Clinical Cancer Research

Ipilimumab plus Lenalidomide after Allogeneic and Autologous Stem Cell Transplantation for Patients with Lymphoid Malignancies

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Abstract

Purpose: Prevention or treatment of relapsed lymphoid malignancies after hematopoietic stem cell transplantation (HSCT) requires novel strategies. We hypothesized that antitumor–cell responses could be enhanced by the addition of lenalidomide to the cytotoxic T-lymphocyte–associated protein 4 inhibitor ipilimumab.

Experimental Design: We conducted a phase II investigator-initiated trial to assess the safety and activity of ipilimumab and lenalidomide in patients with lymphoid malignancies that relapsed after allogeneic HSCT and in high-risk patients after autologous HSCT. Patients received 10 mg of oral lenalidomide daily for 21 days followed by intravenous ipilimumab at 3 mg/kg bodyweight. The regimen was repeated 4 weeks later for a total of four treatments.

Results: We enrolled 17 patients (10 allogeneic and seven autologous transplant recipients). Immune-mediated toxicity was limited to one patient with asymptomatic hypothyroidism and one with dermatitis in the allogeneic and autologous groups, respectively. One allogeneic transplant recipient had a flare of prior GVHD while taking lenalidomide that precluded further treatment. All others finished treatment without GVHD. Four of 10 patients in the allogeneic group had complete responses (three of which were durable at 19+, 21+, and 32+ months), and three had partial responses. The disease in six of seven patients in the autologous group remains in remission. The groups had similar immune responses, including a two- to threefold increase in inducible $ICOS^+CD4^+FoxP3^-$ T-cell number.

Conclusions: Our early-phase data suggested that ipilimumab plus lenalidomide is well tolerated after HSCT. Adverse events did not differ significantly between the allogeneic and autologous groups. *Clin Cancer Res; 24(5); 1011–8. ©2017 AACR.*

Introduction

Hematopoietic stem cell transplantation (HSCT) can produce long-term disease control and cure of various lymphoid malignancies (1, 2). However, despite improvements in conditioning regimens and supportive care (3), relapse remains a risk in 40% to 80% of transplant recipients, with a disappointing prognosis (4, 5). To date, no therapy has improved overall survival durations after HSCT failure, and novel strategies are needed to prevent relapse in high-risk patients after autologous HSCT. Advances in the understanding of immunoregulatory mechanisms have led to the design of new strategies to generate more effective antitumor immune responses in these patients.

A novel therapeutic approach consists of targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4), one of the primary immune checkpoint molecules (6, 7). Inadequate T-cell antitumor responses may be one mechanism triggering failure of HSCT. The inhibitory immune checkpoint CTLA-4 competes with the costimulatory receptor CD28 for the B7 ligands CD80 and CD86, which leads to abrogation of T-cell responses (8). CTLA-4 blockade therefore functions to remove inhibitory signals and enhance antitumor T-cell responses (7).

Ipilimumab is a fully human antibody against CTLA-4 (9, 10) that has demonstrated antitumor activity in patients with various cancers (11), including lymphomas (12). In a randomized trial, patients with advanced melanoma exhibited a dose-dependent increase in immune-related adverse events with increasing ipilimumab doses, as the incidence of grade 3 to 4 events was 3.5-fold higher in patients receiving it at 10 mg/kg compared with those receiving it at 3 mg/kg (13). Researchers confirmed this finding in two clinical trials using ipilimumab in patients with relapsed hematologic malignancies after allogeneic HSCT (14, 15).

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Note: Prior presentation: Presented in part at the American Society of Hematology Annual Meeting, December 2–6, 2016, San Diego, CA (Abstract # 1164).

ClinicalTrials.gov identifier: NCT01919619.

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doi: 10.1158/1078-0432.CCR-17-2777

Translational Relevance

Clinical trials using ipilimumab in patients with relapsed hematologic malignancies after allogeneic hematopoietic stem cell transplantation (HSCT) have disappointing results when the drug is administered at the conventional dose of 3 mg/kg intravenously. Higher doses are associated with a higher risk of immune-mediated toxicity, GVHD, and death. There is a paucity of information about the use of ipilimumab after autologous HSCT to decrease the risk of relapse in high-risk patients. On the basis of its immunomodulatory properties, we hypothesized that lenalidomide can harness the immune effect of ipilimumab to combat lymphomas, at a standard dose with a reduced risk of toxic effects. In this trial, we show that this strategy is safe, with no severe treatment-related toxicities, minimal immune-mediated toxicity, and no GVHD. Surprisingly, our preliminary data indicate similar immune responses in allogeneic and autologous transplant recipients. A greater than expected and durable observed response rate of 70% with just two doses of ipilimumab in patients who relapsed after allogeneic HSCT suggests that this finding may be related to the addition of lenalidomide to ipilimumab. This impact is also suggested by the additive increased numbers of inducible costimulator+CD4$^+$ T cells by lenalidomide. Findings from our study support further investigation of this combination in this setting.

