Regular Article

blood

TRANSPLANTATION

Safety and efficacy of allogeneic hematopoietic stem cell transplant after PD-1 blockade in relapsed/refractory lymphoma

Reid W. Merryman,[1] Haesook T. Kim,[2] Pier Luigi Zinzani,[3] Carmelo Carlo-Stella,[4,5] Stephen M. Ansell,[6] Miguel-Angel Perales,[7] Abraham Avigdor,[8] Ahmad S. Halwani,[9] Roch Houot,[10,11] Tony Marchand,[10] Nathalie Dhedin,[12] Willy Lescaut,[13] Anne Thiebaut-Bertrand,[14] Sylvie François,[15] Aspasia Stamatoullas-Bastard,[16] Pierre-Simon Rohrlich,[17] Hélène Labussière Wallet,[18] Luca Castagna,[4,5] Armando Santoro,[4,5] Veronika Bachanova,[19] Scott C. Bresler,[20] Amitabh Srivastava,[20] Harim Kim,[21] Emily Pesek,[1] Marie Chammas,[1] Carol Reynolds,[1] Vincent T. Ho,[1] Joseph H. Antin,[1] Jerome Ritz,[1] Robert J. Soiffer,[1] and Philippe Armand[1]

[1]Division of Hematologic Malignancies and Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA; [2]Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA; [3]Institute of Hematology "L. e A. Seràgnoli," University of Bologna, Bologna, Italy; [4]Humanitas Clinical & Research Center, Milan, Italy; [5]Department of Oncology and Hematology, University of Milan, Milan, Italy; [6]Mayo Clinic, Rochester, MN; [7]Department of Medicine, Adult Bone Marrow Transplant Service, Memorial Sloan Kettering Cancer Center, New York, NY; [8]Division of Hematology and Bone-Marrow Transplantation, Chaim Sheba Medical Center, Tel Hashomer, Israel; [9]Huntsman Cancer Institute, Salt Lake City, UT; [10]Centre Hospitalier Universitaire (CHU) Rennes, Service Hématologie Clinique, Rennes, France; [11]INSERM, Rennes, France; [12]Hôpital Saint Louis, Service Hématologie Adolescents et Jeunes Adultes, Assistance Publique-Hôpitaux de Paris, Paris, France; [13]Centre Hospitalier Princesse Grace, Service Médecine Interne et Onco-Hématologie, Monaco, France; [14]CHU Grenoble, Clinique Universitaire d'Hématologie, Grenoble, France; [15]CHU Angers, Service Maladies du Sang, Angers, France; [16]Centre Henri Becquerel, Service Hématologie Clinique, Rouen, France; [17]CHU Nice, Service Hématologie et Onco-Hématologie Pédiatrique, Nice, France; [18]Hospices Civils de Lyon, Service d'Hématologie Clinique, Lyon, France; [19]Division of Hematology, Oncology, and Transplantation, University of Minnesota, Minneapolis, MN; [20]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; and [21]University College of Medicine, Seoul National University, Seoul, South Korea

Key Points

- HSCT after PD-1 blockade is feasible, although may be associated with increased early immune toxicity.
- PD-1 blockade may cause persistent depletion of PD1$^+$ T cells and alterations in T-cell differentiation impacting subsequent treatment.

Anti–programmed cell death protein 1 (PD-1) monoclonal antibodies are being increasingly tested in patients with advanced lymphoma. Following treatment, many of those patients are likely to be candidates for allogeneic hematopoietic stem cell transplant (HSCT). However, the safety and efficacy of HSCT may be affected by prior PD-1 blockade. We conducted an international retrospective analysis of 39 patients with lymphoma who received prior treatment with a PD-1 inhibitor, at a median time of 62 days (7-260) before HSCT. After a median follow-up of 12 months, the 1-year cumulative incidences of grade 2-4 and grade 3-4 acute graft-versus-host disease (GVHD) were 44% and 23%, respectively, whereas the 1-year incidence of chronic GVHD was 41%. There were 4 treatment-related deaths (1 from hepatic sinusoidal obstruction syndrome, 3 from early acute GVHD). In addition, 7 patients developed a noninfectious febrile syndrome shortly after transplant requiring prolonged courses of steroids. One-year overall and progression-free survival rates were 89% (95% confidence interval [CI], 74-96) and 76% (95% CI, 56-87), respectively. One-year cumulative incidences of relapse and nonrelapse mortality were 14% (95% CI, 4-29) and 11% (95% CI, 3-23), respectively. Circulating lymphocyte subsets were analyzed in 17 patients. Compared with controls, patients previously treated with PD-1 blockade had significantly decreased PD-1$^+$ T cells and decreased ratios of T-regulatory cells to conventional CD4 and CD8 T cells. In conclusion, HSCT after PD-1 blockade appears feasible with a low rate of relapse. However, there may be an increased risk of early immune toxicity, which could reflect long-lasting immune alterations triggered by prior PD-1 blockade. (*Blood*. 2017;129(10):1380-1388)

Introduction

Programmed cell death protein 1 (PD-1) is an inhibitory receptor expressed on activated T cells and other lymphoid and myeloid cells. The PD-1 synapse is an important mechanism by which malignancies can evade the host immune response.[1,2] Clinical trials of anti-PD-1

monoclonal antibodies (mAbs) have demonstrated promising results in lymphoma patients with advanced disease. Hodgkin lymphoma (HL), which appears to have a unique biologic dependence on the PD-1 pathway, is particularly susceptible to PD-1 blockade,[3,4] and 1 PD-1

inhibitor, nivolumab, was recently approved by the US Food and Drug Administration for treatment of classical HL.[5] Clinical activity has also been observed in several subtypes of non-Hodgkin lymphoma (NHL).[6,7]

Given the success of these agents in early studies, anti-PD-1 mAbs are being tested alone and in combination in a growing number of clinical trials across many hematologic malignancies. Many participants in these trials will at some point become potential candidates for allogeneic hematopoietic stem cell transplantation (HSCT); however, the safety and efficacy of HSCT may be different in patients previously exposed to PD-1 inhibitors, given their immunomodulatory mechanism and their prolonged clinical activity.[8] Specifically, residual PD-1 inhibition peri- and post-HSCT could enhance allogeneic T-cell responses, which could augment the graft-versus-tumor (GVT) effect but also increase the incidence or severity of immune complications. Animal models demonstrate that PD-1 blockade early after allogeneic HSCT may augment GVT in anatomic niches that are susceptible to relapse,[9] but could also result in higher rates of acute graft-versus-host disease (GVHD) and increased GVHD lethality.[10,11] Early clinical trials suggest that checkpoint blockade therapy with nivolumab or ipilimumab (IPI) as treatment of relapse after HSCT could be effective with a tolerable side-effect profile.[12,13] Understanding the impact of prior PD-1 blockade on both the safety and the efficacy of HSCT is critical to select candidates for PD-1 blockade among patients who may be future candidates for HSCT and to select appropriate treatment strategies for HSCT candidates who are responding to PD-1 blockade or who have relapsed after prior PD-1 blockade.

We therefore conducted an international retrospective study of patients treated with an anti-PD1 mAb at some point prior to HSCT, with the goal of describing toxicity and disease control. In addition, immune profiling analysis using flow cytometry was performed on a subset of patients with available samples pre- and post-HSCT to explore the possible basis of observed differences in HSCT outcomes.

