q- tended to be more frequent in EM as compared to BM relapse (35 vs 25%).

The overall distribution of MM stage, cytogenetics, LDH levels, number of pretreatments and staging at transplantation were not significantly different in both subgroups (Tables 1 and 2). While patients relapsing from BM sites had in 55% received CD34 selected PBSC, patients with an EM relapse had only in 17% received a CD34 selected graft (Table 1).  $\chi^2$  analysis indicated that an EM relapse is more common in auto-patients receiving unselected stem cell transplants. This constellation was not observed in the allo-group.

### OS with respect to the site of relapse

As of May 31, 2004, 37/53 (70%) patients relapsing after auto-HSCT and 18/25 (72%) patients relapsing after allo-HSCT have died. The number of deaths after auto-HSCT was 6/6 in the EM group and 31/47 in the BM group. The number of deaths after allo-HSCT was 6/8 in the EM group and 12/17 in the BM group.

The median duration of follow-up among survivors in the auto-HSCT group (A) was 39 months (range 10–77). The overall median survival was 35 months (95% CI 29–41) in A1 and 38 months (95% CI 31–41) in A2 (NS by the log-rank test) (Figure 1).

The median duration of follow-up among survivors in the allo-HSCT group (B) was 25 months (range 6–48). The overall median survival was 16.8 months (95% CI 14–20) in B1 and 17.3 months (95% CI 13–21) in B2 (NS by the log-rank test) (Figure 2).

In addition, the OS calculated from the point of relapse or progression after auto-HSCT and allo-HSCT was not significantly different in the BM group as compared to the EM group.

#### PFS with respect to the site of relapse

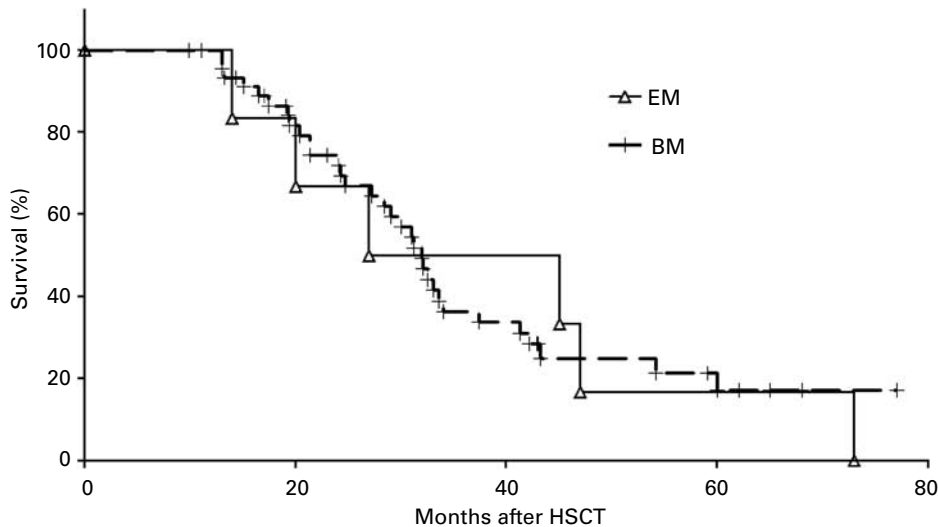
As of May 31, 2004, 46/53 (87%) patients progressed after auto-HSCT and 18/25 (72%) patients progressed after

allo-HSCT. The median PFS was 31 months (95% CI 27–39) in A1 and 34 months (95% CI 29–40) in A2 (NS by the log-rank test). The PFS was 15 months (95% CI 13–18) in B1 and 16 months (95% CI 12–20) in B2 (NS by the log-rank test).

