

Lenalidomide-Induced Immunomodulation in Multiple Myeloma: Impact on Vaccines and Antitumor Responses

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Abstract

Purpose: To show that the immunomodulatory drug lenalidomide can be used in patients with relapsed multiple myeloma to augment vaccine responses.

Experimental Design: Early phase clinical trial of patients with multiple myeloma who received at least one prior therapy. Patients were treated with single-agent lenalidomide and randomized to receive two vaccinations with pneumococcal 7-valent conjugate vaccine (PCV) on different schedules. Cohort A received the first PCV vaccination prior to the initiation of lenalidomide and the second vaccination while on lenalidomide. Cohort B received both vaccinations while on lenalidomide.

Results: PCV-specific humoral and cellular responses were greater in cohort B than A and were more pronounced in the bone marrow than the blood, suggesting that maximal vaccine efficacy was achieved when both vaccines were administered concomitantly with lenalidomide. Patients with a clinical myeloma response showed evidence of a tumor-specific immune response with increases in myeloma-specific IFN- γ ⁺ T cells and reductions in Th-17 cells.

Conclusions: This is the first clinical evidence showing that lenalidomide augments vaccine responses and endogenous antitumor immunity in patients and as such may serve as an adjuvant for cancer and possibly infectious vaccines. *Clin Cancer Res*; 18(5); 1426–34. ©2012 AACR.

Introduction

Thalidomide was the first "novel" drug introduced for the treatment of multiple myeloma and has shown considerable antitumor activity through multiple mechanisms, including via the tumor microenvironment through inhibition of angiogenesis and TNF- α (1). Lenalidomide, an immunomodulatory agent, inhibits myeloid cell-mediated inflammatory immune function through inhibition of proinflammatory cytokines TNF- α and interleukin (IL)-6 (2). It also increases lymphoid immune function by increasing natural killer (NK) cell numbers and antibody-dependent cell-mediated cytotoxicity (3–5) and augments NK T-cell numbers and function through increases in CD1d-mediated presentation of glycolipids

(6). Lenalidomide enhances T-cell cytokine production and proliferation by augmenting activator protein (AP)-1 transcriptional activity (7), reducing the inhibitory effect of cytotoxic T-lymphocyte antigen (CTLA)-4 (8), and possibly reducing the generation of regulatory T cells (T_{regs} ; ref. 9). This activity suggests that a major mechanism of lenalidomide clinical activity is through its immunomodulatory role within the tumor microenvironment (10).

Although used in myeloma, the impact of single-agent lenalidomide on antigen-specific immune responses in patients with myeloma has not been formally examined (11, 12). Previous studies have indicated that lenalidomide has the potential to enhance immune responses both *in vitro* (5, 13) and in patients with advanced tumors (14, 15). In addition, while vaccines can induce immune responses in patients with myeloma, the lack of a measurable clinical benefit is largely due to the profound tumor-associated immune tolerance of patients (16). Thus, current strategies to improve myeloma vaccines must emphasize modulation of the immune system. This study was designed to determine whether lenalidomide could augment vaccine responses and elicit myeloma-specific immune responses when used in combination with the pneumococcal 7-valent conjugate vaccine (PCV; Prevnar, Wyeth Pharmaceuticals Inc.), a vaccine conjugated to the modified diphtheria toxin (DT; CRM197).

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Translational Relevance

Lenalidomide has been developed as an immunomodulatory derivative of the parent compound, thalidomide. Despite its significant clinical efficacy and presumed immune effect, no human studies to date have documented these properties. In this study, we show these immune outcomes in two ways. Lenalidomide elicits a direct immune-mediated antimyeloma effect and augments nonspecific immunity to increase vaccine efficacy of the pneumococcal 7-valent conjugate vaccine (PCV). This study thus establishes the rationale for using lenalidomide as an adjuvant to augment vaccine efficacy in both malignant and non-malignant individuals.

Patients and Methods

Patient eligibility

This was an open-label, 2-cohort study in which all patients received lenalidomide in combination with 2 PCV vaccinations in one of 2 randomly assigned vaccine schedules. PCV was chosen because of its ability to invoke both T-cell-dependent antipneumococcal antibody responses and anti-CRM197 T-cell responses (17).

Lenalidomide-naïve patients with relapsed myeloma following 1 to 3 prior therapies were included in this study. The study was approved by the Institutional Review Board at the Johns Hopkins Medical Institutions (Baltimore, MD), and all patients provided written informed consent.

Patients were enrolled after 1-month of no myeloma treatment. Patients in both cohorts received lenalidomide at a starting dose of 25 mg/d on days 1 to 21 of each 28-day cycle, for a total of 6 cycles. Cohort A received their first vaccination 2 weeks before starting lenalidomide and their second on day 14 of cycle 2 (Fig. 1). Cohort B received their

first vaccination on day 14 of cycle 2 and their second on day 14 of cycle 4. Steroids were prohibited to avoid immunosuppression. Lenalidomide dose reductions were based on standard clinical practice: 20 mg (dose level: -1); 15 mg (level: -2); 10 mg (level: -3); and 5 mg (level: -4). *Candida*-specific, delayed-type hypersensitivity (DTH) was administered at enrollment, prior to each vaccination, and 6 weeks after the last vaccination. Erythema and induration to *Candida* were recorded at 48 hours by measuring the widest diameters in 2 perpendicular directions. For purposes of immune monitoring, blood and bone marrow samples were obtained as indicated in the study schema. Samples were obtained at baseline in both cohorts: prior to the first Prevnar administration in cohort A or prior to initiation of lenalidomide in cohort B. Subsequent sample time points were prior to the second vaccine and 6 weeks after the second vaccine.

Response assessment

The clinical response to lenalidomide was assessed after each cycle. Patients with a 50% or more decrease in the monoclonal paraprotein levels were defined as responders. Patients whose myeloma progressed by an increase in monoclonal paraprotein levels of 25% or more were defined as progressors. Stable disease was defined as a less than 50% decrease in their monoclonal protein levels.

