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Melphalan 140 mg/m² or 200 mg/m² for autologous transplantation in myeloma: results from the Collaboration to Collect Autologous Transplant Outcomes in Lymphoma and Myeloma (CALM) study. A report by the EBMT Chronic Malignancies Working Party

Holger W. Auner,[1] Simona Iacobelli,[2] Giulia Sbianchi,[2] Cora Knol-Bout,[3] Didier Blaise,[4] Nigel H. Russell,[5] Jane F. Apperley,[1] David Pohlreich,[6] Paul V. Browne,[7] Guido Kobbe,[8] Cecilia Isaksson,[9] Stig Lenhoff,[10] Christof Scheid,[11] Cyrille Touzeau,[12] Esa Jantunen,[13] Achilles Anagnostopoulos,[14] Ibrahim Yakoub-Agha,[15] Alina Tanase,[16] Nicolaas Schaap,[17] Wieslaw Wiktor-Jedrzejczak,[18] Marta Krejci,[19] Stefan O. Schönland,[20] Curly Morris,[21] Laurent Garderet[22] and Nicolaus Kröger[23]

[1]Department of Medicine, Imperial College London, UK; [2]Department of Biology, Tor Vergata University of Rome, Italy; [3]EBMT Data Office, Leiden, the Netherlands; [4]Institut Paoli Calmettes, Marseille, France; [5]Nottingham University, UK; [6]Charles University Hospital, Prague, Czech Republic; [7]St. James's Hospital, Trinity College Dublin, Ireland; [8]Heinrich Heine Universität, Düsseldorf, Germany; [9]Umeå University Hospital, Sweden; [10]Skåne University Hospital, Lund, Sweden; [11]University of Cologne, Germany; [12]CHU Nantes, France; [13]Kuopio University Hospital, Finland; [14]George Papanicolaou General Hospital, Thessaloniki, Greece; [15]CHU de Lille, LIRIC, INSERM U995, France; [16]Fundeni Clinical Institute, Bucharest, Romania; [17]Radboud University Medical Centre, Nijmegen, the Netherlands; [18]Medical University, Warsaw, Poland; [19]University Hospital Brno, Czech Republic; [20]University of Heidelberg, Germany; [21]Queens University of Belfast, Northern Ireland; [22]Hôpital Saint-Antoine, Paris, France and [23]University Hospital Hamburg-Eppendorf, Hamburg, Germany

ABSTRACT

Melphalan at a dose of 200 mg/m² is standard conditioning prior to autologous hematopoietic stem cell transplantation for multiple myeloma, but a dose of 140 mg/m² is often used in clinical practice in patients perceived to be at risk of excess toxicity. To determine whether melphalan 200 mg/m² and melphalan 140 mg/m² are equally effective and tolerable in clinically relevant patient subgroups we analyzed 1964 first single autologous transplantation episodes using a series of Cox proportional-hazards models. Overall survival, progression-free survival, cumulative incidence of relapse, non-relapse mortality, hematopoietic recovery and second primary malignancy rates were not significantly different between the melphalan 140 mg/m² (n=245) and melphalan 200 mg/m² (n=1719) groups. Multivariable subgroup analysis showed that disease status at transplantation interacted with overall survival, progression-free survival, and cumulative incidence of relapse, with a significant advantage associated with melphalan 200 mg/m² in patients transplanted in less than partial response (adjusted hazard ratios for melphalan 200 mg/m² *versus* melphalan 140 mg/m²: 0.5, 0.54, and 0.56). In contrast, transplantation in very good partial or complete response significantly favored melphalan 140 mg/m² for overall survival (adjusted hazard ratio: 2.02). Age, renal function, prior proteasome inhibitor treatment, gender, or Karnofsky score did not interact with overall/progression-free survival or relapse rate in the melphalan dose groups. There were no significant survival or relapse rate differences between melphalan 200 mg/m² and melphalan 140 mg/m² patients with high-risk or standard-risk chromosomal abnormalities. In conclusion, remission status at the time of transplantation may favor the use of melphalan 200 mg/m² or melphalan 140 mg/m² for key transplant outcomes (NCT01362972).

Introduction

High-dose chemotherapy followed by autologous hematopoietic stem cell transplantation (ASCT) has been the standard consolidation treatment for patients up to the age of 65 years with newly diagnosed multiple myeloma for over two decades. Initially, high-dose chemotherapy plus ASCT proved superior to conventional chemotherapy.[1,2] More recently, the benefit of upfront high-dose chemotherapy plus ASCT has been confirmed in treatment approaches incorporating thalidomide analogs and proteasome inhibitors.[3-6] High-dose chemotherapy plus ASCT is also commonly used in older patients over the age of 65 years.[7,8] However, the superiority of ASCT over non-intensive therapies in older patients remains to be established.[9,10]

In the trials that demonstrated superiority of autologous transplantation over non-intensive approaches, patients received high-dose chemotherapy with melphalan at a dose of 200 mg/m^2 (Mel200).[1-6] Mel200 was less toxic than other high-dose combination regimens[11,12] and associated with longer progression-free survival and overall survival in patients younger than 60 years, when compared to melphalan 100 mg/m^2 in a tandem transplant approach.[13] Mel200 has therefore been recommended and widely used as standard conditioning therapy for ASCT.[14-16] Some studies have linked Mel200 to excess toxicity in older patients and those with renal impairment.[17-19] Consequently, a dose of 140 mg/m^2 (Mel140) is widely used in clinical practice in older patients and in patients with renal impairment.[20-25] However, Mel140 was associated with inferior response or survival rates compared to Mel200 in two very recent studies.[24,26] It therefore remains to be determined whether Mel140 and Mel200 are equally effective and tolerable across subgroups of patients.

To address this question we analyzed outcomes of almost 2000 first single autologous transplants for multiple myeloma after conditioning with either Mel140 or Mel200 which were reported to the European Society for Blood and Marrow Transplantation (EBMT). The results of the study indicate that the selection of Mel200 *versus* Mel140 may have a significant effect on key transplant outcomes, including overall survival.

