VOLUME 35 · NUMBER 18 · JUNE 20, 2017

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Safety and Activity of Varlilumab, a Novel and First-in-Class Agonist Anti-CD27 Antibody, in Patients With Advanced Solid Tumors

Howard A. Burris, Jeffrey R. Infante, Stephen M. Ansell, John J. Nemunaitis, Geoffrey R. Weiss, Victor M. Villalobos, Branimir I. Sikic, Matthew H. Taylor, Donald W. Northfelt, William E. Carson III, Thomas R. Hawthorne, Thomas A. Davis, Michael J. Yellin, Tibor Keler, and Timothy Bullock

Author affiliations and support information (if applicable) appear at the end of this article.

Published at jco.org on May 2, 2017.

Clinical trial information: NCT01460134.

Corresponding author: Howard A. Burris, MD, Sarah Cannon Research Institute, Tennessee Oncology, 1100 Charlotte Ave, Ste 800, Nashville, TN 37203; e-mail: howard.burris@scresearch.net.

0732-183X/17/3518w-2028w/$20.00

ABSTRACT

Purpose

CD27, a costimulatory molecule on T cells, induces intracellular signals that mediate cellular activation, proliferation, effector function, and cell survival upon binding to its ligand, CD70. Varlilumab is a novel, first-in-class, agonist CD27 antibody that stimulates the CD27 pathway, which results in T-cell activation and antitumor activity in tumor models. This first-in-human, dose-escalation and expansion study evaluated the safety, pharmacology, and activity of varlilumab in patients with advanced solid tumors.

Methods

In a 3 + 3 dose-escalation design (n = 25), patients received a single dose of varlilumab (0.1, 0.3, 1.0, 3.0, or 10 mg/kg intravenously) with a 28-day observation, followed by up to five multidose cycles (one dose per week for 4 weeks), depending on tumor response. Expansion cohorts were initiated at 3.0 mg/kg in patients with melanoma (n = 16) and renal cell carcinoma (RCC; n = 15). Primary objectives were to assess the safety and the maximum tolerated and optimal biologic doses of varlilumab. Secondary objectives were to evaluate the pharmacokinetics, pharmacodynamics, and clinical antitumor activity of varlilumab.

Results

Exposure to varlilumab was linear and dose proportional across dose groups. Only one patient experienced a dose-limiting toxicity—grade 3 transient asymptomatic hyponatremia at the 1.0-mg/kg dose level. Treatment-related adverse events were generally grade 1 or 2 in severity. Evidence of biologic activity consistent with CD27 stimulation—chemokine induction, T-cell stimulation, regulatory T cell depletion—was observed at all dose levels. A patient with metastatic RCC experienced a partial response (78% shrinkage, progression-free survival > 2.3 years). Eight patients experienced stable disease > 3 months, including a patient with metastatic RCC with progression-free survival of > 3.9 years.

Conclusion

Dose escalation of varlilumab to 10 mg/kg was well tolerated without identification of a maximum tolerated dose. Varlilumab was biologically and clinically active.

J Clin Oncol 35:2028-2036. © 2017 by American Society of Clinical Oncology

INTRODUCTION

Immunotherapy that targets T-cell checkpoint pathways has validated the concept of engaging the immune system in the war against cancer and has changed the treatment and outcome for many patients with cancer.[1] This concept of removing the brake relies on an active immune response that is being held back by these powerful regulatory pathways. In many patients with cancer, the immune system's recognition and response to the tumor requires help to initiate and maintain productive antitumor T cells. Agonist antibodies to costimulatory molecules that promote the generation of antitumor T cells have the potential to add to the immunotherapeutic approach to cancer.

CD27, a member of the tumor necrosis factor receptor superfamily, acts as a potent costimulatory molecule that, unlike other related family members, is expressed constitutively on

ASSOCIATED CONTENT

Appendix
DOI: https://doi.org/10.1200/JCO.2016.70.1508

DOI: https://doi.org/10.1200/JCO.2016.70.1508

unstimulated T lymphocytes. CD70, the ligand for CD27, is transiently expressed on antigen-presenting cells. CD27–CD70 mediated costimulation—concomitant with antigen-specific T-cell receptor (TCR) stimulation—results in T-cell activation, proliferation, survival, and maturation of effector capacity and T-cell memory.[2,3] CD27–CD70 interactions also promote B-cell proliferation, generation of plasma cells, production of immunoglobulin and B-cell memory,[4-7] and induction of the cytolytic activity of natural killer (NK) cells.[8] CD27 is also expressed by regulatory T cells (Tregs) that can be associated with the suppression of antitumor immunity[9] and may have a role in their expansion and activation.[10]

Varlilumab (CDX-1127) is a novel, first-in-class, fully human IgG1κ anti-CD27 monoclonal antibody that acts as an agonist of CD27 and reacts with the ligand binding site of CD27 as demonstrated by inhibition of CD70 binding to CD27.[11] Varlilumab enhances the CD27-mediated lymphocyte costimulatory pathway but does not directly activate lymphocytes in the absence of signaling through the TCR as shown with in vitro lymphocyte proliferation and cytokine induction studies. Varlilumab has an unmodified Fc region and can also mediate Fc-dependent effector functions. In human CD27 transgenic mice, varlilumab has potent antitumor immunity in multiple models, which provides the rationale for its clinical development.[12]

This first-in-human, phase I, open-label, dose-escalation and expansion study was conducted to assess the safety, pharmacokinetics, pharmacodynamics, and activity of varlilumab when administered as monotherapy to patients with advanced malignancies. Patients with solid tumors and hematologic malignancies were separately enrolled in parallel dose-escalation and expansion phases, given the potential for differing mechanisms of action, pharmacokinetics, and toxicity profiles of anti–CD27-directed therapy in these populations. This report describes the results of evaluations of patients with solid tumors.

METHODS

Patients

The study was open to patients with metastatic melanoma, renal cell carcinoma (RCC), hormone-refractory prostate adenocarcinoma, ovarian cancer, colorectal adenocarcinoma, or non–small-cell lung cancer progressive subsequent to previous therapies with no remaining alternative approved therapy options. Patients with melanoma who enrolled in the expansion cohort must have received or refused ipilimumab and, if expressing the BRAF V600E mutation, vemurafinib. Additional eligibility requirements are provided in the Appendix (online only).

The study was conducted at each of the participating institutions in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines after approval by a local human investigations committee and in accordance with an assurance filed with and approved by the Department of Health and Human Services, where appropriate. All patients provided written informed consent before any protocol-specific procedures.

Study Design and Treatment

The study consisted of a standard 3 + 3 dose-escalation phase,[13] followed by tumor-specific expansion cohorts to explore the clinical and biologic activity of varlilumab in distinct patient populations. Study design and treatment schema are shown in Appendix Figs A1 and A2 (online only). Primary study objective was to assess the safety and tolerability profile of single and multiple doses of varlilumab and, if possible, to determine the maximum tolerated dose and/or optimal biologic dose in patients with solid tumors. Secondary objectives were to determine the pharmacokinetics, pharmacodynamic profile, and immunogenicity of single and multiple doses of varlilumab.

Varlilumab dose levels of 0.1, 0.3, 1.0, 3.0, and 10 mg/kg were selected for dose escalation on the basis of the no observable effect level of 25 mg/kg that was identified during nonclinical toxicology as well as to bridge the dose ranges with expected clinical activity. Varlilumab was initially administered as a single dose followed by a 4-week evaluation period. Additional multidose treatment (one dose per week for 4 weeks with a 4-week observation) and retreatment (up to four additional cycles, each consisting of one dose per week for 4 weeks, with an 8-week observation) were allowed for patients with stable disease. Varlilumab was permanently discontinued upon confirmed disease progression or dose-limiting toxicity (DLT). Patients with confirmed tumor response discontinued varlilumab, were observed for response duration, and were eligible for additional cycles of treatment at the time of relapse. DLT was defined as any grade $\geq$ 3 treatment-related toxicity, excluding the following: grade 3 inflammation as a result of local therapeutic response persisting $\leq$ 7 days; grade 3 nonmalignant lymphocyte changes that improve to grade $\leq$ 2 or within 20% of baseline within 28 days; and grade 3 nausea, vomiting, or diarrhea that resolve to grade $\leq$ 1 within 48 hours.