Immune analyses

Serologic responses to PCV. Serum IgG levels against 4 (6B, 14F, 19F, and 23F) of the 7 PCV serotypes were measured by ELISA as previously described (18, 19). Titers were reported in $\mu\text{g/mL}$ by interpolating Abs_{450} values in the dose-response curve of the pneumococcal reference standard serum 89SF.

Antigen-specific T-cell responses. Peripheral blood lymphocytes (PBL) and bone marrow cells were thawed in AIM-V media (Invitrogen), labeled with carboxyfluorescein succinimidyl ester (CFSE; Invitrogen) and incubated for 10 minutes at 37°C. CRM-197 responses were determined by adding the DT, CRM197 (Sigma; 10 $\mu\text{g/mL}$) for 5 days at 37°C and staining with anti-CD3 (BD-Biosciences) and anti-IFN- γ (e-Biosciences) prior to analysis by flow cytometry. Data were acquired on a FACSCalibur (BD-Biosciences) and analyzed with CellQuest software. Antigen-specific T cells were identified as $\text{CFSE}^{\text{low}}/\text{IFN-}\gamma^+/\text{CD3}^+$ T cells. To identify myeloma-specific T cells, bone marrow cells were labeled with CFSE (as earlier) and incubated in either AIM-V alone, SW780 (nonspecific bladder carcinoma cell line) lysates, or H929 + U266 (myeloma cell line) lysates each. These cell lines were obtained from the American Type Culture Collection. Bone marrow cells were incubated for 5 days in the presence or absence of the cell lysates, harvested, and stained with anti-CD3 (BD-Biosciences) and IFN- γ (e-Biosciences) prior to analysis by flow cytometry.

Flow cytometry

Cells were stained for cell surface expression of CD3, CD4, CD8, CD40L, CTLA4, CD14, CD19, CD26,

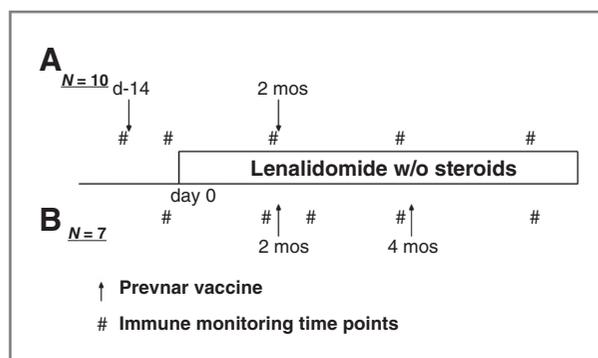


Figure 1. Trial schema. Patients were assigned to either cohort A which received the first PCV 14 days (d-14) prior to initiation of lenalidomide and the second on day 14 of cycle 2 of lenalidomide or cohort B in which the first PCV was administered on day 14 of cycle 2 and the second on day 14 of cycle 4. Blood and bone marrow were obtained at the indicated time points for immune monitoring assays. w/o, without.

CD56, and CD11c (BD-Biosciences). Cells were enumerated using a FACSCalibur and analyzed using CellQuest Pro software. Intracellular staining for FOXP3 (e-Biosciences), IFN- γ , and IL-17 was carried out by adding GolgiPlug (BD-Biosciences) as per manufacturer's recommendations. Extracellular staining was carried out as described earlier.

Statistics

P values were determined using the GraphPad *t* test online software.

Results

A total of 22 patients were enrolled, 11 in each cohort. Patients were deemed evaluable if they received both PCV vaccinations; 1 patient in cohort A and 4 in cohort B showed evidence of disease progression while on study and were not included in this analysis. Characteristics of the patients are summarized in Table 1.

One reliable measure of systemic immunity is the ability of an individual to generate a DTH reaction to the antigen of interest. As such, *Candida* DTH reactions were measured in patients at baseline and upon completion of the study. At baseline, patients in cohort B were more anergic than those in cohort A (mean areas of induration, 6.29 vs. 51.38 mm², respectively; Fig. 2A). DTH reactivity increased 9.8-fold in cohort B whereas cohort A actually showed a decrease (51.38 to 27.75 mm²) in the DTH response.

PCV-specific immune responses

One of the benefits of using the PCV vaccine lies in the ability to measure both humoral responses to pneumococcal antigens as well as the cellular immune response to the

carrier molecule, the diphtheria-derived protein, CRM-197. To examine whether PCV-specific responses could be generated and maintained, or augmented by lenalidomide, antibody titers were examined for 4 of the 7 serotypes present in PCV (Fig. 2B and C). In cohort A, antibody titers were stable or decreased in both blood and bone marrow across the vaccination schedule. In contrast, antibody titers in cohort B showed a continuous increase across the vaccination schedule.

To determine the potential of lenalidomide to augment antigen-specific T-cell immunity, CRM197 responses were determined at baseline and after each vaccination. In the peripheral blood, cohort A showed no increase in CRM197-specific T-cell responses (1.2-fold above baseline; Fig. 2D). In contrast, cohort B displayed increases at both time points, with a maximal 4.7-fold increase observed after the first vaccination (Fig. 2D).

Overall measures of antigen-specific T-cell responses were significantly greater in the bone marrow than in the blood (Fig. 2E). This likely reflects the ability of the bone marrow to serve as a reservoir of antigen-experienced T cells. After the first vaccination, CRM-197-specific T-cell responses were greater in the bone marrow than the blood (cohort A: 7.5% vs. 2.9%, respectively, *P* = 0.001; and cohort B: 11.1% vs. 5.2%, *P* = 0.002; Fig. 2D and E). Consistent with the data obtained in the blood, PCV-specific T-cell responses were greater in cohort B than cohort A. Interestingly, the antigen-specific response to the second vaccination was blunted in cohort A but remained stable in cohort B. To investigate this, we examined the CRM197-specific T-cell responses based on the clinical responses of patient. Patients with progressive disease showed a blunted antigen-specific response to the first vaccination when compared with responders (blood: 2.6% vs. 4.5%, respectively, *P* = 0.32; and bone marrow: 5.8% vs. 9.6%, *P* = 0.006). As expected, responses to the second vaccination were further decreased in progressors, stable in patients with stable disease, and increased in responders. Cohort A had 30% progressors and 60% with stable disease. Cohort B had 14% progressors and 28% with stable disease. This marked difference in clinical response rates to lenalidomide seems to be associated with corresponding differences in antigen-specific immune reactivity where disease progression significantly blunted the CRM-197 immune response. These results are in keeping with previously published work showing the ability of a growing tumor burden to blunt antigen-specific T-cell responses (20).

