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ARTICLE

Autologous tumor cell vaccine induces antitumor T cell immune responses in patients with mantle cell lymphoma: A phase I/II trial

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Here, we report on the results of a phase I/II trial (NCT00490529) for patients with mantle cell lymphoma who, having achieved remission after immunochemotherapy, were vaccinated with irradiated, CpG-activated tumor cells. Subsequently, vaccine-primed lymphocytes were collected and reinfused after a standard autologous stem cell transplantation (ASCT). The primary endpoint was detection of minimal residual disease (MRD) within 1 yr after ASCT at the previously validated threshold of ≥1 malignant cell per 10,000 leukocyte equivalents. Of 45 evaluable patients, 40 (89%) were found to be MRD negative, and the MRD-positive patients experienced early subsequent relapse. The vaccination induced antitumor CD8 T cell immune responses in 40% of patients, and these were associated with favorable clinical outcomes. Patients with high tumor PD-L1 expression after in vitro exposure to CpG had inferior outcomes. Vaccination with CpG-stimulated autologous tumor cells followed by the adoptive transfer of vaccine-primed lymphocytes after ASCT is feasible and safe.

Introduction

Mantle cell lymphoma (MCL) is an incurable non-Hodgkin lymphoma that carries a poor prognosis regardless of therapy. Recently, the focus has centered on the intensification of frontline therapy with either higher doses of conventional cytotoxic chemotherapeutics or the addition of cytarabine (Delarue et al., 2013; Geisler et al., 2008; Romaguera et al., 2005). In fit patients, subsequent consolidation with high-dose chemotherapy and autologous stem cell transplantation (ASCT) in first remission improves progression-free survival (Dreyling et al., 2005). Moreover, maintenance rituximab after ASCT has been shown to improve overall survival (OS; Le Gouill et al., 2017). Alternatively, allogeneic stem cell transplant can yield more durable remissions due to an immune graft-versus-lymphoma effect (Fenske et al., 2014). However, allogeneic transplant is associated with higher treatment-related mortality and chronic graft-versus-host disease. We hypothesized that the addition of a tumor vaccination to ASCT could enhance treatment efficacy without additional toxicity.

Oligodeoxynucleotides with sequences rich in cytosine-phosphate-guanosine (CpG) repeats are characteristic of microbial DNA and are recognized by cells of the innate immune system via their TLR9 (Ohto et al., 2015). This receptor is constitutively expressed in plasmacytoid dendritic cells and in B cells, including B cell lymphomas (Jahrsdörfer et al., 2001). Stimulation through TLR9 induces expression of costimulatory molecules such as CD80/86, antigen-presenting molecules such as MHC II, and cytokine production such as IL-12 and TNF, leading to the recruitment of T cells and adaptive immune responses. PF-3512676 is a 29-basepair, synthetic CpG that has shown antineoplastic activity in vitro and in vivo (Brody et al., 2010; Goldstein et al., 2011; Li et al., 2007). In preclinical models, vaccination with syngeneic murine lymphoma cells activated by CpG induced a robust antitumor T cell immune response (Goldstein et al., 2011). T cells from these vaccinated mice could be adoptively transferred to irradiated syngeneic recipients, where they expanded and mediated the regression

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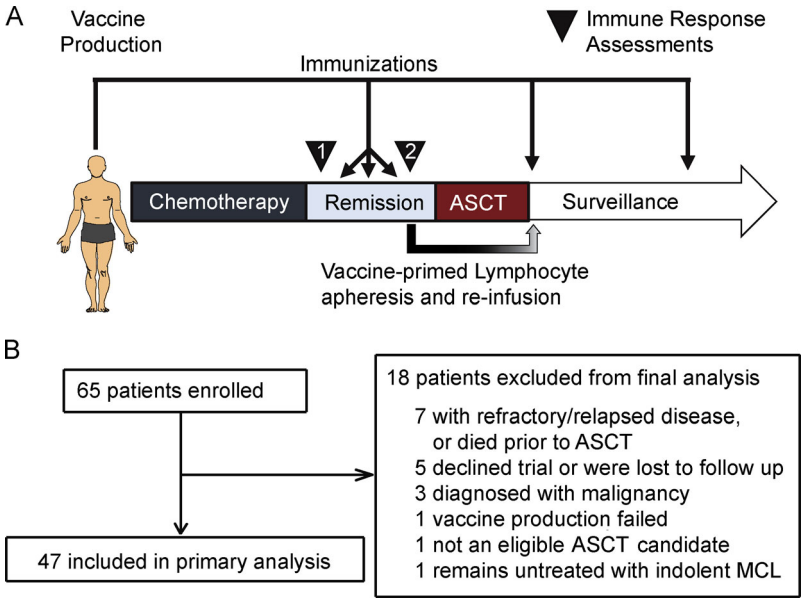

Figure 1. **Schema of trial design and CONSORT diagram. (A)** Prior to chemotherapy, tumor cells were collected by apheresis or biopsy, treated with CpG, radiated, and cyropreserved in single-use aliquots as described in the Materials and methods. Patients achieving at least a partial response to initial chemotherapy received three vaccine doses followed by apheresis for T cell collection. 1 d after infusion of autologous stem cells, collected T cells were reinfused and a fourth vaccination was given. After complete recovery from the ASCT, a final "booster" vaccination was given. PBMCs were collected before and after the initial three vaccine doses for immune response assessments. (**B**) CONSORT diagram of all patients enrolled.

of large, established tumors, resulting in long-lasting immunity against subsequent lymphoma challenge (Goldstein et al., 2011).

Guided by these preclinical results, we designed a phase I/II clinical trial (NCT00490529) to evaluate the therapeutic potential of a CpG-activated whole autologous tumor cell vaccination followed by transfer of immune T cells as an additive to standard ASCT for patients with MCL (Fig. 1 A). The primary endpoints were safety and freedom from minimal residual disease (MRD) 1 yr after ASCT, an endpoint previously correlated with subsequent remission duration (Pott et al., 2010). Secondary endpoints included time to progression (TTP) from the date of ASCT, OS, and immune responses to autologous tumor cells.

Results

Patients

Accrual to this trial occurred between April 2009 and April 2016. All eligible patients at our institution were offered this trial during this time period. 65 patients with previously untreated MCL entered and underwent either excisional biopsy or leukapheresis for vaccine production. 18 patients were excluded after enrollment for various reasons, with only one patient excluded because of failure to produce the vaccine. 47 patients received all intended trial-related treatments and were included in the primary and secondary analyses. The demographics, clinical risk profiles, induction chemotherapy details, and response to induction chemotherapy of the vaccine trial patients are summarized in Table 1.

