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Immunotherapy

A Phase 2 Study of Pembrolizumab during Lymphodepletion after Autologous Hematopoietic Cell Transplantation for Multiple Myeloma

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A B S T R A C T

The programmed death-1 (PD-1) axis can suppress immune surveillance against multiple myeloma (MM). We tested the safety and efficacy of pembrolizumab, an anti-PD-1 antibody, in MM after autologous hematopoietic cell transplantation (AHCT). We enrolled patients with MM who did not achieve a complete response (CR) to induction therapy. The study intervention involved a total of 9 doses of i.v. pembrolizumab, with 1 dose given every 21 days starting on day +14 post-AHCT. The primary endpoint was the rate of CR at end of treatment (EOT) in patients receiving ≥2 pembrolizumab doses. Thirty-two patients were enrolled, but 3 withdrew consent before receiving the first dose. The study was terminated early after failing to meet its interim analysis endpoint to detect a 20% difference in EOT CR rate conversion. The median patient age was 59 years. All but 1 patient received triplet induction for a median of 4 cycles (range, 2 to 7 cycles), with 69% partial response (PR) and 31% very good PR (VGPR). No grade 4/5 toxicities or graft failures occurred. Among 26 evaluable patients, 23 had an EOT evaluation, and 7 of these 23 (31%) achieved CR. Two patients had EOT serologic CR but no bone marrow confirmation (CRu), and 1 patient had no EOT evaluation. Bone marrow was minimal residual disease-negative by flow cytometry in 12 of 16 patients (75%) at day +180. With a median follow-up of 23.7 months (range, 15.1 to 33.5 months), no patient achieving EOT CR/CRu had relapsed, whereas 3 patients progressed before EOT and 1 patient progressed at 8 months after EOT VGPR. The estimated 2-year progression-free rate was 83% (95% confidence interval, 68% to 100%). Our data show that early post-AHCT pembrolizumab with lenalidomide maintenance is feasible; however, the efficacy is uncertain and requires further study. This trial was registered at ClinicalTrials.gov (NCT02331368).

INTRODUCTION

Multiple myeloma (MM) is the second most common adult hematologic malignancy in the Western hemisphere, with an estimated 30,770 new cases and 12,770 expected deaths in 2018 [1]. Although MM remains an incurable cancer, survival outcomes of patients with MM have continued to increase, particularly with the use of novel therapies, such as proteasome inhibitors (PIs) and immunomodulatory drugs (IMIDs) [2], as well as treatment paradigms including upfront high-dose therapy (HDT)/autologous hematopoietic cell transplantation (AHCT) and post-transplantation maintenance treatment [3]. Even though patients with standard-risk MM now experience survival upward of a decade [2–4], those with high-risk or primary refractory disease still have suboptimal outcomes [5]. Improving the treatment of high-risk MM remains a critical unmet need.

The response to induction chemotherapy is a prognostic marker in MM. The achievement of a very good partial response (VGPR) or better was associated with improved progression-free survival (PFS) after HDT/AHCT in multiple Intergroupe Francophone Du Myelome clinical trials [6,7]. Patients achieving a VGPR or better to induction treatment had improved post-transplantation outcomes, even those with higher-stage or poor-risk cytogenetics. As a corollary, attaining stringent complete response (sCR) and bone marrow (BM)

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minimal residual disease (MRD) negativity post-AHCT have been consistently shown to predict prolonged PFS and possibly overall survival (OS) [8–10].