Methods

Patients

To develop this collaboration, we contacted centers that participated in early studies of PD-1 inhibitors for lymphoma, or that informed us of patients at their center who had been transplanted after prior PD-1 blockade. Between July 2013 and March 2016, we identified 39 patients who received pembrolizumab or nivolumab for treatment of HL or NHL and subsequently underwent allogeneic HSCT. Patients underwent transplantation under a variety of treatment plans and investigational protocols. The Institutional Review Boards at all sites approved the protocol prior to patient enrollment. A waiver of informed consent was obtained for this retrospective chart review. Medical records of all patients were reviewed by participating investigators. Assessment of GVHD was performed by each center following their standard grading practice.

Immune profiling analysis

Patients treated at Dana-Farber Cancer Institute/Brigham and Women's Hospital (DFCI/BWH) who had available samples pre- and post-HSCT were included in an immune reconstitution subanalysis. Patients treated at the same institution with reduced-intensity conditioning (RIC) HSCT *without* prior anti-PD-1 treatment between 2011 and 2015 were used as a control cohort. Blood samples from both cohorts were obtained on day 0 (prior to stem cell infusion) and 1, 2, 3, 6, 9, and 12 months after transplant. Whenever possible, fresh samples were analyzed prospectively. When fresh samples had not been analyzed, cryopreserved samples were thawed and tested with the same panel of mAbs. Samples obtained after relapse were not included in this analysis. Written informed consent was obtained from all patients prior to sample collection as part of established research sample collection protocols.

A panel of directly conjugated mAbs was used to define functionally distinct T-cell subsets, as previously described.[14] Three major T-cell populations: CD4$^+$ T-regulatory cells (CD4Treg), conventional CD4 cells (CD4Tcon), and effector CD8$^+$ T cells, were defined as CD3$^+$CD4$^+$Foxp3$^+$, CD3$^+$CD4$^+$Foxp3$^-$, and CD3$^+$CD4$^-$CD8$^+$, respectively. Within each population, subsets were defined as follows: naive T cells (CD45RA$^+$CD62L$^+$); central memory (CD45RA$^-$ CD62L$^+$); effector memory (CD45RA$^-$CD62L$^-$); and CD8 terminal effector memory T cells (CD8$^+$CD45RA$^+$CD62L$^-$). In addition, we quantified the populations of PD-1$^+$ cells among all subsets.

Statistical considerations

Patient baseline characteristics and immunophenotype data were analyzed descriptively. Endpoints of interest included overall survival (OS), progression-free survival (PFS), relapse, nonrelapse mortality (NRM), as well as acute and chronic GVHD. OS was defined as the time from stem cell infusion to death from any cause. Patients who were alive or lost to follow-up were censored at the time last seen alive. PFS was defined as the time from stem cell infusion to disease relapse, progression, or death from any cause, whichever occurred first. Patients who were alive without disease relapse or progression were censored at the time last seen alive and progression-free. OS and PFS were calculated using the Kaplan-Meier method, and the log-rank test was used for comparisons of Kaplan-Meier curves. Cumulative incidence curves for nonrelapse death, relapse with or without death, and GVHD were constructed in the competing risks framework considering relapse, NRM, and death or relapse without developing GVHD, respectively, as competing events. All time to events were measured from the date of stem cell infusion. The difference between cumulative incidence curves in the presence of a competing risk was tested using the Gray method.[15] Immunophenotype data were compared using Wilcoxon rank sum test for unpaired group comparison and Wilcoxon signed rank test for paired comparison. Correlation analysis was performed using the Spearman rank test. All tests are 2-sided at the significance level of 0.05 and multiple comparisons were not considered. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC) and R version 3.1.3 (the CRAN project).

Results

Patients

A total of 39 patients who were treated with PD-1 blockade prior to HSCT (4 in combination with the anti-CTLA-4 mAb IPI) were included in this analysis. Baseline characteristics are summarized in Table 1. Median age at the time of transplantation was 34 years (range, 21-67). Thirty-one patients (79%) were transplanted for classical HL, and the remainder for NHL. Patients received a median of 4 systemic treatments (range 2-8). Eighty-two percent received an autologous HSCT a median of 23 months (range 2-132) prior to allogeneic HSCT. Among the 31 patients with HL, 24 (77%) were treated with brentuximab, in 22 cases prior to PD-1 therapy. Patients received a median of 8 (range, 3-27) cycles of a PD-1 inhibitor. The best response to PD-1 therapy was a complete response (CR) in 14 patients (36%), partial response in 10 patients (26%), stable disease in 7 patients (18%), and progressive disease in 8 patients (21%). The overall response rate was higher among patients with HL (74%) compared with NHL (13%). The median time from last dose of PD-1 therapy to HSCT was 62 days (range, 7-260). Nineteen patients (49%) had intervening salvage therapy between anti-PD-1 treatment and HSCT, all for progression after PD-1 blockade. All patients received T-cell–replete grafts, and all but 1 patient had RIC.

Survival and relapse

Median follow-up for survivors was 12 months (range, 2-33 months). Overall, the 1-year OS and PFS were 89% (95% confidence interval [CI], 74-96) and 76% (95% CI, 56-87), respectively, whereas the 1-year

Table 1. Baseline characteristics of patients

Patient characteristics	N = 39*
Sex	
Male	20 (51%)
Female	19 (49%)
Age at transplant, y (median, range)	34 (21-67)
Institution, N (%)	
DFCI/BWH	19 (49)
Mayo Clinic	3 (8)
MSKCC	3 (8)
University of Bologna	4 (10)
Humanitas Clinical & Research Center	9 (23)
Chaim Sheba	1 (3)
Disease, N (%)	
Classical HL	31 (79)
DLBCL	2 (5)
FL	2 (5)
PMBCL	2 (5)
EATL	1 (3)
MCL	1 (3)
Total number of systemic treatments (median, range)	4 (2-8)
Radiation therapy, N (%)	25 (64)
Prior autologous transplant, N (%)t	32 (82)
Number of cycles of PD-1 inhibitor (median, range)	8 (3-27)
PD-1 inhibitor received, N (%)	
Nivolumab†	28 (72)
Pembrolizumab	11 (28)
Best response to PD-1 inhibitor, N (%)	
Complete response	14 (36)
Partial response	10 (26)
Stable disease	7 (18)
Progressive disease	8 (21)
Number of patients with immune-related adverse events while on PD-1 inhibitor (prior to transplant), N (%)	4 (11)
Colitis	2 (6)
Pneumonitis	2 (6)
Hepatitis	1 (3)
Uveitis	1 (3)
Interval between last PD-1 treatment and HSCT (d)	62 (7-260)
Patients with intervening salvage therapy between PD-1 and HSCT, N (%)	19 (49)
Graft source, N (%)	
PB	28 (72)
BM	11 (28)
Donor type, N (%)	
MRD	9 (23)
MUD	12 (31)
Haploidentical transplant	14 (36)
MMUD‡	4 (10)
Disease status at allogeneic transplant, N (%)	
Complete response	25 (64)
Partial response	11 (28)
Stable disease	2 (5)
Progressive disease	1 (3)
Conditioning regimen, N (%)	
RIC	38 (97)
Cy, Flu, TBI	13 (33)
Bu, Flu	11 (28)
Flu, Mel	5 (13)
TT, CY, Flu, ATG	4 (10)
TT, CY, Flu, TBI	2 (5)
TT, Flu, Mel	2 (5)
TT, Flu	1 (3)
MAC	1 (3)
Bu, Flu	1 (3)

Table 1. (continued)

Patient characteristics	N = 39*
GVHD prophylaxis, N (%)	
PTCY	7 (18)
PTCY, Tacrolimus, MMF	7 (18)
Tacrolimus, Sirolimus, MTX	6 (15)
Tacrolimus and MTX	6 (15)
CSA, MTX	5 (13)
Other§	8 (21)

ATG, antithymocyte globulin; BM, bone marrow; Bu, busulfan; Chaim Sheba, Chaim Sheba Medical Center in Ramat Gan, Israel; CSA, cyclosporine; CY, cyclophosphamide; DLBCL, diffuse large B-cell lymphoma; EATL, enteropathy-associated T-cell lymphoma; FL, follicular lymphoma; Flu, fludarabine; MAC, myeloablative conditioning; MCL, mantle cell lymphoma; Mel, melphalan; MMF, mycophenolate mofetil; MMUD, mismatched-unrelated donor; MRD, matched-related donor; MSKCC, Memorial Sloan Kettering Cancer Center; MTX, methotrexate; MUD, matched-unrelated donor; PB, peripheral blood; PMBCL, primary mediastinal B-cell lymphoma; PTCY, posttransplant cyclophosphamide; TBI, total body irradiation; TT, thiotepa; University of Bologna, Hematology Institute "L. e A. Seragnoli" at the University of Bologna.

