Published OnlineFirst August 28, 2018; DOI: 10.1158/2159-8290.CD-18-0743

RESEARCH ARTICLE

In Situ Vaccination with a TLR9 Agonist and Local Low-Dose Radiation Induces Systemic Responses in Untreated Indolent Lymphoma

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ABSTRACT This multicenter phase I/II clinical trial evaluated intratumoral SD-101, a TLR9 agonist, and low-dose radiation in patients with untreated indolent lymphoma. Twenty-nine enrolled patients received 4 Gy of radiation followed by 5 weekly intratumoral injections of SD-101 at a single tumor site. No treatment-related grade 4 or serious adverse events occurred. Nearly all patients had tumor reduction at their treated site. More importantly, 24 patients had tumor reduction at their nontreated sites, with 5 patients achieving a partial response and one achieving a complete response. Treatment-related increases of $CD8^+$ and $CD4^+$ effector T cells and decreases of T follicular helper and T regulatory cells (Treg) were observed in the tumor microenvironment. Low pretreatment levels of $CD4^+$ Tregs, proliferating $CD8^+$ T cells, and Granzyme B^+ $CD8^+$ T cells were associated with favorable outcomes. Intratumoral SD-101 in combination with low-dose radiation is well tolerated and results in regression of both treated and untreated sites of disease.

SIGNIFICANCE: *In situ* vaccination with the TLR9 agonist SD-101, along with low-dose radiation, was safe and induced systemic responses in patients with indolent lymphoma. Low levels of $CD4^+$ Tregs, proliferating $CD8^+$ T cells, and Granzyme B^+ $CD8^+$ T cells in the tumor microenvironment predicted favorable response to treatment. *Cancer Discov; 8(10); 1258–69. ©2018 AACR.*

INTRODUCTION

A wide variety of therapies are now being used to harness the immune system to treat cancer. Some of these treatments are customized for each patient, including chimeric antigen receptor (CAR) T cells (1), a product of engineered autologous immune cells, or vaccines based on somatic mutations that require the identification of tumor-associated antigens (TAA; ref. 2). Although not requiring customization, immune checkpoint antibodies can enhance existing T-cell responses to endogenous TAA, but can also result in autoimmune toxicities (3). With these clinical challenges in mind, we have developed an alternative approach called *in situ* vaccination, in which immunostimulatory agents are injected locally into

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Note: Supplementary data for this article are available at Cancer Discovery Online (http://cancerdiscovery.aacrjournals.org/).

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the tumor microenvironment, triggering antitumor immune responses that can act against tumors throughout the body (4, 5).

Multiple preclinical studies have validated this *in situ* vaccine approach using short synthetic oligodeoxynucleotides containing cytidine–guanosine motifs (CpG) that stimulate innate immunity through the Toll-like receptor 9 (TLR9; refs. 6–11). Our preclinical studies established that the CpG needs to be injected directly into the tumor microenvironment. Furthermore, the addition of a T-cell stimulatory antibody against OX40 markedly enhanced the therapeutic effect of *in situ* vaccination and resulted in cure of established lymphoma, colon cancer, and even a spontaneous model of breast cancer (11). We previously conducted a clinical trial for patients with relapsed indolent lymphoma, testing the combination of local low-dose radiation and intratumoral CpG (PF-3512676; ref. 12). We observed regressions of both treated and distant, noninjected sites of disease in this small 15-patient trial. Notably, this study was conducted with a CpG sequence that had shown little therapeutic activity when given systemically (13, 14). However, PF-3512676 is no longer in clinical development after it caused increased toxicities without improving outcomes when combined with chemotherapy in non–small cell lung cancer (15, 16).

Based on these findings, we designed a dose-escalating phase I/II clinical trial of *in situ* vaccination in patients with low-grade B-cell lymphoma using a novel CpG compound, SD-101, a class C CpG that induces high levels of IFNα as well as dendritic cell maturation. The primary endpoints were safety and the induction of IFN-regulated gene expression in peripheral blood cells. Secondary endpoints included clinical efficacy and modulation of the immune microenvironment at both treated and untreated sites of tumor.

RESULTS

Patient Characteristics

Twenty-nine patients with untreated low-grade lymphoma were enrolled between October 2014 and October 2016 with baseline characteristics as summarized in Table 1 and were treated as summarized in Fig. 1. Diagnoses included follicular lymphoma (FL; $n = 21$), marginal zone lymphoma

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Table 1. Baseline characteristics

Baseline characteristics					
Dose cohort	1 mg ($n = 10$)	2 mg ($n = 3$)	4 mg ($n = 3$)	8 mg ($n = 13$)	All ($n = 29$)
Age (y)					
Mean (range)	56.9 (22–69)	56 (50–67)	61 (50–80)	62.7 (34–84)	59.8 (22–84)
Sex					
Female	4 (40%)	2 (66.7%)	1 (33.3%)	6 (46.2%)	13 (44.8%)
Male	6 (60%)	1 (33.3%)	2 (66.7%)	7 (53.9%)	16 (55.2%)
ECOG PS at screening					
0	6 (60%)	3 (100%)	3 (100%)	11 (84.6%)	23 (79.3%)
1	43 (40%)	0	0	2 (15.4%)	6 (20.7%)
Disease type					
Cutaneous B cell	0	0	0	1 (7.7%)	1 (3.5%)
Follicular	8 (80%)	3 (100%)	2 (66.7%)	8 (61.5%)	21 (72.4%)
Marginal	1 (10%)	0	0	3 (23.1%)	4 (13.8%)
SLL/CLL	1 (10%)	0	1 (33.3%)	1 (7.7%)	3 (10.3%)
Stage					
I	0	0	0	0	0
II	1 (10%)	0	0	2 (15.4%)	3 (10.3%)
III	3 (30%)	0	1 (33.3%)	2 (15.4%)	6 (20.6%)
IV	6 (60%)	3 (100%)	2 (66.7%)	9 (69.2%)	20 (69%)
Grade (follicular only)					
N	8	3	2	8	21
1	0	3 (100%)	1 (50%)	6 (46.2%)	9 (42.9%)
2	6 (75%)	0	1 (50%)	2 (15.4%)	9 (42.9%)
3A	2 (25%)	0	0	0	2 (9.5%)
FLIPI score (follicular only)					
n	8	3	2	8	21
0	0	0	0	1 (12.5%)	1 (4.8%)
1	2 (25%)	0	0	1 (12.5%)	3 (14.3%)
2	4 (50%)	3 (100%)	1 (50%)	3 (37.5%)	11 (52.4%)
3	2 (25%)	0	1 (50%)	3 (37.5%)	6 (28.6%)

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group Performance Status; FLIPI, Follicular Lymphoma International Prognostic Index.