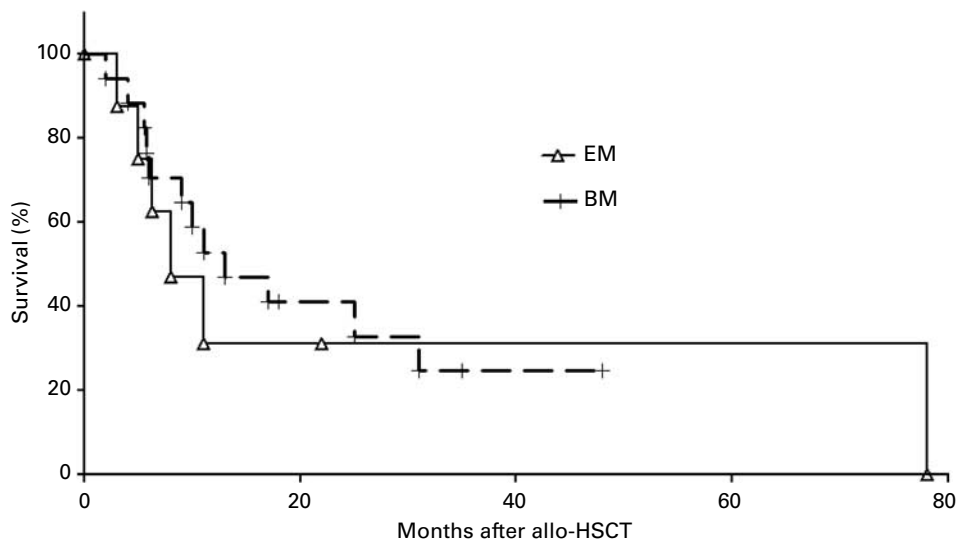
The PFS calculated from the point of response to salvage treatments was not significantly different in the BM group as compared to the EM group.

#### Sites of extramedullary relapse

The extramedullary relapse sites included lungs (2), soft tissue (2), bone (1) and pericardium (1) after auto-HSCT, and lungs (3), soft tissue (2), skin (1), central nervous system (1) and pericardium (1) after allo-HSCT (Tables 1 and 2). The serum monoclonal component (MC) in these patients was extremely low or absent. Relapse was diagnosed with imaging techniques including MRI or computed tomography (CT scan) in 5/6 (83%) after auto-



**Figure 1** OS of relapse patients after auto-HSCT.



**Figure 2** OS of relapse patients after allo-HSCT.

and 6/8 (75%) after allo-HSCT. The extramedullary relapse was diagnosed by biopsy in all EM patients and confirmed by autopsy in 7/7 EM relapse patients.

### Relapse treatment

Therapeutic approaches for relapse after auto-HSCT included a second auto- or allo-HSCT, thalidomide, radiation, standard chemotherapy (melphalan/Prednisolone according to Alexanian and high-dose dexamethasone)<sup>27,28</sup> or best supportive care (Table 1). After allo-HSCT, DLIs with or without radio- or standard chemotherapy, chemotherapy alone or best supportive care was given (Table 2). An antimyeloma response to secondary treatment, defined as CR, PR or at least SD, was achieved in 64% in group A and 52% in group B (Tables 1 and 2). In our patients, 10 with relapse after auto-HSCT were treated with thalidomide and 3/10 (30%) responded. The overall response to treatment after relapse was comparable for BM as compared to EM relapse patients after auto-HSCT (60 vs 67%) and allo-HSCT (54 vs 50%). Interestingly, in MM patients relapsing after allo-HSCT, DLIs used in 9/17 (53%) patients in group B1 and 4/8 (50%) patients in B2 induced temporary responses in 5/9 (56%) and 3/4 (75%), respectively (Table 2).

The three patients with EM relapse responding to DLI had involvement of the EM sites: skin and soft tissue (paravertebral mass, iliac muscle). They received a median cumulative dose of  $3.8 \times 10^6$ /kg b.w. CD3<sup>+</sup> T cells per patient.