*Percentages may not add to 100 because of rounding.

†Among those, 4 patients received nivolumab in combination with ipilimumab.

‡One permissive DR mismatch; 1 DQ mismatch, and unidirectional HvG mismatch.

§One patient received a GVHD regimen that included a single dose of ATG. No patients received alemtuzumab.

cumulative incidences of relapse (CIR) and NRM were 14% (95% CI, 4-29) and 11% (95% CI, 3-23), respectively (Figure 1; Table 2). Among the 31 patients with HL, 1-year OS, PFS, CIR, and NRM were 90% (95% CI, 71-97), 74% (95% CI, 50-88), 16% (95% CI, 3-36), and 10% (95% CI, 3-25), respectively (Table 2).

Toxicity

The 1-year cumulative incidences of grade 2-4, grade 3-4, and grade 4 acute GVHD were 44%, 23%, and 13%, respectively, with a median day of onset of 27 days. The 1-year cumulative incidence of chronic GVHD was 41%. The rates of GHVD were similar for the HL cohort (Table 2). There were 4 treatment-related deaths: 3 from acute GVHD arising within 14 days of HSCT and 1 from hepatic SOS (Table 2). Among the 3 patients with fatal acute GHVD, 2 patients (patients 3 and 8) had a noninfectious febrile syndrome, which began within 1 week of transplant and 4 to 6 days prior to neutrophil engraftment. Patient 3 developed severe acute GVHD, bacteremia, acute kidney injury, and hypoxic respiratory failure leading to death on day 100. Following an initial noninfectious febrile syndrome, patient 8 also developed severe acute GHVD, which was complicated by bacteremia, diffuse alveolar hemorrhage, posterior reversible encephalopathy syndrome, and death on day 123. The third patient with fatal GVHD (patient 18) had a pretransplant course complicated by grade 2 colitis and pneumonitis after prior combination treatment with nivolumab and IPI. After transplantation, she developed severe liver GVHD, hepatic encephalopathy, and hypotension. A liver biopsy prior to her death on day 35 demonstrated acute liver GVHD as well as lobular hepatitis consistent with drug-induced hepatitis (Figure 2). Her last treatment with nivolumab and IPI was 49 days before HSCT and 74 days prior to the biopsy.

Three patients (8%) developed severe hepatic SOS despite RIC. All 3 patients were treated with defibrotide, and 2 required central venovenous hemofiltration, with 1 fatality 51 days after transplant (patient 14; see Table 2). The third patient who developed severe SOS received corticosteroids in addition to defibrotide and recovered rapidly and completely.
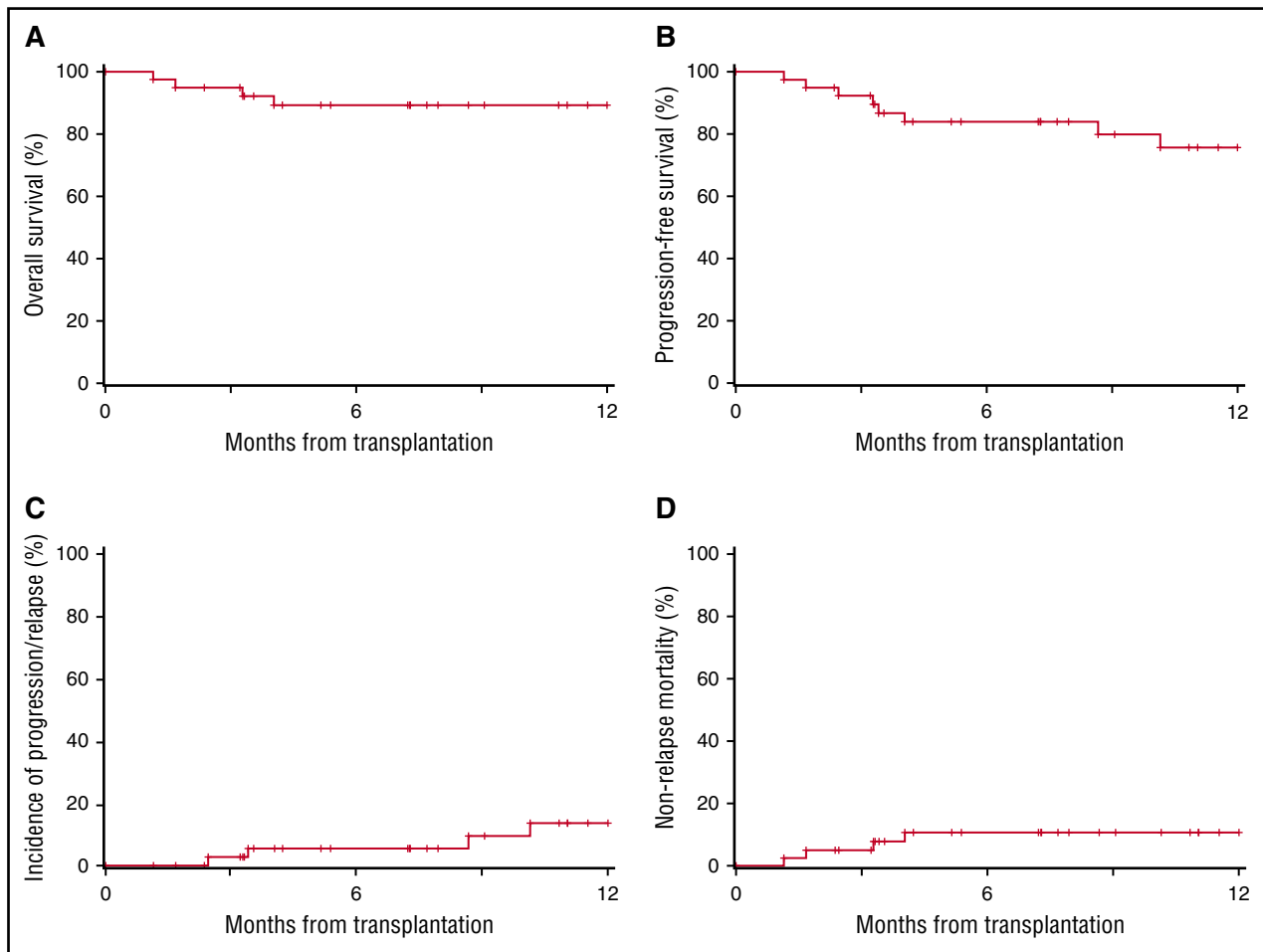
Figure 1. OS, PFS, CIR, and NRM following HSCT in patients previously treated with a PD-1 inhibitor. (A) OS, (B) PFS, (C) CIR, and (D) NRM.