Methods

Study criteria and data management

The Collaboration to Collect Autologous Transplant Outcomes in Lymphoma and Myeloma (CALM) study (NCT01362972) is an observational clinical outcome analysis of a defined cohort of patients with lymphoma or multiple myeloma who underwent ASCT between 2008 and 2012, with data reported retrospectively to the EBMT. Patients were eligible for the CALM study if they were ≥18 years old and received their first autologous peripheral blood stem cell transplant using cells mobilized with one of the following mobilization regimens: plerixafor plus granulocyte colony-stimulating factor (G-CSF), plerixafor plus G-CSF plus chemotherapy, G-CSF plus chemotherapy, or G-CSF alone. For this non-planned subgroup analysis, patients were selected from the CALM study population in the EBMT registry if they had a diagnosis of multiple myeloma and received a first single ASCT. Tandem transplants (defined as an ASCT followed by a second transplant within 6 months of the first and no relapse/progression between the two transplants), and patients who received melpha-

lan doses other than 200 or 140 mg/m^2, were not included. A total of 2253 patients from the CALM study EBMT registry fulfilled these general criteria. We excluded 289 of these patients from further analysis because of missing or inconclusive data regarding subsequent transplants (n=213), relapse date (n=67), or renal function (n=9), resulting in a final study population of 1964 patients. The database for this study was closed on December 14, 2016.

The study was performed in accordance with the principles of the Declaration of Helsinki and approved by the Chronic Malignancies Working Party of the EBMT, a non-profit scientific society representing more than 600 transplant centers mainly located in Europe. Data reported to the EBMT are entered, managed, and maintained in a central database with internet access housed in Leiden University Medical Center, the Netherlands. Each EBMT center is represented in this database, and all patients whose transplant data are reported by participating centers provide informed consent for transplant-related data to be used for research purposes in an anonymous way.

Statistical analysis

Patients' characteristics between the two groups (Mel140 and Mel200) were compared using the χ^2 test for categorical variables and the Mann-Whitney test for continuous variables. *P*-values for variables with more than two levels refer to an overall test for the presence of any difference. Overall survival was defined as the time from the date of ASCT to death from any cause. Patients still alive were censored at their last follow up. Progression-free survival was defined as the time between transplantation and progression of disease or death, censoring patients who did not develop an event. The probabilities of overall survival and progression-free survival were obtained using the Kaplan–Meier estimator and comparisons were made with the log-rank test. The probabilities of relapse (cumulative incidence of relapse) and death without prior relapse (non-relapse mortality) were calculated by the proper non-parametric estimator for outcomes with competing risk and comparisons made with the Gray test. These methods were also used to compute the cumulative incidence of second primary malignancy considering death without such a prior malignancy as a competing event.

Cox proportional hazards models were used to estimate adjusted hazard ratios (HR) for Mel140 compared to Mel200 in terms of overall survival, progression-free survival and the cumulative incidence of relapse. Factors included in the multivariable analysis were age at transplant (<65 *versus* ≥65 years), renal function (normal glomerular filtration rate >50 mL/min *versus* impaired glomerular filtration rate ≤50 mL/min), prior proteasome inhibitor treatment (yes *versus* no), status of disease at transplant (complete response/very good partial response *versus* partial response *versus* less than partial response), Karnofsky performance score (<90 *versus* ≥90) and gender. Age was dichotomized with a cut-off of 65 years for comparability with other studies considering that Martingale residuals analysis did not suggest other cut-off points (*data not shown*). There was no evidence that exclusion of missing values from multivariable analysis induced any bias in the estimation of regression coefficients (*data not shown*).

In order to explore any possible modification of the effect of the melphalan dose in different subgroups, we then fitted a secondary series of Cox models. Each model included melphalan dose, the selected adjustment variables, and the interaction between melphalan dose and one of the factors. This procedure returned estimated adjusted hazard ratios for Mel140 compared to Mel200 in each subgroup defined by the selected factors, and the results are shown in forest plots.

Due to the partial availability of International Staging System (ISS) and cytogenetic data, the interactions of ISS stage and chro-

mosomal abnormalities with melphalan dose were analyzed separately. Chromosomal abnormalities were classified as high-risk [t(4;14), t(14;16), and del(17)] or standard risk (all other cytogenetic findings).

All *P*-values shown are from two-sided tests, and the reported confidence intervals (CI) refer to 95% boundaries. A *P*-value <0.05 was regarded as statistically significant. A value up to 0.2 was used to determine the significance of interaction terms.

Results

Patient- and treatment-related characteristics

Patient-related and treatment characteristics are shown in Table 1. Patients in the Mel140 group (n=245, 12.5%) were older than patients in the Mel200 group (n=1719, 87.5%) at the time of ASCT [median 64 years (range, 27-73) *versus* 59 years (range, 25-76); *P*<0.001]. Compared to the Mel200 patients, those in the Mel140 group more often had light chain myeloma, were more often in ISS III, and were less often transplanted within 12 months of diagnosis. The two groups differed significantly in terms of body mass index, with a higher proportion of normal weight patients in the Mel140 group. Mel140 patients had received proteasome inhibitor-containing induction therapy more often, and a greater proportion had a Karnofsky score of <90. Finally, more Mel140 patients had impaired renal function, defined as a glomerular filtration rate of ≤50 mL/min, and a greater proportion of Mel140 patients underwent ASCT in partial remission or worse.

Efficacy

Overall survival was not significantly different between the two melphalan dose groups (Mel140, median not reached; 95% CI, 70.6 months to indeterminate; Mel200, 78 months; 95% CI, 74.0 months to indeterminate) (Figure 1A). The overall adjusted hazard ratio (HR) for death from all causes was 1.10 (95% CI: 0.79-1.54; *P*=0.56) for the Mel140 group (Figure 1B). Multivariable analysis of different subgroups showed that age, renal function, prior proteasome inhibitor treatment, gender, or Karnofsky score did not interact with overall survival in the melphalan dose groups (Figure 1B). However, disease status at transplant significantly modified the risk of death (*P*=0.006). In patients transplanted in less than partial response, Mel200 was associated with a significant overall survival advantage (adjusted HR 0.5 for Mel200 *versus* Mel140). In contrast, transplantation in very good partial response/complete response significantly favored Mel140 (adjusted HR 2.02). Transplantation in partial response did not modify the effect of melphalan dose on overall survival (adjusted HR 0.98).