Active immunotherapies, such as adoptive T cell transfer and cell-based vaccines, are limited in their efficacy by the presence of immune checkpoints and other immune regulatory components, such as regulatory T cells and myeloid-derived suppressing cells, and of native immune cells competing for space niches and essential homeostasis lymphokines [11–15]. In MM, preclinical and clinical evidence suggests that the immune checkpoint programmed death-1 (PD-1) receptor/PD-1 ligand (PD-L1) axis plays an important role in suppressing immune surveillance against MM [16–21], especially after HDT/AHCT, with the peak PD-1 expression on patient T cells occurring at approximately 30 days post-AHCT [21]. Antibodies that block PD-1/PD-L1 binding have been shown to disrupt the inhibitory signaling axis and restore immune activity against MM. Moreover, synergy between anti-PD-1 therapy and lenalidomide in targeting MM through the enhanced immune reactivity has been reported [22,23]. Unfortunately, 2 recent randomized phase 3 studies of combined pembrolizumab, dexamethasone, and an immunomodulatory agent (lenalidomide or pomalidomide) were terminated early owing to the increased mortality in the pembrolizumab arms [24–26].

The post-AHCT lymphodepletion promotes the proliferation of transferred and recovering T and NK cells and their functions against MM due to decreased presence of immune regulatory components and increased availability of activating lymphokines (hematopoietic cell product infusion) and of space niches [27–31]. We hypothesized that administration of the humanized PD-1-specific monoclonal antibody pembrolizumab during lymphodepletion early after HDT/AHCT, a period when PD-1 expression on immune effector cells peaks and MM tumor burden is at a nadir, would be safe and tolerable, and would leverage immune recognition of MM, resulting in a higher complete response (CR) rate in patients who had suboptimal response to induction therapy.

METHODS

We conducted this prospective 2-site single-arm phase 2 study in patients with MM who did not achieve CR from standard induction therapy. The trial has been registered at ClinicalTrials.gov (identifier NCT02331368). The study was approved by the Institutional Review Boards of the University of Michigan and the Medical College of Wisconsin. Written informed consent was obtained from each patient before enrollment.

Patients

Patients aged 18 to 70 years with biopsy-proven symptomatic MM of any molecular risk group who had failed to achieve CR status after induction therapy following the standard of care with at least 2 cycles of a triplet regimen (IMID + PI + steroid) or at least 4 cycles of a doublet regimen (IMID + steroid or PI + steroid) and with measurable disease at diagnosis (serum monoclonal protein ≥.5 g/dL or urine monoclonal protein ≥200 mg/24 hours or abnormal serum-free light chain [FLC] ratio with involved FLC level ≥10 mg/dL) were eligible. Patients were required to be enrolled within 12 months after diagnosis and to have adequate organ function to proceed with HDT/AHCT in accordance with institutional guidelines.

Exclusion criteria included the following: history of nonsecretory MM; coexistence of amyloidosis or plasma cell leukemia or POEMS syndrome or CNS myeloma; presence of autoimmune disease, noninfectious pneumonitis, or interstitial lung disease; previous therapy with anti-PD-1, anti-PD-L1, anti-cytotoxic T lymphocyte-associated antigen-4 antibody, or any other antibody or drug specifically targeting T cell costimulation or checkpoint pathways; active MM therapy, including dexamethasone within 2 weeks before transplantation or 4 weeks before the first dose of pembrolizumab; active infection including HIV, hepatitis B, or hepatitis C infection; being pregnant or breastfeeding, or expecting to conceive children within the projected duration of the study; significant coagulopathy; symptomatic congestive heart failure, unstable angina pectoris, or cardiac arrhythmia; any type of previous HCT; and receipt of live vaccines within 30 days were excluded.

Standard-of-Care Procedure

Patients received induction therapy in accordance with their institution's standard of care. Peripheral blood hematopoietic cells were collected by use of filgrastim with/without plerixafor per institutional guidelines. The target hematopoietic cell dose was $\geq 2 \times 10^6$ $CD34^+$ cells/kg. Subjects received high-dose melphalan (140 to 200 mg/m^2 i.v.) on day -1, followed by AHCT on day 0. Transfusion support, antibiotic prophylaxis, and filgrastim at 5 μg/kg/day to facilitate engraftment were administered according to institutional guidelines.