In addition, 7 patients (18%) developed a prolonged febrile syndrome beginning 1 to 7 weeks after HSCT with elevated transaminases in 3 patients and rash in 4 patients (Figure 3). Only 1 of 7 patients met Spitzer criteria and 3 of 7 patients met Maiolino criteria for engraftment syndrome.[16,17] All patients were started on corticosteroids (~1 mg/kg) within 3 weeks of fever onset. Two patients with this syndrome (patients 3 and 8) developed severe, fatal acute GVHD shortly after the onset of fevers, as described above. Of the remaining 5 patients, 4 required prolonged steroid courses for management of recurrent fevers, rash, or liver function test abnormalities (Figure 3). Two of those 4 patients developed clear chronic GVHD while on a steroid taper.

Clinical predictors of GVHD, survival, and NRM

PD-1 dosing and timing. Patients who received greater than or equal to 8 doses (the median value in this cohort) of PD-1 blockade appeared to have improved PFS at 1 year compared with those receiving <8 doses (91% [95% CI 70-98] vs 54% [95% CI 25-76], $P = .039$). A slightly larger proportion of these patients had a CR at the time of HSCT, but the difference was not significant (69% vs 54%, $P = .5$). No significant differences were seen in 1-year rates of OS (95% [95% CI 72-99] vs 77% [95% CI 44-92], $P = .064$), CIR (4% vs 23%, $P = .4$), and NRM (5% vs 23%, $P = .070$) when stratified by total doses of PD-1 (8 or more doses vs <8 doses). Duration of time between the last dose of PD-1 blockade and HSCT (>60 days vs <60 days) did not

significantly affect 1-year rates of OS, PFS, CIR, NRM, or the incidence of GVHD or febrile syndrome.

The 4 patients treated concurrently with IPI and a PD-1 inhibitor all developed acute GVHD, including 1 fatal case of grade 4 acute GVHD. The 6-month rate of grade 3-4 acute GVHD was higher in these patients compared with those treated with PD-1 blockade alone (75% vs 17% $P = .010$). There were no significant differences seen in 1-year OS (75% vs 91% $P = .3$), 10-month PFS (75% vs 80% $P = .13$), 10-month CIR (0% vs 11% $P = .4$), 10-month NRM (25% vs 9% $P = .3$), or 1-year incidence of chronic GVHD (25% vs 43%, $P = .8$).

Donor and graft characteristics. Among patients receiving BM grafts, rates of grade 3-4 acute GVHD were lower compared with patients receiving peripheral blood stem cells (PBSCs; 0% vs 32%, $P = .036$), but this did not result in a significant difference in chronic GVHD, NRM, or survival. There were no significant differences in outcomes based on donor source (ie, matched vs unmatched). Among the 14 patients undergoing haploidentical donor transplants, 1-year OS, PFS, CIR, and NRM were similar to the entire cohort (OS 93% [95% CI, 59-99], PFS 70% [95% CI, 30-90], CIR 23% [95% CI, 2-56], and NRM 7% [95% CI, 0-28]). Likewise, there were no significant differences based on GVHD prophylaxis regimen, treatment with a salvage regimen between PD-1 blockade and HSCT, or timing of prior autologous HSCT.

Immune reconstitution after HSCT

Seventeen of 19 patients who underwent HSCT at DFCI/BWH had blood samples available and were included in a subgroup analysis.

Table 2. Toxicity, outcomes, and deaths

Toxicity and outcomes (1-y cumulative incidence)		
	All patients (N = 39) % (range, 95% CI)	HL subset (N = 31) % (range, 95% CI)
Acute GVHD		
Grade 2-4	44 (28-59)	45 (27-62)
Grade 3-4	23 (11-37)	26 (13-55)
Grade 4	13 (5-25)	13 (4-27)
Chronic GVHD	41 (22-60)	33 (13-55)
Hepatic SOS (3-mo cumulative incidence)	8 (2-19)	6 (1-19)
OS	89 (74-96)	90 (71-97)
PFS	76 (56-87)	74 (50-88)
CIR	14 (4-29)	16 (3-36)
NRM	11 (3-23)	10 (3-25)

Deaths					
Pt. no.	Histology	Donor source	Graft source	Interval between PD-1 and HSCT, d	Day of death	Complications
3	Mixed cellularity HL	MMUD	PB	17	100	Febrile syndrome, hyperacute grade 4 acute GVHD, bacteremia, acute kidney injury, hypoxic respiratory failure
8	Nodular sclerosing HL	MUD	PB	100	123	Febrile syndrome, hyperacute grade 4 acute GVHD, bacteremia, diffuse alveolar hemorrhage, posterior reversible encephalopathy syndrome
14	Nodular sclerosing HL	Haploidentical	BM	156	51	Severe SOS and renal failure requiring central veno-venous hemofiltration (CVVH)
18	Enteropathy-associated T-cell lymphoma	MUD	PB	49*	35	Grade 4 acute GVHD, hepatic encephalopathy, and hypotension

PB, peripheral blood; SOS, sinusoidal obstruction syndrome.

*Received ipilimumab concurrently with anti-PD-1 therapy. The last dose of ipilimumab was also 49 d prior to HSCT.