(MZL; $n = 4$), small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL; $n = 3$), and cutaneous B-cell lymphoma (CBCL; $n = 1$). At the time of enrollment, patients' mean age was 59.8 years (range, 22–84), with the majority of patients having stage III/IV disease (90%).

Safety

No treatment-related dose-limiting toxicities (DLT) were observed. Drug-related adverse events (AE) of grades 1 and 2 were reported by all patients, with 8 patients having grade 3 drug-related AEs (Table 2; Supplementary Table S1). No drug-related grade 4 or serious AEs were experienced by any patients. The most common treatment-related side effect was a flu-like systemic reaction consisting of malaise, chills, headache, fatigue, and fever lasting typically between 24 and 48 hours after the injections, a rate similar to that observed in prior studies of TLR9 agonists (12). More grade 3 drug-related AEs were seen at the 8-mg dose (46.2%) compared with the 1-mg dose (20%); more nausea and vomiting was seen at the 8-mg dose. Four patients required a delay in treatment due to treatment-related AEs; 3 patients were delayed due to neutropenia and 1 patient was delayed due to pain at the treatment site. Only 1 patient discontinued treatment because of a treatment-related AE, due to fever and confusion that rapidly improved.

Clinical Responses

All 29 enrolled patients were evaluable for clinical response with a median follow-up of 12 months. Tumor response at the treated site was expected due to the low-dose radiation and occurred in virtually all the patients (Supplementary Fig. S1). Twenty-six of 29 treated patients demonstrated a reduction in overall tumor burden, with 7 patients achieving a partial response and 1 patient achieving a complete response. The best overall clinical response is shown in the waterfall plots by disease subtype and dose (Fig. 2A; Supplementary Fig. S1). Systemic responses at the distant, nonirradiated lesions were

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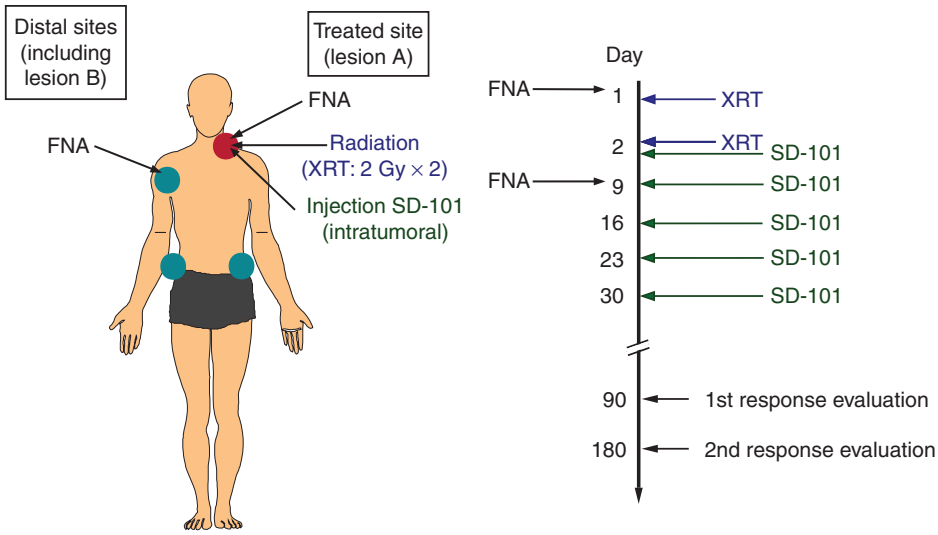

Figure 1. Schema of clinical trial. Patients underwent treatment at lesion A with radiation (XRT) with 2 Gy per day on days 1 and 2. Starting after XRT on day 2, patients received 5 weekly intratumoral injections at the treated site (lesion A) with the TLR9 agonist SD-101. Treatment response was evaluated 90 and 180 days after treatment and every 6 months thereafter. Fine-needle aspiration (FNA) biopsies were collected pretreatment (day 1) and after initial treatment (day 9) at lesion A and, if available, at a single distal site, lesion B. Red circle, treated site; green circles, distal sites.

seen in 24 of 29 patients (Fig. 2B; Supplementary Fig. S1). The response of distant, nonirradiated lesions correlated with response at the treated site (Supplementary Fig. S2). Figure 2C shows examples of the pretreatment and posttreatment responses at treated and untreated sites in 2 patients with FL. Tumor responses were typically durable and could deepen over time, as has been described in previous studies of tumor immunotherapy (Fig. 2D and E; Supplementary Fig. S3; ref. 12). Neither initial tumor burden, stage, FL international prognostic indices (for those patients with FL), nor the development of the flu-like symptoms during therapy correlated with clinical response (Supplementary Table S2). During the expansion phase of the trial, patients were permitted to receive a second cycle at the same doses they had received initially. Four patients who initally had a minimal response received a second cycle of treatment, again with minimal clinical response. In these 4 patients, the treatment-related AEs were similar for both cycles.

Pharmacokinetics

The majority of postdose samples were below the limit of quantitation, indicating that there is little systemic SD-101 following intratumoral injections at these doses. For those few samples that had values above the lower limit of quantitation, half were at the 1-hour postdose time point, with the rest scattered among different time points (Supplementary Table S3).