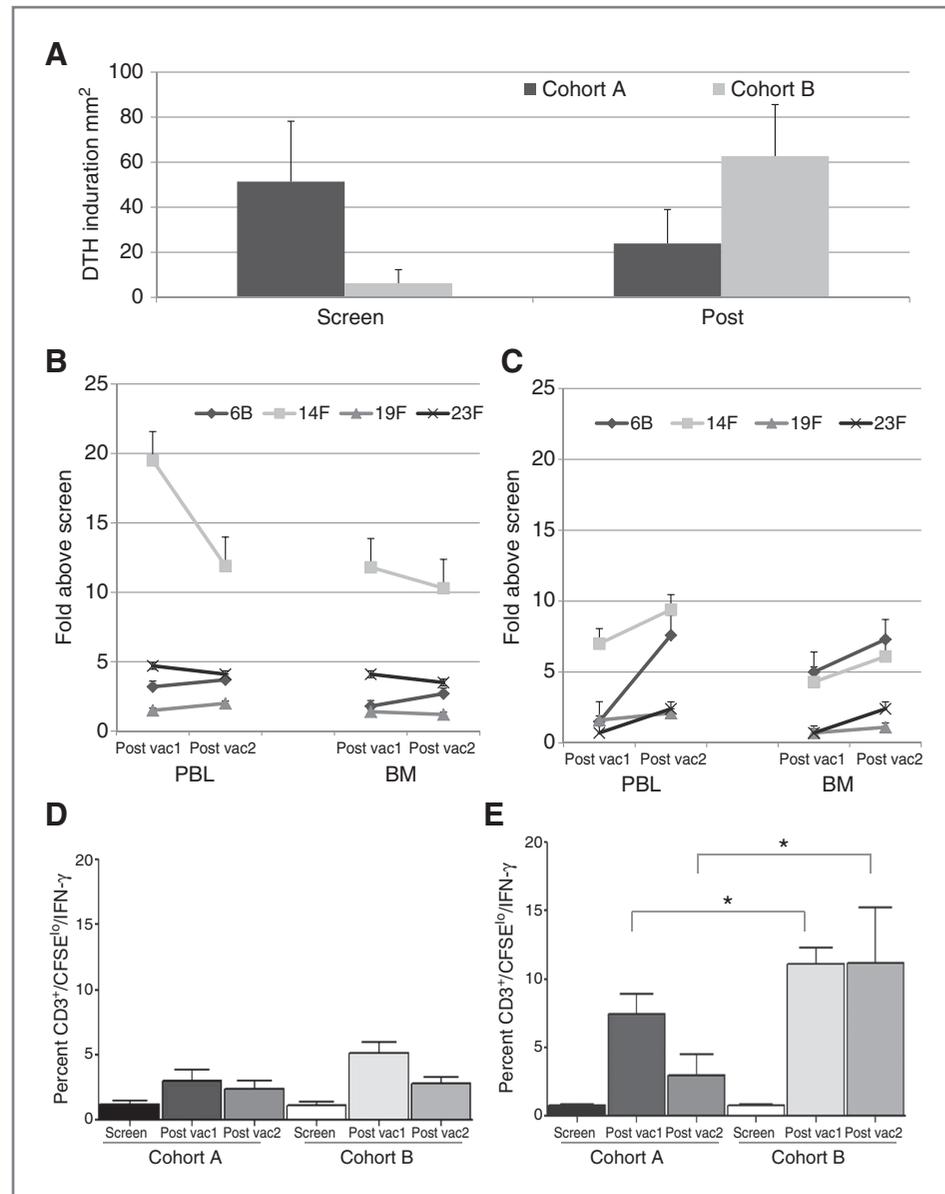
T-cell function

As an immunomodulatory agent with effects on T-cell function, we sought to examine the effect of lenalidomide on various immune parameters in both peripheral blood and bone marrow. Flow cytometric analyses were conducted for all evaluable patients. At baseline, the only significant differences observed in T-cell parameters were a greater percentage of central memory T cells (T_{CM}) characterized as CD45RO⁺/CD62L⁺ (Fig. 3B and C) and fewer T_{regs} in cohort B in the bone marrow. All other parameters were similar in

Table 1. Patient characteristics

Characteristic	Cohort A	Cohort B
Total number	10	7
Median age (range)	67 (54–80)	65 (53–77)
Gender, %		
Male	40	71
Female	60	28
Ethnicity, %		
African Americans	30	28
Caucasian	70	57
Other	0	14
Prior therapies (1–3)	1.8	1.4
Myeloma subtypes, %		
IgA	50	14
IgG	40	57
Light chain	10	28
Percentage plasma cells	26	35.20
Overall response rate	10	57

Figure 2. PCV-specific responses. A, DTH responses to *Candida* administered at baseline and 6 weeks after the last vaccine. B, cohort A and (C) cohort B pneumococcal antibody response averages to 4 subtypes (6B, 14F, 19F, and 23F) in PBL and bone marrow plasma obtained prior to initiation of the first intervention (PCV vaccination for cohort A or lenalidomide for cohort B), 8 weeks after the first vaccine (post vac1) or 8 weeks after the second vaccine (post vac2). Data are graphed as fold difference compared with screen sample. D, PBL and (E) bone marrow T-cell responses to CRM197; CFSE-labeled PBLs, or bone marrow cells were incubated with CRM197 for 3 days after which antigen-specific T cells were analyzed by flow cytometry as CD3⁺/CFSE^{low}/IFN- γ ⁺. Data shown are for CD3⁺/CFSE^{low} averages from cohort A and B pre- and post-PCV vaccination 1 and 2. *, comparisons in which the *P* value is less than 0.05.



both compartments (blood and bone marrow) for both cohorts. Lenalidomide treatment increased the percentage of T_{CM} in both compartments whereas no changes were noted in the effector memory T-cell population. It also increased the T_{reg} population in cohort B in the bone marrow, whereas no significant changes to T_{regs} were appreciable in the blood in either group. Additional statistically significant changes in immune parameters were observed primarily in cohort B and were most evident in the bone marrow. Specifically, we observed an increase in IFN- γ and in CD40L expression in the CD4⁺T cells but not CD8⁺ (Fig. 3B, F, and H). These changes suggest that antigen-specific T-cell activation correlates with overall disease response which was greater in cohort B. Th-17 cells were also reduced in the bone marrow of cohort B whereas their levels in cohort A remained unchanged (Fig. 3B).