Specifically, Davids and colleagues observed a 23% risk of GVHD and immune-related events when they gave patients 10 mg/kg of ipilimumab (15).

Combination strategies for treatment of lymphoid malignancies with targeted agents, such as the addition of lenalidomide to ipilimumab, may result in the release of tumor antigens from cancerous lymphoid cells, which then become presented by antigen-presenting cells to tumor-specific T cells and enhance the activity of ipilimumab (16). Moreover, lenalidomide is an immunomodulatory agent that can enhance immune responses both *in vitro* (17, 18) and in patients with advanced tumors (19, 20). Lenalidomide use has been shown to augment vaccine responses and endogenous antitumor immunity in patients with relapsed multiple myeloma (21). We therefore hypothesized that lenalidomide can enhance the immunomodulatory effect of ipilimumab by harnessing the immune system to eradicate residual disease and decrease the risk of recurrence after autologous HSCT, and combat relapsed lymphoma after allogeneic HSCT with a reduced risk of toxic effects and GVHD at a standard dose of 3 mg/kg. The primary objective of this study was to assess the safety of treatment with lenalidomide alternating with ipilimumab after autologous and allogeneic HSCT in patients with lymphoid malignancies.

Patients and Methods

Study design and eligibility criteria

Patients at our institution who underwent allogeneic HSCT were included if they had persistent or relapsed lymphoid disease after their transplant, had engrafted donor cells, and had been off immunosuppression for 3 to 4 weeks without symptoms of GVHD.

In the absence of the graft-versus-malignancy effect to prevent disease relapse after autologous transplantation, and the lack of risk for GVHD, patients with lymphoma who underwent autologous HSCT were included in this study anytime within 6 months of undergoing their transplantation if they had no signs of progression.

Other inclusion criteria assessed at study entry for both autologous and allogeneic groups included: age 18 to 70 years; an Eastern Cooperative Oncology Group performance status score of 0 to 2; adequate liver (bilirubin and liver enzyme concentrations up to two times the upper limit of normal), renal (serum creatinine <1.6 mg/dL and creatinine clearance $\geq$30 mL/minute according to the Cockcroft–Gault equation), cardiac (ejection fraction $\geq$45%), and pulmonary (diffusing capacity of the lung for carbon monoxide $\geq$40% of predictive value) function; no active infections; an absolute neutrophil count of at least 1.5×10^9 cells per L; and a platelet count of at least 75×10^9 cells per L in the peripheral blood.

This investigator-initiated pilot trial (ClinicalTrials.gov number NCT01919619) was conducted at The University of Texas MD Anderson Cancer Center (Houston, TX) from February 2014 to July 2016. The study was approved by our Institutional Review Board and was conducted in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent.

Procedures

Patients received lenalidomide for 21 days (cycle 1), then within 3 days after the last dose of lenalidomide, one dose of ipilimumab was administered (cycle 2). Thirty days after the cycle 2 ipilimumab dose, the treatment was repeated with lenalidomide (cycle 3) followed by ipilimumab (cycle 4; Fig. 1). This schedule was chosen for the purpose of avoiding overlapping toxicity between the two drugs. The starting and highest allowable dose of lenalidomide in this study was 10 mg given orally daily. This dose was chosen in accordance with other published reports

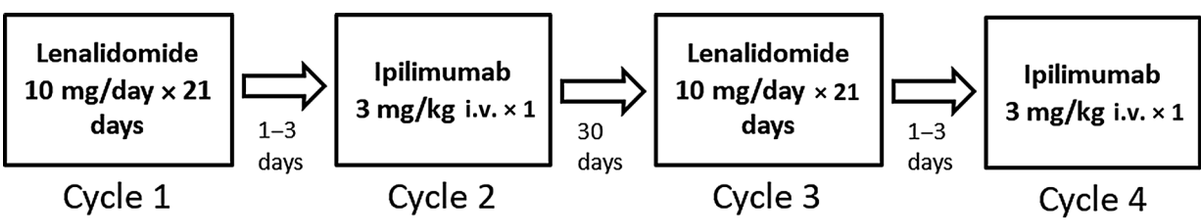

Figure 1.
Ipilimumab and lenalidomide dosing schedule after HSCT.