The median progression-free survival was 29 months (95% CI: 24.6-33.7) in the Mel140 group and 26.3 months (95% CI: 24.6-28.1) in the Mel200 group (Figure 2A). The adjusted HR for disease progression or death was 1.0 (95% CI: 0.79-1.25; *P*=0.98) for the Mel140 group (Figure 2B). The multivariable models with interaction terms indicated that the HR of Mel200 *versus* Mel140 was not significantly modified by age, renal function, prior proteasome inhibitor treatment, gender, or Karnofsky score. However, in line with the overall survival analysis, there was a statistically significant change (*P*=0.043) according to disease status at transplantation. Among the patients transplanted in partial response or less, Mel200 was associated with a

significant progression-free survival advantage (adjusted HR 0.54 for Mel200 *versus* Mel140), while Mel140 was linked to a numerically better outcome in those trans-

Table 1. Patient- and transplant-related characteristics.

Patients' characteristics	Mel200 n=1719 (87.5%)	Mel140 n=245 (12.5%)	P value
Gender			
Male	997 (58.0%)	144 (58.8%)	0.818
Female	722 (42.0%)	101 (41.2%)	
Age at ASCT (median and range)			
years	59 (25–76)	64 (27–73)	0.001*
Body mass index (BMI)			
Underweight (BMI <18.5)	15 (1.1%)	4 (2.0%)	0.004*
Normal weight (18.5 ≤ BMI < 25)	484 (35.2%)	95 (48.5%)	
Overweight (25 ≤ BMI < 30)	587 (42.7%)	66 (33.7%)	
Obese (30 ≤ BMI < 35)	216 (15.7%)	22 (11.2%)	
Severely obese (BMI ≥ 35)	72 (5.2%)	9 (4.6%)	
Karnofsky score			
≥ 90	1117 (71.9%)	139 (61.8%)	0.002*
< 90	436 (28.1%)	86 (38.2%)	
Estimated GFR at ASCT			
>50 mL/min	1316 (96.3%)	123 (63.1%)	0.001*
≤50 mL/min	51 (3.7%)	72 (36.9%)	
Myeloma characteristics			
Myeloma type			
Common type	1328 (78.9%)	159 (65.7%)	0.001*
Light chain	328 (19.5%)	79 (32.6%)	
Non-secretory	27 (1.6%)	4 (1.7%)	
International Staging System stage			
I	453 (43.1%)	31 (23.5%)	0.001*
II	373 (35.5%)	48 (36.4%)	
III	225 (21.4%)	53 (40.2%)	
High-risk chromosomal abnormalities#			
No	466 (85.8%)	78 (85.7%)	0.979
Yes	77 (14.2%)	13 (14.3%)	
Treatment-related parameters			
Pre-transplant treatment			
Alkylating agent(s)	155 (10.6%)	7 (3.4%)	0.001*
Alkylating agent(s) + proteasome inhibitor	236 (16,2%)	35 (17.1%)	
Alkalyting agent(s) + IMiD(s)	322 (22.1%)	37 (18.0%)	
Alkylating agent(s) + proteasome inhibitor + IMiD(s)	88 (6.0%)	14 (6.8%)	
Proteasome inhibitor	233 (16.0%)	44 (21.5%)	
IMiD(s)	92 (6.3%)	4 (2.0%)	
Proteasome inhibitor + IMiD(s)	280 (19.2%)	54 (26.3%)	
Other	50 (3.4%)	10 (4.9%)	
Time from diagnosis to ASCT			
< 12 months	1366 (79.5%)	179 (73.1%)	0.022*
> 12 months	353 (20.5%)	66 (26.9%)	
Disease status at ASCT			
CR/VGPR	765 (45.2%)	105 (43.9%)	0.020*
PR	797 (47.1%)	103 (43.1%)	
<PR	130 (7.7%)	31 (13.0%)	
CD34+ cells infused (per kg)			
< 3 x 10^6	405 (33.4%)	79 (40.9%)	0.123
3 − 5 x 10^6	508 (41.9%)	71 (36.8%)	
> 5 x 10^6	299 (24.7%)	43 (22.3%)	

*Denotes statistically significant *P* values (<0.05); # includes t(4;14), t(14;16), or del(17p). ASCT: autologous stem cell transplantation; GFR: glomerular filtration rate; IMiD: immunomodulatory drug; CR: complete response; VGPR: very good partial response; PR: partial response.

planted in very good partial response/complete response (adjusted HR 1.19).

The cumulative incidence of relapse at 3 years was not significantly different between the Mel140 (55.1%; 95% CI: 48.6-61.6) and Mel200 (59.9%; 95% CI: 57.5-62.3) groups (Figure 3A). The adjusted HR for relapse was 0.99 (95% CI: 0.78-1.25; *P*=0.935) for Mel140 (Figure 3B). Subgroup analysis again showed a significant interaction of melphalan dose with disease status at the time of ASCT (*P*=0.07), in that transplantation in partial response or less significantly favored Mel200 (adjusted HR 0.56 for Mel200 *versus* Mel140). The adjusted HR for transplantation in partial response was 0.98 while that for transplantation in very good partial response/complete response was 1.2.

Patients with high-risk chromosomal abnormalities had poorer overall and progression-free survival, and a higher cumulative incidence of relapse, compared with those with other chromosomal aberrations, but we observed no statistically significant differences between Mel140 and Mel200 in high-risk or standard-risk patients (Figure 4). Similarly, while ISS stage was associated with overall and progression-free survival, and cumulative incidence of relapse (*Online Supplementary Figure S1*), there was no interaction between melphalan dose and ISS stage (*Online Supplementary Figure S2*).