Safety

Vaccination and transfer of vaccine-primed lymphocytes were well tolerated by the 47 patients who received all trial-related treatments (Fig. 2). One additional patient was included in the safety analysis since this individual received the initial three vaccinations but relapsed just before ASCT and did not receive the subsequent trial-related treatments (Table 2). The CpG-MCL vaccination caused transient neutropenia and thrombocytopenia; these abnormalities resolved quickly and did not prevent the planned post-vaccine apheresis. No toxicities were attributed to the autologous lymphocyte infusion. Patients experienced the expected side effects of ASCT, including fevers and infections. The time to engraftment of trial patients averaged 10.6 d (range, 9–13 d), similar to routine ASCT. Two patients died without clinical evidence of relapse within 1 yr after ASCT due to infectious complications. Patient 11 developed respiratory failure from metapneumovirus diagnosed on day +9 after ASCT, complicated by vancomycin-resistant pneumonia and possible carmustine-induced pneumonitis, and ultimately died on day +50. A second patient (patient 36) developed cellulitis on day +256 complicated by septic shock and died on day +270. Neither of these deaths was considered related to the vaccination or the autologous lymphocyte infusion. Additionally, two patients had delayed recovery of platelets and received infusion of a backup stem cell product and subsequently recovered normal blood counts. The prespecified safety endpoints, which would have required halting the trial, were not triggered, as no patient experienced nonengraftment and only one patient (patient 11) experienced early mortality.

Primary endpoint

MRD, the prespecified primary endpoint, was considered positive if a tumor-specific VDJ sequence was detected in the peripheral blood cells by Ig high-throughput sequencing (Ig-HTS) at a frequency of ≥1 molecule per 10,000 input leukocyte equivalents of DNA within 1 yr after ASCT. Previous reports using an allele-specific oligonucleotide PCR (ASO-PCR) method had validated this threshold as predicting remission duration after ASCT (Pott et al., 2010). As described above, two patients (patients 11 and 36) died without evidence of disease before 1 yr after ASCT and were excluded from the MRD analysis. The 45 evaluable patients had a 1-yr freedom from MRD rate of 89%

Table 1. **Patient characteristics**

Demographics	***n* = 47**
Median age, yr (range)	58 (34–70)
Gender, *n*	28 male; 19 female
Stage	
Stage III	3 (6%)
Stage IV	44 (94%)
ECOG PS	
0	35 (74%)
1	11 (23%)
2	1 (2%)
Treatment received	
R-CHOP	7 (15%)
R-HyperCVAD	15 (32%)
R-CHOP/R-DHAP	23 (49%)
Dose-adjusted R-EPOCH	1 (2%)
R-Bendamustine	1 (2%)
Response to immunochemotherapy	
PR	5 (11%)
CR	42 (89%)
MIPI	
Low risk	26 (55%)
Intermediate risk	15 (32%)
High risk	6 (13%)
Ki-67	
≥30%	18 (38%)
≤30%	22 (47%)
Unknown	7 (15%)
B-MIPI	
Low risk	8 (17%)
Intermediate risk	20 (43%)
High risk	12 (26%)
Unknown	7 (15%)

Data are presented as *n* (%) unless specified otherwise. ECOG PS, Eastern Cooperative Oncology Group performance status; R-HyperCVAD, rituximab, cyclophosphamide, vincristine, doxorubicin, and dexamethasone; R-DHAP, rituximab, dexamethasone, cytarabine, and cisplatin; R-EPOCH, rituximab, etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin; PR, partial response; CR, complete response.

(*n* = 40). One patient, patient 24, had an isolated central nervous system relapse within 2 mo after ASCT, before the first MRD assessment. This patient and the four patients (patients 13, 18, 32, and 38) who were MRD positive had early relapse and/or death (Fig. 2). The average time to relapse for the four MRD-positive patients was 4.5 mo (range, 0–13.3 mo) after their first positive MRD signal. Two of these five patients were found to have mutated *TP53*, and three of these five patients had a high biologic Mantle Cell Lymphoma International Prognostic Index (B-MIPI) risk score.

Clinical significance of MRD positivity and low-level MRD

Since the sensitivity of the Ig-HTS test exceeds our prespecified threshold of 1 per 10^4 leukocyte equivalents, we evaluated the clinical significance of detection above (MRD positive) and below this threshold (low-level MRD). Of the 45 evaluable patients, 22 had detectable tumor cells at some point in time: 14 patients (patients 1, 13, 14, 15, 18, 22, 23, 26, 29, 30, 32, 35, 38, and 43) had their first MCL clonotype detected at or before the 1-yr post-ASCT assessment and eight patients (patients 2, 4, 7, 10, 21, 24, 34, 39) after the 1-yr post-ASCT assessment (Fig. 2). Detection of MRD (MRD positive) compared with those who were below the threshold for MRD positivity was significantly associated with an increased hazard of clinical relapse hazard ratio (HR = 6.44; 95% confidence interval [CI]: 2.38–17.41). In an exploratory analysis, we evaluated the clinical significance of MRD positivity, low-level MRD, or no detectable tumor. MRD positivity was associated with an increased hazard of clinical relapse relative to low-level MRD (HR = 3.76; 95% CI: 1.35–10.44). The hazard of clinical relapse for undetectable tumor cells versus low-level MRD was similar (HR = 0.29; 95% CI: 0.08–1.09). We conclude that for MCL, lower levels of MRD may not be a significant risk factor for relapse, as opposed to what has been previously reported for diffuse large B cell lymphoma (Roschewski et al., 2015).

Secondary endpoints

The median TTP and OS from ASCT were 6.9 yr and not reached, respectively (Fig. 2), with a median follow-up of 4.6 yr. The median TTP and OS from initial chemotherapy were 8.1 yr and not reached, respectively, with a median follow-up of 5.4 yr (Fig. S1, A and B). Clinical outcomes were not different between patients with or without initial leukemic involvement of MCL or by initial chemotherapy received (data not shown). Patients with high B-MIPI scores had inferior outcomes compared with those with intermediate/low-risk scores. Patients with high B-MIPI scores had a median TTP from ASCT of 6.9 yr versus not reached (P = 0.0260) and a median OS of 4.1 yr versus not reached (P = 0.0143; Fig. S1, C and D). The source of vaccine, lymph node–derived versus circulating tumor cell–derived, made no difference in outcomes (Fig. S2).

Immune response

Antitumor T cell immune responses were evaluated by an in vitro evocative test on patients' peripheral blood mononuclear cell (PBMCs) before and after vaccination (Fig. 1 A). For the 35 patients with available material, as described in the Materials and methods, PBMCs were co-cultured with CpG-activated autologous MCL tumor cells and evaluated for tumor-specific immune responses as measured by CD137 expression on their T cells, a sensitive marker for antigen engagement (Wolfl et al., 2007; Khodadoust et al., 2017). Tumor-specific memory $CD8^+$ and $CD4^+$ T cells were found in 89% (*n* = 31) and 57% (*n* = 20), respectively, at baseline before vaccination. The range of tumor-specific $CD8^+$ cells was between 0.1% and 5.3% of $CD8^+$ T cells, and the range of tumor-specific $CD4^+$ cells was between 0.08%