IFN-Responsive Gene Signature

SD-101 is a "C" class CpG, optimized to induce high-level IFNα after engaging TLR9 on plasmacytoid dendritic cells. Blood samples were collected before and 24 hours after the second intratumoral injection of SD-101 to measure induction of mRNA transcripts of well-characterized IFN-responsive genes. As Supplementary Fig. S1C shows, IFN-responsive genes were upregulated at 24 hours at all dose levels.

Treatment-Related Immune Changes in Patients with FL

Fine-needle aspiration (FNA) biopsies were collected from patients at pretreatment (day 1) and posttreatment (day 9) from the treated site (lesion A) and, if available, at a second uninjected, distal site (lesion B). Because the microenvironment of each lymphoma subtype is different, we focused our analysis on the most prevalent histology, FL. Sixteen of 21 patients with FL had sufficient day 1 and 9 samples for paired analysis from lesion A, and 2 additional patients had just a day 9 sample. The pretreatment intratumoral immune cell composition was remarkably similar between different tumor sites of the same patient but varied considerably among patients (Supplementary Fig. S4). We observed a significant reduction in malignant cells and an increase in $CD3^+$, $CD8^+$, and $CD4^+$ T cells after treatment (day 9) in the treated site (Fig. 3A and B; Supplementary Fig. S5). Further investigation of $CD4^+$ T-cell subsets in the treated site revealed a marked decrease in T follicular helper cells (TFH) and T regulatory cells (Treg), and a significant increase in effector $CD4^+$ T cells (Fig. 3C; Supplementary Fig. S5B).

Next, we investigated if any baseline (day 1) characteristics of $CD4^+$ or $CD8^+$ T-cell subsets correlated to distal and overall tumor responses. Interestingly, we found that a low baseline percentage of $CD4^+$ Tregs correlated with better clinical responses (Fig. 4A). Similarly, within the $CD8^+$ subsets, we found a low initial percentage of proliferating ($Ki67^+$) and granzyme B^+ (GzB^+) $CD8^+$ T cells correlated with better clinical outcomes (Fig. 4B and C). Finally, we noted a significant relationship between the expression of MHC II on the tumor cells at the treated site and the clinical outcomes. High tumor-expressing MHC class II posttreatment was associated with improved clinical outcomes (Fig. 4D).

DISCUSSION

The ultimate goal of cancer immunotherapy is to harness the immune system to trigger durable antineoplastic immune responses without inducing significant autoimmune toxicities. We now know that patients with B-cell lymphomas possess tumor-specific $CD4^+$ T cells that are capable of recognizing MHC class II presented peptides derived from the idiotype, renewing an interest in creating antilymphoma vaccines (17). Therefore, it is possible that such preexisting immune

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Table 2. Drug-related AEs that occurred in at least 10% of patients

Drug-related AEs												
SD-101 dose	1 mg ($n = 10$)		2 mg ($n = 3$)		4 mg ($n = 3$)		8 mg ($n = 13$)			Total ($n = 29$)		
Grade	1/2	3	1/2	3	1/2	3	1/2	3	1/2	3	All
Malaise	9 (90)	0	2 (67)	0	3 (100)	0	8 (62)	5 (38)	22 (76)	5 (17)	27 (93)
Chills	8 (80)	0	2 (67)	0	3 (100)	0	9 (69)	4 (31)	22 (76)	4 (14)	26 (90)
Fatigue	7 (70)	0	3 (100)	0	3 (100)	0	10 (77)	2 (15)	23 (79)	2 (7)	25 (86)
Headache	7 (70)	1 (10)	3 (100)	0	3 (100)	0	7 (54)	4 (31)	20 (69)	5 (17)	25 (86)
Myalgia	6 (60)	0	3 (100)	0	3 (100)	0	10 (77)	3 (23)	22 (76)	3 (10)	25 (86)
Fever	4 (40)	0	1 (33)	0	2 (67)	0	12 (92)	0	19 (66)	0	19 (66)
Nausea	2 (20)	0	1 (33)	0	0	0	8 (62)	0	11 (38)	0	11 (38)
Diarrhea	3 (30)	0	0	0	1 (33)	0	4 (31)	0	8 (28)	0	8 (28)
Injection-site erythema	4 (40)	0	0	0	1 (33)	0	3 (23)	0	8 (28)	0	8 (28)
Vomiting	0	0	1 (33)	0	0	0	5 (38)	0	6 (21)	0	6 (21)
Neutropenia	1 (10)	1 (10)	0	0	0	0	2 (15)	1 (8)	3 (10)	2 (7)	5 (17)
Decreased appetite	2 (20)	0	0	0	0	0	2 (15)	0	4 (14)	0	4 (14)
Injection-site swelling	3 (30)	0	1 (33)	0	0	0	0	0	4 (14)	0	4 (14)
Night sweats	2 (20)	0	0	0	1 (33)	0	0	0	3 (10)	0	3 (10)
Sore throat	1 (10)	0	0	0	0	0	2 (15)	0	3 (10)	0	3 (10)
Thrombocytopenia	0	0	1 (33)	0	0	0	2 (15)	0	3 (10)	0	3 (10)