Other immune parameters such as dendritic cell populations and NK populations did not seem to be affected by treatment. However, increases in NK-mediated cell lysis were observed in both cohorts A and B (Supplementary Fig. S1).

Myeloma-specific immunity

We examined whether tumor-specific immunity could be detected in our patients. Because of the paucity of autologous tumor available in this study and the abundance of antigen-presenting cells (APC) in the bone marrow capable of capturing, processing, and presenting antigen, myeloma-specific immunity was determined using APCs pulsed with allogeneic myeloma cell lysates, and the specificity of this response was assessed by comparing the T-cell reactivity towards APCs pulsed with the irrelevant bladder

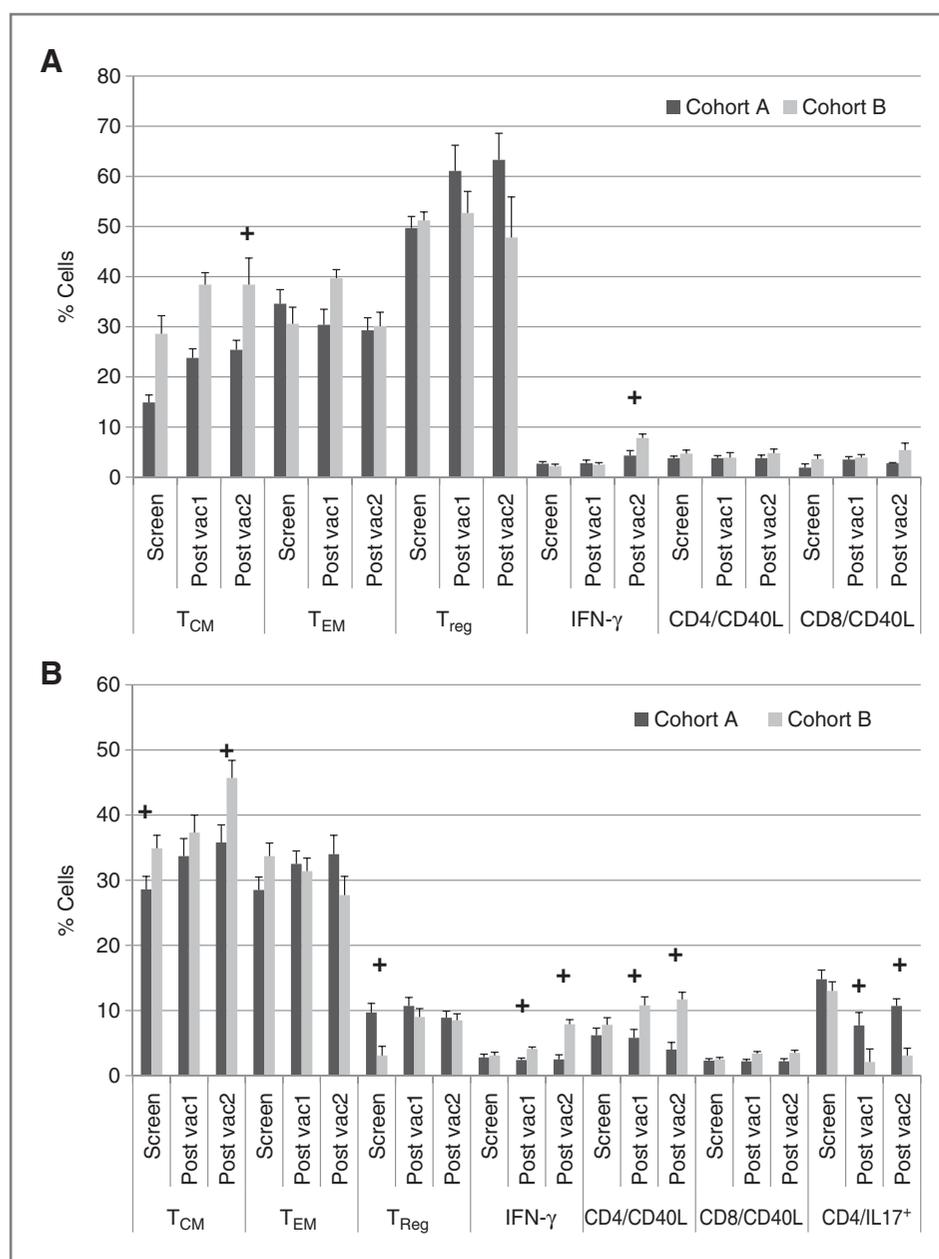


Figure 3. Flow cytometric analysis. A, PBLs or (B) bone marrow obtained at the indicated time points were labeled with the indicated antibodies and analyzed by flow cytometry. Comparisons in which the *P* value is less than 0.05 is indicated by (+).

cancer cell line (SW780). Absence of nonspecific IFN- γ production in the presence of SW780 confirms the absence of nonspecific alloreactivity and the utility of this assay. The tumor-specific immune response increased in cohort B upon completion of the study with an average antigen-specific CD3 cell percentage of 7.7% up from a baseline of 2.25% ($P = 0.003$; Fig. 4A–C). In contrast, cohort A showed no significant induction of a tumor-specific response.