using maintenance lenalidomide after autologous HSCT (22). Higher doses of lenalidomide have been associated with unacceptable toxicity after allogeneic HSCT (23). Dose reduction to 5 mg within patient was permitted for later cycles based on clinical practice. The ipilimumab dose was fixed at 3 mg/kg actual weight and given intravenously over 90 minutes.

Acute and chronic GVHD in the study patients were assessed according to standard criteria (24, 25). Patients underwent evaluation for toxicity at the end of each cycle.

Clinical responses were scored using standard criteria for patients with lymphoma (26). Responses in patients with chronic lymphocytic leukemia were scored according to the recommendations of the NCI-Sponsored Working Group (27). Disease extent was further assessed by CT scans of the chest, abdomen, and pelvis. In addition, functional imaging with ^{18}F-fluoro-deoxyglucose PET scans was repeated during follow-ups in patients with avid scans at study entry. Initial responses were assessed after 12 weeks of induction therapy and every 12 weeks for 2 years, and then every 6 months thereafter.

Peripheral blood mononuclear cells (PBMCs) obtained from the study patients were analyzed by members of the MD Anderson Immunotherapy Platform. Blood samples were drawn for immunophenotypic analysis of PBMCs at baseline and before and after administration of lenalidomide and ipilimumab. Following whole blood collection, PBMCs were separated using a Ficoll gradient at room temperature.

Multiparametric flow cytometry analysis of PBMCs was performed using fluorescently conjugated mAbs recognizing: CD4 AF532 (SK3, eBioscience), CD3 PerCP-Cy5.5 (UCHT1, BioLegend), CD8 AF700 (RPA-T8, BD Biosciences), CD127 BV711 (HIL-7R-M21, BD Biosciences), ICOS PE-Cy7 (ISA-3, eBioscience), and FoxP3 PE-e610 (PCH101; eBioscience). Live/Dead fixable yellow stain was obtained from Thermo Fisher Scientific. Samples were run using an LSRFortessa (BD Biosciences) and analyzed using the FlowJo software program. After appropriate forward/side scatter and live cell gating, we determined the frequency of total $CD3^+$ T cells, $CD8^+$ T cells ($CD3^+CD8^+$), and CD4+ T cells ($CD3^+CD4^+$). ICOS expression was evaluated within $CD4^+$ effector T cells ($CD3^+CD4^+FoxP3^-$).

Statistical analysis

All patients who received the study drugs were included in safety and efficacy analyses. Both transplant groups were monitored separately for toxicity according to the Common Terminology Criteria for Adverse Events (version 4.0). A Bayesian sequential monitoring scheme was employed for interim safety monitoring, separately targeting a toxicity rate of less than 30% at 30 days following cycle 4 of treatment in both groups. Toxic effects were (i) any grade 4 hematologic toxic effect of a study drug that did not resolve within 2 weeks; (ii) any immune-mediated reaction to ipilimumab that required permanent cessation of administration of the drug; (iii) any other grade 3 to 5 organ toxic effect of a study drug; and (iv) grade 3 or 4 acute GVHD in the allogeneic transplant group.

Patients were monitored in cohorts of one separately by study group. We assumed a beta (0.6, 1.4) prior distribution for the toxicity rate in each group, which has a mean of 0.30 corresponding to the 30% target toxicity rate. Accrual into either study group would have been stopped if at any time during the study the probability that the toxicity rate at day 30 in that arm exceeded 0.30 was greater than 0.95.

Statistical significant differences in flow cytometry data, between allogeneic and autologous groups, were analyzed using nonparametric two-tailed tests: Wilcoxon signed-rank test (paired) and Mann–Whitney U test (unpaired). $P < 0.05$ was considered statistically significant.

Results

Allogeneic group

We enrolled a total of 10 patients in the allogeneic HSCT group. Patient characteristics at study entry are listed in Tables 1 and 2A. All patients had biopsy-proven relapsed aggressive ($n = 6$) or indolent ($n = 4$) lymphoid malignancies after receiving matched unrelated ($n = 7$), matched related ($n = 2$), or syngeneic ($n = 1$) transplants.