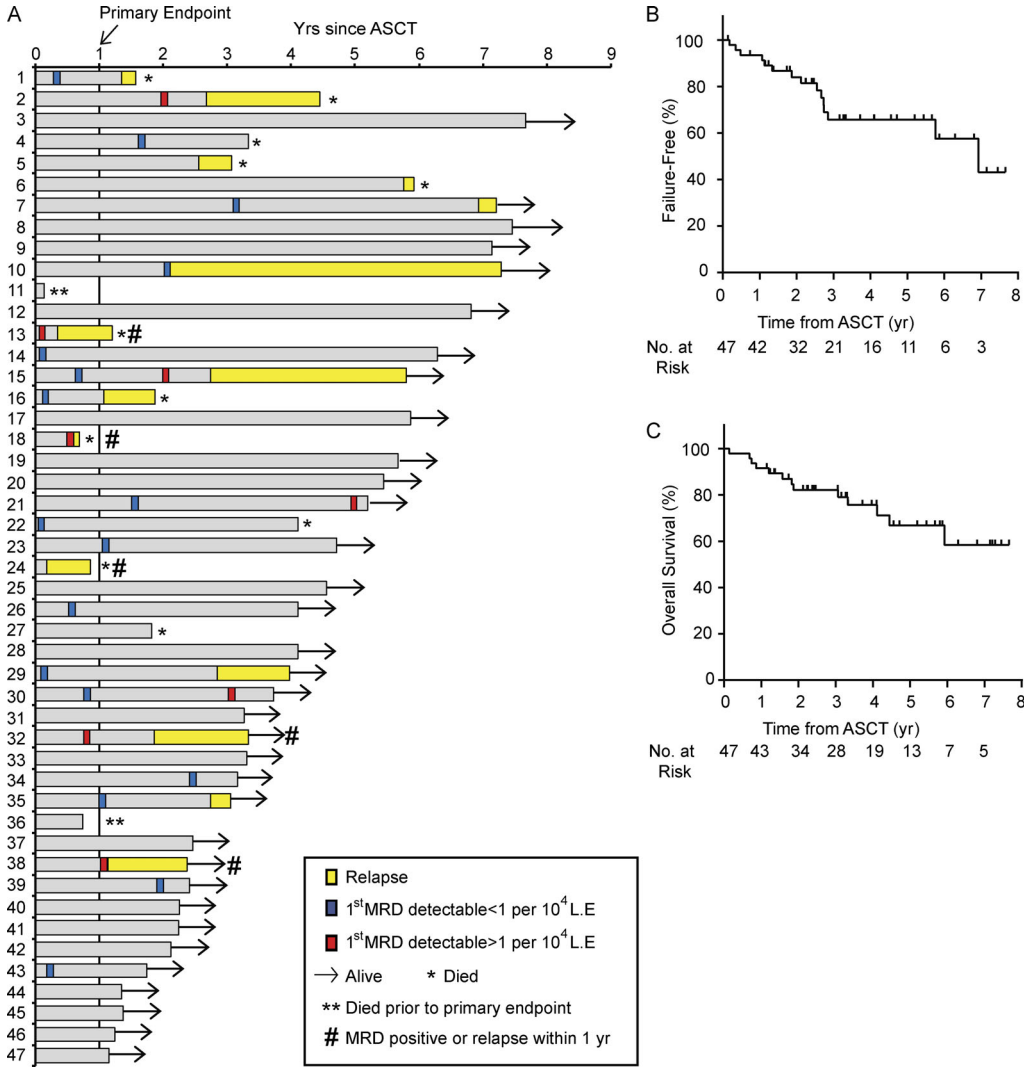

Figure 2. **Clinical trial outcomes: TTP, OS, and patient outcomes by MRD analysis. (A)** Swimmers plot shows time to first MRD event(s) and time to relapse from the time of ASCT. The first detectable MRD event is shown in either blue or red, and the time of relapse is shown in yellow. The time of first MRD detection >1 molecule per 10^4 is red, and time of first MRD detection less than this threshold is blue. **(B and C)** TTP (B) and OS (C) examined by Kaplan-Meier analysis and log-rank test were assessed from the date of ASCT in all patients who received the CpG-MCL vaccination and adoptive T cell transfer. L.E., leukocyte equivalents.

and 4.95% of $CD4^+$ T cells. An example of a patient with baseline antitumor memory $CD8^+$ T cells is shown in Fig. 3 A. After vaccination, 40% (n = 14) of these patients demonstrated a $CD8^+$ T cell vaccine response by expanding tumor-specific $CD137^+$ $CD8^+$ T cells (Fig. 3 A). The source of the vaccine made no difference in $CD8^+$ T cell responses, with 40% (4 of 10) of patients with circulating tumor cell–derived vaccine and 40% (10 of 25) of patients with lymph node–derived vaccine generating a memory CD8 T cell response. Patients generating such a memory $CD8^+$ T cell vaccine response had a significantly longer TTP than those who did not (not reached vs. 5.8 yr, P = 0.0246). We found 40% (n = 14) of patients developed a memory $CD4^+$ T cell vaccine response; however, this response was not related to clinical outcomes (Fig. S3 A).

Detection of late immune response

Previously, we showed that the lymphoma Ig is a prominent tumor-specific antigen for $CD4^+$ T cells from patients with MCL (Khodadoust et al., 2017). We also found that stimulation with CpG to create the vaccine product significantly boosted presentation of Ig neoantigens and theorized that Ig neoantigens may be a key antigen of the vaccine response (Khodadoust et al., 2019). We therefore searched for the presence of Ig neoantigen-specific T cells after vaccination. We identified a patient with a high-risk B-MIPI score and TP53 mutation who continued to have no detectable MRD over 43 mo after her ASCT. Over 2 yr after completion of her vaccination and reinfusion of vaccine-primed lymphocytes, we could detect both $CD8^+$ and $CD4^+$ T cells in her peripheral blood targeting her lymphoma Ig neoantigens (Fig. S4 A). After ex vivo expansion, these neoantigen-targeting T cells were able to directly kill her lymphoma cells when co-cultured (Fig. S4 B).

Vaccine characteristics

The vaccine consisted of tumor cells activated ex vivo by CpG. CpG induced the expression of costimulatory (e.g., CD80, CD86)

Table 2. **Vaccine-associated adverse events (*n* = 48)**

Adverse event	Grade 1/2	Grade 3/4	Total *n* (%)
Injection site reaction/Rash (maculopapular)	47 (98)	0	47 (98)
Myalgia	27 (56)	0	27 (56)
Fever	22 (46)	0	22 (46)
Fatigue	22 (46)	0	22 (46)
Chills	21 (44)	0	21 (44)
Hematologic			
Neutropenia	8 (17)	8 (17)	18 (38)
Thrombocytopenia	13 (27)	0	13 (27)
Anemia	1 (2)	0	1 (2)
Pain (local site)	17 (35)	0	17 (35)
Arthralgia	16 (33)	0	16 (33)
Headache	15 (31)	0	15 (31)
Pruritus	13 (27)	0	13 (27)
Diarrhea	3 (6)	0	3 (6)
Insomnia	2 (4)	0	2 (4)
Anorexia	2 (4)	0	2 (4)
Vomiting	2 (4)	0	2 (4)
Nausea	2 (4)	0	2 (4)
Neutropenic fever	0	1 (2)	1 (2)

Data are presented as *n* (%) unless specified otherwise.