Discussion

This is the first study in humans to examine both the general and antigen-specific immunomodulatory properties of lenalidomide. Vaccine-specific humoral and cellular responses were greater in the cohort receiving

both vaccinations concomitantly with lenalidomide (cohort B), thus supporting the immunostimulatory role of lenalidomide. These data show the multifaceted mechanisms of lenalidomide. It augments global systemic immunity as showed by increases in *Candida* DTH reactions and augments NK cell activity (although not necessarily NK cell numbers). In addition, we show increases in IFN- γ -producing T cells, decreases in Th-17 cells, and increases in antigen-specific T-cell, responsiveness which correlate with clinical responses. Taken together, these data strongly support an immune-mediated antitumor effect of lenalidomide.

This study was designed to show whether vaccine responses could be augmented through the addition of

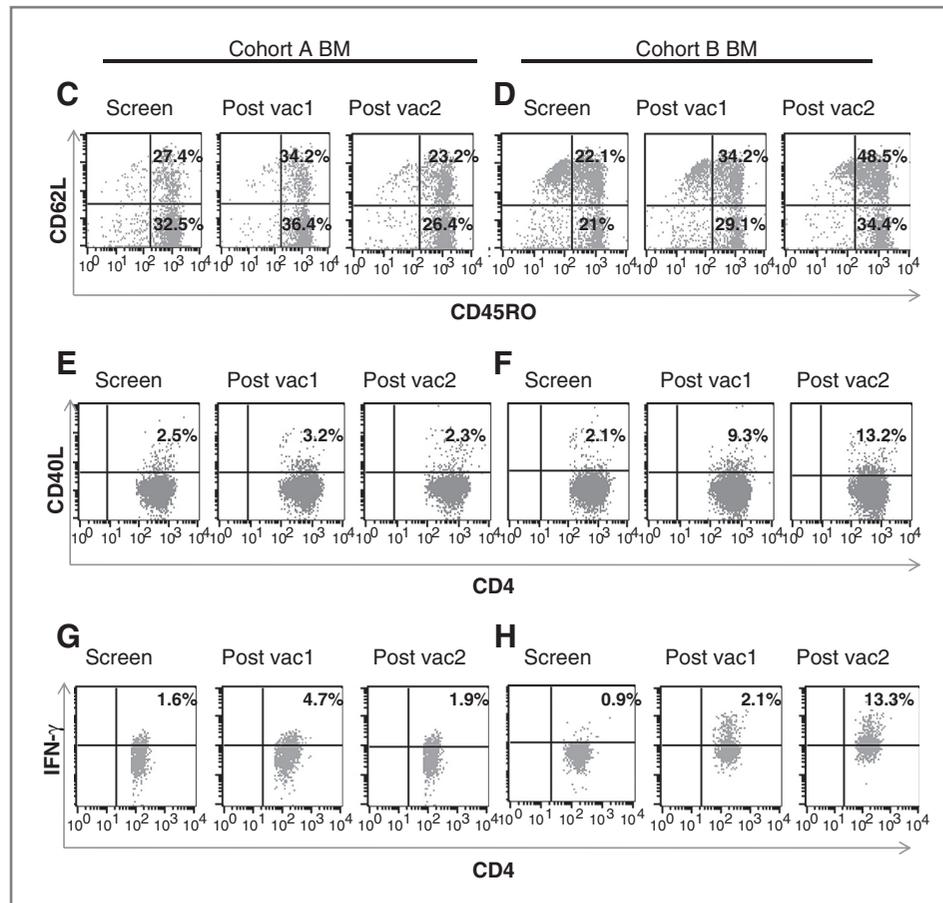


Figure 3. (Continued) C and D, representative flow cytometric analysis of bone marrow (BM) T cells using CD45RO versus CD62L staining of a representative patient in cohort A and B, respectively. E and F, representative flow cytometric analysis for CD4⁺ versus CD40L⁺ of bone marrow T cells for a patient in cohort A and B, respectively. G and H, representative staining of CD4⁺ versus IFN- γ of bone marrow T cells for representative patients in cohort A and B.

the immunomodulatory drug, lenalidomide. The study used the polyvalent pneumococcal vaccine, Prevnar, because of our ability to measure both humoral and cellular responses. In this study, we were able to confirm this synergy by showing increases in the antibody titers and higher antigen-specific T-cell responses with simultaneous administration of vaccine and lenalidomide. These *in vivo* findings confirm the numerous reports describing the immunomodulatory effects of lenalidomide (8, 21, 22). Considering the profound immune dysfunction associated with myeloma (23), strategies to overcome these obstacles should increase immune responsiveness to infectious vaccines. This could reduce infectious complications which currently represent a major morbidity in myeloma (24). In addition, the addition of lenalidomide to immune-based antimyeloma strategies could augment their efficacy.

Unlike most other studies published to date, our immune analysis primarily focused on the bone marrow for 2 major reasons. First, this represents the tumor microenvironment and as such, the site in which changes in immune function will have the most significant biologic and clinical effects. Second, the bone marrow is unique site that enriches for antigen-specific T-cell responses (25, 26). This is confirmed in our study by the greater percentage of antigen-specific T cells in both cohorts in the bone marrow than blood

(Fig. 2D and E) and by the greater changes in overall T-cell function seen in the bone marrow in response to lenalidomide (Fig. 3B).

Vaccine-specific immune responses appeared greater when vaccine was administered concomitantly with lenalidomide (cohort B). The potential explanation for this lies in the ability of lenalidomide to augment global immune responsiveness. One parameter critical to the successful maintenance of immune response is the ability of T cells to persist long-term *in vivo*. T_{CM} have been shown to possess the ability to rapidly proliferate upon antigen rechallenge and to migrate to peripheral tissues as compared with effector memory T cells (27). Lenalidomide increased the T_{CM} population in both groups although in the bone marrow the effect was more dramatic in cohort B. Still unclear is why differences in global immune responsiveness are observed in both groups considering that the extent of lenalidomide therapy was the same and, if anything, cohort B had slightly more aggressive disease going into the study. The presence of a significant T_{CM} population could prove critical to generating effective vaccine responses.