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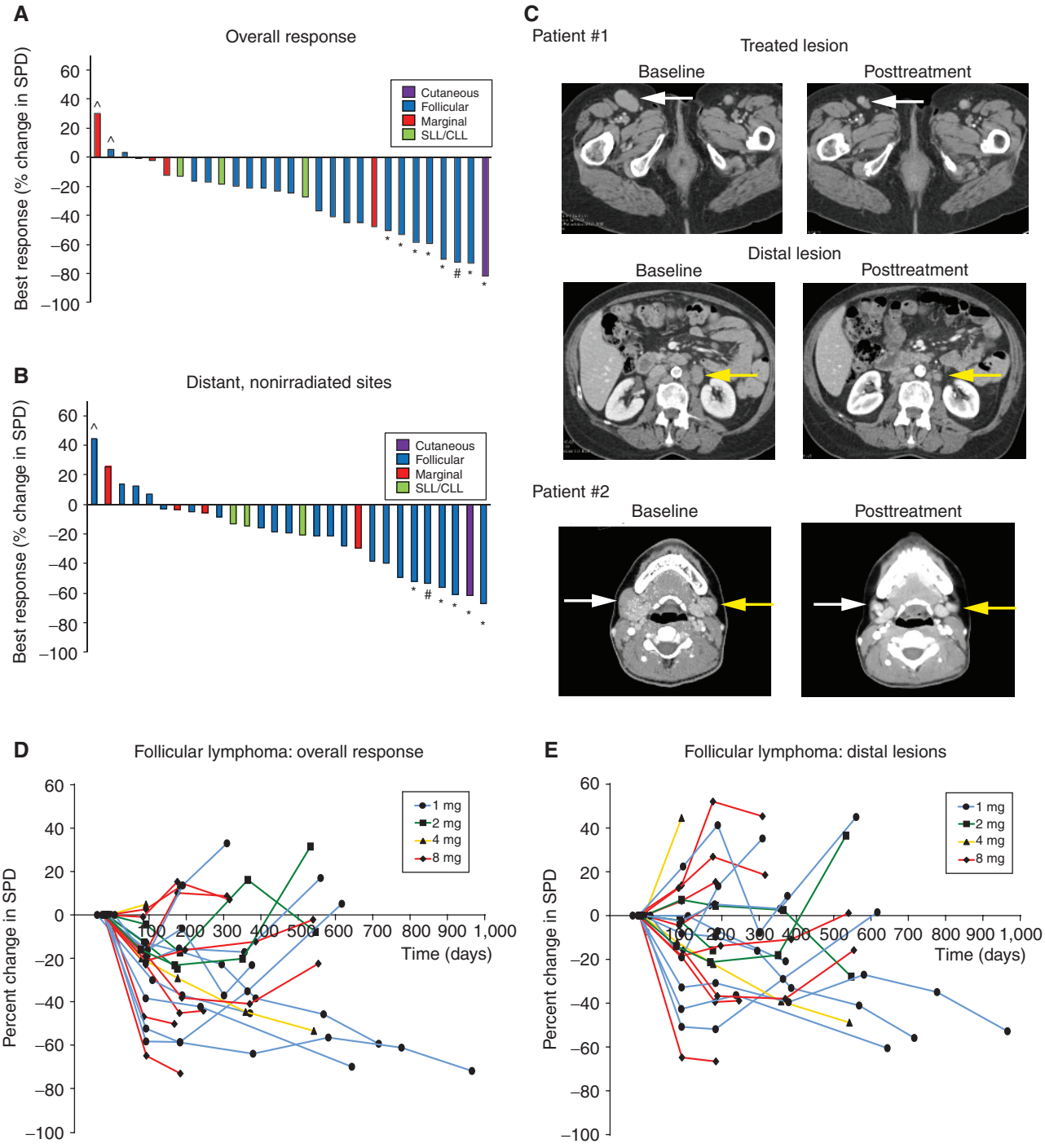

Figure 2. SD-101 and low-dose radiation induces responses in patients with indolent lymphoma. Waterfall plot showing the best overall change in the sum of the product of the diameters (SPD) in all target lesions **(A)** and distal sites **(B)** by lymphoma subtype. Patients achieving a partial response (*), complete response (#), or progression (^) by the Revised 2007 International Working Group criteria are shown. **C,** A patient with FL treated in the right inguinal lesion (white arrow, top) has both local and systemic responses (para-aortic lesion; yellow arrow, middle) as seen in the initial pretreatment imaging and 6 months after treatment. A second patient with FL treated in the right cervical lesion (white arrow, bottom) has both local and systemic responses (left cervical lesion; yellow arrow, bottom) as seen in the initial pretreatment imaging and 21 months after treatment. **D** and **E,** Spider plot showing change over time in the sum of the product of the diameters in all lesions **(D)** or just distal sites (**E;** excluding lesion A) by dose in patients with FL.

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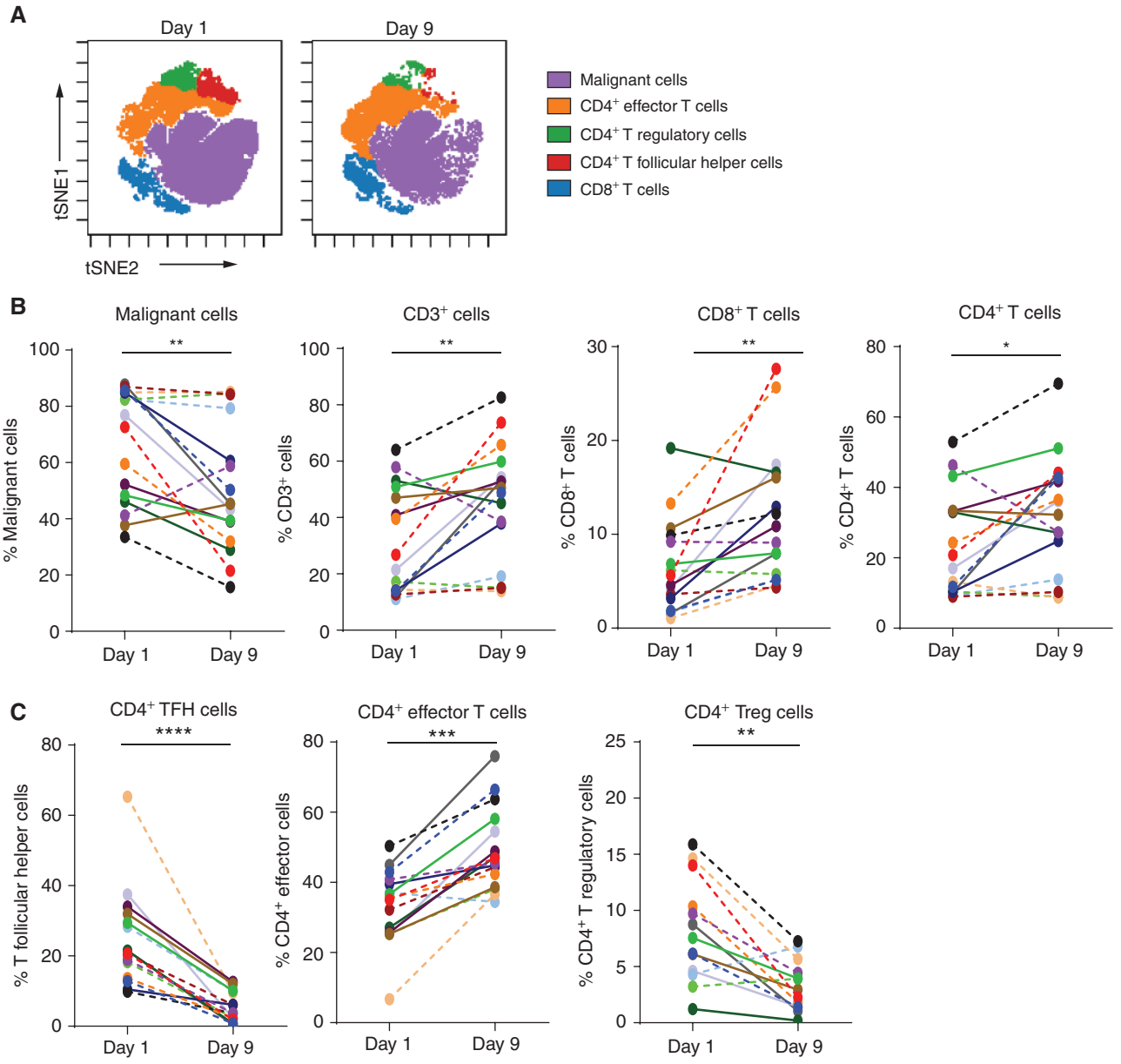