Twenty-four patients treated with RIC HSCT during the same time period were assembled as a control cohort. They were matched as able for disease, conditioning intensity, sex, and GVHD prophylaxis regimen (supplemental Table 1, available on the *Blood* Web site). Patients treated with PD-1 blockade prior to HSCT had similar absolute counts of CD4$^+$ and CD8$^+$ T cells across studied time points (0, 1, 2, 3, and 6 months) after transplantation (Figure 4). Likewise, there were no significant differences in subset populations of naive T cells, central memory, effector memory, or CD8 terminal effector memory T cells between the PD-1 and control cohort. However, among patients previously treated with PD-1 blockade, the ratio of CD4Treg:CD4Tcon cells was significantly decreased at 0 and 1 months and the ratio of CD4Treg:CD8 T cells was significantly reduced at 0, 1, and 2 months after transplantation (Figure 4). Further examination of CD4Treg, CD4Tcon, and CD8 T-cell subsets revealed that the expression of PD-1 was significantly decreased across all examined T-cell subsets at the time of transplant (0 months) and 1, 2, 3, and 6 months after HSCT (Figure 5), despite a median time from last PD-1 treatment to transplant of 148 days in this subgroup (range, 7-260).
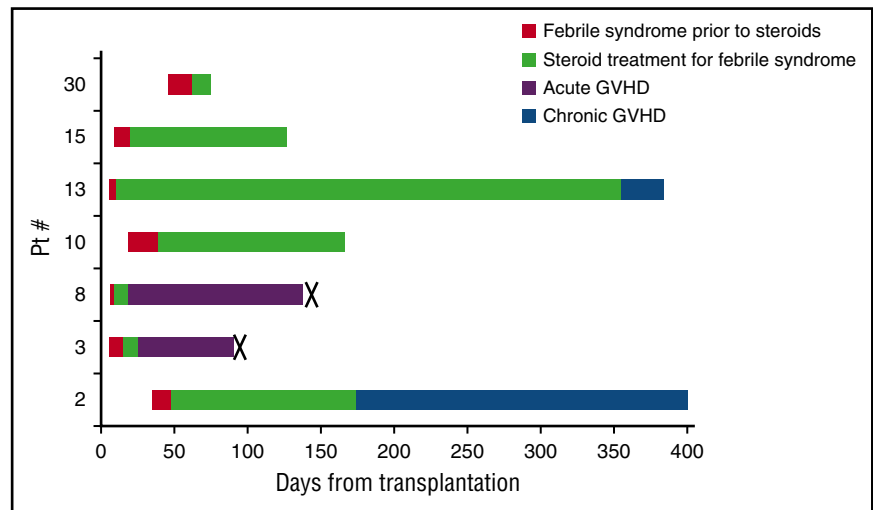
Total leukocyte donor chimerism values were slightly but significantly lower in the control group compared with the PD-1 cohort at 30 days after HSCT (median donor chimerism 95% [range 34% to 100%] vs 99% [range 0% to 100%], $p = .036$). A similar trend was seen for day 30 CD3 chimerism (75% [range 6% to 100%] vs 87% [range 1% to
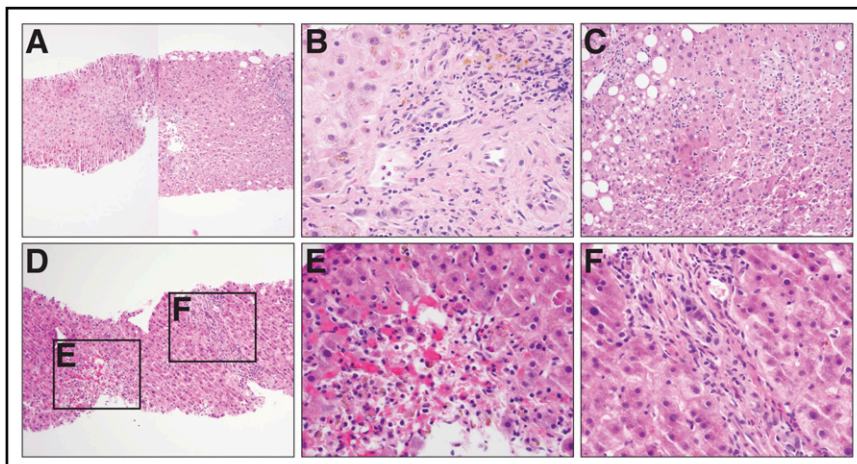
Figure 2. Liver biopsy from patient with acute liver failure after HSCT compared with example cases. (A) Example case, low power image of GVHD (left) with minimal lobular inflammation in contrast to IPI-associated hepatitis (right) showing pronounced lobular hepatitis (original magnification ×50; hematoxylin and eosin stain). (B) Example case, high power view of a portal tract in a patient with GVHD exhibiting cholestasis and bile duct injury, evidenced by cells with nuclear pleomorphism, eosinophilic cytoplasm, and intracytoplasmic vacuoles (original magnification ×400; hematoxylin and eosin stain). (C) Example case, high power image of IPI-associated hepatitis exhibiting a pan-lobular, predominantly lymphocytic infiltrate with spotty hepatocellular necrosis (original magnification ×200; hematoxylin and eosin stain). (D-F) Patient case demonstrating striking centrizonal hemorrhage and hepatocellular dropout as well as bile duct injury. (D) Original magnification ×50; hematoxylin and eosin stain. (E) Original magnification ×400; hematoxylin and eosin stain. (F) Original magnification ×400; hematoxylin and eosin stain.

Figure 3. Febrile syndrome: time course among affected patients. Seven patients (18%) developed prolonged prolonged noninfectious febrile syndromes shortly after HSCT. They received allografts from 2 MMURD, 2 MURD, 2 haploidentical donors (haplo), and 1 MRD. Five grafts were PB and 2 were BM. (Patient 2: PB graft, MMUD; patient 3: PB graft, MMUD; patient 8: PB graft, MUD; patient 10: BM graft, haplo; patient 13: PB graft, MUD; patient 15: PB graft, MRD; patient 30: BM graft, haplo.) Patient 10 and 30 received PTCY. Red, febrile syndrome prior to steroids; green, treatment with steroids for febrile syndrome; purple, acute GVHD; blue, chronic GVHD; X, death. Both deaths were from complications of severe acute GVHD, described in detail in Table 2.


100%], $p=.40$). At 100 days, both total leukocyte and CD3 donor chimerism values were significantly lower in the control cohort compared with PD-1 cohort (total leukocyte 95% [range 15% to 100%] vs 100% [range 60% to 100%], $p = .027$; CD3 64% [range 25% to 100%] vs 97% [range 56% to 100%], $p = .015$).

Discussion

Preclinical studies have suggested that immune activation from checkpoint blockade could lead to both increased immune-related toxicity and antitumor activity.[9-11] Our results strongly support the