BM flow cytometry-based MRD testing was performed at day +100. The flow cytometry methods were 9-color, 11-parameter panel combining surface staining for CD19, CD20, CD38, CD45, CD56, CD117, CD138, and cytoplasmic staining for light chains, with a sensitivity of 1 in 30,000 (University of Michigan) and 8-color panel combining surface staining for CD3, CD4, CD5, CD8, CD10, CD11b, CD14, CD16, CD19, CD20, CD38, CD45, CD56, CD138, and CD200 and cytoplasmic staining for light chains, with a sensitivity of 1 in 10,000 (Medical College of Wisconsin).

Post-AHCT maintenance therapy with lenalidomide 5 to 15 mg/day was started at 100 to 120 days after transplantation following the standard of care. No other immunomodulatory agents were allowed. The protocol was amended on February 17, 2016, to allow maintenance to start as early as 45 days after transplantation according to the updated standard of care, and 8 patients started lenalidomide earlier than 100 days after transplantation (median, 84 days; range, 60 to 98 days).

Pembrolizumab

Pembrolizumab was started on evidence of adequate hematologic recovery, including neutrophils ≥1000/μL, platelets ≥20,000/μL, and hemoglobin ≥8 g/dL. Pembrolizumab was administered at a fixed dose of 200 mg i.v. every 3 weeks, starting on day 14 (±4 days) after AHCT for a total of 9 doses, with the last dose given on approximately day +180 (±4 days). Subsequent doses could be postponed and restarted without dose reduction for immunologic adverse events in accordance with the investigational brochure, with all doses being given within 200 days after transplantation (Figure 1).

Statistical Considerations

Primary and Secondary Endpoints

The study's primary endpoint was the CR rate, defined as the observed proportion of complete responders at the end-of-treatment (EOT) evaluation, which occurred approximately 4 weeks after day +180 or the last dose of pembrolizumab in evaluable subjects (ie, those receiving ≥2 doses). Secondary endpoints of OS and PFS were modeled using the Kaplan-Meier method. Treatment-related mortality, graft failure, immune-related adverse reactions (IrAEs), response, and relapse are reported descriptively.

The estimated historical CR rate post-AHCT of the randomized cohorts receiving lenalidomide maintenance starting at day +100 was approximately 30% [32,33]. The association of IrAE development with CR and with MRD-negative status at EOT was analyzed using Fisher's exact test.

Study Design and Interim Analysis

We hypothesized that CR rate at day 180 post-AHCT would be increased by at least 20% with the administration of pembrolizumab during lymphodepletion post-AHCT. Assuming a 30% baseline CR rate conversion in the historical cohort, this study aimed to enroll 46 evaluable subjects to detect a 20% increase in CR rate conversion (30% to 50%) at EOT, with an 80% statistical power and a 5% 1-sided type I error. Patients were planned to enroll in 2 cohorts according to an optimal Simon 2-stage design. The first cohort consisted of 15 patients; if more than 5 of them should achieve CR at EOT, then an additional cohort of 31 patients would be enrolled. We would then require more than 18 complete responses in 46 evaluable subjects for the study to reach its primary aim.

The interim analysis revealed a 30% (5/15) CR rate at EOT, though 47% (7/15) of subjects achieved BM CR by morphology and MRD status. The enrollment was placed on hold after 32 subjects had been enrolled as the observed CR rate in the first 15 patients did not reach the expected (at least 6

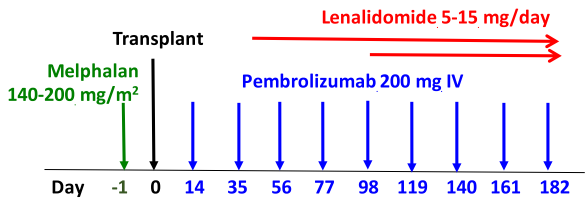

Figure 1. Treatment schema. Pembrolizumab was started on day +14 after engraftment and given every 21 days for a total of 9 doses. Lenalidomide maintenance was later allowed to start as early as day +45.

patients or 40%), and the study was thus terminated. Here we report the outcomes of the evaluable patient cohort.