After evaluation of safety, pharmacodynamics, and early activity data from the dose-escalation component of the study, two expansion cohorts, one consisting of 16 patients with melanoma and one consisting of 15 patients with RCC, were treated with varlilumab 3.0 mg/kg to further evaluate its antitumor activity. The dose of 3 mg/kg was selected to ensure receptor saturation and was based on a consistent immune activation profile in patients who were treated at the dose level. These patients received up to five cycles of varlilumab, each administered as one dose per week for 4 weeks with an 8-week observation.

Varlilumab was administered as a 90-minute intravenous infusion without prophylactic premedication. Dose-escalation patients received the first dose of varlilumab as a split dose, in which a 10% test dose that was infused over 10 minutes was followed by a 1-hour observation before administration of the remaining dose. All patients were monitored for 4 to 6 hours after the first two infusions and for 2 hours after each subsequent dose.

Assessments

Safety assessments included physical exams, vital signs, hematology, blood chemistry, urinalysis, thyroid function, C-reactive protein, immunoglobulin, autoimmune panel, and ECG. Patients were monitored through 70 days post-treatment for such events as diarrhea, colitis, rash, endocrinopathies, and hepatitis, which may occur secondary to activation of the immune system.[14] Toxicity was graded according to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.

Antitumor activity was assessed by investigators according to the Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST 1.1).[15] Restaging of assessments were performed every 12 weeks.

Serum samples were obtained at various time points for pharmacokinetic, antidrug antibodies, cytokine and chemokine, and soluble CD27 analysis. Whole blood was collected and processed into peripheral blood mononuclear cells (PBMCs) and frozen for subsequent analysis by flow cytometry and ELISpot as previously described[16,17] (Appendix Fig A3, online only). Paired fresh tumor biopsy specimens was optional and obtained from a single patient.

Pharmacokinetics and Statistics

Noncompartmental analysis was performed on individual patient serum drug levels by using NCA Model 202 (Phoenix WinNonlin 6.4; Princeton, NJ). In brief, elimination half-life ($T_{1/2}$) and exposure parameters were estimated after visual inspection of time versus concentration profiles to identify the terminal phase, and goodness-of-fit criteria

were used to refine the terminal phase for each patient. Area under the curve was calculated by the linear trapezoidal rule with linear interpolation. All calculations were based on actual sampling times and infusion durations. For pharmacodynamic end points, significant differences were calculated by two-tailed *t* tests using paired or unpaired data as indicated in figure legends.

RESULTS

Patient Characteristics

Fifty-six patients were enrolled at nine centers from October 31, 2011, to March 5, 2014. The dose escalation component of the study included 25 patients, whereas 16 patients with melanoma and 15 patients with RCC were enrolled in expansion cohorts. The most frequent tumor type in the dose-escalation phase was colorectal cancer (40%) followed by melanoma (28%). All patients had stage IV disease. Patients, in general, were heavily pretreated. Patients in the dose-escalation phase had received a median of five prior lines of anticancer therapy. Most patients (81%) in the melanoma expansion cohort had received prior checkpoint blockade (primarily anti–cytotoxic T-cell lymphocyte-4 targeted therapy), whereas all patients in the RCC expansion cohort had received tyrosine kinase inhibitors (Appendix Table A1, Online only).

Study Treatments and Tolerability

Varlilumab was well tolerated. Patients received a median of four (range, one to 21) varlilumab doses on study, with 10 patients receiving more than one cycle of treatment. Dose escalation completed through 10 mg/kg without identification of a maximum tolerated dose. A single DLT—grade 3 asymptomatic hyponatremia (129 mmol/L)—occurred at the 1.0-mg/kg dose level. The event, with onset 14 days after the single dose of varlilumab, spontaneously resolved. No additional patients discontinued varlilumab as a result of toxicity. Treatment-related toxicities—generally grade 1 and 2—included fatigue, rash, nausea, and diarrhea (Table 1). Grade 3 treatment-related events were limited to one case each of hyponatremia (the DLT discussed above), decreased

Table 1. Toxicity

	Dose-Escalation Cohort (n = 25)		Melanoma Expansion Cohort (n = 16)		Renal Cell Carcinoma Expansion Cohort (n = 15)		All Patients (N = 56)	
	All Grades	Grade 3-4	All Grades	Grade 3-4	All Grades	Grade 3-4	All Grades	Grade 3-4
Any event	25 (100)	14 (56)	16 (100)	8 (50)	15 (100)	10 (67)	56 (100)	32 (57)
Fatigue	9 (36)	0	10 (63)	0	11 (73)	0	30 (54)	0
Nausea	5 (20)	0	4 (25)	0	8 (53)	0	17 (30)	0
Dyspnea	7 (28)	0	3 (19)	0	4 (27)	1 (7)	14 (25)	1 (2)
Decreased appetite	6 (24)	0	3 (19)	0	4 (27)	0	13 (23)	0
Edema peripheral	5 (20)	0	3 (19)	0	3 (20)	1 (7)	11 (20)	1 (2)
Rash	3 (12)	0	6 (38)	0	2 (13)	2 (13)	11 (20)	2 (4)
Diarrhea	6 (24)	0	0	0	4 (27)	0	10 (18)	0
Arthralgia	3 (12)	2 (8)	3 (19)	0	2 (13)	0	8 (14)	2 (4)
Vomiting	2 (8)	0	1 (6)	0	5 (33)	0	8 (14)	0
Cough	3 (12)	0	1 (6)	0	4 (27)	0	8 (14)	0
Constipation	5 (20)	2 (8)	2 (13)	0	1 (7)	0	8 (14)	2 (4)
Chills	4 (16)	0	3 (19)	0	0	0	7 (13)	0
Asthenia	4 (16)	0	1 (6)	0	2 (13)	0	7 (13)	0
Headache	1 (4)	0	4 (25)	0	2 (13)	0	7 (13)	0
Pruritus	0	0	5 (31)	0	2 (13)	1 (7)	7 (13)	1 (2)
Anxiety	2 (8)	0	2 (13)	0	2 (13)	0	6 (11)	0
Insomnia	1 (4)	0	2 (13)	0	3 (20)	0	6 (11)	0
Any treatment-related adverse event	14 (56)	2 (8)	12 (75)	1 (6)	10 (67)	1 (7)	36 (64)	4 (7)
Fatigue	4 (16)	0	8 (50)	0	5 (33)	0	17 (30)	0
Rash	3 (12)	0	6 (38)	0	2 (13)	0	11 (20)	0
Nausea	1 (4)	0	3 (19)	0	5 (33)	0	9 (16)	0
Diarrhea	2 (8)	0	0	0	4 (27)	0	6 (11)	0
Decreased appetite	3 (12)	0	1 (6)	0	1 (7)	0	5 (9)	0
Pruritus	0	0	3 (19)	0	2 (13)	0	5 (9)	0
Vomiting	1 (4)	0	0	0	4 (27)	0	5 (9)	0
Pyrexia	1 (4)	0	2 (13)	0	1 (7)	0	4 (7)	0
Edema peripheral	2 (8)	0	1 (6)	0	1 (7)	0	4 (7)	0
Headache	0	0	3 (19)	0	1 (7)	0	4 (7)	0
Chills	2 (8)	0	1 (6)	0	0	0	3 (5)	0
Hypertension	0	0	2 (13)	1 (6)	0	0	2 (4)	1 (2)
Asthma	0	0	0	0	1 (7)	1 (7)	1 (2)	1 (2)
Lymphocyte count decreased*	1 (4)	1 (4)	0	0	0	0	1 (2)	1 (2)
Hyponatremia	1 (4)	1 (4)	0	0	0	0	1 (2)	1 (2)

NOTE. Data shown as No. (%). Table presents all-causality adverse events with incidence of > 10% and treatment-related adverse events with incidence of > 5% overall or with any instance of severity grade ≥ 3. No grade 5 treatment-related adverse events were reported.