Patient 1 was a 53-year-old male, relapsing with subcutaneous plasmacytoma lesions day +330 after allo-HSCT. After cessation of the immunosuppression, a total of  $6.2 \times 10^7$ /kg b.w. CD3<sup>+</sup> T cells derived from the donor were transfused on 4 consecutive days. To enhance the T-cell effect, six doses of 5 million units IFN- $\alpha$  were given subcutaneously. After 5 days, the tumors started to shrink and have completely vanished since day +400 after BMT. The patient developed acute GVHD grade III of the liver and gut, which was treated by reinduction of steroids and cyclosporin A. At 54 months after relapse, the patient was in CR, but eventually died of pneumonia.

Patient 2 was a 52-year-old male relapsing day +351 with a paravertebral plasmacytoma lesion. Treatment consisted of  $3.8 \times 10^6$ /kg b.w. CD3<sup>+</sup> donor T cells divided into four doses. MRI demonstrated a PR 5 weeks after the fourth DLI. Unfortunately, the patient developed acute GVHD of the gut grade III at day +390, requiring intensive immunosuppression, and later died due to infectious complications.

Patient 3 was a 60-year-old male, relapsing with a plasmacytoma lesion in the iliac muscle occurring on day +210 after allo-HSCT. A total of  $3.2 \times 10^6$ /kg b.w. CD3<sup>+</sup> T cells derived from the donor were transfused on 2 days with an interval of 1 week. To enhance the T-cell effect, six doses of 6 million units IFN- $\alpha$  were given subcutaneously. MRI demonstrated a CR 8 weeks after the second DLI. The patient developed GVHD grade III of the gut and GVHD grade II of the liver and died on day +320 due to respiratory failure due to busulfan-induced toxicity in CR.

Acute GvHD  $\geq$  grade II was observed in group B1 in 7/17 and in B2 in 4/8 patients, while chronic GvHD  $\geq$  grade II was observed in group B1 in 5/17 and in B2 in 3/8 patients (Table 2).

### Discussion

Auto- and/or allo-HSCT have been proven to prolong OS and PFS in patients suffering from MM.<sup>1,2,6,29,30</sup> Nevertheless, despite HSCT, MM relapse is still a major cause of treatment failure, indicating that neoplastic plasma cells may display remarkable resistance to intensive conditioning regimens and exhibit immune escape mechanisms.<sup>6,29</sup> Interestingly, unlike other non-Hodgkin lymphoma, where an autograft is most successfully performed in best remission (CR, PR), most patients with MM, achieving CR or PR do so after transplantation, and refractoriness to induction treatment does not necessarily indicate a poor prognosis after HSCT.<sup>30</sup> DLIs have also in MM shown to exert a potent and durable graft-versus-myeloma effect (GvM) in patients with relapse after allo-HSCT.<sup>31–33</sup> However, the few case reports on extramedullary MM relapse after allo-HSCT suggest that malignant and highly proliferative plasma cells may escape the GvM effect of immunocompetent alloreactive T cells in the extramedullary sites.<sup>7,8,9</sup> Zomas *et al*<sup>8</sup> reported on a case of extramedullary MM relapse after allo-HSCT, responding to DLI in terms of BM infiltration and serum immunoglobulin levels, but not in extramedullary manifestations. These failed to respond to interferon and continued to grow in the absence of marrow plasmacytosis or other evidence of systemic disease. In contrast to these reports, we observed that patients relapsing in EM sites after allo-HSCT and receiving DLIs responded, as well as the BM relapse subgroup. Treatment responses to DLIs were observed in 56% in group B1, and 75% in group B2. Especially one patient relapsing with subcutaneous plasmacytoma responded well to DLI, reaching a long-lasting CR after EM relapse, again demonstrating the potential impact of the GvM effect also in EM sites.<sup>33</sup> Although our DLI response was noteworthy, this was observed in a yet relatively small patient group, with some also receiving additional antimyeloma therapy and thereby increasing the response to DLIs. Acute and chronic GvHD  $\geq$  grade II were equally present in both subgroups, suggesting that BM and EM relapse patients were comparably immunosuppressed. Immunosuppression, required to treat GvHD, may also hamper antimyeloma-specific T cells to eradicate their targets. The equal clinical course of patients relapsing in EM sites after HSCT as reflected by PFS and OS, which is in contrast to the hypothesis that EM relapse is associated with selection of a highly proliferative, therapy-resistant plasma-cell clone.