RESULTS

Thirty-two patients were enrolled, but 3 withdrew consent before receiving pembrolizumab. The 29 patients who received pembrolizumab included 11 patients from the University of Michigan and 18 from the Medical College of Wisconsin. Patient and MM characteristics are summarized in Table 1. All patients except 1 received a triplet-regimen primary therapy, with a median of 4 cycles (range, 2 to 7 cycles). In addition, all but 1 patient received 4 or more cycles of induction therapy. Twenty patients (69%) achieved partial response (PR) and 9 (31%) achieved very good PR (VGPR) before AHCT. The median post-AHCT day of pembrolizumab initiation was day +14 (range, days +13 to +19). The median number of doses received was 9 (range, 1 to 9). The median days of neutrophil engraftment and platelet engraftment were day +11 (range, days +6 to +13) and day +12 (range, days +8 to +39), respectively.

Toxicities

Among the 29 patients who received at least 1 dose of pembrolizumab, nineteen patients (66%) developed 29 IrAEs attributed to pembrolizumab, without deaths or grade 4 toxicities (Table 2). Five events led to the discontinuation of pembrolizumab, including grade 3 infusion reaction in 1 patient, grade 2 and 3 colitis in 1 patient each, grade 2 rash in 1 patient, and grade 2 radiculopathy in 1 patient (Table 2). The other 24 events that did not lead to discontinuation were 12 grade 1, 10 grade 2, and 2 grade 3 in severity. These included hyperthyroidism and hypothyroidism in 5 patients each, infusion reaction in 4 patients, rash in 3 patients and colitis in 2 patients.

All grade 3 events, except glomerulonephritis, resolved with systemic steroid therapy. Of interest, a patient with late-onset acute kidney injury developed hypertension, edema, elevated serum creatinine (3.2 mg/dL), and proteinuria at approximately 350 days post-AHCT. Pathological evaluation revealed proliferative glomerulonephritis and acute tubular injury, with immunogranular and semilinear capillary and mesangial staining of IgG_k and C3. There was no evidence of MM relapse; thus, this was consistent with atypical hemolytic uremic syndrome and improved with prolonged eculizumab therapy.

Nineteen of the 29 IrAEs (66%) occurred with pembrolizumab treatment alone, with all 5 infusion reactions with first doses of pembrolizumab. The other 10 reactions (34%) emerged during lenalidomide treatment at a median of 42 days (range, 16 to 232 days) after the initiation of lenalidomide maintenance therapy. One patient developed a grade 3 non-IrAE *Salmonella* gastroenteritis, which was attributed to pembrolizumab and occurred simultaneously with a grade 3 IrAE colitis, leading to removal from the study. There were no primary or secondary graft failures; the median times to achieving neutrophil and platelet engraftment were 11 days (range, 6 to 13 days) and 12 days (range, 8 to 39 days), respectively. There were no reported second primary malignancies during the follow-up period.

Table 1
Baseline Patient and Disease Characteristics

Characteristic	Value
Institution, UM/MCW, n	11/18
Sex, male/female, n	19/10
Age, yr, median (range)	59 (49-70)
Light chain disease, n (%)	4 (14)
Serum β-2-microglobulin, mg/dL, median (range)	3.3 (1.5-9.3)
Serum albumin, g/dL, median (range)	3.8 (2-4.6)
Revised-ISS stage, I/II/unknown, n (%)	10 (34)/15 (52)/4 (14)
Cytogenetic studies: conventional method, FISH, n (%)	
Trisomies, tetrasomies	3 (10), 6 (21)
Normal karyotype	18 (62), 3 (10)
+1q	1 (3), 4 (14)
+1q and t(4;14)	0, 1 (3)
+1q and trisomies	0, 1 (3)
-17p	1 (3), 0
t(11;14) alone	0, 5 (17)
Others	2 (7), 5 (18)
Unknown, not performed	4 (14), 4 (14)
Primary therapy	
Number of cycles, median (range)	4 (2-7)
Triplet regimen, n (%)	
VRD	20 (69)
CyBorD	5 (18)
CRD	1 (3)
RD and VRD	1 (3)
CyBorD and VRD	1 (3)
Doublet regimen, n (%)	
RD	1 (3)
MM status at AHCT, n (%)	
VGPR	9 (31)
PR	20 (69)