*Laboratory abnormalities were reported as adverse events if clinically significant. According to laboratory data, 16 (51%) of 31 patients in expansion cohorts had at least one grade worsening in lymphocyte count.

appetite, and decreased lymphocyte count. An additional patient with history of asthma, lung metastases, and previous grade 4 anti–programmed death-1 monoclonal antibody–associated infusion reaction, including bronchospasm, experienced grade 4 asthma and bronchospasm that led to hospitalization. Two patients experienced grade 2 infusion reaction associated with varlilumab administration and received additional varlilumab infusions with premedication, including corticosteroid, antihistamine, and acetaminophen without further reactions.

Pharmacokinetics and Pharmacodynamics

After initial dosing, mean peak serum levels of varlilumab ranged from 3.6 μg/mL (0.1 mg/kg) to 357 μg/mL (10 mg/kg; Fig 1A and Appendix Table A2, online only). Exposure was dose proportional, with mean $T_{1/2}$ ranging from 2.7 days (0.3 mg/kg) to 10.5 days (10 mg/kg) after day 1 dosing. After steady-state dosing, $T_{1/2}$ ranged from 4.8 days (0.3 mg/kg) to 19.3 days (10 mg/kg). Only the lowest dose level of varlilumab (0.1 mg/kg) resulted in complete clearance of the antibody within 28 days, which also corresponded to a loss of detectable surface-bound antibody on circulating T cells (Fig 1B). Doses of ≥ 1 mg/kg resulted in near saturation of T cells for at least 1 month. Furthermore, samples from a patient with pre- and on-treatment biopsies (3-mg/kg dose) showed evidence of varlilumab bound to T cells that were isolated from the tumor (Fig 1C). Patients showed a significant and dose-dependent increase in levels of soluble CD27, which likely reflects stabilization of shed CD27 in serum (Fig 1D). Antidrug antibodies were not detected.

The effect of varlilumab on the levels of circulating cytokines, chemokines, and growth factors was determined by cytokine bead array. Significant changes in a broad spectrum of molecules that generally peaked within a few hours after administration were observed at all dose levels (Fig 2A and Appendix Fig A4, online only). The full panel for the nine patients who received 1 mg/kg is shown as a heat map in Fig 2A and indicates the transient and predominantly proinflammatory nature of the response. Among molecules tested, interferon gamma (IFN-γ) –induced protein 10 (IP-10 or CXCL10) was most prominently increased and without a clear dose relationship (data not shown). Increases in IP-10 were more profound after the initial dose compared with the final dose of the cycle, which suggested potential desensitization (Fig 2B).

Varlilumab administration was associated in some patients with generally low-grade lymphopenia that was stable during continued treatment and not associated with a clear dose effect. In expansion cohorts, 16 (51%) of 31 patients had at least one grade worsening in lymphocyte count. There was no consistent effect observed in $CD8^+$ T- or B-cell numbers, whereas $CD4^+$ T cells were consistently reduced among most patients, regardless of dose level (Fig 3A). Decline in $CD4^+$ T cells was correlated with a significant decrease in $CD4^+$ Tregs that was maintained or further decreased through the treatment cycle (Figs 3B and 3C). Approximately one half of patients had a marked and sustained increase in NK cells,

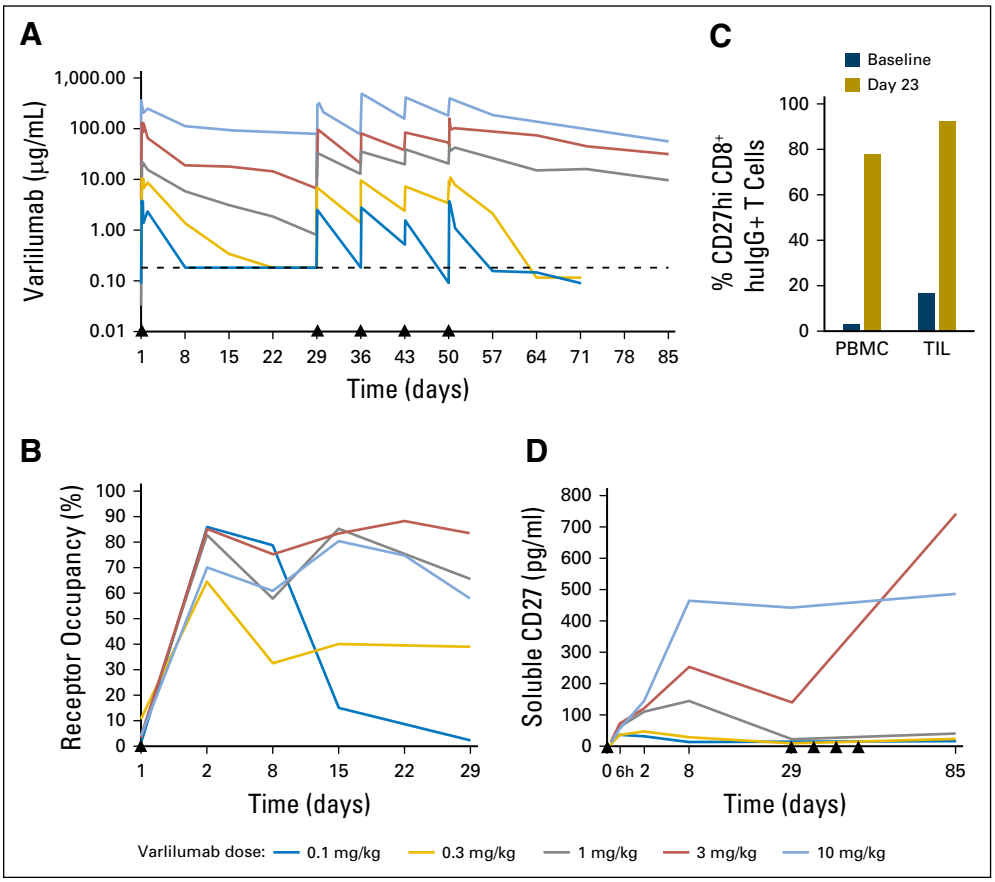

Fig 1. Varlilumab pharmacokinetics and receptor occupancy. (A) Serum concentrations of varlilumab were determined by ELISA by using recombinant CD27-coated plates and anti-human IgG-horseradish peroxidase conjugate as a detection probe. Values represent the mean for patients in each cohort. (B) Percent receptor occupancy was determined by flow cytometry using anti-human IgG conjugate for detection of varlilumab and a noncompeting anti–CD27-labeled monoclonal antibody for identifying CD27 expression. Values respresent the percentage of $CD8^+/CD27^+$ T cells that stained positive with anti-human IgG treated at different dose levels. (C) Comparison of receptor occupancy in samples collected from blood and tumor biopsies from a single patient treated with 3 mg/kg. Tissue was digested with a mixture of collagenase IV and DNase I and 2.5 U/mL hyaluronidase. (D) Mean level of soluble CD27 as using a sandwich ELISA that is not obstructed by the presence of bound varlilumab. Black triangles (▲) represent varlilumab dosing. ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; PBMC, peripheral blood mononuclear cell; TIL, tumor-infiltrating lymphocytes.