The median time since HSCT and study entry was 29 months in patients with aggressive histologies. The median time that these patients were off immunosuppressive therapy prior to study enrollment was 1 month. This included two patients who had prior extensive GVHD and were off systemic immunosuppressive therapy for 4 and 6 weeks, respectively.

A longer median time since HSCT and study entry of 52 months was observed in patients with indolent histologies. The median time that these patients were off immunosuppressive therapy prior to study enrollment was 36 months. This was expected, considering the delayed patterns of relapses that these patients experience after allogeneic HSCT compared with patients with aggressive lymphoma histologies. Furthermore, the standard therapeutic approach of donor lymphocyte infusions (DLIs) in these patients further contributed to this longer time interval between relapse after allogeneic HSCT to study entry. Patients with indolent histologies who were enrolled on this study have all received DLIs after their last recurrence prior study entry, with no clinical response.

Patients were heavily pretreated prior to their allogeneic HSCT. The median number of therapies received, outside their transplant, was 3. Prior autologous transplants failed in two patients, a prior syngeneic transplant failed in one patient, and two prior matched unrelated hematopoietic stem cell transplants failed in

Table 1. Baseline patient characteristics

Characteristics	Allogeneic ($n = 10$)	Autologous ($n = 7$)
Male sex, No. (%)	5 (50)	5 (71)
Median age (range) at enrollment, years	54.5 (44–66)	56 (33–68)
HCT-CI, No. (%)		
0–2	4 (40)	N/A
≥3	6 (60)	N/A
Donor type		
Unrelated	7 (70%)	N/A
Related	2 (20%)	N/A
Syngeneic	1 (10%)	N/A
Graft source		
Peripheral blood	10 (100%)	7 (100%)
Conditioning intensity		
Myeloablative	1 (10%)	7 (100%)
Nonmyeloablative	8 (80%)	
Reduced intensity	1 (10%)	
PET-positive before immunotherapy	4/7 (57%)	1 (14%)
Median (range) % donor T cells at SE	100 (31–100)	N/A
Prior GVHD cases		
Acute	0/9 (0%)	N/A
Chronic, extensive	4/9 (44%)	N/A

Abbreviations: HCT-CI, hematopoietic cell transplantation-comorbidity index; N/A, not applicable; SE, study entry.

Table 2A. Clinical outcomes in allogeneic hematopoietic stem cell transplant recipients

Patient No.	Histology	Therapies prior alloSCT	Response to lenalidomide and ipilimumab	Current status[a]
1	FL	2	CR	Alive, CR, 32 months
2	MCL	3	CR, 12 months	Alive, PD, 31 months
3	FL	3	PR, 5 months	Alive, PD, 28 months
4	THL	4, ASCT	CR	Alive, CR, 21 months
5	MCL	3, 2 prior alloSCT	PR	Dead, PR, 21 months
6	MCL	2, syngeneic SCT	CR	Alive, CR, 19 months
7	DLBCL	4, ASCT	PR	Alive, PD, 19 months
8	CLL	3	SD	Alive, PD, 18 months
9	CLL	2	SD	Alive, SD, 17 months
10	ALCL	7	NE	Alive, 3 months[b]

Abbreviations: ALCL, anaplastic large T-cell lymphoma; AlloSCT, allogeneic stem cell transplantation; ASCT, autologous stem cell transplantation; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; NE, not evaluable; PD, progressive disease; SCT, stem cell transplantation; THL, triple-hit lymphoma.

[a]Time from initiation of treatment.

[b]Lost follow-up after 3 months.

one patient. The median hematopoietic cell transplantation-comorbity index at study entry was 3 (range, 0–10).

Four patients had a history of extensive chronic GVHD. One case each involved the liver and gastrointestinal tract, skin and gastrointestinal tract, and skin and joints. The fourth patient had liver, skin, and mouth GVHD involvement. Steroid-based therapy was discontinued for this patient just 4 weeks prior to study entry.