UM indicates University of Michigan; MCW, Medical College of Wisconsin; VRD, bortezomib/ lenalidomide/ dexamethasone; CyBorD, cyclophosphamide/ bortezomib/ dexamethasone; CRD, carfilzomib/ lenalidomide/ dexamethasone; RD, lenalidomide/ dexamethasone.

Response

Twenty-six patients receiving $\geq$2 doses of pembrolizumab were deemed evaluable for the efficacy assessment (Table 3). Twenty-four patients started lenalidomide maintenance at a median of 103 days (range, 60 to 154 days) post-AHCT. One patient had progressive disease at the day +100 evaluation, and 1 patient withdrew consent after receiving 3 doses. Among the 23 patients who had a complete EOT response evaluation at 4 weeks after the last dose, the CR rate was 31% (7 of 23) by both serology and BM study (morphology and flow cytometry-based MRD testing). In the remaining 3 patients, 2 patients with serologic CR at EOT did not have a confirmatory BM study, and 1 patient did not have an EOT evaluation. The BM MRD-negative rates by flow cytometry were 44% (11 of 25) at day +100 and 75% (12 of 16) at day +180. Among the 15 patients who had MRD testing completed at both the day +100 and day +180 evaluations, 8 patients attained MRD negativity at day +100, and 7 of these remained MRD-negative at day +180. The persistent serologic positivity despite MRD negativity in some IgG_k MM patients might have been due to testing interference by pembrolizumab.

There was no association between the development of IrAEs and either CR status at EOT ($P = .18$) or MRD-negative status at EOT ($P = .60$). Three of 7 patients with CR (43%) and 4 of 5 MRD-negative patients (80%) developed IrAEs, compared with 12 of 16 non-CR patients (75%) and 7 of 13 MRD-positive patients (54%).

Outcomes and Survival

With a median duration of follow-up of 23.7 months (range, 15.1 to 33.5 months), none of 9 patients achieving either CR or

Table 2
IrAEs

CTC AEs version 4 (in 19 of 29 patients)	**Number of Events (N = 29)**		
	Grade 1 (n = 12)	**Grade 2** (n = 13)	**Grade 3** (n = 4)
Infusion reaction	1	3	1*
Hyperthyroidism	4	1	
Hypothyroidism		5	
Colitis	2	1*	1*
Rash/pruritus	3	1*	
Arthralgia	1		
Neuropathy	1	1*	
Leg muscle spasm		1	
Hepatitis			1
Glomerulonephritis			1

* Resulting in removal from the study.

serologic CR only (CRu) at EOT subsequently relapsed, whereas 3 patients had progressed by EOT and 1 patient later progressed at 8 months after initial achievement of VGPR at EOT (Table 3). The estimated 1-year and 2-year PFS rates were 88% (95% confidence interval [CI], 77% to 100%) and 83% (95% CI, 68% to 100%), respectively (Figure 2). Owing to the short duration of follow-up, the estimated OS rates are not reported.

DISCUSSION

The depth of MM response achieved after HDT/AHCT, especially stringent CR, has been consistently demonstrated to predict better MM-free survival [7-10]. Thus, our study aimed to deepen the response of HDT/AHCT, particularly in patients who had a suboptimal serologic response to induction therapy with novel doublets or triplets, by disrupting the PD-1/PD-L1 checkpoint axis, the effect of which is leveraged by HDT/AHCT (ie, lymphodepletion and transfer of essential lymphokines).