Figure 3. Treatment-induced immune cell changes. Initial (day 1) and posttreatment (day 9) lymphoma-infiltrating immune subsets were gated and visualized in t-distributed stochastic neighbor embedding (tSNE) space using Cytobank software. **A,** Malignant cells, CD3+ cells, CD8+ T cells, and CD4+ T cells were evaluated pretreatment (day 1) and posttreatment (day 9). **B,** As a percentage of all cells, the percentage of intratumoral malignant cells decreased ($P = 0.0052$) and CD3+, CD8+, and CD4+ T cells increased posttreatment ($P = 0.0079$, $P = 0.0036$, and $P = 0.0248$, respectively, using a two-tailed paired *t* test). **C,** CD4+ T-cell subsets' percentages of T cells were analyzed pre- and posttreatment. TFH cells (CD4+ FOXP3− CXCR5+ PD-1hi ICOS+) and Tregs (CD4+ CD25+ CD127−) decreased ($P < 0.0001$ and $P = 0.0012$, respectively) and effector cells (remaining CD4+ FOXP3− cells) increased ($P = 0.0010$) using a two-tailed paired *t* test. Each patient is tracked by a specific color, and patients achieving at least an overall partial response are connected by solid lines and those who did not by dashed lines. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

potential can be enhanced by delivering the appropriate immunostimulatory signals directly into the tumor microenvironment, where the T-cell repertoire may be enriched for tumor-reactive T cells. *In situ* vaccination may have the advantage of inducing such immune responses to the tumor while avoiding the induction of autoimmunity.

This multicenter clinical trial tested an *in situ* vaccination strategy using a novel "C" class CpG, SD-101, in contrast to a previous clinical trial that used a "B" class CpG, PF- 3512676, that is no longer available for clinical testing (12). Whether these class differences in CpGs translate to meaningful differences in therapeutic efficacy or toxicity is unknown. In addition, the current trial tested treatment-naïve patients, in contrast to the previous trial that tested only patients with relapsed disease. Despite these differences, the rate of overall and distant tumor responses was remarkably similar between

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these two trials. In the current trial, 26 of 29 patients had a reduction in total tumor volume, with 7 patients achieving an overall partial response and 1 patient achieving a complete response. Abscopal responses to radiation are known to occur rarely in patients with lymphoma (18, 19). Therefore, the benefit of intratumoral CpG plus radiation over radiation alone in the current study, although suggestive, is not proven. Additionally, patients with indolent lymphoma can occasionally have spontaneous remissions, but again, the response rate seen in this trial surpasses what has been previously observed (20).

A variety of preclinical studies have demonstrated that potent systemic antitumor immune responses can be generated without the use of cytotoxic chemotherapy/radiotherapy by combining local CpG with other immunostimulating agents. Treatment with local CpG combined with ibrutinib or with monoclonal antibodies (mAb) targeting GITR, CTL4, and/or OX40 generates potent systemic antineoplastic T-cell responses in multiple transplantable and spontaneous mouse tumor models (8–11). Similarly, intratumoral SD-101 combined with anti–PD-1 mAbs induces strong, systemic antitumor responses, even in tumor models unresponsive to anti–PD-1 alone (21). Ongoing studies continue to determine the optimal therapeutic partner to combine with intratumoral CpG for the treatment of lymphoma and other malignancies. Trials evaluating intratumoral SD-101 and local radiation with intratumoral ipilimumab (NCT02254772) or with oral ibrutinib (NCT02927964) have been initiated; and trials combining SD-101 with an anti-OX40 mAb in both indolent lymphomas (NCT03410901) and solid malignancies are planned, given encouraging preclinical data (11). Additionally, SD-101 in combination with pembrolizumab without the use of radiation (NCT02521870) has shown early promise in patients with melanoma (22). Beyond TLR9 agonists, other immunostimulatory agents such as TLR3 agonists (23, 24), TLR4 agonists (25), TLR7/8 agonists (26–28), and STING agonists (29) have shown early therapeutic promise, and ongoing/future studies will be needed to determine optimal *in situ* vaccination combinations.