Toxicity

Non-relapse mortality was not significantly different between the Mel140 and Mel200 groups [1-year non-relapse mortality 1.3% (95% CI: 0.0-2.7) and 0.9% (95% CI: 0.4 – 1.3), respectively; *P*=0.20]. The early non-relapse mortality rate at 3 months after ASCT was not significantly different either (0.8 and 0.5%, respectively; *P*=0.198). The main cause of death within 12 months of the transplant was relapse/progression, being the cause in 77.8% of patients in the Mel140 group and 80.0% of patients in the Mel200 group. Median times to neutrophil and platelet recovery were not significantly different between the Mel140 and Mel200 groups, being 12 (Mel140 95% CI: 12-13; Mel200 95% CI: 12-12) days in both groups for neutrophil recovery (*P*=0.283) and 16 (95% CI: 15-17) and 15 (95% CI: 15-16) days for platelet recovery (*P*=0.468). Second primary malignancy rates 5 years after ASCT were
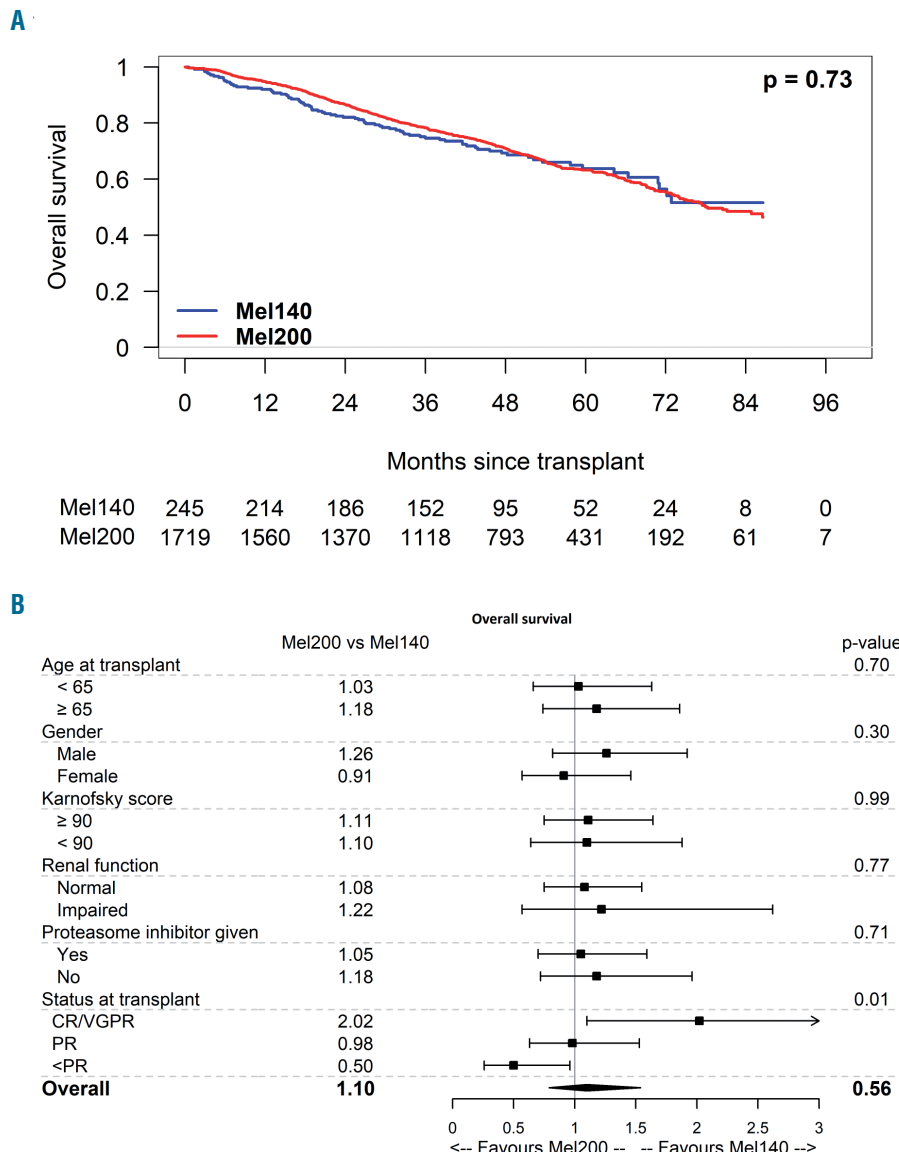
A

B

Figure 1. Overall survival after autologous stem cell transplantation for patients who received conditioning with melphalan 140 mg/m² (Mel140) or 200 mg/m² (Mel200). (A) Kaplan-Meier curves. (B) Multivariate analyses. The overall hazard ratio and corresponding *P* value (bottom line) refers to the comparison between Mel140 and Mel200 performed in a Cox model without interactions; the other hazard ratios and *P* values refer to interaction terms between melphalan dose and the indicated factors. All comparisons are adjusted for: age at transplant, renal function, prior proteasome inhibitor treatment, gender, status of disease, and Karnofsky score. CR/VGPR: complete response/very good partial response; PR: partial response.

very similar between the Mel140 (4.8%; 95% CI: 1.1-8.5) and Mel200 groups (4.8%; 95% CI: 3.6-6.0) (*P*=0.61).

Discussion

While Mel200 is generally recommended as standard conditioning prior to ASCT for multiple myeloma,[27] Mel140 is often used in clinical practice in those perceived to be at risk of excess toxicity from Mel200. However, the effect of melphalan dose on transplant outcomes remains undetermined. Here we present data from a large number of patients undergoing ASCT as part of real-world treatment practice. These data suggest that remission status at the time of transplantation may favor Mel200 or Mel140 for key transplant outcomes including overall survival.

One of the key findings of the study is that transplantation in less than partial response favored Mel200 over Mel140 in terms of overall survival, progression-free survival, and relapse risk. This may be explained by a greater dose-dependency of melphalan-induced anti-myeloma effects in cells with limited chemosensitivity. However, we observed no benefit of Mel200 over Mel140 for patients with high-risk chromosomal aberrations or higher ISS stage. Thus, while the better outcomes with Mel200 may at least partly be explained by the ability of the higher dose to overcome clinical resistance to induction therapies, Mel200 does not overcome the effects of poor-risk cytogenetics or advanced ISS stage. While the number of patients with known high-risk aberrations in our study was limited, the findings may be considered in line with preliminary data from an ongoing study which suggest a possible benefit of tandem ASCT for high-risk patients.[28] It remains to be determined whether molecular risk profiles other than those based on cytogenetic findings, or clinical features such as extramedullary disease, favor Mel200. These data were not available for analysis in this study.