In this investigator-initiated study, we make the following observations: (1) administration of pembrolizumab in the early post-AHCT period was feasible without undue graft toxicity; (2) the combination of pembrolizumab and low-dose lenalidomide was associated with increased immunologic reactions, although no grade 4 or 5 toxicity was noted; and (3) the study resulted in a 31% CR rate at EOT but a promising 75% BM MRD-negative rate at day +180.

Checkpoint inhibitors have demonstrated efficacy in a number of hematologic malignancies [34]. In patients with MM, preliminary data have reported response rates >60% and a similar incidence of IrAE of 58% to 68% [35] at the median duration of >14 months with the combination of pembrolizumab, dexamethasone, and an immunomodulatory drug—lenalidomide or pomalidomide—in the relapsed refractory setting [36,37]. More recently, however, the US Food and Drug Administration placed holds on 2 randomized phase 3 clinical trials evaluating pembrolizumab in combination with dexamethasone and an immunomodulatory agent, owing to the increased mortality seen in the experimental arm [24–26]. Subsequently, all ongoing studies testing pembrolizumab in combination with an immunomodulatory agent have been discontinued. Our present study demonstrates the feasibility and safety of using pembrolizumab early after AHCT, with no negative effects on engraftment.

Of note, most reported direct pembrolizumab-related adverse events have been infusion reactions after the first dose. There was an increase in the incidence of immunologic toxicities, mostly thyroid function abnormalities, with the initiation of lenalidomide maintenance as the standard of care. These reactions occurred at a median time of 42 days (range, 16 to 232 days) after combined treatment with low-dose lenalidomide and pembrolizumab, supporting recent data indicating that the combination of these agents with immunomodulatory imides increases toxicity.

MRD negativity is increasingly recognized as a surrogate for prolonged PFS and OS [9,10]. In particular, recent data

Table 3
MM Response

Baseline	**D100**	**EOT**
VGPR	**CR**	**CR**
PR	**CR**	**CR**
VGPR	CRu	**CR**
PR	VGPR	**CR**
PR	VGPR	**CR**
PR	VGPR	**CR**
VGPR	VGPR	**CR**
VGPR	VGPR	CRu
PR	VGPR	CRu
PR	VGPR	VGPR
PR	VGPR	VGPR
PR	VGPR	VGPR
PR	VGPR	VGPR
PR	PR	VGPR
PR	PR	VGPR
PR	PR	VGPR
PR	PR	VGPR
PR	PR	PR
VGPR	VGPR	VGPR
VGPR	VGPR	VGPR
VGPR	VGPR	VGPR
VGPR	VGPR (EOT)	
PR	PR (EOT)	
VGPR	VGPR	PD
PR	PD	
PR	PD	

D100 indicates day +100 evaluation; PD, progressive disease.

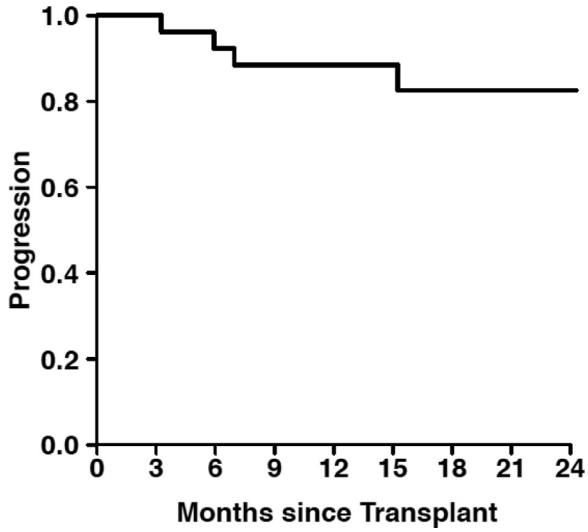

Figure 2. Kaplan-Meier PFS estimate. The estimated 1-year and 2-year PFS rates were 88% and 83%, respectively.