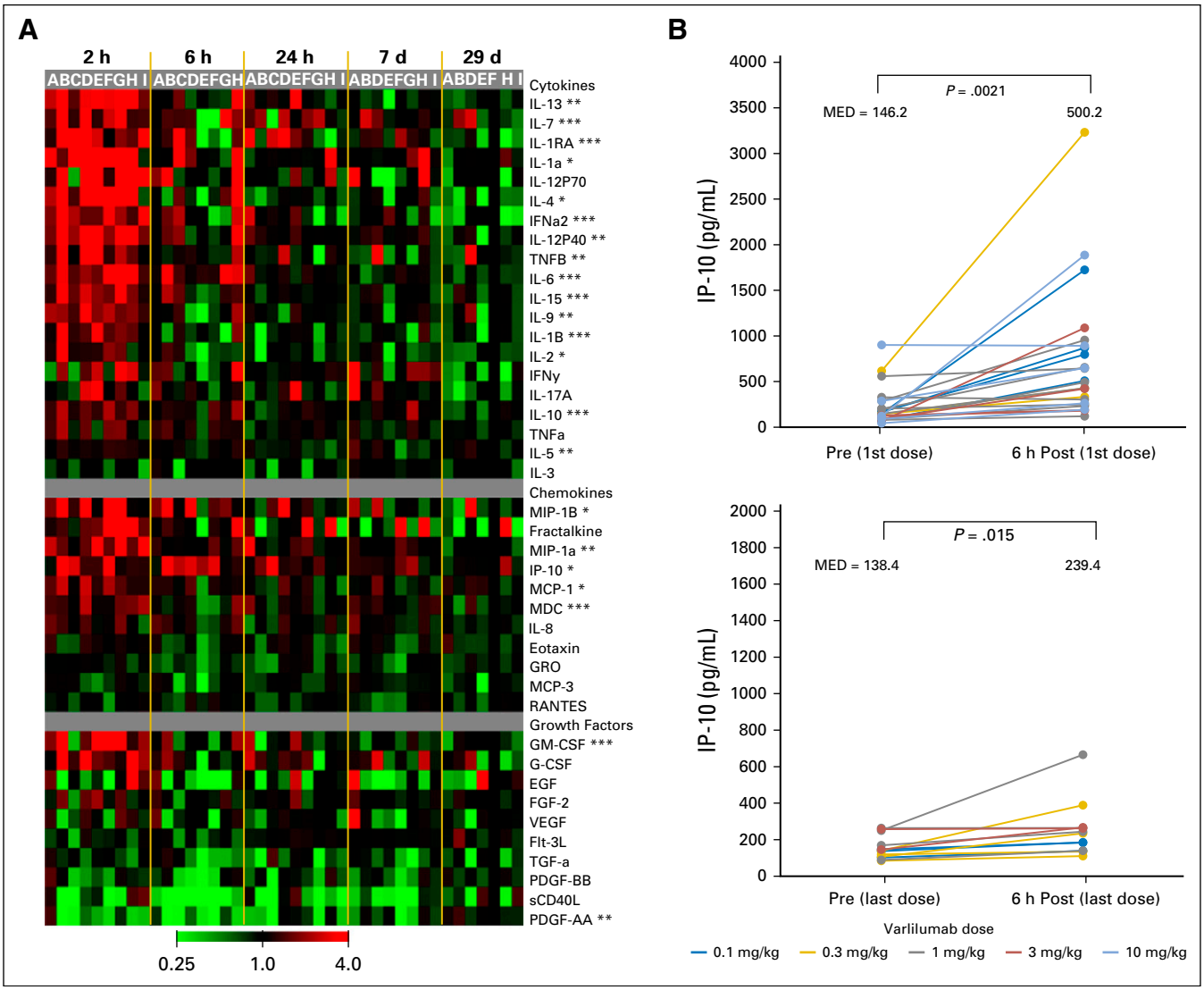

Fig 2. Changes in serum factors after varlilumab administration. (A) Heatmap illustration of changes in cytokines, chemokines, and growth factors in serum from nine patients who received varlilumab at 1 mg/kg. *P* values for changes at the 2-hour timepoint versus baseline by two-tailed paired *t* test are indicated. $*P \leq .05$; $**P \leq .01$; $***P \leq .001$. (B) Interferon gamma–induced protein 10 (IP-10) serum levels at baseline and 6 hours after the first or last varlilumab dose from patients with available samples.

which were CD56 dim, a phenotype associated with cytotoxic NK cells (Fig 3A and data not shown).

Varlilumab treatment changed the relative ratios and activation status of naïve, memory, and effector T cells. Among $CD8^+$ T cells, there was a decrease in the percentage of naïve cells and an increase in the terminally differentiated effector memory subset (Appendix Fig A5, online only). Similarly, $CD4^+$ T cells had lower numbers of cells with a naïve phenotype, with increased numbers of effector memory cells. These changes correlated with an increased expression of the activation marker, HLA-DR, that was most prominent on circulating $CD4^+$ T cells (Fig 4), but also apparent on tumor infiltrating T cells (not shown) and confirmed by upregulation of other activation makers, including CD69, 4-1BB (CD137), and ICOS (CD278) (data not shown). Consistent with changes in the T-cell activation profile, we also observed increased responses to recall antigens and phytohemagglutinin in a number of patients as monitored by IFN-γ ELISpot assays (Appendix Fig A6, online only).

To investigate the effect of varlilumab on antigen-specific T-cell responses, we looked for evidence of T-cell reactivity by IFN-γ ELISpot with suspected melanoma antigens in patients with melanoma with relevant HLA type. Of interest, we observed both augmentation of preexisting and induction of de novo responses in a subset of patients (Fig 5). Of particular interest was the development of a de novo response to a MAGE-A1 peptide, which was also confirmed by using pentamer staining (patient 04-9002).

Antitumor Activity

One patient in the RCC expansion cohort who had previously received three lines of treatment for stage IV disease experienced durable and significant tumor regression (Appendix Fig A7, online only). After a partial response after the first cycle of varlilumab and discontinuation of varlilumab at 5.5 months, tumor volume was further reduced with 78% shrinkage of target lesions, including