Correlation of the graft with the site of relapse indicated that a BM relapse was independent of unselected vs CD34<sup>+</sup> selected cells, an observation that is consistent with the Intergroupe Francophone du Myelome (IFM) 90 trials, showing no consistent OS and PFS advantage with selected stem cell grafts as compared to unselected cells in MM.<sup>34–36</sup> This finding indicates that – although several papers have

convincingly shown that CD34<sup>+</sup> selection of PBSCs markedly reduces tumor contamination in MM and provides effective hematopoietic support for patients receiving myeloablative regimens<sup>37</sup> – endogenous relapse from residual tumor mass is more likely to influence disease recurrence than residual tumor cells in stem cell grafts. This is in line with our data, not detecting a difference in BM relapse with un- or CD34-selected cells; nevertheless, it was of interest that EM relapse was more common in auto-patients receiving unselected peripheral blood stem cells. It is therefore tempting to speculate that CD34 selection may influence EM relapse in auto-patients.

The features -13q, a high plasma-cell labeling index, mitoses, high LDH and plasma-cell leukemia are associated with poor prognosis in MM. We did not detect significant differences when comparing the EM and BM relapse groups with respect to these features. This corresponds to our observation that OS and PFS were not significantly different in both groups. However, our patient numbers, absence of plasma-cell leukemia patients and cytogenetics not being available for all patients limit our single-center analysis.

With the development of EM relapse – besides bearing potential therapeutic difficulties – the diagnosis of MM relapse is often delayed or missed, due to the unusual presentation of the disease. This may especially occur, when skeletal radiological studies do not reveal progression of lytic lesions, nonsecretory disease and nonexistent BM infiltration. In our EM relapse group, a measurable monoclonal paraprotein was absent in group A2 in 5/6 patients and in B2 in 7/8 patients. Thus, in the context of clinical suspicion of relapse after HSCT, imaging techniques, such as CT, MRI and positron-emission tomography (PET) studies, are useful in detecting EM MM relapse, in the evaluation of response and are currently being evaluated in larger studies.<sup>38,39</sup>

New options for salvage treatment for MM, especially after treatment intensification, include agents, such as thalidomide and bortezomib, that target the marrow microenvironment, angiogenesis, NFκB targets and MM cells.<sup>10,40,41</sup> Both have proven to be effective in about 35% of relapsed or refractory MM patients, showing increased efficacy in combination with other MM-effective drugs, such as dexamethasone, melphalan, cyclophosphamide and others.<sup>40,41</sup> In our patients, 10 with relapse after auto-HSCT were treated with thalidomide and showed a response in 30%. The overall response to treatment at relapse was comparable for BM as compared to EM relapse patients after auto- (60 vs 67%) and allo-HSCT (54 vs 50%). Since more patients with EM relapse underwent an allo-HSCT after auto-HSCT than patients with BM relapse (67 vs 40%), the outcome of EM vs BM relapse after auto-HSCT may be biased by the different salvage treatment. Therefore, the comparable outcome after allo-HSCT in EM and BM relapse patients may not be directly transferable in the auto-HSCT setting.

In summary, our data demonstrate comparable PFS and OS after EM as compared to BM relapse, and therefore do not support the hypothesis that an extramedullary plasma-cell clone develops with specific therapy resistance. However, we found that EM relapse after HSCT is common,

suggesting that selection of plasma cells with an atypical homing behavior and absent immunoglobulin secretion takes place after HSCT. The observation that EM relapse patients respond to DLI suggests that this relapse pattern is not unfavorable for cell-mediated immunotherapy. This study encourages individualized diagnostic and therapeutic strategies to deal with the phenomena of EM MM relapse after auto- and allo-HSCT and demonstrates that cell-mediated immunotherapy can be effective in EM relapse.

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