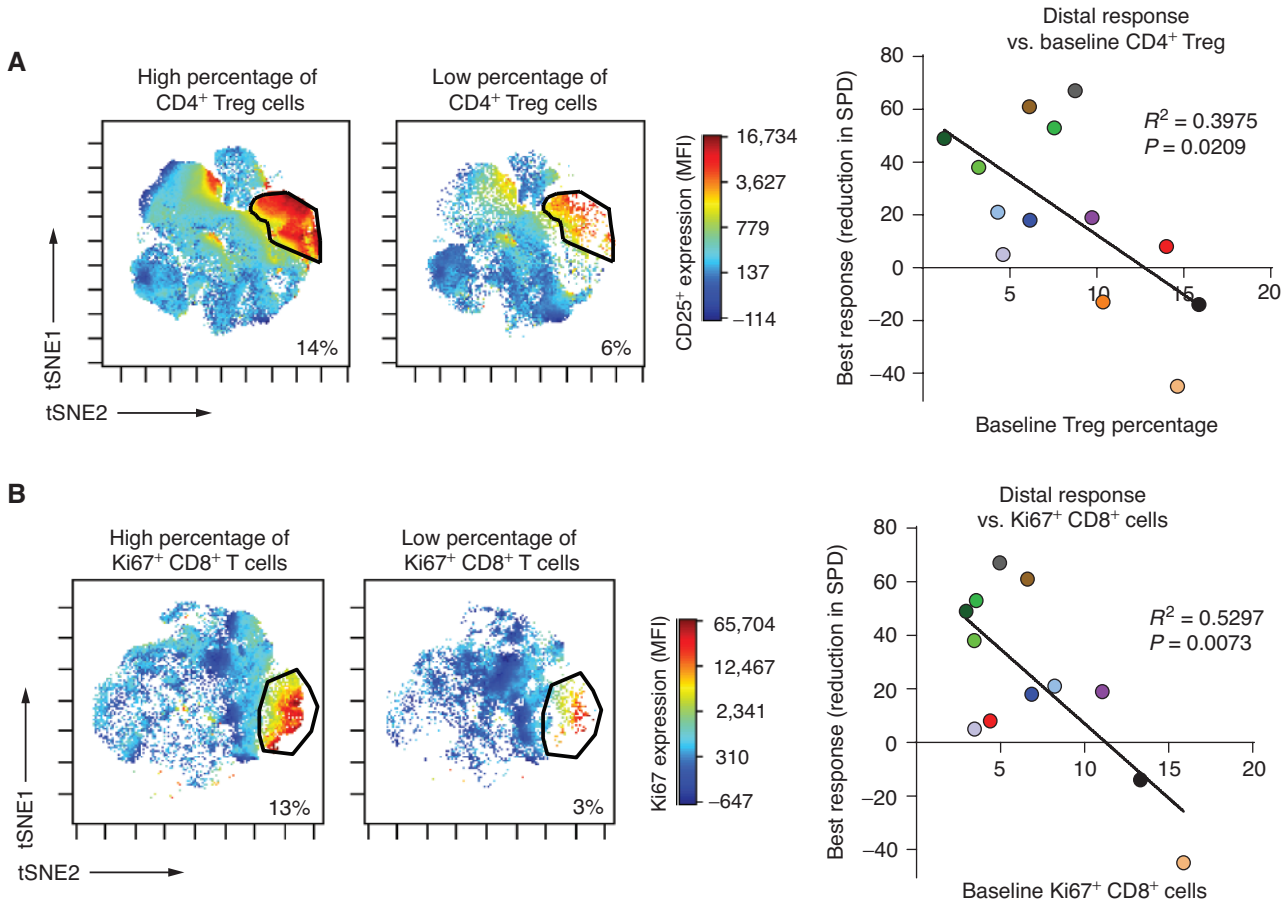

Figure 4. Low initial levels of CD4+ Tregs, proliferating CD8+, and Granzyme B+ CD8+ T cells and high posttreatment tumor MHC class II expression predict better response to treatment. **A,** Baseline percentages of CD4+ Treg of all T cells were gated (CD3+ CD4+ CD25+ CD127-) and visualized in tSNE space using Cytobank software. A low baseline percentage of CD4+ Tregs correlated to a better distal clinical response ($P = 0.0209$) by linear regression analysis. **B,** Baseline percentages of proliferating (Ki67+) CD8+ cells as a total of CD8+ T cells were gated and visualized in tSNE space. A lower percentage of proliferating CD8+ T cells correlated to better distal response ($P = 0.0073$) by linear regression analysis. (*continued on next page*)