Vaccine-specific responses were primed with PCV to a greater extent when administered concomitantly with lenalidomide (cohort B; Fig. 2C and D). Interestingly, the T-cell responses to the second vaccine administered 8 weeks

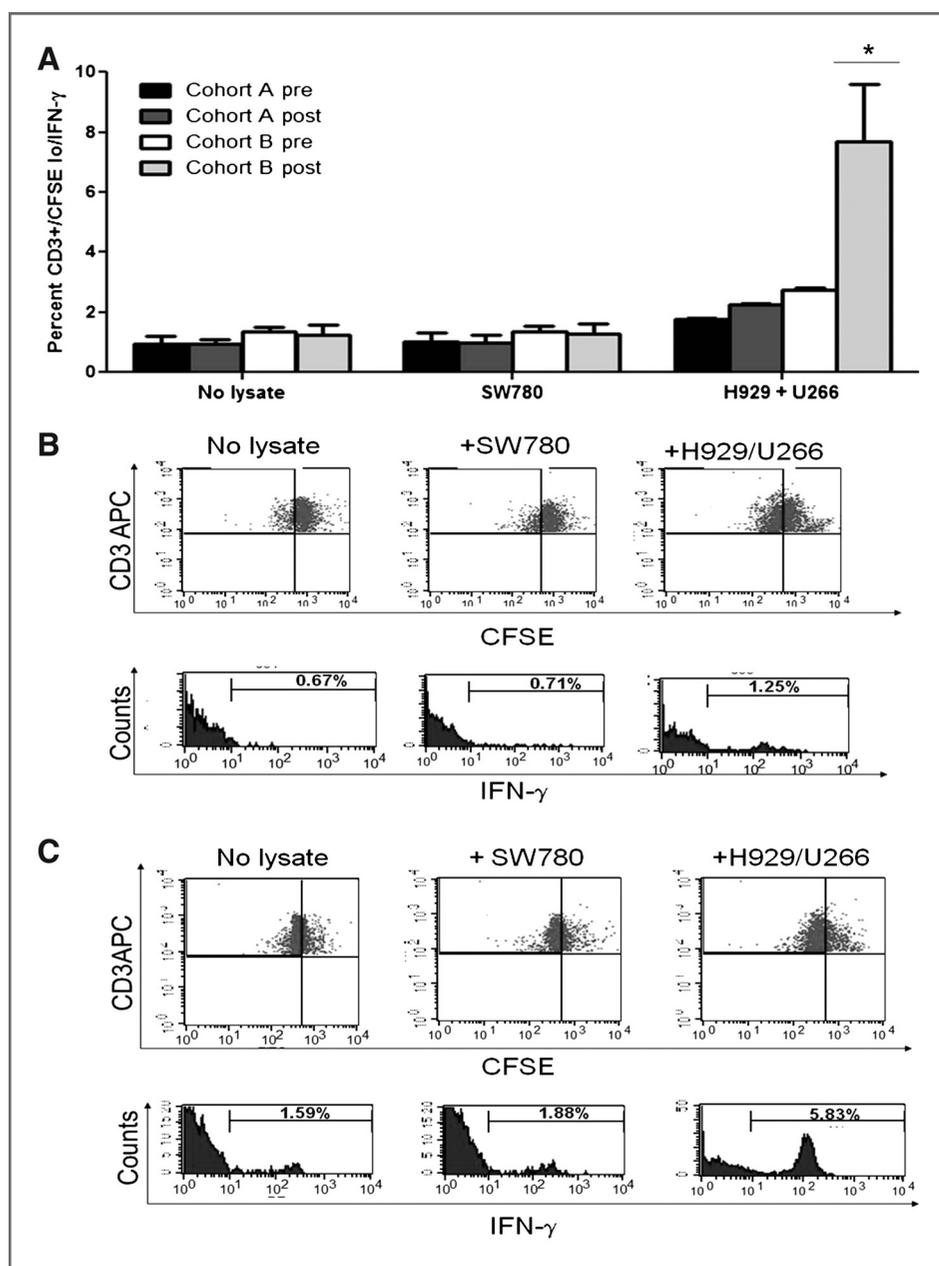


Figure 4. Myeloma-specific responses. **A**, CFSE-labeled bone marrow cells in either media alone, pulsed with SW780 (nonspecific bladder carcinoma cell line) lysate (as negative control), or with H929 + U266 (myeloma cell line) lysate, were incubated for 5 days. Cells were stained for CD3⁺ and IFN- γ . Averages of CD3⁺/CFSE^{low}/IFN- γ -producing cells were analyzed for patients with cohort A and cohort B pretreatment and 6 weeks following their last vaccination. **B**, FACS example of a postvaccine response to media alone, SW780 lysate, and H929 + U266 lysate of a patient with progressive disease or **(C)** responsive disease. *, comparisons in which the *P* value is less than 0.05.

later was reduced in cohort A and stable in cohort B. This is likely explained by the accompanying clinical response. More patients showed stable or progressive disease in cohort A compared with cohort B (90% vs. 43%). In fact, an analysis of the patients based on response rates showed a reduction in T-cell responses to PCV in progressors and an increase in PCV T-cell responses in patients achieving at least a partial response (data not shown). Similarly, IFN- γ and CD40L expression was also increased in cohort B as would be expected with priming of an antigen-specific T-cell response.

The role of Th-17 cells within the bone marrow micro-environment also warrants discussion. Cohort B showed a significant decline in Th-17 cells in the bone marrow

whereas these cells initially decreased and then increased in cohort A. Myeloma-induced production of IL-6 in the presence of TGF- β skews naive CD4 cells away from T_{regs} towards a Th-17 phenotype (28). As proinflammatory agents, Th-17 cells facilitate the establishment of a chronic inflammatory state that enhances tumor growth and activated osteoclasts leading to worsening of bone disease (26). We, thus, conclude that an increase in Th-17 cells in the bone marrow likely contributes to disease progression in myeloma. Less clear is whether lenalidomide itself can directly reduce the generation of Th-17 cells through the alteration of cytokine expression by the tumor and/or T cells or whether the reduction is the result of a negative feedback loop simply due to a diminishing tumor size.