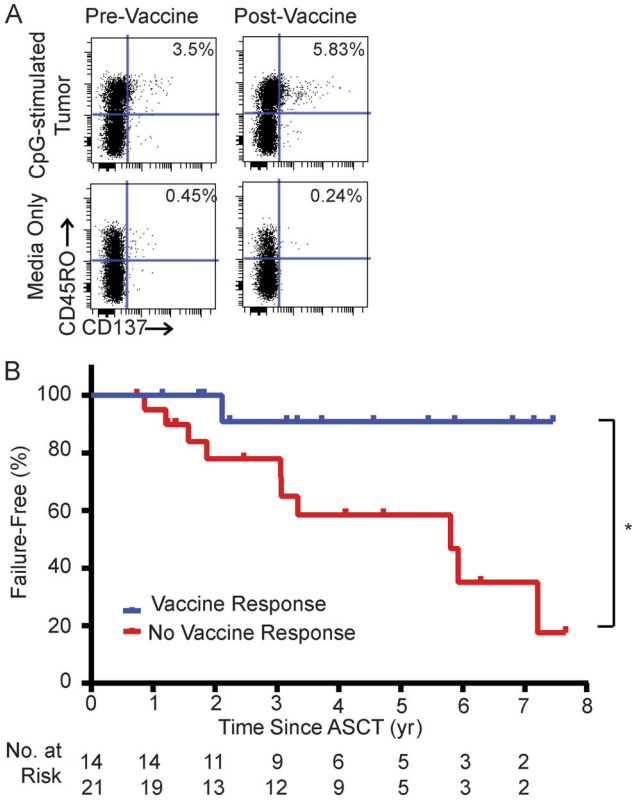

Figure 3. **Patients who generate a memory CD8 T cell vaccine response have favorable outcomes. (A)** An example of a patient with an induction of tumor-reactive memory CD8 T cells after vaccination is shown. PBMCs, collected before or after vaccine administration, were simultaneously co-incubated with or without CpG-stimulated tumor cells, as described in the Materials and methods. Each plot is gated on $CD8^+$ T cells, and the percentage $CD137^+CD45RO^+$ T cells are shown. The percentage of tumor-reactive memory $CD8^+$ T cells is determined by subtracting the percentage of $CD137^+CD45RO^+$ T cells found in the media-only sample from the percentage found in the sample with CpG-stimulated tumor cells. This patient was determined to have had a memory $CD8^+$ T cell vaccine response since there was a higher percentage of tumor-reactive memory $CD8^+$ T cells after vaccination compared with baseline. **(B)** TTP from ASCT was examined by Kaplan-Meier analysis and log-rank test in patients with a memory CD8 T cell vaccine response (blue) or no vaccine response (red). Trial patients with a memory CD8 T cell vaccine response had a significantly longer TTP than those who did not (median TTP was not reached vs. 5.8 yr; *, P = 0.0246).

and activation (e.g., CD40) molecules on the surface of the tumor cells (Fig. S5) as previously reported (Goldstein et al., 2011; Jahrsdörfer et al., 2001). In addition, we found that CpG induced the expression of programmed death ligand 1 (PD-L1) to a variable degree on the tumor cells (Fig. S5). We found a significant correlation between PD-L1 expression on tumor cells after CpG activation with both the TTP and OS. When analyzed as a continuous variable, PD-L1 expression with respect to the TTP had an HR = 1.244 (95% CI: 1.038–1.491; P = 0.018) and with respect to OS had an HR = 1.163 (95% CI: 1.002–1.350; P = 0.047). While the optimal cutoff point is not known for this exploratory endpoint, we found that patients with tumor samples that exhibited more than the mean expression of PD-L1 had worse TTP (median TTP of 5.8 yr vs. not reached; P = 0.0453) and OS (median OS of 4.1 yr vs. not reached; P = 0.0462; Fig. 4). None of the deaths in the low PD-L1 group were due to lymphoma relapse, whereas six of the seven patients who died in the high–PD-L1 group were from relapsed disease. The baseline MIPI and B-MIPI were similar between these two groups. Interestingly, there may have been a relationship between PD-L1 induced on the tumor cell used as the vaccine and the resulting CD8 T cell immune response in patients. 45% (*n* = 9 of 20) of patients with low PD-L1–expressing tumor vaccines generated a memory $CD8^+$ T cell vaccine response, whereas only 25% (*n* = 3 of 12) of patients with high PD-L1–expressing tumor vaccine products generated a memory $CD8^+$ T cell vaccine response (Fig. 4 A).

Discussion

Therapeutically targeting a patient's immune system affords an opportunity to create a highly specific response to malignancies. Induction of tumor immune responses can be triggered in numerous ways, some of which have now made it to clinical practice, such as immune checkpoint inhibition. Herein, we report analysis of a phase I/II trial promoting anti-lymphoma T cell responses through vaccination with CpG-activated autologous tumor cells followed by adoptive transfer of autologous vaccine-primed T cells. We show that vaccine-primed T cell infusion after ASCT is feasible and safe. Importantly, production of this vaccine was almost universally successful. This study met its primary endpoint by achieving an 89% freedom from MRD at the landmark of 1 yr after ASCT.