In contrast to transplantation in poor clinical responders to induction therapy, transplantation in very good partial response/complete response appeared to favor Mel140. Considering that Mel200 was not linked to delayed hematopoietic recovery, increased early or late non-relapse mortality, or second primary malignancy rate, an explanation for these findings is not apparent. It is conceivable that Mel200 resulted in moderately increased toxicities that were not clinically apparent or were not captured in our study, such as delayed physical recovery, or organ-specific toxicities such as cardiac arrhythmias.[26]

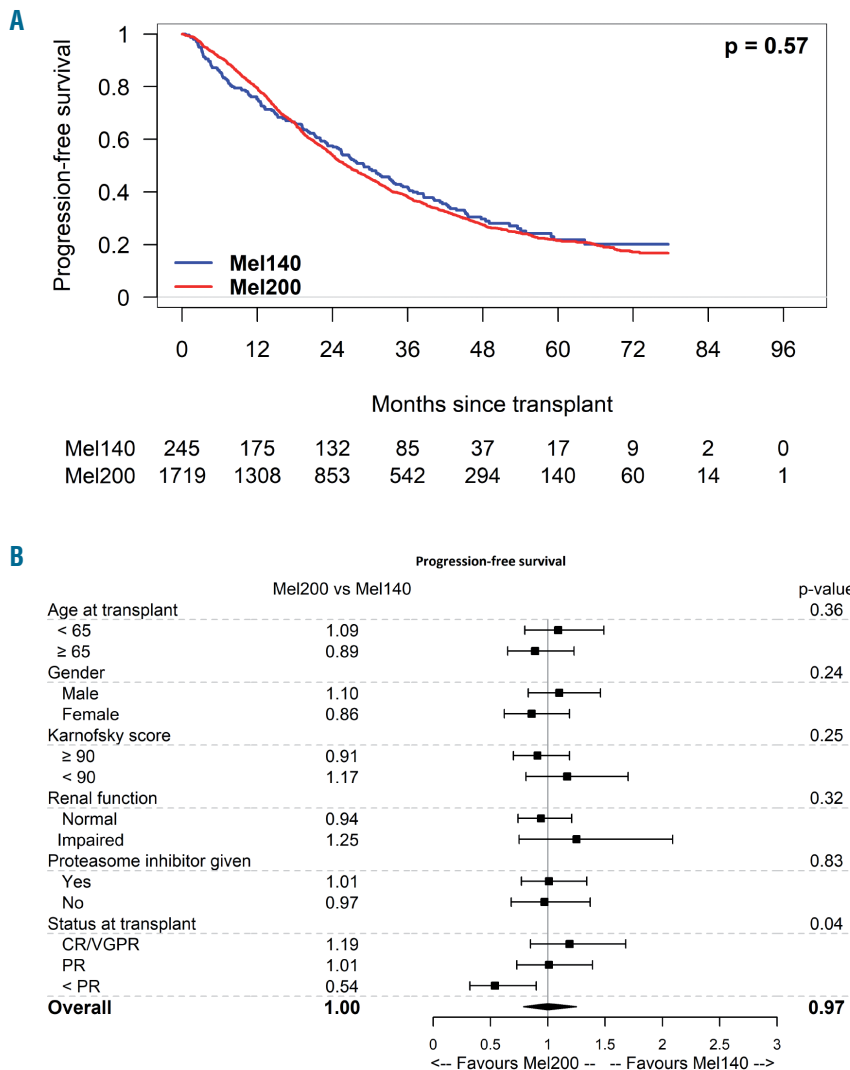|									
Mel140	245	175	132	85	37	17	9	2	0
Mel200	1719	1308	853	542	294	140	60	14	1

Figure 2. Progression-free survival after autologous stem cell transplantation for patients who received conditioning with melphalan 140 mg/m² (Mel140) or 200 mg/m² (Mel200). (A) Kaplan-Meier curves. (B) Multivariate analyses. The overall hazard ratio and corresponding *P* value (bottom line) refers to the comparison between Mel140 and Mel200 performed in a Cox model without interactions; the other hazard ratios and *P* values refer to interaction terms between melphalan dose and the indicated factors. All comparisons are adjusted for: age at transplant, renal function, prior proteasome inhibitor treatment, gender, status of disease, and Karnofsky score. CR/VGPR: complete response/very good partial response; PR: partial response.

Such effects may have affected physicians' and patients' attitude towards the nature, intensity, or duration of post-transplant treatment. However, they are not likely to fully explain the favorable outcomes linked to Mel140 in patients transplanted in very good partial response/complete response. Differences in melphalan pharmacokinetics in certain subgroups of patients may also have accounted for some of the effects we observed, given that diverse factors such as creatinine clearance, fat free mass and hematocrit influence melphalan exposure.[29] Melphalan exposure can vary considerably in myeloma patients treated with high-dose melphalan and ASCT, and higher exposure has been linked to greater toxicity and better disease responses.[30] In a recent study, high melphalan exposure was associated with significantly improved overall survival in myeloma patients undergoing ASCT.[31] However, despite the clear survival benefit, melphalan exposure was not associated with time to progression or progression-free survival, suggesting a possible link between melphalan exposure and long-term outcomes that is not directly attributed to immediate anti-myeloma effects.