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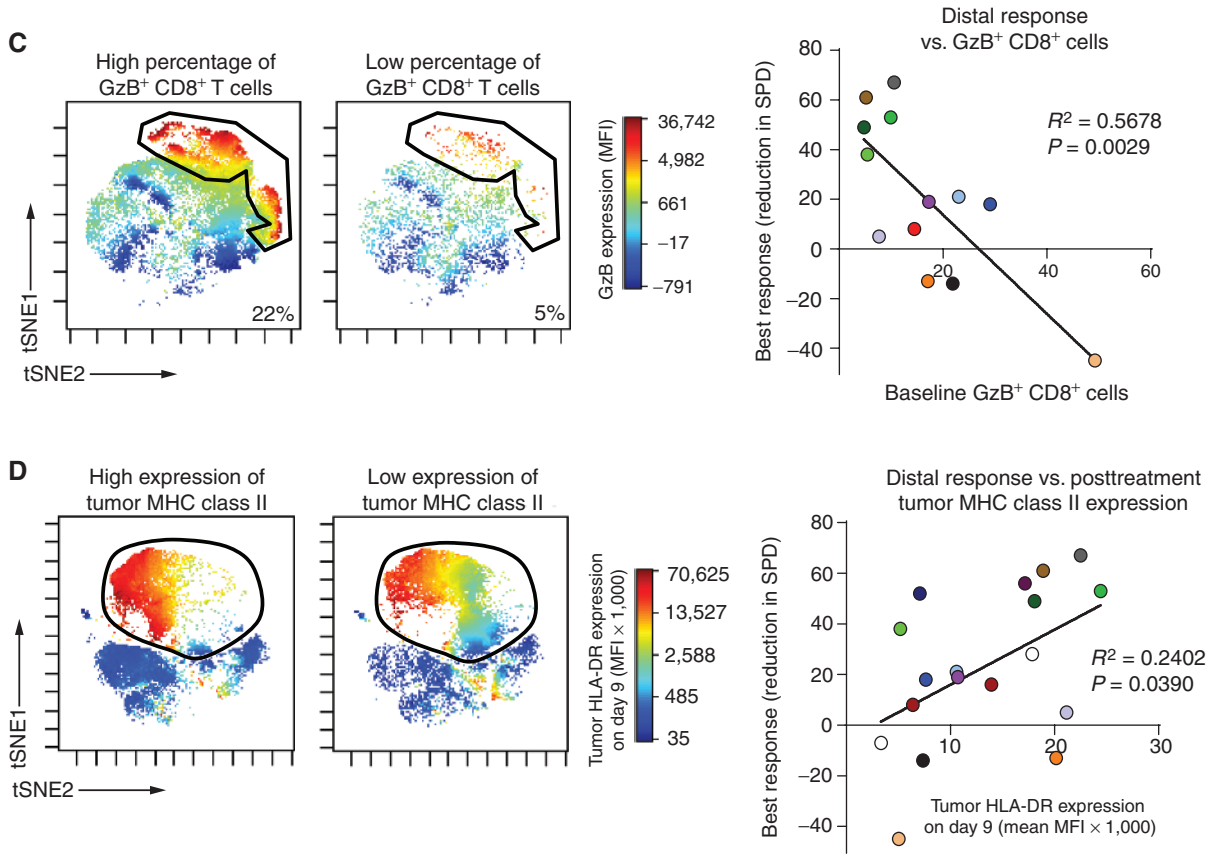

Figure 4. ***(Continued)*** **C,** Baseline percentage of Granzyme B+ (GzB) CD8+ cells as a total of CD8+ T cells were gated and visualized in tSNE space. A lower percentage of GzB+ CD8+ T cells correlated to better distal response ($P = 0.0029$) by linear regression analysis. **D,** Posttreatment (day 9) tumor cells were gated and visualized in tSNE space to evaluate MHC class II (HLA-DR) expression. Tumor cell MHC class II expression as measured by mean fluorescence intensity (MFI) positively correlated to distal response ($P = 0.0390$) by linear regression analysis. Each patient is tracked by a specific color.

The combination of SD-101 and local radiotherapy rapidly induced changes, including a reduction in tumor percentage associated with an increase of CD8+ T cells and CD4+ effector cells. This treatment combination also substantially reduces the percentage of TFH cells, cells thought to promote FL immune escape by inducing an immunosuppressive tumor microenvironment (30). Additionally, we observed a treatment-induced reduction of CD4+ Treg in the treated site. Interestingly, patients who exhibited low initial levels of CD4+ Tregs had better responses to treatment. Although substantial data suggest that Tregs contribute to tumor escape from host immune surveillance, the relationship between Tregs and patient outcomes is idiosyncratic. Low levels of Tregs can be associated with either an improved or unfavorable prognosis depending on the treatment modality and tumor type (31). Intriguingly, high induced levels of CD4+ Tregs in an *ex vivo* assay were associated with poor response to TLR9 agonists in indolent lymphoma (12). In addition, in our current trial, low levels of proliferating CD8+ and GzB+ CD8+ T cells correlated with better response to treatment. Similar findings have been seen for other tumor types (32, 33). These findings are the result of an initial exploratory analysis and will need to be validated.

Preclinical studies evaluating TLR9 agonists have demonstrated that systemic tumor clearance was partially CD4+ T-cell dependent (11). Here, we find that high expression of tumor MHC class II was associated with a better overall clinical response, further supporting a CD4+ T cell–dependent antitumor mechanism. Additionally, the observed variability in MHC class II expression on FL cells may have a genetic basis. Mutations in *CREBBP*, which is commonly mutated in FL, are associated with a downregulation of MHC class II (34, 35). Therefore, it will be of interest to examine the relationship between *CREBBP* mutation status and outcomes of *in situ* vaccination in future trials.

Overall, these results and associated preclinical studies provide the rationale for expanded studies of *in situ* vaccination with TLR9 agonists in conjunction with other immune-modulating agents in patients with lymphoma.

METHODS

Study Design

This multicenter, open-label, dose-escalation phase I/II study (ClinicalTrials.gov Identifier: NCT02266147) was designed to evaluate safety, pharmacodynamics (PD), and preliminary efficacy of intratumoral SD101 together with low-dose radiotherapy. Part 1 consisted of four cohorts of escalating doses of SD-101 (1, 2, 4, and 8 mg) in a standard 3 + 3 design, and part 2 studied expanded cohorts to further evaluate the 1-mg and 8-mg doses. Subjects in part 2 had

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the option to undergo a second cycle of treatment at the same dose level they received in cycle 1. All subjects underwent safety, pharmacokinetic (PK), and PD assessments, tumor response determinations, and sampling of the treated (lesion A) and untreated (lesion B) tumor sites just prior to treatment and at day 9 by FNA for correlative biomarker analysis.

All investigators obtained written informed consent from patients prior to participation in the study, and this study was conducted according to the Declaration of Helsinki principles. This study was approved by institutional review boards prior to enrollment. This study was conducted in accordance with good clinical practice as defined in International Conference on Harmonisation guidelines and US Code of Federal Regulations Title 21, Parts 11, 50, 54, 56, 312, and Title 45 Parts 46, 160, and 164.

Patient Selection

Patients had untreated low-grade B-cell lymphomas, including grade 1–3A FL, MZL, CLL/SLL, and CBCL with multiple lymph node involvement that could be managed by a "watch and wait" approach. Patients were required to have at least two sites of disease amenable for injection and/or FNA sampling. One of these sites was used for treatment and the second site outside of the treatment field was used for FNA sampling and for response assessment. Patients were ≥18 years with adequate hematopoietic, renal, and hepatic function; and were excluded for the presence of central nervous system lymphoma involvement, hepatitis B or C, human immunodeficiency virus, or any active infection; for clinically significant cardiovascular disease; or for previous diagnosis with another cancer requiring treatment within the past 3 years or autoimmune disease, pregnancy, or an Eastern Cooperative Oncology Group performance status of ≥2.