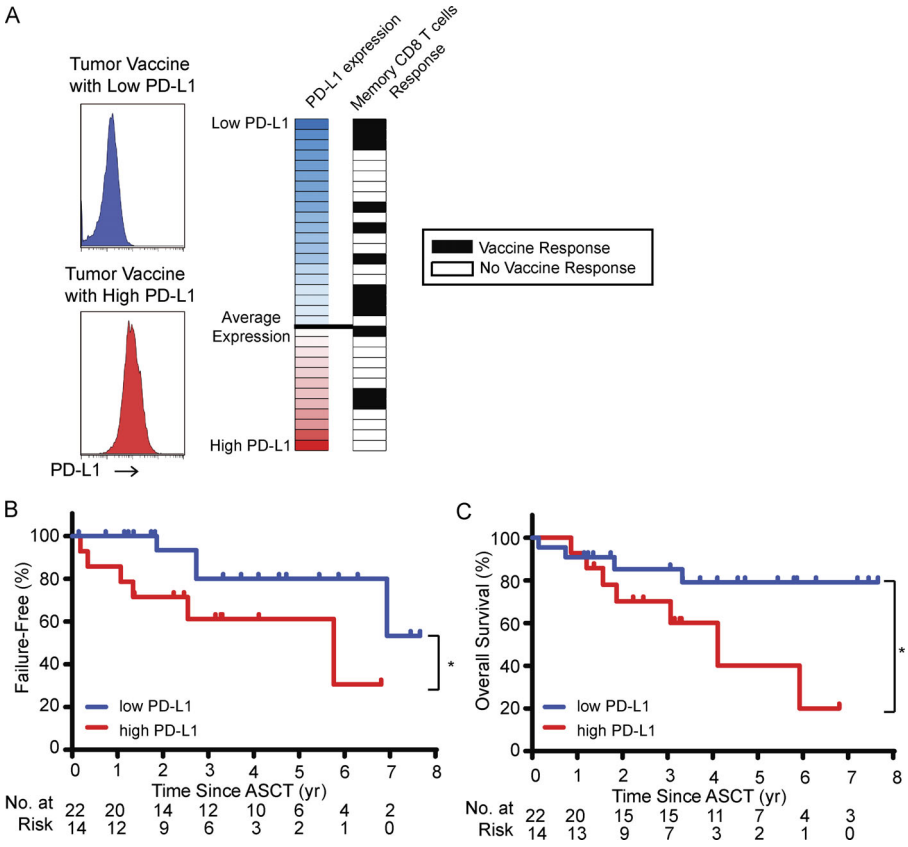

Figure 4. **High CpG–induced PD-L1 expression is associated with poor outcomes. (A)** Mantle cell tumor suspensions were incubated with CpG as described in the Materials and methods and assayed for PD-L1 expression. Examples of low (upper panel) and high (lower panel) tumor PD-L1 expression are shown. Each row shows a patient's CpG-induced tumor PD-L1 expression from lowest to highest expression level in relation to whether that patient had a CD8 T cell vaccine response (black box) or no response (white box). Expression level was determined by mean fluorescence intensity (MFI) normalized to an isotype control. The average expression level, as measured by MFI, is shown for the 36 patients with available material for analysis. Patients with expression levels above this level were considered to have high PD-L1 expression, and those below this level were considered to have low PD-L1 expression. **(B and C)** TTP and OS examined by Kaplan-Meier analysis and log-rank test was assessed from ASCT stratified by PD-L1 expression (red line represents patients with tumor PD-L1 expression above the mean, and blue line represents patients with tumor PD-L1 expression below the mean). Patients with lower PD-L1 expression had a significantly longer TTP (median TTP was not reached vs. 5.76 yr; *, P = 0.0453) and OS (median OS was not reached vs. 4.11 yr; *, P = 0.0462) than those who did not.

Because of evolving standard induction regimens, our study allowed for any rituximab-based induction therapy, making it challenging to compare clinical outcomes to prior reports that used a uniform induction chemoimmunotherapy regimen. The evaluated experimental interventions, specifically the vaccinations and vaccine-primed lymphocyte infusions, were intended only for patients who achieved either a partial or complete response to initial chemotherapy. Therefore, we have reported outcomes from the time of those interventions that began at the time of ASCT. In an exploratory analysis, we found that the subgroup of trial patients who generated a $CD8^+$ antitumor immune response had superior clinical outcomes compared with those who did not generate an immune response. However, causality is difficult to establish because patients who were able to generate a $CD8^+$ vaccine response may have had superior outcomes because of a yet unidentified favorable biology unrelated to the immune response per se. However, our data show evidence that the vaccine induces expansion of antitumor T cells that have cytotoxic activity against the autologous lymphoma cells. We previously demonstrated in two patients from this trial that expanded neoantigen-targeting T cells were able to kill autologous lymphoma cells (Khodadoust et al., 2017). Here, we demonstrated in a third patient with high-risk TP53-mutated MCL who remained MRD negative over 2 yr after treatment that an expanded subset of neoantigen-targeting T cells retained cytotoxic activity against her autologous lymphoma cells in vitro. Collectively, these data show direct evidence that the vaccine induces expansion of neoantigen-targeting T cells and raise the question of how these immune responses might be further enhanced.

We noted that CpG incubation induced PD-L1 expression on tumors and this induction showed a negative correlation with both TTP and OS. It is possible that the high PD-L1 expression inhibited T cell responses to the vaccine, making the vaccine less effective. Patients with low induced tumor PD-L1 levels were more likely to generate a vaccine response than those with high induced tumor PD-L1 levels. It was recently reported that $CD8^+$ T cells undergo activation and homeostatic inhibition manifested by up-regulation of PD1, resulting in functional suppression. The addition of checkpoint blockade, including anti-PD1 therapy, prevented homeostatic inhibition and improved antitumor T cell responses in murine preclinical models (Marshall et al., 2019). Collectively, these data suggest that the addition of a PD1-blocking checkpoint antibody to the vaccine maneuver might improve the immune response rate and subsequent therapeutic effects.

MRD testing was previously shown to have prognostic value in MCL (Pott et al., 2010; Geisler et al., 2008). These prior studies used the approach of ASO-PCR (Andersen et al., 2002; Geisler et al., 2008; Pott et al., 2010). In the current study, we applied Ig-HTS, a highly sensitive marker of MRD. Ig-HTS has been shown to have a high concordance with ASO-PCR, but it has not been evaluated as a predictor of outcomes in MCL (Ladetto et al., 2014). We found that MRD detection by Ig-HTS is associated with a short remission duration in MCL when a threshold of one malignant clone per 10^4 leukocyte equivalents was applied; patients with tumor cells in their blood above this threshold have a significantly higher risk of clinical relapse. In contrast, we found that treated MCL patients with lower levels of MRD below this threshold by Ig-HTS had a similar risk of clinical relapse

compared with those with no detectable tumor cells. In contrast, a positive Ig-HTS MRD test at its limit of sensitivity was reported to be associated with early clinical relapse in DLBCL (Roschewski et al., 2015).

Overall, our data demonstrate that the addition of a CpG-activated whole cell tumor vaccination followed by adoptive transfer of vaccine-primed lymphocytes to the treatment of MCL is feasible and safe and can induce $CD8^+$ T cell immune responses that are associated with a superior clinical outcome. Vaccinated MCL patients demonstrated freedom from MRD at 1 yr after ASCT that surpasses previously reported rates.

Materials and methods

Patient eligibility

Eligible patients were newly diagnosed with MCL, were between 18 and 75 yr of age, were in need of treatment, and were candidates for ASCT. Patients were required to be negative for HIV and to have an Eastern Cooperative Oncology Group performance status of ≤2. Exclusion criteria included any prior therapy for their lymphoma, recent secondary malignancy, concurrent requirement for immunosuppressive medications, or pregnancy. Furthermore, patients had to have a disease site accessible for excisional biopsy or sufficient circulating tumor cells to allow acquisition by leukapheresis.

Study design and treatment

Following screening, evaluation, and enrollment, patients underwent either excisional biopsy or leukapheresis to collect at least 1.5×10^9 autologous tumor cells that were used to create a patient-specific whole tumor cell vaccine. A total of 10^9 tumor cells were cultured in AIM-V media and human AB-serum containing PF-3512676 (CpG) at a concentration of 3 µg/ml at 37°C and 5% CO_2 for 72 h. The tumor cells were then irradiated with a single fraction of 200 Gy and cryopreserved in media containing saline, human serum albumin, hydroxyethyl starch, and 10% DMSO in aliquots of 10^8 cells for single-use vaccine doses.