hypothesis that prior checkpoint blockade affects HSCT outcomes, based on several aspects of this cohort. In terms of toxicity, we observed a higher than expected rate of early severe transplant-related complications. Although the overall incidences of acute and chronic GVHD did not appear very different from that expected in a population of patients undergoing T-cell replete RIC HSCT for lymphoma, the incidence of grade 4 acute GVHD appears higher than prior studies (13% vs 3% to 4%)[18,19]; in addition, the 3 patients who succumbed to GVHD developed a particularly rapid and virulent form of acute GVHD, which is rare after RIC HSCT. These early deaths prompted a US Food and Drug Administration label warning for use of HSCT after prior PD-1 blockade. In further support of the possible role of prior checkpoint blockade, the liver biopsy of one of the patients with fatal
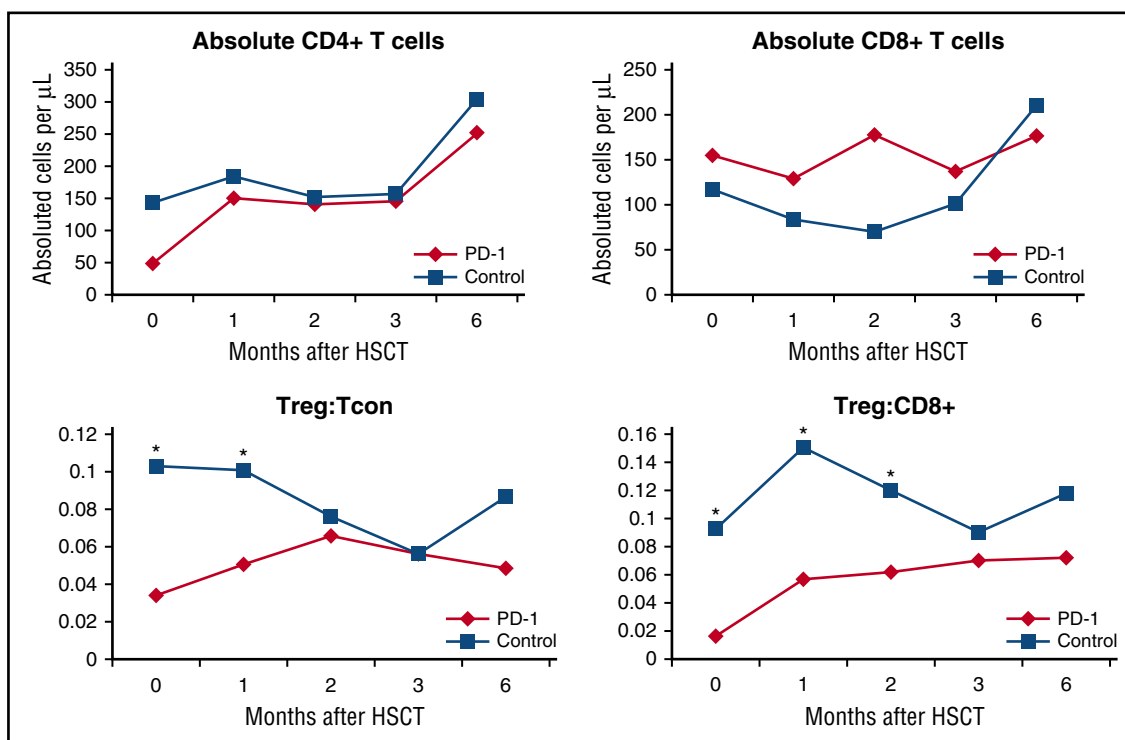
Figure 4. Immune reconstitution analysis of T-cell subsets. T-cell subsets were quantified from blood samples from the PD-1 and control cohorts on day 0 (baseline) as well as 1, 2, 3, and 6 months after HSCT. Tcon, conventional CD4 cells (*$P < .05$); Treg, T-regulatory cell.
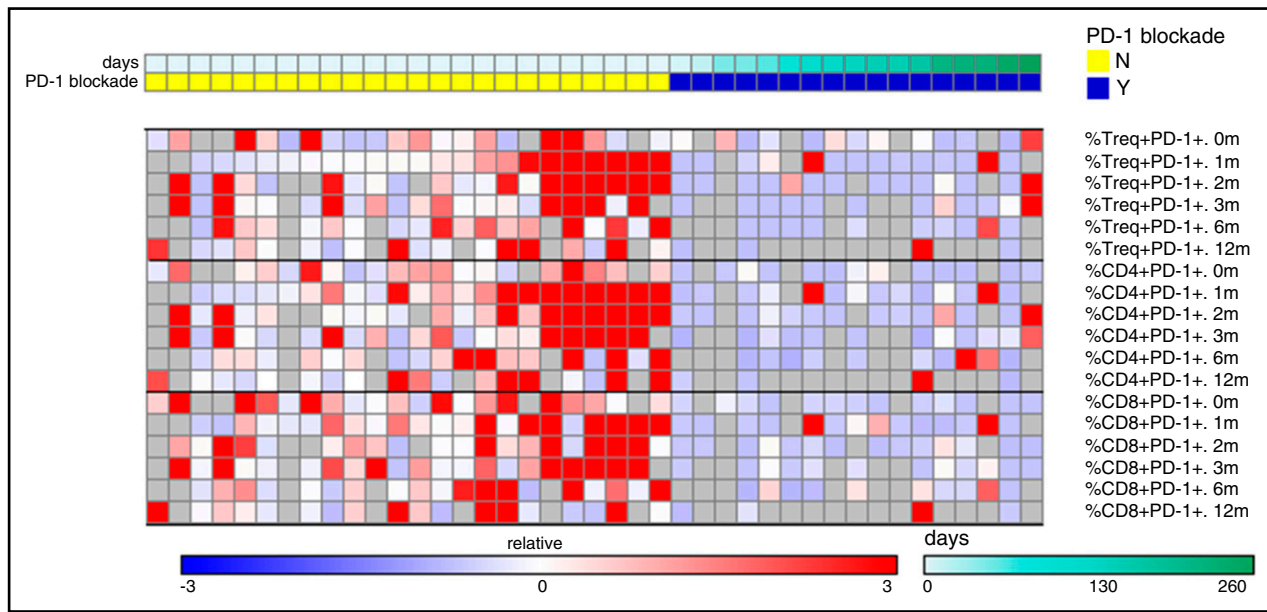
Figure 5. PD-1 expression among case and control patients displayed as heatmap. PD-1 expressing cells were quantified at the time of HSCT (0 months) and at multiple post-HSCT time points (1, 2, 3, 6, and 12 months after HSCT) in a control cohort (left columns, yellow top bar) and in patients previously treated with PD-1 (right columns, blue top bar). Each column represents an individual patient. The PD-1 cohort is arranged by time from last PD-1 treatment to HSCT (see legend). The percentage of PD-1$^+$ cells among various T-cell subsets is displayed as a heatmap where blue, white, and red represent low-, intermediate-, and high-subset frequency, respectively. Gray indicates missing value. Values in each row were subtracted from row median and divided by row median absolute deviation. Treg, T-regulatory cells; Tcon, conventional CD4 cells. PD-1 expression was significant lower on Treg cells, Tcon cells, and CD8$^+$ cells at 0, 1, 2, 3, and 6 months. N, no; Y, yes.

GVHD (Figure 2) provides histologic evidence of both GVHD and drug-induced hepatitis, strongly suggesting that prior checkpoint blockade may have magnified the severity of liver injury. In addition, severe SOS is a rare event in the RIC setting, and the rate of severe SOS in this cohort appeared greater than that seen in a prior large retrospective series of RIC allogeneic transplants (8% vs 2.1%).[20] We also report an atypical noninfectious febrile syndrome that occurred in a sizeable minority of patients. The majority of these patients did not meet criteria for engraftment syndrome and required much longer courses of steroid treatment than is typically seen in engraftment syndrome. The syndrome also appeared to be distinct from reports of cytokine-release syndrome in patients receiving PTCY after a haploidentical PBSC. None of the 7 patients we report received PTCY after a haploidentical PBSC, and the onset of fevers is delayed in our cohort (median, 15 days; range, 6-47 days) compared with those reported with cytokine-release syndrome, which occurred within 72 hours of HSCT.[21] As many of these patients went on to develop GVHD, this febrile syndrome may lie on the same immunologic/inflammatory spectrum as GVHD. Prospective assessment with measurements of cytokines and immune activation is needed to better define this phenomenon.

Despite these toxicities, the rate of NRM was not appreciably higher than previously published series in similar patient populations.[22-25] Furthermore, HSCT after PD-1 blockade may be associated with lower than expected relapse rates. CIR at 1 year in this study compares favorably to that expected for this cohort based on the Disease Risk Index mix of patients (14% vs 26%).[26] Among the HL subgroup, the 1-year relapse rate appears lower than that observed in many historical series of HL patients undergoing RIC allogeneic transplant (16% vs 26% to 41%)[18,19,22,24,27]; however, patients in most of these series likely had a higher disease burden at the time of HSCT compared with our cohort. Rates of relapse were similar to that seen in 1 small phase 2 trial of RIC for HL in which patients had a comparable rate of CR prior to transplant.[28] Clearly longer-term follow-up of larger populations is necessary (which is ongoing, as we continue to expand this

collaboration and follow the patients included). Nonetheless, our results at least lend credence to a possible accentuation of GVT by prior PD-1 blockade.

Immune profiling of circulating T-cell subsets also supports an immunologic difference in patients treated with PD-1 blockade. Although it was not possible to create a control cohort for this analysis that was perfectly matched with respect to all possible salient characteristics (eg, age, disease, sex, donor, graft source, GVHD prophylaxis), we observed clear patterns of differences in immune reconstitution after PD-1 exposure that warrant additional investigation. Specifically, the increased rates of early immune complications and potentially decreased relapse rates may be due to imbalanced T-cell recovery after transplantation. Prior checkpoint blockade therapy did not appear to affect the overall recovery of CD4 or CD8 T cells after transplant, but patients treated with anti-PD-1 mAbs had a decreased Treg:CD4Tcon ratio at 0 and 1 months after transplant and a decreased Treg:CD8 ratio at 0, 1, and 2 months after HSCT when compared with matched controls (Figure 4). In prior studies, a ratio of Treg:CD4Tcon cells of <9% predicted both increased incidence and severity of acute GVHD, and the ratio was 3% to 5% in our PD-1 cohort vs 10% to 11% in controls.[29,30] In addition, decreased Treg:CD4Tcon ratios may also predict an augmented GVT effect as observed in a trial of IPI for relapse after HSCT where there was a trend among clinical responders of lower Treg:CD4Tcon.[13]