Autologous group

We enrolled a total of seven patients in the autologous HSCT group. Their characteristics at study entry are listed in Tables 1 and 2B. All had high-risk disease, with three patients having double-/triple-hit lymphoma including one with relapsed disease who received four lines of prior therapy; two others received their transplants in first complete remission following completion of first-line therapy with a conventional regimen of rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP), rather than a more intensive first-line therapy. Two patients had Mantle cell lymphoma with an elevated proliferation index (Ki67 > 30%), including one patient who twice underwent prior autologous HSCT. One patient with Hodgkin disease experienced disease progression 1 month after standard first-line chemotherapy and then received salvage chemotherapy, with maintenance of stable disease (SD) prior to transplant. After transplantation, the patient had a positive PET imaging. One patient had follicular lymphoma grade 3 who initially presented with bulky disease and superior vena cava syndrome; this patient was refractory to R-CHOP, lenalidomide plus obinutuzumab, R-bendamustine; the patient then received R-GDP (gemcitabine, dexamethasone, cisplatin) prior to having a partial response (PR) along with maintenance of persistent PET positivity before transplantation.

Table 2B. Clinical outcomes in autologous hematopoietic stem cell transplant recipients

Patient No.	Histology	Prior therapies	Current status†
11	THL	1	Alive, CR, 38 months
12	DHL	1	Alive, PD, 28 months
13	MCL	3, 2 ASCT	Alive, CR, 27 months
14	HD	2, PET+ post-ASCT	Alive, CR, 17 months
15	DHL	4	Alive, CR, 15 months
16	MCL	1	Alive, CR, 14 months
17	FL	4	Alive, CR, 10 months

Abbreviations: ASCT, autologous stem cell transplantation; DHL, double-hit lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HD, Hodgkin disease; MCL, mantle cell lymphoma; PD, progressive disease; THL, triple-hit lymphoma.

[a]Time from initiation of treatment.

Safety

In the allogeneic group, all patients completed treatment with the exception of one patient who had a flare of chronic liver and mouth GVHD 1 week after starting the first cycle of lenalidomide. The patient's steroid-based therapy for chronic GVHD was tapered 3 weeks earlier. This precluded further treatment, and this patient was discontinued from the study without exposure to ipilimumab. The remaining nine patients finished the study treatment without any cases of GVHD. Grade 3 and 4 toxic effects (Table 3) were limited to four episodes of neutropenia (two at grade 3 and 2 at grade 4), requiring a reduction of the lenalidomide dose in the second cycle of lenalidomide in one patient to 5 mg. Immune-mediated toxicity was limited to one patient with asymptomatic grade 2 hypothyroidism. All toxic effects were resolved.

All seven patients in the autologous group finished their planned treatment. In this group, we observed three episodes of grade 4 neutropenia, requiring a reduction in the lenalidomide dose to 5 mg in one patient. The other two episodes occurred after ipilimumab use and resolved. Another patient had an incidental pulmonary embolism detected using routine computed tomographic imaging of the chest, which was managed with anticoagulation without any complications. One patient had immune-mediated dermatitis, which resolved promptly with steroid-based therapy.

Clinical outcomes

Four patients (40%) and three patients (30%) in the allogeneic group had complete responses (CRs) and PRs, respectively, after just two cycles of lenalidomide and two cycles of ipilimumab, resulting in an overall response rate of 70%. One patient was not evaluable for response, and two patients had SD. These responses were irrespective of the percentage of donor T cells in blood at the time of therapy initiation, as one patient had 31% donor cells and is now in continuous CR.

At a median follow-up time of 20.5 (range, 3–32) months, 90% of allogeneic patients are alive (Fig. 2A). CRs in three patients and a PR in one patient continued at 21, 19, 32, and 21 months, respectively, after initiation of therapy (Table 2A). The PR patient died due to an unrelated cause. Four other patients had recurrent or progressive disease after therapy initiation: one patient with SD experienced progression at 4.6 months; two patients with PRs

Table 3. Adverse events

Variable	No. of episodes	
	Allogeneic	Autologous
GVHD		
Chronic GVHD of liver, mouth	1	N/A
Immune-related adverse events		
Dermatitis, grade 2	—	1
Hypothyroid, grade 2	1	—
Hyperthyroid, grade 1	—	1
Other hematologic toxic effects		
Neutropenia		
Grade 4	2	3
Grade 3	2	1
Grade 2	2	2
Thrombocytopenia, grade 2	1	—
Anemia, grade 2	2	0
Other adverse events		
Pulmonary embolism, grade 3	—	1
Nausea, grade 2	1	—
Headache, grade 2	1	—
Diarrhea, grade 2	1	1
Elevated transaminase, grade 2	1	—
Hypertension, grade 2	1	—

Abbreviation: N/A, not applicable.

relapsed at 5.4 months and 10 months; and one CR patient experienced a relapse at 12 months. For this allogeneic cohort, the 4- and 12-month relapse-free survival rates were 100% and 56%, respectively.