Patients returned to their treating physician and received a rituximab-chemotherapy of their physician's choosing. To remain eligible, patients must have attained a partial or complete response based on the 2007 Revised Response Criteria for Malignant Lymphoma and remain a candidate for ASCT (Cheson et al., 2007). After recovery from induction therapy, patients received three subcutaneous priming vaccinations 5–7 d apart with their CpG-activated MCL vaccine mixed at the bedside with 18 mg of PF-3512676. Approximately 4 wk from the third vaccination, patients received intravenous rituximab (375 mg/m^2) to deplete tumor B cells, followed by leukapheresis within 72 h to collect at least 5×10^9 vaccine-primed lymphocytes, which were cryopreserved. 10% of the leukapheresis product was analyzed for activation markers and for determining the baseline for immune response assays described in Fig. 1 A. Patients subsequently continued to stem cell mobilization, collection, and ASCT per institutional standard conditioning with carmustine 15 mg/kg (capped at 550 mg/m^2), cyclophosphamide 100 mg/kg, and etoposide 60 mg/kg. After a protocol amendment on December 21, 2014, the carmustine dosing cap was reduced to 300 mg/m^2. Within 72 h after ASCT, patients received their vaccine-primed lymphocytes and a fourth CpG-MCL vaccination. At ∼3 mo after ASCT, after hematologic recovery, a fifth and final vaccination was given. Delays of up to 3 mo to allow for recovery from ASCT-related complications were allowed for this final vaccination.

The study was approved by the Stanford Institutional Review Board, and written informed consent was obtained from all patients before study-specific procedures per the Declaration of Helsinki. Data analyzed here were collected through January 1, 2018.

Study assessments

Patients were examined every 3 mo after ASCT. Positron emission tomography/computed tomography evaluation was repeated every 6 mo or as clinically indicated until disease progression. Adverse events were graded using the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 4.0.3.

The ClonoSEQ Ig-HTS test was employed to elucidate MRD (Faham et al., 2012; Kurtz et al., 2015). Detection of this patient-specific tumor sequence was assessed in PBMC samples every 3 mo in the first year after transplant and every 6 mo thereafter for an additional 2 yr or until evidence of clinical disease progression. For the primary trial endpoint, MRD positivity was defined as a detectable tumor-specific VDJ sequence at a frequency of ≥1 molecule per 10,000 leukocyte equivalents of DNA anytime within 1 yr after ASCT. This disease landmark and MRD threshold was previously validated as a predictor of subsequent clinical outcome (Pott et al., 2010). We also performed exploratory analyses of Ig-HTS results at lower frequencies of malignant cells down to the limit of sensitivity of the assay.

Tumor phenotype analysis and immune response assay

MCL tumor cells were purified by negative selection (EasySep Human B Cell Enrichment Kit without CD43 depletion; Stem Cell Technologies), cultured in RPMI-1640 supplemented with 5% fetal calf serum, L-glutamine, and penicillin-streptomycin at 37°C and 5% CO_2 for 72 h with or without CpG at a concentration of 1 µg/ml, and then irradiated with 100 Gy to prevent tumor cell proliferation. Patient samples processed into the tumor vaccines were radiated to 200 Gy. However, after the initiation of the trial, we found that 100 Gy was sufficient to inhibit tumor cell proliferation; therefore, this dose was used for the immune response assay. After radiation, tumor cells were stained for CD80, CD86, CD40 (BD Biosciences and PD-L1; Biolegend) and analyzed by flow cytometry on a FACScan (BD Immunocytometry Systems).

The method to determine vaccine responses was previously described (Wolfl et al., 2007; Brody et al., 2010) Briefly, irradiated CpG-activated lymphoma cells were incubated with autologous PBMCs, which had been collected before and after the initial three vaccinations. The cryopreserved PBMCs from both time points were thawed simultaneously, purified by Ficoll density gradient, and co-cultured in RPMI-1640 with 5% FCS for 5 d with or without an equal number of autologous irradiated CpG-activated MCL cells. After 5 d, cells were washed, and the cultures were restimulated with fresh CpG-activated lymphoma cells for an additional 18 h. The resulting cells were stained with

fluorochrome-conjugated antibodies against CD4, CD8, CD137, and CD45RO (BD Biosciences) and analyzed by flow cytometry on an LSR II cytometer (BD Immunocytometry Systems). The data were analyzed using Cytobank software. The percentage of tumor-reactive memory CD4 or CD8 T cells was determined as follows: After gating on $CD4^+$ or $CD8^+$ T cells, the percentage of $CD137^+$ $CD45RO^+$ T cells was measured. To determine the percentage of tumor-reactive T cells, the percentage of $CD137^+$ $CD45RO^+$ T cells found in the media-only sample was subtracted from the percentage of $CD137^+$ $CD45RO^+$ T cells found in the CpG-stimulated tumor–containing sample. A patient was determined to have made an immune vaccine response if the percentage of tumor-reactive memory T cells after vaccination exceeded the percentage measured at baseline.

Ig neoantigens from the lymphoma tumors were identified in the peptides eluted from their HLA-DR molecules and subjected to mass spectrometry. We previously reported the results from two of the patients in this trial (Khodadoust et al., 2017). In these cases, we found T cells in the patients that recognized their own Ig-derived peptides and that could kill their autologous lymphoma tumor cells. We report here the results from a third patient in this trial. From this third patient, a 30mer Ig-derived peptide (LQMNSLRAEDTAVYYCARASRITIFGVVRK) was synthesized (Elim Biopharmaceuticals) and used for the immune response assay. PBMCs from this patient were treated for 16 h with either the neoantigenic peptide or with a pool of peptides from other known HLA class I– and class II–restricted T cell epitopes (JPT Peptides Technologies). Antigen-specific CD4 cells were isolated by flow cytometry according to their expression of the CD137 activation marker and expanded in the presence of 30 ng/ml anti-CD3 antibody (OKT3; eBiosciences), 1 µg/ml anti-CD28 antibody (CD28.2; eBiosciences), and recombinant IL-2 (Peprotech). The expanded T cells were co-cultured for 24 h with autologous MCL cells labeled with CellTrace Violet dye (Thermo Fisher Scientific), and tumor cell killing was detected by flow cytometry after staining with 7-amino-actinomycin D.

Statistical analysis

The primary endpoints of this trial were safety and freedom from MRD within 1 yr after ASCT. The prespecified safety endpoints, which would have required halting the trial, were excessive rates of nonengraftment or excessive rates of early death (within 100 d from transplant) from any causes. Specifically, after 8, 16, or 24 subjects, the trial would have stopped if two, four, or five subjects experienced nonengraftment, respectively, or three, five, or six patients experienced early mortality, respectively. This rule would have stopped the study if the statistics indicated the possibility with 80% certainty that the nonengraftment rate was >10% or the early mortality rate was >15%.