While some studies have suggested that older age and renal impairment can be linked to excess toxicity with Mel200, others have not reported such an association.[19,20,22-24,26,32-34] The results of this study support the notion that older age and impaired renal function do not favor the use of a lower melphalan dose with regards to non-relapse mortality, hematopoietic recovery, or second primary malignancy rate. Moreover, we found no interaction of Karnofsky performance score with melphalan dose. However, the paucity in our study of data on comorbidities and frailty scores, and on the nature and grading of specific adverse events such as mucositis, means that we cannot exclude that Mel200 may be linked to an increase in some toxic effects in certain patients, or that Mel140 may have avoided such effects. Nonetheless, the data provide further support for the notion that ASCT is safe and effective in fit, older patients, and they are in line with those of a recent study demonstrating the value of Mel140 tandem ASCT as an independent component of therapy in older patients.[35] The application of objective criteria to determine patients' fitness in the context of co-morbidi-
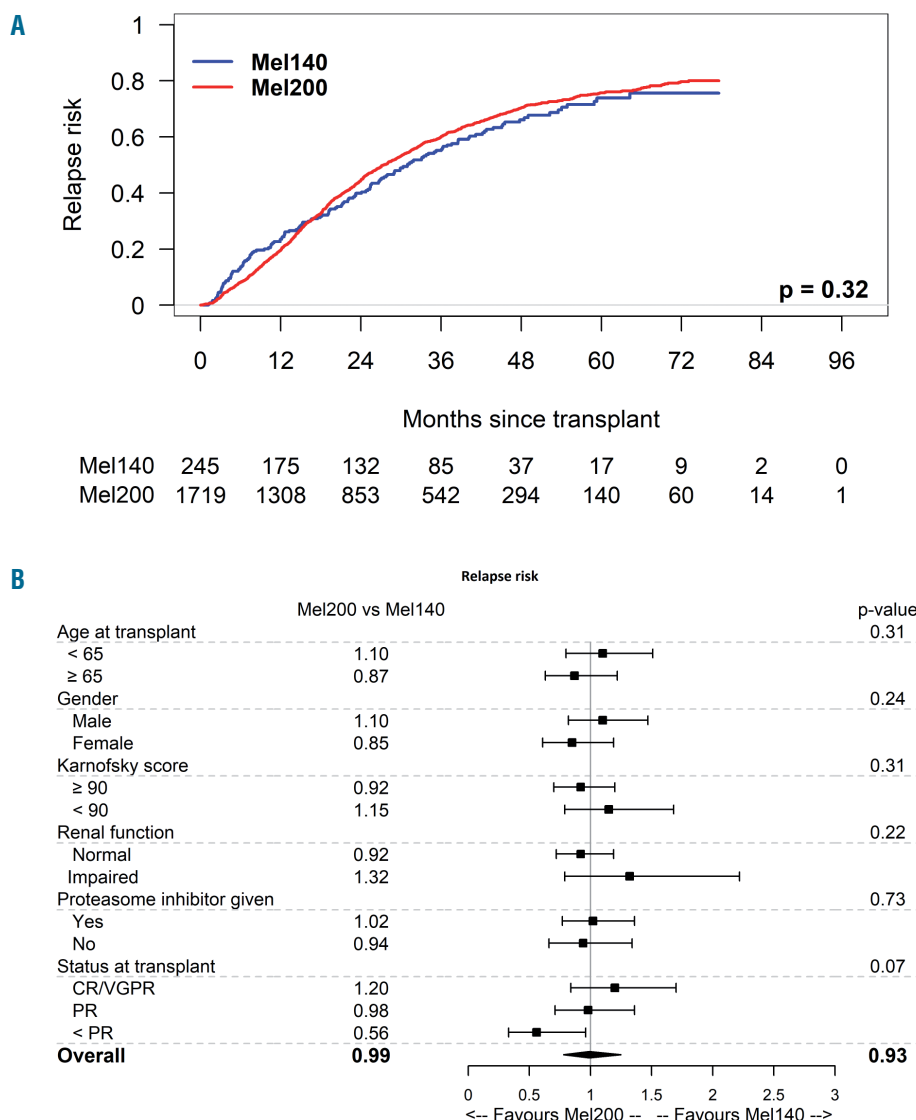

Figure 3. Cumulative incidence of relapse after autologous stem cell transplantation for patients who received conditioning with melphalan 140 mg/m² (Mel140) or 200 mg/m² (Mel200). (A) Kaplan-Meier curves. (B) Multivariate analyses. The overall hazard ratio and corresponding *P*-value (bottom line) refers to the comparison between Mel140 and Mel200 performed in a Cox model without interactions; the other hazard ratios and *P* values refer to interaction terms between melphalan dose and the indicated factors. All comparisons are adjusted for: age at transplant, renal function, prior proteasome inhibitor treatment, gender, status of disease, and Karnofsky score. CR/VGPR: complete response/very good partial response; PR: partial response.
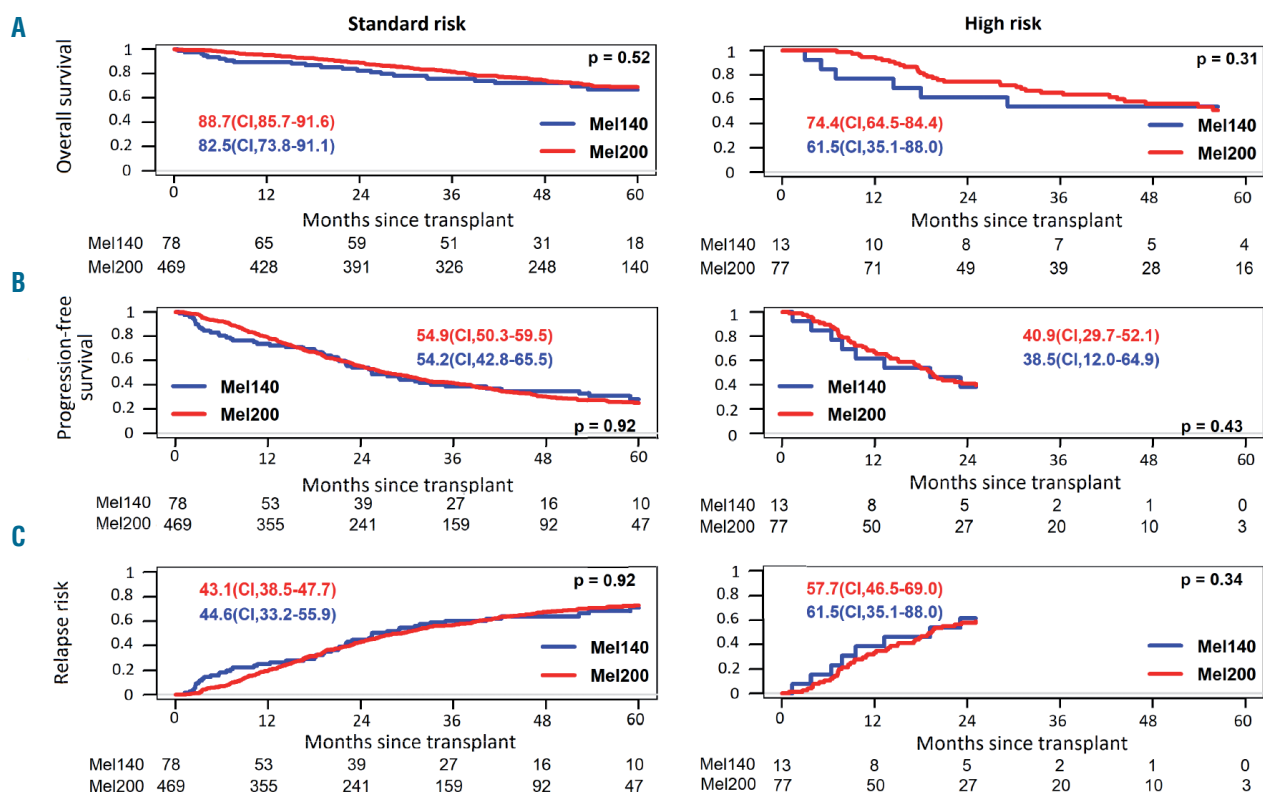
Figure 4. Survival and relapse risk by cytogenetic risk. (A) Overall survival, (B) progression-free survival and (C) relapse risk estimates and confidence intervals at 2 years after ASCT are shown for patients with high-risk or standard-risk chromosomal abnormalities.