Treatment Schema

A single palpable site of disease, lesion A, was irradiated with 4 Gy, a standard treatment for single sites of low-grade lymphoma, known to kill some tumor cells while sparing the antigen-presenting cells in the tumor microenvironment (24). The patients received this treatment over 2 consecutive days (days 1 and 2) and then were injected 5 times at 1-week intervals with SD-101 at the assigned dose (Fig. 1). Blood was collected for measurement of IFN-responsive gene induction, a PD endpoint. FNAs were performed at both the treated lesion (A) and at a second, untreated lesion (B), before and 1 week after the initiation of therapy, and the resulting cell suspensions were shipped overnight at 4°C in RPMI medium containing 5% fetal calf serum to Stanford University, where flow cytometry analysis was performed.

Study Assessments

Safety. AEs were graded according to the NCI Common Terminology Criteria for Adverse Events (version 4.0). Treatment delays occurred for grade ≥2 neutropenia (absolute neutrophil count < 1,500/mm^3). DLTs were defined as any nonhematologic toxicity grade ≥3 except for alopecia or nausea uncontrolled by medical management; grade 4 thrombocytopenia or grade 3 thrombocytopenia with bleeding or any requirement for platelet transfusion; febrile neutropenia; grade 4 neutropenia lasting >5 days; grade 4 anemia, unexplained by underlying disease; and/or any grade ≥2 toxicity related to SD-101 that does not resolve to grade ≤1 with standard treatment by the time of the next treatment.

Efficacy. Disease assessment included CT (or PET/CT) scans at screening, at 3 and 6 months after treatment, and then every 6 months for the remainder of the trial. There was an additional CT scan at 9 months for subjects who received cycle 2. Overall tumor responses were assessed according to the Revised 2007 International Working Group criteria (36). In addition, we scored tumor responses separately at the treated site and at untreated sites of disease. Patients were not permitted to receive lymphoma-directed therapy, including steroids, during the follow-up period and were off study if they received any such treatments.

Pharmacokinetics. Plasma samples were collected for PK analysis before and 24 hours after the day 9 injection and analyzed for SD-101 level by LC/MS-MS.

IFN-Responsive Gene Signature

Before and 24 hours after treatment on day 9, whole blood was collected in PAXgene tubes (Qiagen) and frozen until RNA was isolated. The expression of IFN-responsive genes (MCP1, GBP1, ISG54, and MxB) was performed via quantitative PCR and normalized to the expression level of ubiquitin. To assess the engagement of TLR9, a composite score was generated by calculating the geometric mean of the fold activity of each of the four genes on day 10 relative to the day 9 baseline.

FNA Analysis by Flow Cytometry

Serial FNA samples were collected on days 1 and 9 from both a treated lesion (lesion A) and a single distal lesion (lesion B), if available. A single cell suspension was stained with three panels of fluorochrome-conjugated antibodies, fixed and permeabilized using Fix/Perm solution (BD Biosciences), and then stained for intracellular proteins. Panel 1 included antibodies against CD3, CD4, CD8, CD25, CD27, CD127, CD134, CD278(ICOS), CD279(PD-1), CXCR5, HLA-DR, and intracellular FOXP3 (BD Biosciences). Panel 2 included antibodies against CD3, CD8, CD25, CD45RO$^+$, CD62L, CD127, CD279, and intracellular Eomes, Ki67, and Granzyme B (GzB). Panel 3 included antibodies against CD3, CD19, CD20, and lambda light chain. Flow cytometry was performed with an LSR II cytometer (BD Immunocytometry Systems), and the data were analyzed using Cytobank software. Relationships between individual markers on T, B, and myeloid cell subsets were interrogated in relation to therapy and to clinical outcomes. Statistical significance in the differences in cell populations between days 1 and 9 was determined using Prism 6.0 (GraphPad). P values of <0.05 were considered statistically significant.

Disclosure of Potential Conflicts of Interest

P.M. Reagan reports receiving a commercial research grant from Seattle Genetics. J.W. Friedberg is a consultant/advisory board member for Bayer. R. Janssen has ownership interest (including stock, patents, etc.) in Dynavax Technologies. A.F. Candia has ownership interest (including stock, patents, etc.) in Dynavax Technologies. R.L. Coffman has ownership interest (including stock, patents, etc.) in Dynavax Technologies. R. Levy reports receiving a commercial research grant from Dynavax and is a consultant/advisory board member for Checkmate and Immune Design. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M.J. Frank, L.I. Gordon, J.W. Friedberg, R.T. Hoppe, R. Janssen, A.F. Candia, R.L. Coffman, R. Levy

Development of methodology: M.J. Frank, D.K. Czerwinski, R. Janssen, A.F. Candia, R. Levy

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.J. Frank, P.M. Reagan, L.I. Gordon, J.W. Friedberg, D.K. Czerwinski, S.R. Long, R.T. Hoppe, R. Levy

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.J. Frank, P.M. Reagan, D.K. Czerwinski, A.F. Candia, R. Levy

Published OnlineFirst August 28, 2018; DOI: 10.1158/2159-8290.CD-18-0743

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Acknowledgments

The authors thank all of the participating investigators, patients, and their families. This work was supported by grants from the NCI R35CA19735301 and Dynavax Corporation. This work was supported by a grant to M.J. Frank from the American Cancer Society's Grand View League.

Received June 29, 2018; revised July 13, 2018; accepted August 9, 2018; published first August 28, 2018.

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Published OnlineFirst August 28, 2018; DOI: 10.1158/2159-8290.CD-18-0743

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Published OnlineFirst August 28, 2018; DOI: 10.1158/2159-8290.CD-18-0743

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CANCER DISCOVERY

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Cancer Discov 2018;8:1258-1269. Published OnlineFirst August 28, 2018.