Historically, 68% of patients remain MRD negative within 1 yr after ASCT after receiving R-CHOP (rituximab, cyclophosphamide, hydroxydaunomycin, vincristine, and prednisone) induction immunochemotherapy, and MRD negativity correlates to better response duration (Pott et al., 2010). To allow for early stopping for futility, a Simon two-stage optimum design was used with a desired significance level (α) of 0.1 and desired power ($1-\beta$) of 0.9. The first stage required evaluation of the first 20 patients using a MRD-free rate of 70% as the null hypothesis to trigger trial discontinuation and a MRD-free rate of 85% as the alternative hypothesis to allow trial continuation. Futility was not met. The prespecified total target size was 59 evaluable patients; however, the trial was stopped at 47 evaluable patients at the time PF-3512676 reached expiration and no additional PF-3512676 was available to continue the trial. Preplanned secondary endpoints of TTP, the time from ASCT to objective disease relapse, and OS from time of ASCT to death from any cause were examined by Kaplan-Meier analysis and log-rank test with plots generated using Prism 6.0 (GraphPad). IBM SPSS Statistics Version 21 was used to calculate the HR of PD-L1 related to TTP and OS.

To assess the risk associated with clinical relapse and MRD level, we conducted a univariable extended Cox regression to accommodate the time-varying nature of MRD level at each time point that MRD was obtained, as previously described (Cox, 1972). Our primary analysis provides an estimate of the HR for clinical relapse of MRD positive (defined as ≥1 molecule per 10^4 leukocyte equivalents of DNA) versus not positive. As a sensitivity analysis, we allowed MRD level to be either positive, low-level (defined as detectable below 1 per 10^4 leukocyte equivalents of DNA), or undetectable tumor cells. For both analyses, the observation period for all patients began at the time of ASCT until data cutoff. Patients who did not experience clinical relapse during their observation period were censored at death or last follow-up visit.

Online supplemental material

Fig. S1 shows the TTP and OS from the start of initial immunochemotherapy and the TTP and OS by B-MIPI scores from ASCT. Fig. S2 shows the TTP based on the source of the vaccine. Fig. S3 shows patient outcomes by memory CD4 T cell vaccine response. Fig. S4 demonstrates a vaccine-associated immune response in patients with a durable response after treatment. Fig. S5 demonstrates CpG-induced tumor characteristics for CD80, CD86, CD40, and PD-L1.

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Author contributions: J.D. Brody and R. Levy designed the study; D.K. Czerwinski, M.S. Khodadoust, and O.A.W. Haabeth performed tumor phenotyping and immune response evaluation; M.J. Frank, M.S. Khodadoust., M.P. Chu, R.H. Advani, A.A. Alizadeh, N.K. Gupta, L.S. Maeda, S.A. Reddy, G.G. Laport, E.H. Meyer, D.B. Miklos, R.S. Negrin, A.R. Rezvani, W.-K. Weng, I.L. Wapnir, J.D. Brody, and R. Levy treated the patients; and K. Sheehan produced vaccine products. A. Okada, A.H. Moore, and D.L. Phillips coordinated the trial; M. Faham performed Ig-HTS;

M.J. Frank, M.S. Khodadoust, and D.K. Czerwinski analyzed results; and M.J. Frank, M.P. Chu, and R. Levy wrote the manuscript.

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Supplemental material

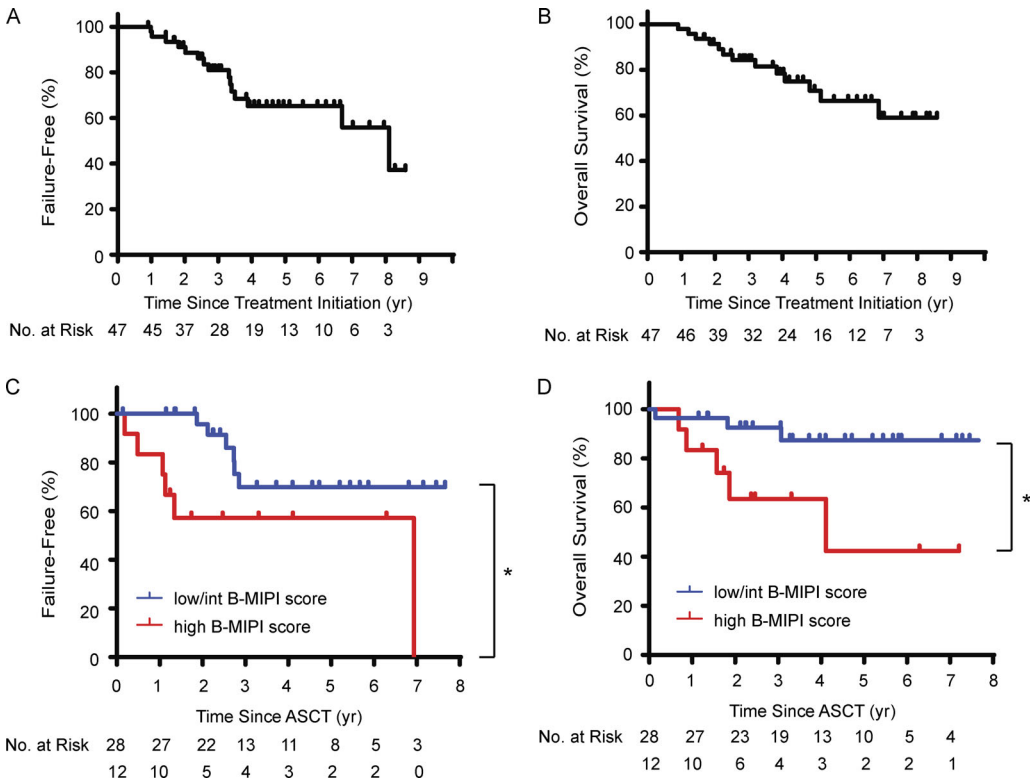

Figure S1. **Clinical trial outcomes from the start of immunochemotherapy and by B-MIPI scores from ASCT. (A and B)** TTP (A) and OS (B) examined by Kaplan-Meier analysis and log-rank test was assessed from the initial day of immunochemotherapy in all patients who received the CpG-MCL vaccination and adoptive T cell transfer. **(C and D)** TTP (C) and OS (D) examined by Kaplan-Meier analysis and log-rank test was assessed from ASCT stratified by high versus low/intermediate (low/int) B-MIPI scores. Patients with low/intermediate B-MIPI scores compared with high B-MIP scores had longer median TTP (not reached vs. 6.93 yr; *, P = 0.0260) and median OS (not reached vs. 4.11; *, P = 0.0143).

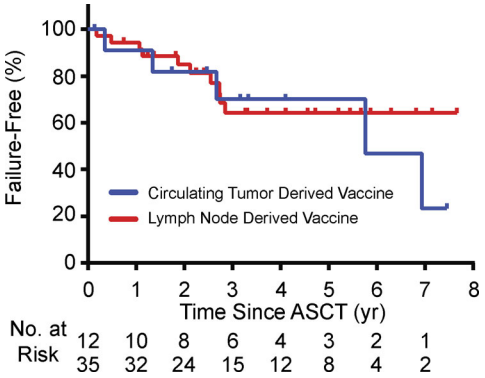

Figure S2. **TTP based on source of the vaccine.** TTP examined by Kaplan-Meier analysis and log-rank test was assessed from ASCT stratified by source of vaccine.