Notably, the immune profiling results also demonstrate severe and persistent depletion of PD1$^+$ T cells in patients exposed to prior PD-1 blockade, a finding that to our knowledge has not been published previously. Decreased PD-1 expression could contribute to the relative decrease in Treg cell populations, as the PD-1/PD-L1 pathway appears to be important in the conversion of type 1 T helper cells to Treg cells and maintenance of Treg populations.[31,32] Of particular practical relevance, the depletion of PD-1$^+$ T cells was strongly apparent despite a median interval from PD-1 blockade to HSCT of over 4 months and persisted for at least 6 months after HSCT. Chimerism data confirm that prior PD-1 inhibition does not negatively affect engraftment; moreover, the high

T-cell chimerism among PD-1–pretreated patients confirms that the differences we observed in PD-1 expression and T-cell subsets must indeed involve donor T cells. For patients with a short interval between PD-1 therapy and HSCT, residual circulating antibodies may explain decreased PD-1 detection early after transplant; however, the mechanism of prolonged decreased PD-1 expression after HSCT is not clear. The PD-1 pathway is critical for both positive and negative selection of T cells in the thymus and is necessary for development of systemic self-tolerance.[33-36] As such, previous PD-1 blockade could have long-lasting effects on the thymus, resulting in altered PD-1 expression and imbalanced processing of the large number of T cells infused during HSCT. In addition, PD-1 blockade may alter posttransplant immune reconstitution through its effects on circulating cytokines and antigen-presenting cells.[10,11] Alternatively, the pattern of prolonged PD-1 depletion in combination with evidence of immunelike liver toxicity (Figure 2) in a patient who had last received checkpoint blockade >10 weeks earlier might suggest that PD-1 inhibitors have longer effective half-lives than measured by pharmacokinetic analyses in blood (roughly 4 weeks).[5,37] Regardless of the mechanism of PD-1 depletion, our results imply that anti-PD-1 therapy creates a long-lasting disturbance in the composition of circulating T-cell populations. These findings would explain the absence of any apparent association between the time interval from PD-1 to transplantation and early toxicity and suggests that the use of a window period to delay HSCT for even several months after PD-1 therapy may not mitigate the impact of this therapy on HSCT outcomes.

Although the associations we examined are only hypothesis generating in nature and in no way intended to support definitive conclusions, they suggest that patients receiving PD-1 blockade who are considering allogeneic HSCT could possibly benefit from receiving at least 8 doses of a PD-1 inhibitor, when clinically possible; this hypothesis should be validated in future studies. In addition, we observed a dramatic decrease in the rate of severe acute GVHD among patients receiving BM compared with PBSC grafts. This pattern has been reported previously, but to a lesser degree than our preliminary findings.[38] Although rates of NRM and PFS were not significantly reduced among recipients of BM grafts, this approach may be beneficial for GVHD control. We also noted significantly higher rates of acute GVHD in patients who received combined therapy with IPI and PD-1 blockade prior to HSCT, suggesting that HSCT in such patients likely carries increased risk and should be performed cautiously. Additional studies of a larger patient cohort are clearly needed to validate and refine these findings.

We acknowledge that this analysis has several limitations. In order to report all known cases of allogeneic HSCT after prior PD-1 therapy, we included patients regardless of their transplant regimens. The heterogeneity of transplant strategies makes interpretation of subset analyses more challenging. Although we directly contacted all centers involved in the early phase studies of PD-1 inhibitors for lymphoma, the growing use of PD-1 blockade on- and off-label makes it impossible to include all patients worldwide who received HSCT after PD-1 blockade. We therefore acknowledge the possibility of selection bias in reporting patient outcomes, although our recruitment strategy, which was center based rather than patient based, should effectively minimize this bias. Finally, the control cohort for the immune reconstitution analysis was older and could not be perfectly matched for all important transplant-related variables, as discussed above.

There are currently many questions regarding the role and appropriateness of HSCT after PD-1 blockade. The sample size and follow-up of this study are limited, which precludes examination of long-term outcomes and limits our ability to study predictive markers of efficacy or toxicity. Despite these limitations, with a 1-year PFS of over 75% in a population of heavily pretreated patients with advanced disease, our study does demonstrate that HSCT after PD-1 blockade is feasible in appropriately selected patients and may be associated with increased immune toxicity but also good disease control. We conclude that prior PD-1 blockade should not be considered a contraindication to HSCT in this patient population.

Acknowledgments

Authorship

Contribution: R.W.M. designed the research, collected and analyzed data, and wrote the manuscript; H.T.K. designed the research, collected and analyzed data, and edited the manuscript; P.L.Z., C.C.-S., S.M.A., M.-A.P., A.A., A.S.H., R.H., T.M., N.D., W.L., A.T.-B., S.F., A.S.-B., P.-S.R., H.L.W., L.C., A. Santoro, J.R., S.C.B., A. Srivastava, H.K., E.P., M.C., C.R., V.T.H., and J.H.A. collected data and edited the manuscript; V.B. edited the manuscript; R.J.S. designed the research, collected data, and edited the manuscript; and P.A. designed the research, collected and analyzed data, and edited the manuscript.

Conflict-of-interest disclosure: P.L.Z. received consulting fees from Roche, Celegene, MSK, Janssen Pharmaceuticals, and AbbVie Inc. C.C.-S. received consulting fees from Boehringer Ingelheim, research support from Rhizen Pharmaceuticals, and travel reimbursement from Takeda and Janssen Pharmaceuticals. S.M.A. received institutional research funding from BMS, Merck, Seattle Genetics, and Affimed Pharmaceuticals. M.-A.P. received consulting fees from Merck, Incyte, and Seattle Genetics. R.H. received an honorarium and consulting fees from BMS. P.-S.R. received an honorarium from Sanofi Aventis, consulting fees from Amgen, and travel reimbursement from Pierre Fabre. A. Santoro received consulting fees from Amgen, Takeda, Novartis, and ArQule. V.B. received research funding from Alopexx, Oxis Inc, and Novartis. J.H.A. received royalties from Wiley-Thomas Hematopoietic Cell Transplantation. R.J.S. received an honorarium from Gilead, consulting fees from Juno and Jazz Pharmaceutical, and travel reimbursement from Gilead, Kiadis, and Jazz Pharmaceuticals. He served on a supervisory board at Kiadis. P.A. received consulting fees from BMS, Merck, and Infinity Pharmaceuticals, and institutional research support from BMS, Merck, Affimed, Pfizer, Roche, Tensha Therapeutics, Sequenta, Otsuka, and Sigma Tau. The remaining authors declare no competing financial interests.

Correspondence: Philippe Armand, Dana-Farber Cancer Institute, 450 Brookline Ave, Boston MA 02215; e-mail: parmand@partners.org.

References

1. Ahmadzadeh M, Johnson LA, Heemskerk B, et al. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. *Blood*. 2009;114(8): 1537-1544.

2. Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med*. 2002;8(8):793-800.

3. Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med*. 2015;372(4): 311-319.

4. Armand P, Shipp MA, Ribrag V, et al. Programmed death-1 blockade with pembrolizumab in patients with classical Hodgkin lymphoma after brentuximab vedotin failure [published online ahead of print 27 June 2016]. *J Clin Oncol*. 2016;JCO673467.

5. Highlights of Prescribing Information - Nivolumab. *US Food Drug Adm*. 2014;1-20.

6. Ding W, Dong H, Call TG, et al. PD-1 blockade with pembrolizumab (MK-3475) in relapsed/ refractory CLL including richter transformation: an early efficacy report from a phase 2 trial (MC1485) [abstract]. *Blood*. 2015;126(23). Abstract 834.

7. Lesokhin AM, Ansell SM, Armand P, et al. Nivolumab in patients with relapsed or refractory hematologic malignancy: preliminary results of a phase Ib study. *J Clin Oncol*. 2016;34(23): 2698-2704.