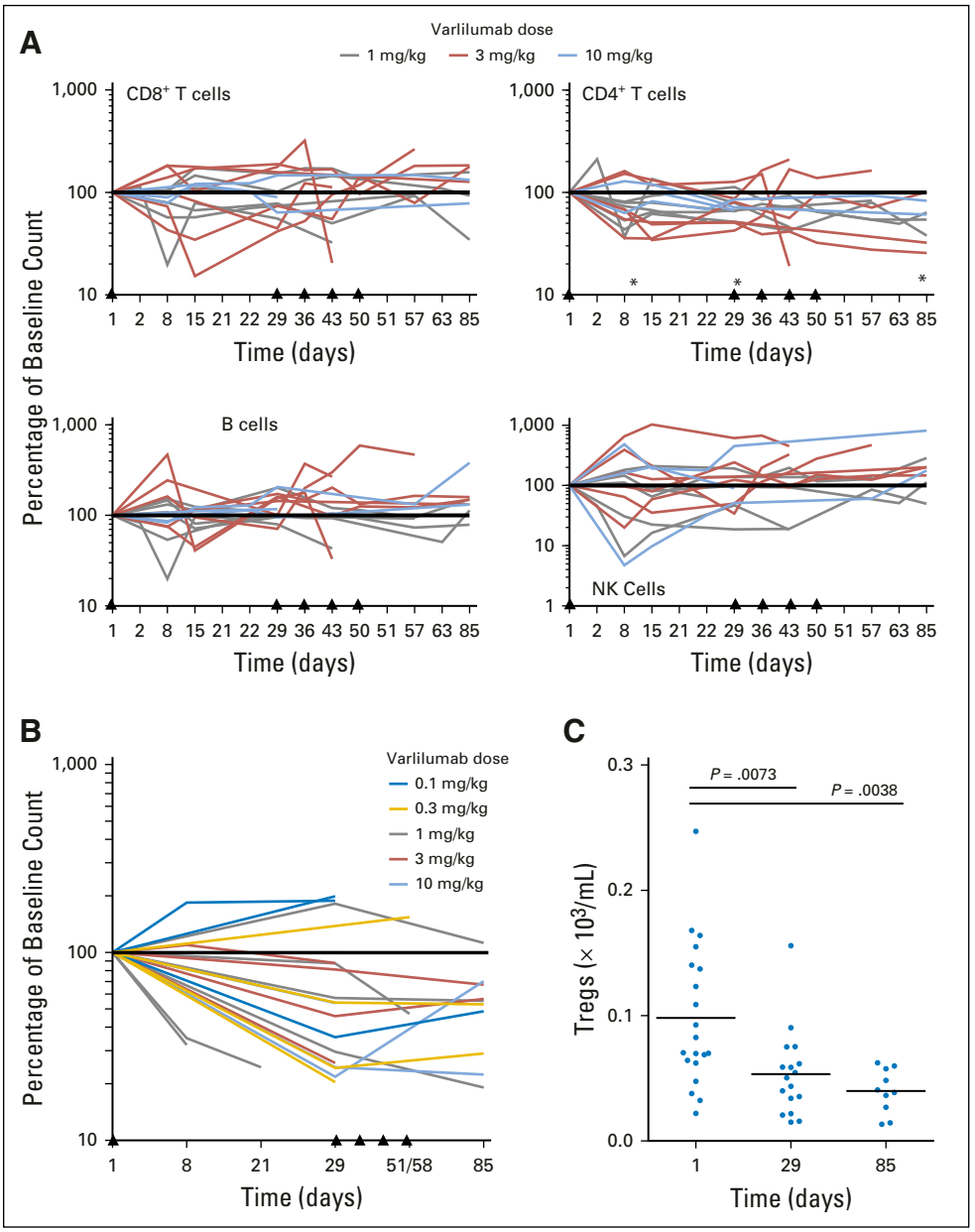

Fig 3. Changes in major lymphocyte population numbers after varlilumab administration. Peripheral blood mononuclear cells purified from whole blood were stored frozen and analyzed together by flow cytometry, and the relative change from baseline for individual patients is shown. (A) Results depict the percentage of absolute cell numbers relative to pretreatment sample for individual patients who were treated at the higher dose levels. Statistical analysis was performed for days 8, 29, and 85 relative to baseline by two-tailed paired *t* test as indicated. *$P \leq .05$. (B) Percentage of absolute counts of regulatory T cells (Tregs; $CD3^+/CD4^+/CD25^+/CD127^-/FoxP3^+$) relative to pretreatment sample for individual patients treated. (C) Absolute counts of Tregs for all patients with samples at the indicated time points. Black triangles (▲) represent varlilumab dosing. NK, natural killer.

complete resolution of lung and paraortic lymph lesions. Partial response persists at 2.3 years without further anticancer therapy. A second patient with RCC completed five cycles of varlilumab (3 mg/kg), experienced 18% shrinkage of target lesions, and has maintained stable disease for 3.9 years without additional therapy. The patient had previously experienced disease progression after 3 months on everolimus and sorafenib and after 9 months on capecitabine.

Eight patients experienced stable disease for > 3 months on study, including four patients with RCC (duration of stable disease: 5.3, 5.6, 9.3, and ≥ 47.3 months), three patients with melanoma (3.8, 7.3, and 11.5 months), and one patient with colorectal adenocarcinoma (5.7 months). A patient with metastatic uveal melanoma with stable disease underwent resection of an enlarging axillary node at 7.5 months, then continued to receive varlilumab with remaining lesions stable until 11.5 months. The patient had previously lost benefit from ipilimumab at 6 months and temozolomide at 4 months.

DISCUSSION

In this first-in-human study of varlilumab—a novel, first-in-class, agonist anti-CD27 monoclonal antibody—dosed at 0.1 to 10 mg/kg was well tolerated by patients with advanced solid tumor malignancies. Pharmacokinetic analysis demonstrated significant and sustained exposure to varlilumab during treatment cycles. Pharmacokinetics did not seem to be impacted by an increase in circulating sCD27 associated with higher doses of varlilumab. The increase in sCD27, which is presumed to be complexed with the

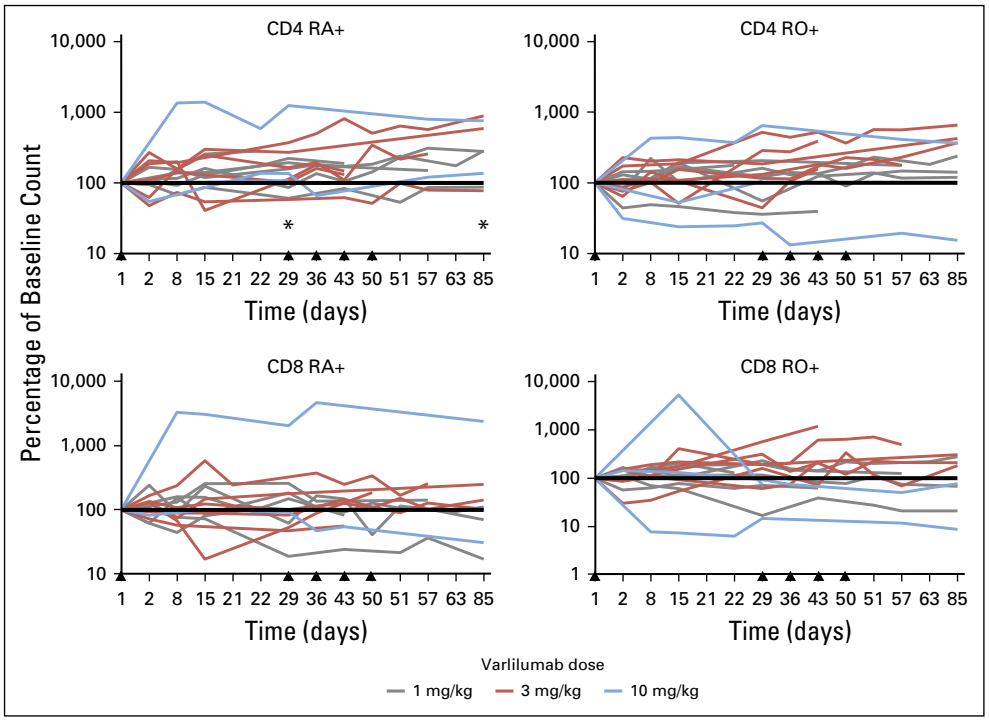

Fig 4. Increase in HLA-DR expression on T cells after varlilumab administration. Peripheral blood mononuclear cells purified from whole blood were stored frozen and analyzed together by flow cytometry. Results represent the percentage of absolute counts relative to pretreatment sample for individual patients who were treated at the higher dose levels. Statistical analysis was performed for days 8, 29, and 85 relative to baseline by two-tailed paired *t* test as indicated. $*P \leq .05$. Black triangles (▲) represent varlilumab dosing. RA, CD45RA; RO, CD45RO.

much higher levels of varlilumab, likely represents slower clearance mechanisms, perhaps similar to those described for the increase in vascular endothelial growth factor during bevacizumab treatment.[18] Of importance, there was no evidence of high-grade hepatitis, colitis, or pneumonitis or other high-grade immune-related adverse events that have been associated with immune stimulatory monoclonal antibodies that target costimulatory or checkpoint pathways.[19] This favorable safety profile is consistent with the restricted lymphocyte-specific expression of CD27, the requirement for concomitant TCR engagement to induce T-cell