ties should aid optimal selection of both younger and older patients.[36-40]

The CALM study is based on the retrospective analysis of registry data that were collected in a defined cohort of patients. Thus, the choice of Mel140 or Mel200 was made by transplant physicians and influenced or determined by local practice, thereby introducing a potential for biased treatment decisions. The paucity of data on post-transplant treatments, including maintenance, is another limitation of the analysis. However, the large number of patients from multiple centers across Europe is likely to have formed a representative 'real-world' sample of myeloma patients undergoing up-front ASCT. This notion is supported by the distribution of baseline clinical and cytogenetic features and the outcomes of patients with high-risk compared to standard-risk disease. Moreover, we applied robust statistical methods for the estimation of hazard ratios.

Our data indicate that the vast majority of patients undergoing upfront ASCT in a real-world setting receive Mel200 conditioning, and that patients with poor clinical responses to induction therapies derive more benefit from Mel200 than from Mel140. However, the results of this study also indicate that transplantation in very good partial response/complete response may favor Mel140 over Mel200. While the reasons for this unexpected finding remain to be determined, the data raise the challenging question of whether more patients should receive Mel140. This is relevant given that modern induction regimens achieve high very good partial response/complete response rates. The data presented here suggest that a randomized trial to define the optimal melphalan dose is warranted. Such a trial could also investigate the use of alternative conditioning approaches that incorporate novel agents[41-44] and the potential role of melphalan dosing in tandem transplant approaches, which was not feasible in this analysis. In the meantime, although Mel200 should remain the standard of care for ASCT conditioning, Mel140-based transplants could be considered as a valid alternative to offer patients an effective combination of ASCT plus novel therapies.[45]

In conclusion, our findings indicate that remission status at the time of a first ASCT may need to be considered when deciding the melphalan dose.

References

1. Attal M, Harousseau JL, Stoppa AM, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Francais du Myelome. N Engl J Med. 1996;335(2):91-97.
2. Child JA, Morgan GJ, Davies FE, et al. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. N Engl J Med. 2003;348(19):1875-1883.
3. Palumbo A, Cavallo F, Gay F, et al. Autologous transplantation and maintenance therapy in multiple myeloma. N Engl J Med. 2014;371(10):895-905.
4. Gay F, Oliva S, Petrucci MT, et al. Chemotherapy plus lenalidomide versus autologous transplantation, followed by lenalidomide plus prednisone versus

lenalidomide maintenance, in patients with multiple myeloma: a randomised, multicentre, phase 3 trial. Lancet Oncol. 2015;16(16): 1617-1629.

5. Attal M, Lauwers-Cances V, Hulin C, et al. Lenalidomide, bortezomib, and dexamethasone with transplantation for myeloma. N Engl J Med. 2017;376(14):1311-1320.

6. Gay F, Oliva S, Petrucci MT, et al. Autologous transplant vs oral chemotherapy and lenalidomide in newly diagnosed young myeloma patients: a pooled analysis. Leukemia. 2017;31(8):1727-1734.

7. Auner HW, Szydlo R, Hoek J, et al. Trends in autologous hematopoietic cell transplantation for multiple myeloma in Europe: increased use and improved outcomes in elderly patients in recent years. Bone Marrow Transplant. 2015;50(2):209-215.

8. Costa LJ, Zhang MJ, Zhong X, et al. Trends in utilization and outcomes of autologous transplantation as early therapy for multiple myeloma. Biol Blood Marrow Transplant. 2013;19(11):1615-1624.

9. Facon T, Mary JY, Hulin C, et al. Melphalan and prednisone plus thalidomide versus melphalan and prednisone alone or reduced-intensity autologous stem cell transplantation in elderly patients with multiple myeloma (IFM 99-06): a randomised trial. Lancet. 2007;370(9594):1209-1218.

10. Palumbo A, Bringhen S, Petrucci MT, et al. Intermediate-dose melphalan improves survival of myeloma patients aged 50 to 70: results of a randomized controlled trial. Blood. 2004;104(10):3052-3057.

11. Lahuerta JJ, Mateos MV, Martinez-Lopez J, et al. Busulfan 12 mg/kg plus melphalan 140 mg/m2 versus melphalan 200 mg/m2 as conditioning regimens for autologous transplantation in newly diagnosed multiple myeloma patients included in the PETHEMA/GEM2000 study. Haematologica. 2010;95(11):1913-1920.

12. Moreau P, Facon T, Attal M, et al. Comparison of 200 mg/m(2) melphalan and 8 Gy total body irradiation plus 140 mg/m(2) melphalan as conditioning regimens for peripheral blood stem cell transplantation in patients with newly diagnosed multiple myeloma: final analysis of the Intergroupe Francophone du Myelome 9502 randomized trial. Blood. 2002;99(3):731-735.

13. Palumbo A, Bringhen S, Bruno B, et al. Melphalan 200 mg/m(2) versus melphalan 100 mg/m(2) in newly diagnosed myeloma patients: a prospective, multicenter phase 3 study. Blood. 2010;115(10):1873-1879.

14. Jagannath S, Vesole DH, Glenn L, Crowley J, Barlogie B. Low-risk intensive therapy for multiple myeloma with combined autologous bone marrow and blood stem cell support. Blood. 1992;80(7):1666-1672.

15. Shah N, Callander N, Ganguly S, et al. Hematopoietic stem cell transplantation for multiple myeloma: guidelines from the American Society for Blood and Marrow Transplantation. Biol Blood Marrow Transplant. 2015;21(7):1155-1166.

16. Giralt S. 200 mg/m(2) melphalan--the gold standard for multiple myeloma. Nat Rev Clin Oncol. 2010;7(9):490-491.

17. Badros A, Barlogie B, Siegel E, et al. Autologous stem cell transplantation in elderly multiple myeloma patients over the age of 70 years. Br J Haematol. 2001;114(3):600-607.

18. Badros A, Barlogie B, Siegel E, et al. Results of autologous stem cell transplant in multiple myeloma patients with renal failure. Br J Haematol. 2001;114(4):822-829.