8. Hoos A, Eggermont AMM, Janetzki S, et al. Improved endpoints for cancer immunotherapy trials. *J Natl Cancer Inst*. 2010;102(18): 1388-1397.

9. Michonneau D, Sagoo P, Breart B, Garcia Z, Celli S, Bousso P. The PD-1 axis enforces an anatomical segregation of CTL activity that creates tumor niches after allogeneic hematopoietic stem cell transplantation. *Immunity*. 2016;44(1):143-154.

10. Saha A, Aoyama K, Taylor PA, et al. Host programmed death ligand 1 is dominant over programmed death ligand 2 expression in regulating graft-versus-host disease lethality. *Blood*. 2013;122(17):3062-3073.

11. Blazar BR, Carreno BM, Panoskaltsis-Mortari A, et al. Blockade of programmed death-1 engagement accelerates graft-versus-host disease lethality by an IFN-gamma-dependent mechanism. *J Immunol*. 2003;171(3):1272-1277.

12. Herbaux C, Gauthier J, Brice P, et al. Nivolumab is effective and reasonably safe in relapsed or refractory Hodgkin's lymphoma after allogeneic hematopoietic cell transplantation: a study from the Lysa and SFGM-TC [abstract]. *Blood*. 2015; 126(23). Abstract 3979.

13. Davids MS, Kim HT, Bachireddy P, et al. Ipilimumab for patients with relapse after allogeneic transplantation. *N Engl J Med* 2016; 375(2):143-153.

14. Alho AC, Kim HT, Chammas MJ, et al. Unbalanced recovery of regulatory and effector T cells after allogeneic stem cell transplantation contributes to chronic GVHD. *Blood*. 2016;127(5): 646-657.

15. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat*. 1988;16:1141-1154.

16. Spitzer TR. Engraftment syndrome following hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2001;27(9):893-898.

17. Maiolino A, Biasoli I, Lima J, Portugal AC, Pulcheri W, Nucci M. Engraftment syndrome following autologous hematopoietic stem cell transplantation: definition of diagnostic criteria. *Bone Marrow Transplant*. 2003;31(5):393-397.

18. Sureda A, Robinson S, Canals C, et al. Reduced-intensity conditioning compared with conventional allogeneic stem-cell transplantation in relapsed or refractory Hodgkin's lymphoma: an analysis from the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation. *J Clin Oncol*. 2008;26(3):455-462.

19. Robinson SP, Sureda A, Canals C, et al; Lymphoma Working Party of the EBMT. Reduced intensity conditioning allogeneic stem cell transplantation for Hodgkin's lymphoma: identification of prognostic factors predicting outcome. *Haematologica*. 2009;94(2):230-238.

20. Carreras E, Díaz-Beyá M, Rosiñol L, Martínez C, Fernández-Avilés F, Rovira M. The incidence of veno-occlusive disease following allogeneic hematopoietic stem cell transplantation has diminished and the outcome improved over the last decade. *Biol Blood Marrow Transplant*. 2011; 17(11):1713-1720.

21. Abboud R, Keller J, Slade M, et al. Severe cytokine-release syndrome after T cell-replete peripheral blood haploidentical donor transplantation is associated with poor survival and anti-IL-6 therapy is safe and well tolerated. *Biol Blood Marrow Transplant*. 2016;22(10): 1851-1860.

22. Armand P, Kim HT, Sainvil M-M, et al. The addition of sirolimus to the graft-versus-host disease prophylaxis regimen in reduced intensity allogeneic stem cell transplantation for lymphoma: a multicentre randomized trial. *Br J Haematol*. 2016;173(1):96-104.

23. Kanate AS, Mussetti A, Kharfan-Dabaja MA, et al. Reduced-intensity transplantation for lymphomas using haploidentical related donors vs HLA-matched unrelated donors. *Blood*. 2016;127(7): 938-947.

24. Armand P, Kim HT, Ho VT, et al. Allogeneic transplantation with reduced-intensity conditioning for Hodgkin and non-Hodgkin lymphoma: importance of histology for outcome. *Biol Blood Marrow Transplant*. 2008;14(4):418-425.

25. Ghosh N, Karmali R, Rocha V, et al. Reduced-intensity transplantation for lymphomas using haploidentical related donors versus HLA-matched sibling donors: a Center for International Blood and Marrow Transplant Research Analysis. *J Clin Oncol*. 2016;34(26):3141-3149.

26. Armand P, Kim HT, Logan BR, et al. Validation and refinement of the Disease Risk Index for allogeneic stem cell transplantation. *Blood*. 2014; 123(23):3664-3671.

27. Sureda A, Canals C, Arranz R, et al. Allogeneic stem cell transplantation after reduced intensity

conditioning in patients with relapsed or refractory Hodgkin's lymphoma. Results of the HDR-ALLO study—a prospective clinical trial by the Grupo Español de Linfomas/Trasplante de Médula Osea (GEL/TAMO) and the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation. *Haematologica*. 2012;97(2): 310-317.

28. Anderlini P, Saliba RM, Ledesma C, et al. Gemcitabine, fludarabine, and melphalan for reduced-intensity conditioning and allogeneic stem cell transplantation for relapsed and refractory Hodgkin lymphoma. *Biol Blood Marrow Transplant*. 2016;22(7):1333-1337.

29. Pang N, Duan X, Jiang M, et al. Reconstitution and clinical significance of T cell subsets in the early stage after related HLA-mismatched peripheral blood hematopoietic SCT without T-cell depletion in vitro. *Int J Clin Exp Pathol*. 2015;8(8): 8892-8901.

30. Fujioka T, Tamaki H, Ikegame K, et al. Frequency of CD4(+)FOXP3(+) regulatory T-cells at early stages after HLA-mismatched allogeneic hematopoietic SCT predicts the incidence of acute GVHD. *Bone Marrow Transplant*. 2013; 48(6):859-864.

31. Amarnath S, Mangus CW, Wang JCM, et al. The PDL1-PD1 axis converts human TH1 cells into regulatory T cells. *Sci Transl Med*. 2011;3(111): 111ra120.

32. Asano T, Kishi Y, Meguri Y, et al. PD-1 signaling has a critical role in maintaining regulatory T cell homeostasis; implication for Treg depletion therapy by PD-1 blockade [abstract]. *Blood*. 2015;126(23). Abstract 848.

33. Keir ME, Latchman YE, Freeman GJ, Sharpe AH. Programmed death-1 (PD-1):PD-ligand 1 interactions inhibit TCR-mediated positive selection of thymocytes. *J Immunol*. 2005; 175(11):7372-7379.

34. Thangavelu G, Parkman JC, Ewen CL, Uwiera RR, Baldwin TA, Anderson CC. Programmed death-1 is required for systemic self-tolerance in newly generated T cells during the establishment of immune homeostasis. *J Autoimmun*. 2011; 36(3-4):301-312.

35. Blank C, Brown I, Marks R, Nishimura H, Honjo T, Gajewski TF. Absence of programmed death receptor 1 alters thymic development and enhances generation of CD4/CD8 double-negative TCR-transgenic T cells. *J Immunol*. 2003;171(9):4574-4581.

36. Daley SR, Hu DY, Goodnow CC. Helios marks strongly autoreactive CD4+ T cells in two major waves of thymic deletion distinguished by induction of PD-1 or NF-κB. *J Exp Med*. 2013;210(2):269-285.

37. Highlights of Prescribing Information - Pembrolizumab. *US Food Drug Adm*. 2014;1-16.

38. Eapen M, Logan BR, Confer DL, et al. Peripheral blood grafts from unrelated donors are associated with increased acute and chronic graft-versus-host disease without improved survival. *Biol Blood Marrow Transplant*. 2007;13(12):1461-1468.