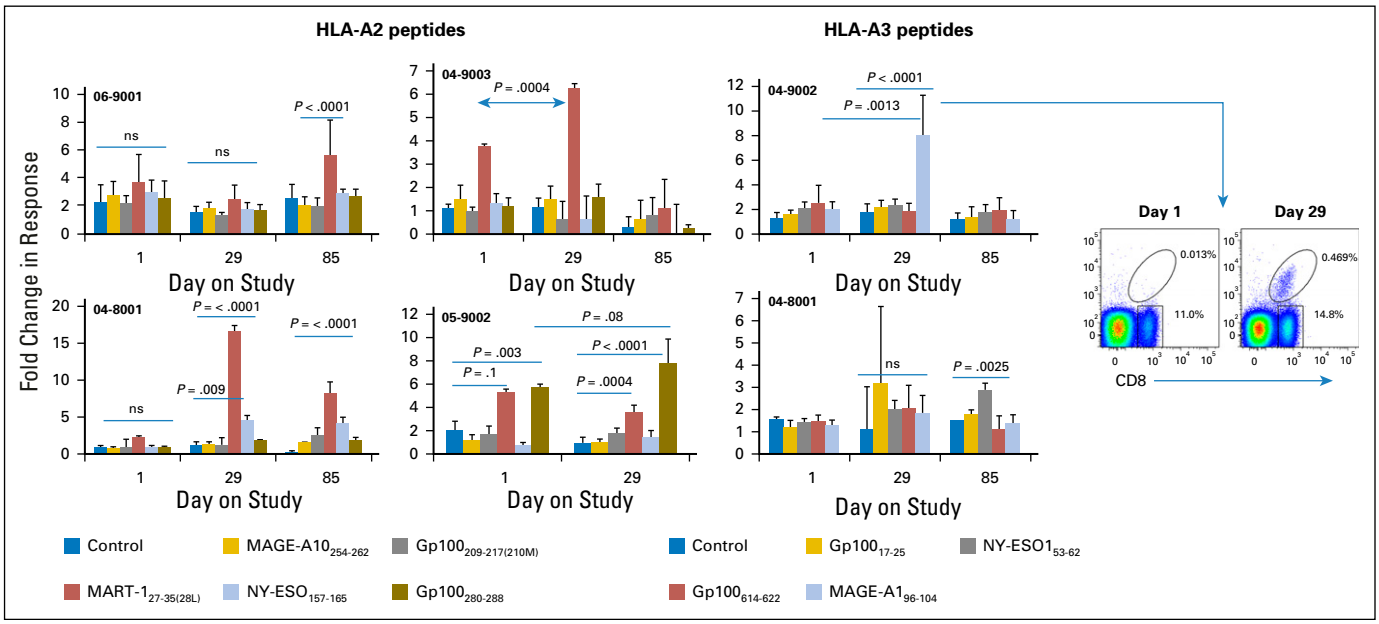

Fig 5. Increase in responses to melanoma antigen in select patients who were treated with varlilumab. Enumeration of $CD8^+$ T-cell responses in HLA-A2– or HLA-A3–positive patients with melanoma to the indicated melanoma-associated antigens. Peripheral blood mononuclear cells (PBMCs) from days 1, 29, and 85 were assayed for interferon-gamma production in response to antigen-presenting cells that were pulsed with selected melanoma antigen peptides after 2 weeks in vitro stimulation with a peptide cocktail. Values shown are the fold change on the basis of dividing the absolute counts for each peptide by the counts with control HIV gag–derived peptides. *P* values were calculated by using one-way analysis of variance for individual timepoints or unpaired Student *t* tests across timepoints. For patient 04-9002, PBMCs were directly stained for major histocompatibility complex multimer binding and representative dot plots are presented. ns, not significant.

activation in conjunction with CD27 stimulation, and the safety of varlilumab in preclinical studies.[11]

Varlilumab demonstrated evidence of biologic activity that was aligned with targeting and activation of the CD27 pathway. Consistent with our previous results using varlilumab for in vitro activation of human T cells,[20] a transient increase in proinflammatory mediators, particularly IP-10, was observed across all dose levels (data not shown). IP-10 has been recognized as an important cytokine whose expression is upregulated by CD27 costimulation in $CD8^+$ T cells.[21] In addition, we observed an increase in circulating T cells with an activated phenotype and a change to fewer naïve T cells and more effector T cells. In selected patients with melanoma, increases in T cells that recognize melanoma-related antigens were observed by ELISpot and confirmed by pentamer staining. Most notable was a consistent decrease in circulating Tregs, which is also observed in our preclinical studies and is linked to its antitumor activity in some models (Wasiuk A, manuscript in preparation). Although we understand that Fc receptor interaction is required for efficient cross-linking and activation of CD27 by varlilumab,[11] it is unclear whether the decrease in circulating Treg is the result of Fc receptor–mediated effector function or an alternative effect. Collectively, these data support the concept that varlilumab is effectively engaging its target, CD27, which leads to activation of this pathway.

One patient with RCC achieved a durable partial response, with post-treatment tumor regression continuing beyond 2 years, a pattern characteristic of immunotherapy. Another patient with RCC continues to experience stable disease at nearly 4 years, and several additional patients with various cancer types demonstrated tumor regression or stable disease. The modest clinical activity of varlilumab monotherapy is similar to previously reported data with antibodies that targeted related costimulatory molecules, 4-1BB[22] and OX40,[23] and underscores the importance of combination therapy to engage multiple aspects of the cancer-immunity cycle. It should also be noted that the immunologic assessments did not identify an optimal biologic dose for varlilumab, and potentially better activity could be observed with less frequent or lower dosing as there was a trend toward more consistent biomarker changes in the dose-escalation phase of the study. Likewise, less frequent dosing correlated with better outcomes for a CD40 agonist antibody in patients with cancer.[24,25]

This phase I study of varlilumab provides proof of concept and a rationale for further study in combination with immunotherapies and traditional therapies. Therapy that targets multiple nonredundant pathways that regulate immune responses may be synergistic and enhance antitumor immune responses. Similarly, combining varlilumab with agents that induce the release of tumor antigens from dying cancer cells can promote new antitumor T-cell responses. On the basis of preclinical models that support synergy of varlilumab with either checkpoint blockade or chemotherapy regimens,[26] a broad clinical evaluation of varlilumab with combination therapy is underway.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Howard A. Burris, Jeffrey R. Infante, Thomas R. Hawthorne, Thomas A. Davis, Michael J. Yellin, Tibor Keler
Administrative support: Howard A. Burris, John J. Nemunaitis
Provision of study materials or patients: Howard A. Burris, Jeffrey R. Infante, John J. Nemunaitis, Geoffrey R. Weiss, Victor M. Villalobos, Branimir I. Sikic, Matthew H. Taylor, Donald W. Northfelt, William E. Carson III
Collection and assembly of data: Howard A. Burris, Jeffrey R. Infante, John J. Nemunaitis, Geoffrey R. Weiss, Victor M. Villalobos, Branimir I. Sikic, Matthew H. Taylor, Donald W. Northfelt, William E. Carson III, Thomas R. Hawthorne, Michael J. Yellin, Tibor Keler, Timothy Bullock
Data analysis and interpretation: Howard A. Burris, Jeffrey R. Infante, Stephen M. Ansell, John J. Nemunaitis, Branimir I. Sikic, Matthew H. Taylor, Donald W. Northfelt, Thomas R. Hawthorne, Thomas A. Davis, Michael J. Yellin, Tibor Keler, Timothy Bullock
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