The role of T_{regs} in hematologic malignancies, and specifically myeloma, is less clear. Decreased numbers of presumptive T_{regs} have been reported in myeloma patients compared with normal individuals (29). Beyer and Schultze showed a direct correlation between T_{regs} in the blood and disease status (30) which is consistent with our data in the blood. The role of T_{regs} in antitumor immunity likely depends upon their role and function within the tumor microenvironment. In colorectal (31) and nasopharyngeal cancers (32), high levels of tumor-infiltrating T_{regs} were associated with improved survival. These studies underscore the importance of examining the immune response within the tumor microenvironment—which in myeloma is the bone marrow. Specifically, cohort B which had a greater number of responders, we observed an expected increase in IFN- γ^+ -producing Th1 cells and T_{regs} with an associated decrease in Th-17 cells in the bone marrow. Although seemingly at odds with previously published clinical data, the increase in T_{regs} in patients with clinical responses suggests a potential beneficial role of T_{regs} in myeloma.

Extensive preclinical data suggest that a major component of the activity of lenalidomide is in augmenting immune responsiveness. Because of the intrinsic immune-mediated antimyeloma activity of lenalidomide, it is difficult to separate the antitumor effects from its effect as a vaccine immune modulator. However, we showed that increased immune responsiveness correlated with increased vaccine-specific immunity (Fig. 2D). This strongly implies an immunomodulatory effect of lenalidomide and not just a tumor-specific cytotoxic effect of the drug. We also observed other immunomodulatory aspects of lenalidomide that underscore its positive immune effects, including an increase in NK cytolytic activity and T_{CM} in both the blood and bone marrow.

PCV in combination with lenalidomide generated interesting and unexpected results. This vaccine was chosen because of its ability to prime both humoral and cellular responses that would enable us to examine both arms of the immune response in patients treated with lenalidomide. We have shown greater increases in both pneumococcal antibody titers as well as CRM197-specific T-cell responses in cohort B, which received both vaccinations while on lenalidomide. However, other findings warrant discussion. First, the T-cell-specific responses were greater in the bone marrow than in the blood with more than double the percentage of antigen-specific T cells. This finding significantly underscores the uniqueness of the bone marrow as an immune niche of antigen-specific T cells (33, 34). Considering that the operative immunosuppressive mechanisms are likely to be greatest within the tumor microenvironment, the augmented immune responses

in the bone marrow compared with the blood of myeloma patients are even more significant. Second, the generation of myeloma-specific immunity upon completion of vaccination was statistically significant in cohort B whereas it was negligible in cohort A. The average daily dose of lenalidomide was equivalent in both groups (cohort A: 21.6 mg/d vs. cohort B: 19.2 mg/d) as was the overall tumor burden. The only appreciable differences between these 2 groups were the vaccination schedules and clinical responses to lenalidomide treatment.

In summary, several conclusions can be made from this initial pilot study. This study is the first to show the *in vivo* immunomodulatory properties of lenalidomide in patients manifest as increases in both global and vaccine-specific immunity as well as provide evidence of myeloma-specific immunity. To further expand and confirm these observations, a clinical trial is open using the lenalidomide platform upon which cancer vaccines will be integrated. We will also determine whether lenalidomide could be used as an adjuvant for the administration of infectious vaccines in a population of patients without cancer—a use that could increase vaccine efficacy especially in situations of limited vaccine supply.

Disclosure of Potential Conflicts of Interest

The authors are fully responsible for all content and editorial decisions for this manuscript. A. Emerling: honoraria from Speaker's Bureau and consultant/advisory board member, Celgene. I. Borrello: commercial research grant and paid consultant, Celgene. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

K. Noonan designed, conducted, and analyzed immune monitoring experiments.

K. Noonan and I. Borrello contributed to writing of the manuscript.

L. Rudraraju carried out the experiments.

L. Rudraraju and I. Borrello analyzed the data.

A. Ferguson was research nurse for the trial.

A. Emerling and C.A. Huff contributed patients to the study.

M.F. Pasetti conducted and analyzed laboratory data.

C.A. Huff reviewed the manuscript.

I. Borrello designed the clinical trial.

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References

- Zangari M, Elice F, Tricot G. Immunomodulatory drugs in multiple myeloma. *Expert Opin Investig Drugs* 2005;14:1411–8.
- Corral LG, Haslett PA, Muller GW, Chen R, Wong LM, Ocampo CJ, et al. Differential cytokine modulation and T cell activation by two

distinct classes of thalidomide analogues that are potent inhibitors of TNF-alpha. *J Immunol* 1999;163:380–6.

- Davies FE, Rajee N, Hideshima T, Lentzsch S, Young G, Tai YT, et al. Thalidomide and immunomodulatory derivatives augment