All patients in the autologous group are alive with a median follow-up duration of 17 (range, 10–39) months (Fig. 2B). One patient with double-hit diffuse large B-cell lymphoma (Table 2B, patient 12) experienced a relapse after one cycle of lenalidomide and 2 weeks after the first cycle of ipilimumab and did not receive further study treatment but continued to be followed. All other patients remain in remission. For this autologous cohort, the 4- and 12-month relapse-free survival rates were both 86% (Fig. 2B).

Immunologic effects

We conducted multiparametric flow cytometric analyses of PBMCs before and after treatment, including after monotherapy with lenalidomide and after combination therapy with lenalidomide plus ipilimumab. We did not observe any significant differences in the frequency of $CD3^+$ (Fig. 3A), $CD4^+$ (Fig. 3B), and $CD8^+$ (Fig. 3C) T cells during treatment in the autologous or allogeneic group.

We previously identified an increase in the number of inducible costimulatory $(ICOS)^+CD4^+$ T cells as a pharmacodynamic biomarker for ipilimumab-based therapy (28). In the current trial, we observed a statistically significant increase in the number of $ICOS^+CD4^+FoxP3^-$ T-cell number in both the autologous and allogeneic group, with a notable increase after the first cycle of lenalidomide and a further increase after the first dose of ipilimumab, suggesting an additive effect to these two agents when combined together ($P = 0.007$; Fig. 3D).

Discussion

Prevention and treatment of relapse of a lymphoid malignancy after HSCT requires novel strategies. Unpublished analysis from our center showed a median OS rate of less than 1 year in 95 patients with aggressive lymphomas who experienced a relapse after allogeneic stem cell transplantation. One remarkable

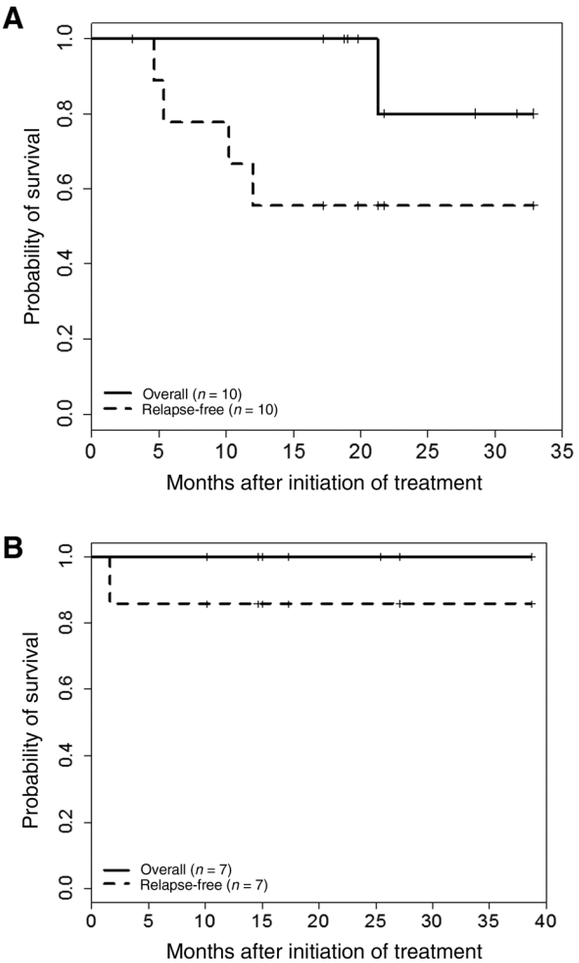

Figure 2.
Treatment with ipilimumab and lenalidomide after HSCT: overall survival and relapse-free survival in patients who received ipilimumab and lenalidomide as therapy for relapsed lymphoid malignancies after allogeneic HSCT (**A**) or to prevent relapse after autologous transplantation (**B**).

advance in cancer immunotherapy has been immune checkpoint blockade. Targeting CTLA-4 with ipilimumab at the conventional dose of 3 mg/kg in patients who have relapses after allogeneic HSCT have not resulted in CRs for patients with non-Hodgkin lymphoma (14, 15). In addition, this strategy has yet to be tested after autologous HSCT to prevent relapse of lymphoid malignancies in high-risk patients. We hypothesized that combination therapy with ipilimumab and lenalidomide could enhance the immune activity of ipilimumab via its known immunomodulatory effect.