REFERENCES

1. Postow MA, Callahan MK, Wolchok JD: Immune checkpoint blockade in cancer therapy. J Clin Oncol 33:1974-1982, 2015
2. Hendriks J, Xiao Y, Borst J: CD27 promotes survival of activated T cells and complements CD28 in generation and establishment of the effector T cell pool. J Exp Med 198:1369-1380, 2003
3. Hendriks J, Gravestein LA, Tesselaar K, et al: CD27 is required for generation and long-term maintenance of T cell immunity. Nat Immunol 1: 433-440, 2000
4. Agematsu K, Kobata T, Yang FC, et al: CD27/CD70 interaction directly drives B cell IgG and IgM synthesis. Eur J Immunol 25:2825-2829, 1995
5. Kobata T, Jacquot S, Kozlowski S, et al: CD27-CD70 interactions regulate B-cell activation by T cells. Proc Natl Acad Sci USA 92:11249-11253, 1995
6. Agematsu K, Nagumo H, Oguchi Y, et al: Generation of plasma cells from peripheral blood memory B cells: Synergistic effect of interleukin-10 and CD27/CD70 interaction. Blood 91:173-180, 1998
7. Klein U, Rajewsky K, Küppers R: Human immunoglobulin (Ig)M^+IgD^+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. J Exp Med 188:1679-1689, 1998
8. Yang FC, Agematsu K, Nakazawa T, et al: CD27/CD70 interaction directly induces natural killer cell killing activity. Immunology 88:289-293, 1996
9. Nishikawa H, Sakaguchi S: Regulatory T cells in cancer immunotherapy. Curr Opin Immunol 27:1-7, 2014
10. Claus C, Riether C, Schürch C, et al: CD27 signaling increases the frequency of regulatory T cells and promotes tumor growth. Cancer Res 72: 3664-3676, 2012
11. Vitale LA, He LZ, Thomas LJ, et al: Development of a human monoclonal antibody for potential therapy of CD27-expressing lymphoma and leukemia. Clin Cancer Res 18:3812-3821, 2012
12. He LZ, Prostak N, Thomas LJ, et al: Agonist anti-human CD27 monoclonal antibody induces T cell activation and tumor immunity in human CD27-transgenic mice. J Immunol 191:4174-4183, 2013
13. Simon R, Freidlin B, Rubinstein L, et al: Accelerated titration designs for phase I clinical trials in oncology. J Natl Cancer Inst 89:1138-1147, 1997
14. Korman A, Yellin M, Keler T: Tumor immunotherapy: Preclinical and clinical activity of anti-CTLA4 antibodies. Curr Opin Investig Drugs 6: 582-591, 2005
15. Eisenhauer EA, Therasse P, Bogaerts J, et al: New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 45:228-247, 2009
16. Slingluff CL Jr, Petroni GR, Olson WC, et al: Effect of granulocyte/macrophage colony-stimulating

factor on circulating CD8+ and CD4+ T-cell responses to a multipeptide melanoma vaccine: Outcome of a multicenter randomized trial. Clin Cancer Res 15: 7036-7044, 2009

17. Slingluff CL Jr, Petroni GR, Chianese-Bullock KA, et al: Randomized multicenter trial of the effects of melanoma-associated helper peptides and cyclophosphamide on the immunogenicity of a multipeptide melanoma vaccine. J Clin Oncol 29: 2924-2932, 2011

18. Alidzanovic L, Starlinger P, Schauer D, et al: The VEGF rise in blood of bevacizumab patients is not based on tumor escape but a host-blockade of VEGF clearance. Oncotarget 7:57197-57212, 2016

19. Friedman CF, Proverbs-Singh TA, Postow MA: Treatment of the immune-related adverse effects of immune checkpoint inhibitors: A review. JAMA Oncol 2:1346-1353, 2016

20. Ramakrishna V, Sundarapandiyan K, Zhao B, et al: Characterization of the human T cell response to in vitro CD27 costimulation with varlilumab. J Immunother Cancer 3:37, 2015

21. Peperzak V, Veraar EA, Xiao Y, et al: CD8+ T cells produce the chemokine CXCL10 in response to CD27/CD70 costimulation to promote generation of the CD8+ effector T cell pool. J Immunol 191: 3025-3036, 2013

22. Sznol M, Hodi FS, Margolin K, et al: Phase I study of BMS-663513, a fully human anti-CD137 agonist monoclonal antibody, in patients (pts) with advanced cancer (CA). J Clin Oncol 26, 2008 (suppl 15S, abstr 3007)

23. Curti BD, Kovacsovics-Bankowski M, Morris N, et al: OX40 is a potent immune-stimulating target in late-stage cancer patients. Cancer Res 73: 7189-7198, 2013

24. Rüter J, Antonia SJ, Burris HA, et al: Immune modulation with weekly dosing of an agonist CD40 antibody in a phase I study of patients with advanced solid tumors. Cancer Biol Ther 10:983-993, 2010

25. Drabick JJ, Schell TD: Poking CD40 for cancer therapy, another example of the Goldilocks effect. Cancer Biol Ther 10:994-996, 2010

26. He L-Z, Thomas LJ, Testa J, et al: Combination therapies augment the anti-tumor activity of agonist CD27 mAb in human CD27 transgenic mouse models. J Immunother Cancer 1:P76, 2013

Affiliations

Howard A. Burris and **Jeffrey R. Infante**, Sarah Cannon Research Institute, Tennessee Oncology, Nashville, TN; **Stephen M. Ansell**, Mayo Clinic, Rochester, MN; **John J. Nemunaitis**, Mary Crowley Cancer Research Center, Dallas, TX; **Geoffrey R. Weiss** and **Timothy Bullock**, University of Virginia, Charlottesville, VA; **Victor M. Villalobos** and **Branimir I. Sikic**, Stanford Cancer Institute, Stanford, CA; **Matthew H. Taylor**, Oregon Health & Science University, Portland, OR; **Donald W. Northfelt**, Mayo Clinic, Scottsdale, AZ; **William E. Carson III**, The Ohio State University, Columbus, OH; **Thomas R. Hawthorne**, **Thomas A. Davis**, **Michael J. Yellin**, and **Tibor Keler**, Celldex Therapeutics, Hampton, NJ.

Support

Supported by Celldex Therapeutics.

Prior Presentation

Presented previously at the 2014 Society for Immunotherapy of Cancer Annual Meeting, National Harbor, MD, November 6-9, 2014, the 2014 American Society of Clinical Oncology Annual Meeting, Chicago, IL, May 30-June 3, 2014, and the 2013 Society for Immunotherapy of Cancer Annual Meeting, National Harbor, MD, November 8-10, 2013.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Safety and Activity of Varlilumab, a Novel and First-in-Class Agonist Anti-CD27 Antibody, in Patients With Advanced Solid Tumors

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/jco/site/ifc.