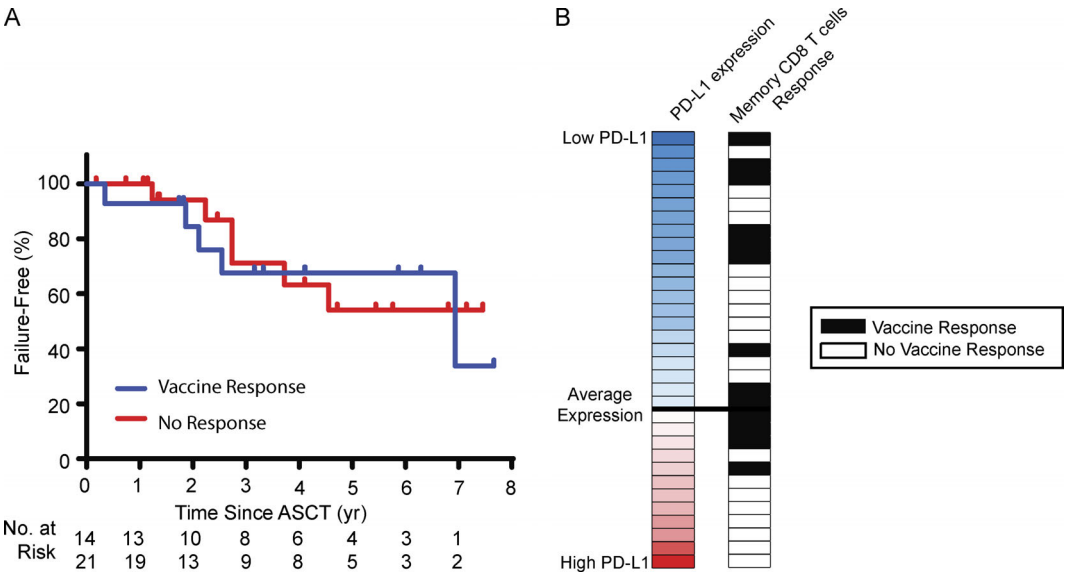

Figure S3. **Patient outcomes by memory CD4 T cell vaccine response. (A)** TTP was examined by Kaplan-Meier analysis and log-rank test from the ASCT in patients with a memory CD4 T cell vaccine response (blue) or no vaccine response (red). **(B)** Mantle cell tumors were incubated with CpG as described in the Materials and methods and assayed for PD-L1 expression. Each row shows a patient's CpG-induced tumor PD-L1 expression from lowest to highest expression level in relation to whether that patient had a CD4 T cell vaccine response (black box) or no response (white box).

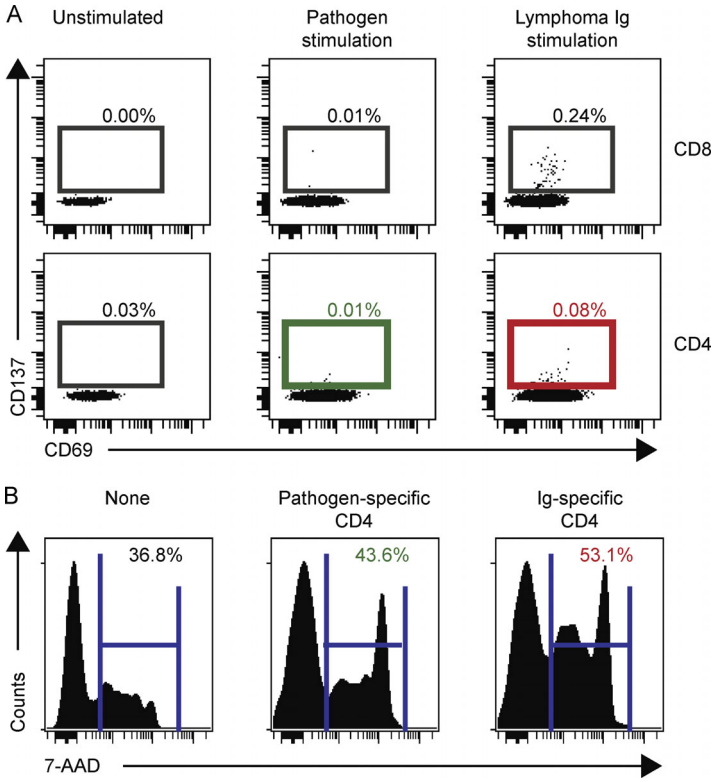

Figure S4. **Vaccine-associated immune responses. (A)** Immune responses to lymphoma Ig neoantigens were tested in a patient 2 yr after completion of ASCT. PBMCs were stimulated with either a pool of T cell epitopes from common pathogens or with a peptide corresponding to a lymphoma-specific Ig neoantigen. Antigen-specific CD8 and CD4 responses were detected by CD137 expression. **(B)** Antigen-specific CD4 T cells as determined in A were FACS sorted and expanded ex vivo. Expanded T cells were co-cultured with labeled autologous lymphoma cells for 24 h, and killing of lymphoma cells was determined by staining with 7-amino-actinomycin D.

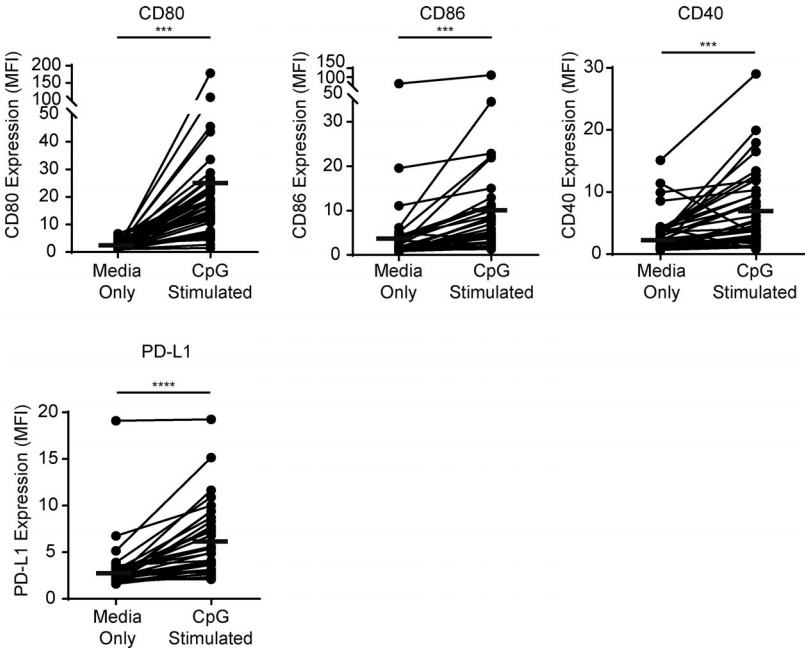

Figure S5. **CpG-activated tumor characteristics.** Mantle cell tumors were incubated with media only or CpG as described in the Materials and methods and assayed for the expression of the indicated molecules. The mean for each indicated molecule is indicated by the horizontal line. ***, P = 0.0001–0.0003; ****, P < 0.0001 by a paired two-tailed *t* test. MFI, mean fluorescence intensity.