In our trial, alternating lenalidomide (10 mg daily for 21 days) and ipilimumab (3 mg/kg) resulted in no significant autoimmune toxic effects, and no new cases of GVHD. One patient with a known history of GVHD had a flare of his symptoms after the first cycle of lenalidomide and did not receive ipilimumab. The overall response rate was 70% in patients with active disease in the allogeneic group after two cycles of both ipilimumab and lenalidomide. Four of these responses were durable beyond 1 year.

Seven patients received the combination after autologous HSCT. These patients had a high risk of relapse at the study entry.

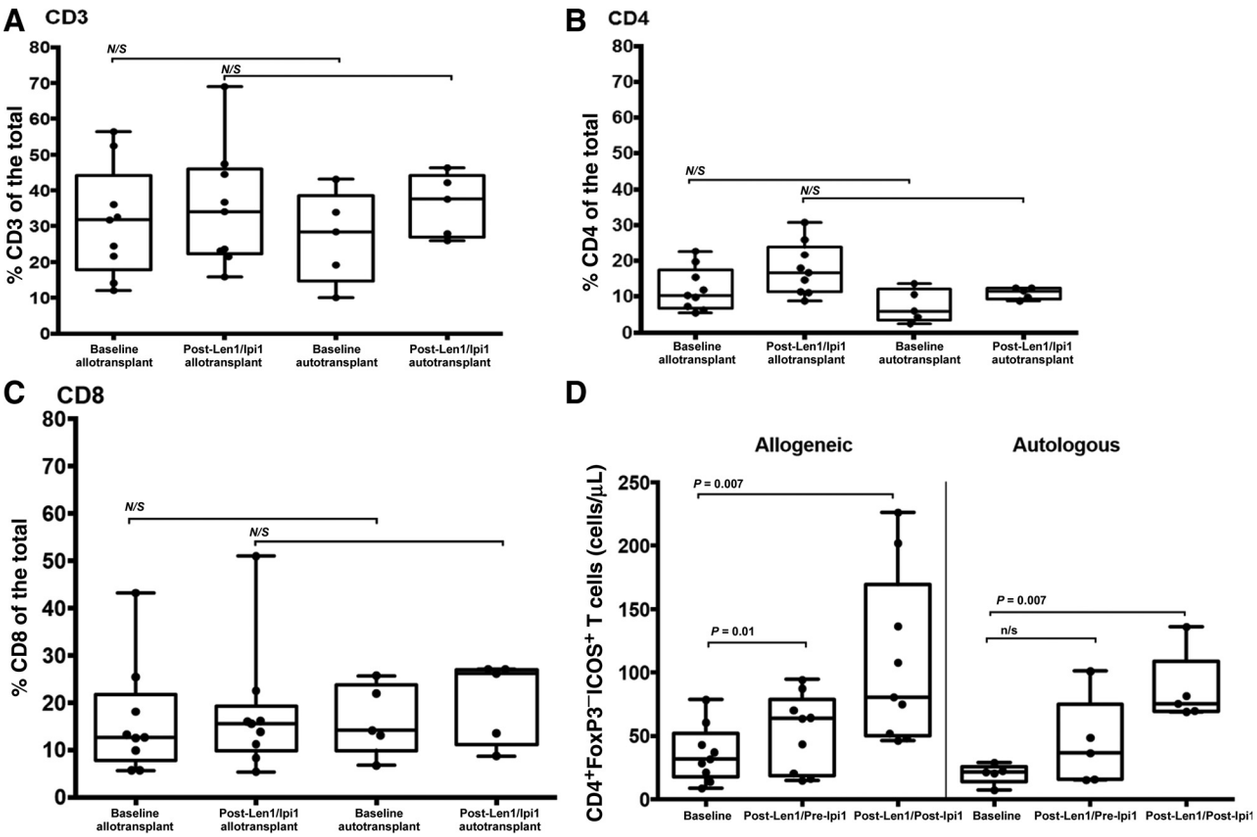

Figure 3.

T-cell immune responses to treatment. Distributions of $CD3^+$ (**A**), $CD4^+$ (**B**), $CD8^+$ (**C**), and number of $ICOS^+CD4^+FoxP3^-$ (**D**) T cells, in PBMCs of study patients after the first cycles of lenalidomide (Len) and ipilimumab (Ipi).