- natural killer cell cytotoxicity in multiple myeloma. *Blood* 2001;98:210–6.
4. Bartlett JM, Ellis IO, Dowsett M, Mallon EA, Cameron DA, Johnston S, et al. Human epidermal growth factor receptor 2 status correlates with lymph node involvement in patients with estrogen receptor (ER) negative, but with grade in those with ER-positive early-stage breast cancer suitable for cytotoxic chemotherapy. *J Clin Oncol* 2007;25:4423–30.
 5. Wu L, Adams M, Carter T, Chen R, Muller G, Stirling D, et al. Lenalidomide enhances natural killer cell and monocyte-mediated antibody-dependent cellular cytotoxicity of rituximab-treated CD20+ tumor cells. *Clin Cancer Res* 2008;14:4650–7.
 6. Chang DH, Liu N, Klimek V, Hassoun H, Mazumder A, Nimer SD, et al. Enhancement of ligand-dependent activation of human natural killer T cells by lenalidomide: therapeutic implications. *Blood* 2006;108:618–21.
 7. Schafer PH, Gandhi AK, Loveland MA, Chen RS, Man HW, Schnetz-kamp PP, et al. Enhancement of cytokine production and AP-1 transcriptional activity in T cells by thalidomide-related immunomodulatory drugs. *J Pharmacol Exp Ther* 2003;305:1222–32.
 8. LeBlanc R, Hideshima T, Catley LP, Shringarpure R, Burger R, Mitsiades N, et al. Immunomodulatory drug costimulates T cells via the B7-CD28 pathway. *Blood* 2004;103:1787–90.
 9. Galustian C, Meyer B, Labarthe MC, Dredge K, Klaschka D, Henry J, et al. The anti-cancer agents lenalidomide and pomalidomide inhibit the proliferation and function of T regulatory cells. *Cancer Immunol Immunother* 2009;58:1033–45.
 10. Quach H, Ritchie D, Stewart AK, Neeson P, Harrison S, Smyth MJ, et al. Mechanism of action of immunomodulatory drugs (IMiDs) in multiple myeloma. *Leukemia* 2010;24:22–32.
 11. Baz R, Patel M, Finley-Oliver E, Lebovic D, Hussein MA, Miller KC, et al. Single agent lenalidomide in newly diagnosed multiple myeloma: a retrospective analysis. *Leuk Lymphoma* 2010;51:1015–9.
 12. Richardson P, Jagannath S, Hussein M, Berenson J, Singhal S, Irwin D, et al. Safety and efficacy of single-agent lenalidomide in patients with relapsed and refractory multiple myeloma. *Blood* 2009;114:772–8.
 13. Dredge K, Marriott JB, Todryk SM, Muller GW, Chen R, Stirling DI, et al. Protective antitumor immunity induced by a costimulatory thalidomide analog in conjunction with whole tumor cell vaccination is mediated by increased Th1-type immunity. *J Immunol* 2002;168:4914–9.
 14. Bartlett JB, Michael A, Clarke IA, Dredge K, Nicholson S, Kristeleit H, et al. Phase I study to determine the safety, tolerability and immunostimulatory activity of thalidomide analogue CC-5013 in patients with metastatic malignant melanoma and other advanced cancers. *Br J Cancer* 2004;90:955–61.
 15. Ayello J, Berg SL, Krailo M, Ven CVD, Ingle AM, Lewis D, et al. Lenalidomide (LMiD) significantly enhances circulating serum levels of IL-2 and IL-15 levels, NK expansion and activation and NK and LAK cytotoxicity in children with refractory/recurrent solid tumors: A Children's Oncology Group Phase I Consortium Report. In: Proceedings of the 50th Annual Meeting and Exposition of ASH; 2008 Dec 6–9; San Francisco, CA; Washington, DC: ASH; 2008. Abstract nr 107.
 16. Hollander N. Current vaccination strategies for the treatment of B-cell lymphoma and multiple myeloma. *Crit Rev Immunol* 2009;29:399–418.
 17. Rapoport AP, Stadtmauer EA, Aquilino N, Badros A, Cotte J, Chrisley L, et al. Restoration of immunity in lymphopenic individuals with cancer by vaccination and adoptive T-cell transfer. *Nat Med* 2005;11:1230–7.
 18. Concepcion N, Frasch CE. Evaluation of previously assigned antibody concentrations in pneumococcal polysaccharide reference serum 89SF by the method of cross-standardization. *Clin Diagn Lab Immunol* 1998;5:199–204.
 19. Concepcion NF, Frasch CE. Pneumococcal type 22f polysaccharide absorption improves the specificity of a pneumococcal-polysaccharide enzyme-linked immunosorbent assay. *Clin Diagn Lab Immunol* 2001;8:266–72.
 20. Staveley-O'Carroll K, Sotomayor E, Montgomery J, Borrello I, Hwang L, Fein S, et al. Induction of antigen-specific T cell anergy: An early event in the course of tumor progression. *Proc Natl Acad Sci U S A* 1998;95:1178–83.
 21. Görgün G, Calabrese E, Soydan E, Hideshima T, Perrone G, Bandi M, et al. Immunomodulatory effects of lenalidomide and pomalidomide on interaction of tumor and bone marrow accessory cells in multiple myeloma. *Blood* 2010;116:3227–37.
 22. Ramsay AG, Clear AJ, Kelly G, Fatah R, Matthews J, Macdougall F, et al. Follicular lymphoma cells induce T-cell immunologic synapse dysfunction that can be repaired with lenalidomide: implications for the tumor microenvironment and immunotherapy. *Blood* 2009;114:4713–20.
 23. Dasanu CA. Immune alterations in untreated and treated multiple myeloma. *J Oncol Pharm Pract* 2011 Aug 22. [Epub ahead of print].
 24. Nucci M, Anaissie E. Infections in patients with multiple myeloma in the era of high-dose therapy and novel agents. *Clin Infect Dis* 2009;49:1211–25.
 25. Feuerer M, Beckhove P, Garbi N, Mahnke Y, Limm A, Hommel M, et al. Bone marrow as a priming site for T-cell responses to blood-borne antigen. *Nat Med* 2003;9:1151–7.
 26. Noonan K, Marchionni L, Anderson J, Pardoll D, Roodman GD, Borrello I. A novel role of IL-17 producing lymphocytes in mediating lytic bone disease in multiple myeloma. *Blood* 2010;116:3380–2.
 27. Berger C, Jensen MC, Lansdorp PM, Gough M, Elliott C, Riddell SR, et al. Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates. *J Clin Invest* 2008;118:294–305.
 28. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006;441:235–8.
 29. Prabhala RH, Neri P, Bae JE, Tassone P, Shamma MA, Allam CK, et al. Dysfunctional T regulatory cells in multiple myeloma. *Blood* 2006;107:301–4.
 30. Beyer M, Schultze JL. Regulatory T cells in cancer. *Blood* 2006;108:804–11.
 31. Salama P, Phillips M, Griew F, Morris M, Zeps N, Joseph D, et al. Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. *J Clin Oncol* 2009;27:186–92.
 32. Zhang YL, Li J, Mo HY, Qiu F, Zheng LM, Qian CN, et al. Different subsets of tumor infiltrating lymphocytes correlate with NPC progression in different ways. *Mol Cancer* 2010;9:4.
 33. Di Rosa F. T-lymphocyte interaction with stromal, bone and hematopoietic cells in the bone marrow. *Immunol Cell Biol* 2009;87:20–9.
 34. Noonan K, Matsui W, Serafini P, Carbley R, Tan G, Khalili J, et al. Activated marrow-infiltrating lymphocytes effectively target plasma cells and their clonogenic precursors. *Cancer Res* 2005;65:2026–34.