Table 4
Comparative Responses in Contemporaneous Clinical Trials

Series	Number of Patients	Age, yr, median (range)	Induction Therapy	Postinduction CR Rate, %	HDT/AHCT	Consolidation and/or Maintenance	Approximate CR Rate at 180 d Post-AHCT, %
Cavo et al [39]	236	58 (52-62)	VTD × 3	19	Tandem	VTD × 2 and D maintenance	42
Gay et al [40]	127	57 (53–62)	RD × 4	2	Tandem	Maintenance LPr or L	13-30
Moreau et al [7]	199	58 (52-62)	VTD or VD × 4	12	Single	None	29-31
Roussel et al [41]	31	58 (33-65)	VRD × 3	23	Single	VRD × 2 and L maintenance	50
Palumbo et al [42]	102	67 (46-74)	PAD × 4	12	100 mg/m^2 × 2	LPr × 4 and L maintenance	33

V or P indicates bortezomib; T, thalidomide; LPr or L, lenalidomide; D, dexamethasone; Pr, prednisone; A, pegylated doxorubicin.

demonstrate that MRD-negative status surpasses the prognostic value of conventional CR achievement for both PFS and OS across various risk types, regardless of treatment [10]. Thus, the achievement of BM flow cytometry-based MRD negativity of 44% at day +100 and 75% at EOT was highly encouraging, even with the low CR conversion rate. Because our study was powered not based on MRD negativity, but rather by conventional CR, we did not meet the preset criteria of our interim analysis. The preset criteria of an expected 30% absolute increase in CR rate on the study was overambitious for testing our hypothesis. The historical CR rate from the McCarthy study that we used as our historical comparison [33] does not truly represent the valid expected baseline CR conversion for our study, considering that some of the patients on that study likely had already achieved CR before AHCT, whereas in our study, not achieving CR before AHCT was a prerequisite. Nonetheless, the baseline pretransplantation CR rate in that study might not have been high, given the limited efficacy of induction therapy at that time.

More recently, the Spanish Myeloma Group conducted a phase 3 study that included induction with 6 cycles of bortezomib, lenalidomide, and dexamethasone (VRD), followed by HDT/AHCT, post-transplantation consolidation with 2 cycles of VRD, and maintenance [38], and reported CR rates of 39% at pre-AHCT, 49% at post-AHCT, and 58% after the consolidation phase, implying a rate of improvement in CR of only 10% to 18%. The corresponding MRD negativity rates were 35%, 54% and 58%, respectively. The recently reported improvement of CR rates at ~180 days post-AHCT ranged from 18% to 27%, as summarized in Table 4.

We acknowledge some limitations of the present study. First, the small patient size and the very short follow-up period due to early termination precluded any possible meaningful comparisons with recent contemporaneous series. Second, correlative studies of PD-1/PD-L1 expression and clinical response were not performed.

Given recent data, the future of checkpoint inhibition therapies in MM is not promising. Our study, which focused on patients early in the course of treatment, demonstrated that the addition of pembrolizumab in the early post-AHCT period was generally safe, as was the combined administration with standard-of-care low-dose lenalidomide maintenance, albeit with increased IrAEs. Post-AHCT pembrolizumab with lenalidomide resulted in a CR conversion rate of 31% at 6 months, including an encouraging 75% rate of BM MRD-negative state, in patients not in CR before AHCT.

We conclude that the concurrent administration of pembrolizumab and low-dose lenalidomide maintenance after AHCT in MM was feasible but ineffective in this study. Nonetheless, the results are sufficiently intriguing to warrant further study with superior design and immune-related AE monitoring.

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