19. Sweiss K, Patel S, Culos K, Oh A, Rondelli D, Patel P. Melphalan 200 mg/m2 in patients with renal impairment is associated with increased short-term toxicity but improved response and longer treatment-free survival. Bone Marrow Transplant. 2016;51(10):1337-1341.

20. Katragadda L, McCullough LM, Dai Y, et al. Effect of melphalan 140 mg/m(2) vs 200 mg/m(2) on toxicities and outcomes in multiple myeloma patients undergoing single autologous stem cell transplantation-a single center experience. Clin Transplant. 2016;30(8):894-900.

21. Dhakal B, Nelson A, Guru Murthy GS, et al. Autologous hematopoietic cell transplantation in patients with multiple myeloma: effect of age. Clin Lymphoma Myeloma Leuk. 2017;17(3):165-172.

22. Gertz MA, Lacy MQ, Dispenzieri A, et al. Impact of age and serum creatinine value on outcome after autologous blood stem cell transplantation for patients with multiple myeloma. Bone Marrow Transplant. 2007;39(10):605-611.

23. Kumar SK, Dingli D, Lacy MQ, et al. Autologous stem cell transplantation in patients of 70 years and older with multiple myeloma: results from a matched pair analysis. Am J Hematol. 2008;83(8):614-617.

24. Garderet L, Beohou E, Caillot D, et al. Upfront autologous stem cell transplantation for newly diagnosed elderly multiple myeloma patients: a prospective multicenter study. Haematologica. 2016;101(11):1390-1397.

25. Dimopoulos MA, Terpos E, Chanan-Khan A, et al. Renal impairment in patients with multiple myeloma: a consensus statement on behalf of the International Myeloma Working Group. J Clin Oncol. 2010;28(33):4976-4984.

26. Muchtar E, Dingli D, Kumar S, et al. Autologous stem cell transplant for multiple myeloma patients 70 years or older. Bone Marrow Transplant. 2016;51(11):1449-1455.

27. Moreau P, San Miguel J, Sonneveld P, et al. Multiple myeloma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2017;28(suppl_4):iv52-iv61.

28. Cavo M, Petrucci MT, Di Raimondo F, et al. Upfront single versus double autologous stem cell transplantation for newly diagnosed multiple myeloma: an intergroup, multicenter, phase III study of the European Myeloma Network (EMN02/HO95 MM Trial). Blood. 2016;128(22):991.

29. Nath CE, Shaw PJ, Trotman J, et al. Population pharmacokinetics of melphalan in patients with multiple myeloma undergoing high dose therapy. Br J Clin Pharmacol. 2010;69(5):484-497.

30. Shaw PJ, Nath CE, Lazarus HM. Not too little, not too much-just right! (better ways to give high dose melphalan). Bone Marrow Transplant. 2014;49(12):1457-1465.

31. Nath CE, Trotman J, Tiley C, et al. High melphalan exposure is associated with improved overall survival in myeloma patients receiving high dose melphalan and autologous transplantation. Br J Clin Pharmacol. 2016;82(1):149-159.

32. Merz M, Neben K, Raab MS, et al. Autologous stem cell transplantation for eld-erly patients with newly diagnosed multiple myeloma in the era of novel agents. Ann Oncol. 2014;25(1):189-195.

33. Bashir Q, Shah N, Parmar S, et al. Feasibility of autologous hematopoietic stem cell transplant in patients aged >/=70 years with multiple myeloma. Leuk Lymphoma. 2012;53(1):118-122.

34. Auner HW, Garderet L, Kroger N. Autologous haematopoietic cell transplantation in elderly patients with multiple myeloma. Br J Haematol. 2015;171(4):453-462.

35. Straka C, Liebisch P, Salwender H, et al. Autotransplant with and without induction chemotherapy in older multiple myeloma patients: long-term outcome of a randomized trial. Haematologica. 2016;101(11):1398-1406.

36. Engelhardt M, Ihorst G, Caers J, Gunther A, Wasch R. Autotransplants in older multiple myeloma patients: hype or hope in the era of novel agents? Haematologica. 2016;101(11):1276-1278.

37. Zweegman S, Engelhardt M, Larocca A, EHA SWG on "Aging and Hematology". Elderly patients with multiple myeloma: towards a frailty approach? Curr Opin Oncol. 2017;29(5):315-321.

38. Engelhardt M, Domm AS, Dold SM, et al. A concise revised Myeloma Comorbidity Index as a valid prognostic instrument in a large cohort of 801 multiple myeloma patients. Haematologica. 2017;102(5):910-921.

39. Bruno B, Auner HW, Gahrton G, et al. Stem cell transplantation in multiple myeloma and other plasma cell disorders (report from an EBMT preceptorship meeting). Leuk Lymphoma. 2016;57(6):1256-1268.

40. Palumbo A, Bringhen S, Mateos MV, et al. Geriatric assessment predicts survival and toxicities in elderly myeloma patients: an International Myeloma Working Group report. Blood. 2015;125(13):2068-2074.

41. Roussel M, Moreau P, Huynh A, et al. Bortezomib and high-dose melphalan as conditioning regimen before autologous stem cell transplantation in patients with de novo multiple myeloma: a phase 2 study of the Intergroupe Francophone du Myelome (IFM). Blood. 2010;115(1):32-37.

42. Blanes M, Lahuerta JJ, Gonzalez JD, et al. Intravenous busulfan and melphalan as a conditioning regimen for autologous stem cell transplantation in patients with newly diagnosed multiple myeloma: a matched comparison to a melphalan-only approach. Biol Blood Marrow Transplant. 2013;19(1):69-74.

43. Rodriguez TE, Hari P, Stiff PJ, Smith SE, Sterrenberg D, Vesole DH. Busulfan, melphalan, and bortezomib versus high-dose melphalan as a conditioning regimen for autologous hematopoietic stem cell transplantation in multiple myeloma. Biol Blood Marrow Transplant. 2016;22(8):1391-1396.

44. Nieto Y, Valdez BC, Pingali SR, et al. High-dose gemcitabine, busulfan, and melphalan for autologous stem-cell transplant in patients with relapsed or refractory myeloma: a phase 2 trial and matched-pair comparison with melphalan. Lancet Haematol. 2017;4(6):e283-e292.

45. Morgan GJ, Rasche L. Haematological cancer: Where are we now with the treatment of multiple myeloma? Nat Rev Clin Oncol. 2017;14(8):461-462.