We have previously reported on the negative prognostic significance of an elevated Ki67 in patients with Mantle cell lymphoma (29) and the poor outcomes in patients with this disease undertaking an autologous HSCT beyond first remission (30). Two patients in our study fall within this category. Poor outcomes have also been reported in patients with follicular lymphoma who are refractory to conventional chemotherapy (31), or in patients with Hodgkin lymphoma who experienced early posttransplantation PET positivity after HSCT (one patient in our study falls within each of these two categories, respectively; ref. 32). Recent reports also described inferior outcomes in patients with double-hit lymphoma who were treated with R-CHOP compared with those who received intensive first-line chemotherapy to achieve a first CR prior autologous HSCT consolidation, or those who have been heavily pretreated (two and one patient in our study fall within these categories, respectively; ref. 33). As most relapses after autologous HSCT usually occur within 18 months of transplantation, we are encouraged that with a median follow-up duration of 17 (range, 10–39) months, only one progression was observed at the time of this analysis. This also occurred in a patient in whom the full efficacy of the treatment could not have been fully accessed as relapse was experienced immediately after the first dose of ipilimumab. Accrual continues at our center to validate these early data in a larger number of patients.

Although we expected to see significant differences in the immunologic impact of ipilimumab-based therapy in allogeneic and autologous transplant recipients, our preliminary data indicated similar immune responses in the two groups. Specifically, our immunologic analysis demonstrated a significant increase in the numbers of $ICOS^+CD4^+FoxP3^-$ T cells after combination therapy with ipilimumab and lenalidomide in both groups. ICOS, a member of the immunoglobulin gene family, is structurally related to CD28 and CTLA-4. $CD4^+$ and $CD8^+$ T cells express ICOS on their surface following activation (34). ICOS functions as a costimulatory molecule on activated T cells, and its expression has been associated with increased effector T-cell survival (35). A retrospective analysis of melanoma patients given ipilimumab identified increased frequency of $CD4^+$ T cells with high ICOS expression that was sustained over 7 weeks and correlated with overall survival (36). The additive impact of lenalidomide on the increased $ICOS^+CD4^+$ T cells may also explain the higher than expected clinical responses to just two doses of 3 mg/kg of ipilimumab observed in our trial. Although lenalidomide has a known clinical activity as a single agent in relapsed aggressive lymphomas (37), the CR rates were 12% when the drug was given at a daily dose of 25 mg; the median time to achieve CR was 4.3 months. The lower dose of lenalidomide used in our trial for just two cycles makes it unlikely that the responses observed were due to

lenalidomide alone. These initial observations need to be verified in a larger cohort of patients.

Single-agent ipilimumab was given at the conventional dose of 3 mg/kg after allogeneic HSCT in two other trials (14, 15). The median intervals from allogeneic HSCT to initiation of therapy in these two trials were 21 (14, 38), and 22.5 months (15), respectively, compared with 29 months in our trial for patients with aggressive histologies. Neither of these two other trials reported, however, clinical CRs in patients with non-Hodgkin lymphoma. To improve responses, Davids and colleagues (15) increased the ipilimumab dose to 10 mg/kg every 3 weeks for four doses, with additional doses every 12 weeks for 60 weeks in patients who exhibited clinical benefit of the treatment. Of the 22 patients treated at such dose and schedule, immune-related adverse events occurred in five patients (23%), one of whom died 42 days after receiving the initial dose of ipilimumab. Five patients (23%) also had severe GVHD. Higher clinical response rates were described in patients with myeloid disease but not in those with relapsed non-Hodgkin lymphoma. Our data lead us to believe that selectively activating the antitumor immunity effect without precipitating clinically significant GVHD is possible.

Given its favorable safety profile, treatment with ipilimumab and lenalidomide is a promising approach to preventing relapse of high-risk lymphoma after autologous HSCT and to treating recurrent disease after allogeneic HSCT. Because duration of therapy may be important to ensure optimal clinical effect and longer lasting antitumor activity, the protocol has been amended to provide patients four cycles of each drug.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: I.F. Khouri, R.L. Bassett Jr, L.M. Vence, A.M. Gulbis
Development of methodology: I.F. Khouri, R.L. Bassett Jr, P. Sharma
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Acknowledgments

This work was supported by the NIH/NCI under award number P30CA016672.

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Received September 24, 2017; revised November 8, 2017; accepted December 8, 2017; published OnlineFirst December 15, 2017.

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