Howard A. Burris
No relationship to disclose

Jeffrey R. Infante
Research Funding: Celldex Therapeutics (Inst), ARMO BioSciences (Inst), BioMed Valley Discoveries (Inst), Novartis (Inst), Janssen Oncology (Inst), GlaxoSmithKline (Inst), Immunocare Therapies (Inst), Calithera Biosciences (Inst), Phosplatin Therapeutics (Inst), Genentech (Inst), Roche (Inst), Aileron Therapeutics (Inst), AstraZeneca (Inst), eFFECTOR Therapeutics (Inst), MedImmune (Inst), Pfizer (Inst), Bristol-Myers Squibb (Inst), Tesaro (Inst), Merck (Inst)

Stephen M. Ansell
Honoraria: WebMD, Research to Practice
Research Funding: Bristol-Myers Squibb (Inst), Celldex Therapeutics (Inst), Seattle Genetics (Inst), Merck (Inst), Affimed Therapeutics (Inst)

John J. Nemunaitis
Employment: Gradalis
Leadership: Gradalis
Stock or Other Ownership: Gradalis
Honoraria: Amgen, AstraZeneca, Foundation Medicine
Consulting or Advisory Role: Amgen, AstraZeneca, Foundation Medicine
Speakers' Bureau: Amgen, AstraZeneca
Patents, Royalties, Other Intellectual Property: Gradalis
Travel, Accommodations, Expenses: Takeda, Amgen, Baxalta, AstraZeneca, Foundation Medicine

Geoffrey R. Weiss
No relationship to disclose

Victor M. Villalobos
Consulting or Advisory Role: Janssen Pharmaceuticals, Eli Lilly, Novartis
Travel, Accommodations, Expenses: Eli Lilly

Branimir I. Sikic
Consulting or Advisory Role: Threshold Pharmaceuticals, Immune Design
Research Funding: Forty Seven (Inst), CellDex Therapeutics (Inst), Gilead Sciences (Inst), Basilea (Inst), Genentech (Inst), Sanofi (Inst)

Matthew H. Taylor
Honoraria: Eisai, Bristol-Myers Squibb, Trillium Therapeutics, Blueprint Medicines, Roche
Consulting or Advisory Role: Eisai, Genentech, Blueprint Medicines, Bristol-Myers Squibb, Trillium Therapeutics
Speakers' Bureau: Eisai
Travel, Accommodations, Expenses: Eisai, Bristol-Myers Squibb, Blueprint Medicines

Donald W. Northfelt
No relationship to disclose

William E. Carson III
No relationship to disclose

Thomas R. Hawthorne
Employment: Celldex Therapeutics
Stock or Other Ownership: Celldex Therapeutics

Thomas A. Davis
Employment: Celldex Therapeutics
Leadership: Celldex Therapeutics
Stock or Other Ownership: Celldex Therapeutics
Travel, Accommodations, Expenses: Celldex Therapeutics

Michael J. Yellin
Employment: Celldex Therapeutics
Stock or Other Ownership: Celldex Therapeutics
Patents, Royalties, Other Intellectual Property: CD40L patent held by Columbia University and licensed to Biogen
Travel, Accommodations, Expenses: Celldex Therapeutics

Tibor Keler
Employment: Celldex Therapeutics
Leadership: Celldex Therapeutics
Stock or Other Ownership: Celldex Therapeutics
Travel, Accommodations, Expenses: Celldex Therapeutics

Timothy Bullock
Consulting or Advisory Role: Innocrin
Research Funding: Celldex Therapeutics
Patents, Royalties, Other Intellectual Property: Patent pending for use of PDL1 and PDL2 for melanoma patient prognosis
Travel, Accommodations, Expenses: Celldex Therapeutics

Acknowledgment

Assistance with clinical trial management was provided by Elsa Paradise (Celldex Therapeutics) and assistance with correlative data analysis was provided by Laura Vitale and Venky Ramakrishna (Celldex Therapeutics). An earlier version of this manuscript was prepared with medical writing assistance provided by Mary-Ann Zalman (MA Zalman and Associates).

Appendix

Eligibility Criteria

The study was open to patients with metastatic melanoma, renal cell carcinoma, hormone-refractory prostate adenocarcinoma, ovarian cancer, colorectal adenocarcinoma, or non–small-cell lung cancer progressive subsequent to previous therapies with no remaining alternative approved therapy options. Patients with melanoma who were enrolled in the expansion cohort must have received or refused ipilimumab and, if expressing the BRAF V600E mutation, vemurafinib. Additional eligibility requirements included age $\geq$ 18 years; measurable or evaluable disease; life expectancy $\geq$ 12 weeks; Eastern Cooperative Oncology Group performance status of 0 or 1; adequate renal, hepatic, and bone marrow function; and resolution of toxicity related to prior therapy—excluding alopecia—to grade $\leq$ 1. A washout of $\geq$ 4 weeks was required for chemotherapy, monoclonal-based therapies, systemic radiation therapy, and immunosuppressive medications, including systemic corticosteroids. Other immunotherapy, investigational drugs, and prior focal radiotherapy were prohibited within 2 weeks of study entry. Patients who were pregnant, breastfeeding, had other prior malignancies within the last 5 years, active brain metastases, autoimmune disease, active infection, significant cardiovascular disease, or any other significant, active, concurrent medical illness that would have precluded study treatment were ineligible.

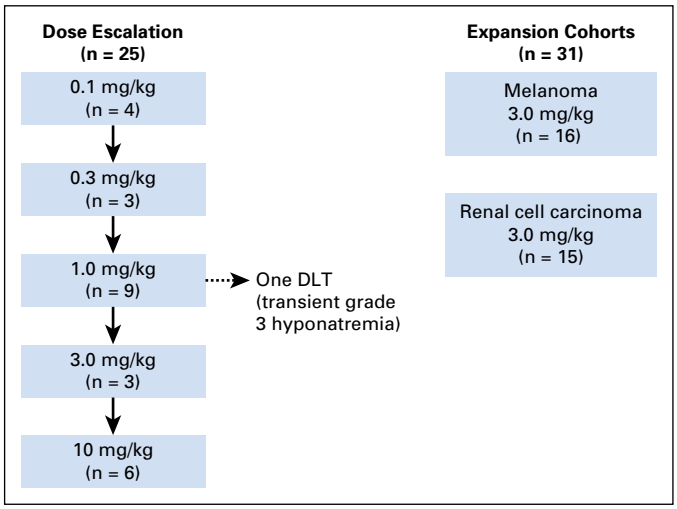

Fig A1. Study design. Dose escalation followed a standard 3 + 3 design in which three patients were initially enrolled in each cohort. In the event of one dose-limiting toxicity (DLT), the cohort was to be expanded to six patients. With two or more DLTs, dosing would be halted and the maximum tolerated dose (MTD) exceeded. MTD would be the highest dose where zero of three, or one or fewer of six patients in a cohort experienced a DLT. Dose escalation for the single-dose and multidose phases proceeded separately, and patients who prematurely discontinued treatment for reasons other than DLT before completion of the multidose phase were replaced as necessary. After the dose escalation component of the study, with consideration to safety, pharmacodynamic, and early activity data from the dose-escalation phase, two expansion cohorts were enrolled to further explore the clinical and biologic activity of varlilumab (at 3 mg/kg) in patients with melanoma and renal cell carcinoma.

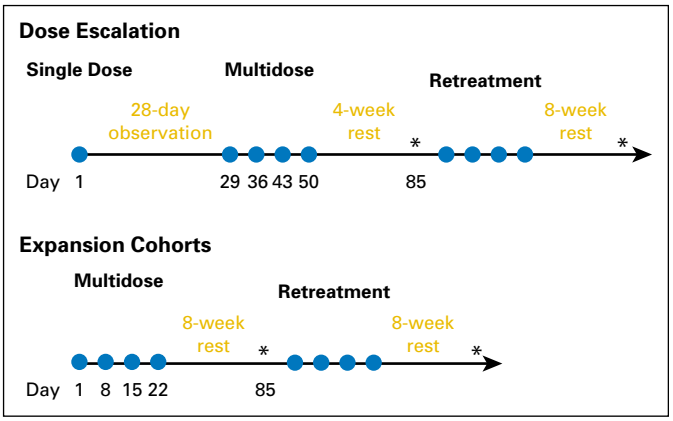

Fig A2. Treatment schema. During the dose-escalation phase, varlilumab (represented by blue circles) was administered as a single dose with 28-day observation, followed by one dose per week for 4 weeks with a 4-week rest. Patients in the expansion cohorts began treatment with one dose per week for 4 weeks doses and an 8-week rest. Diagnostic imaging and restaging was repeated every 12 weeks (as indicated by asterisks). Patients with stable disease were eligible to receive up to four retreatment cycles for a total of five treatment cycles.

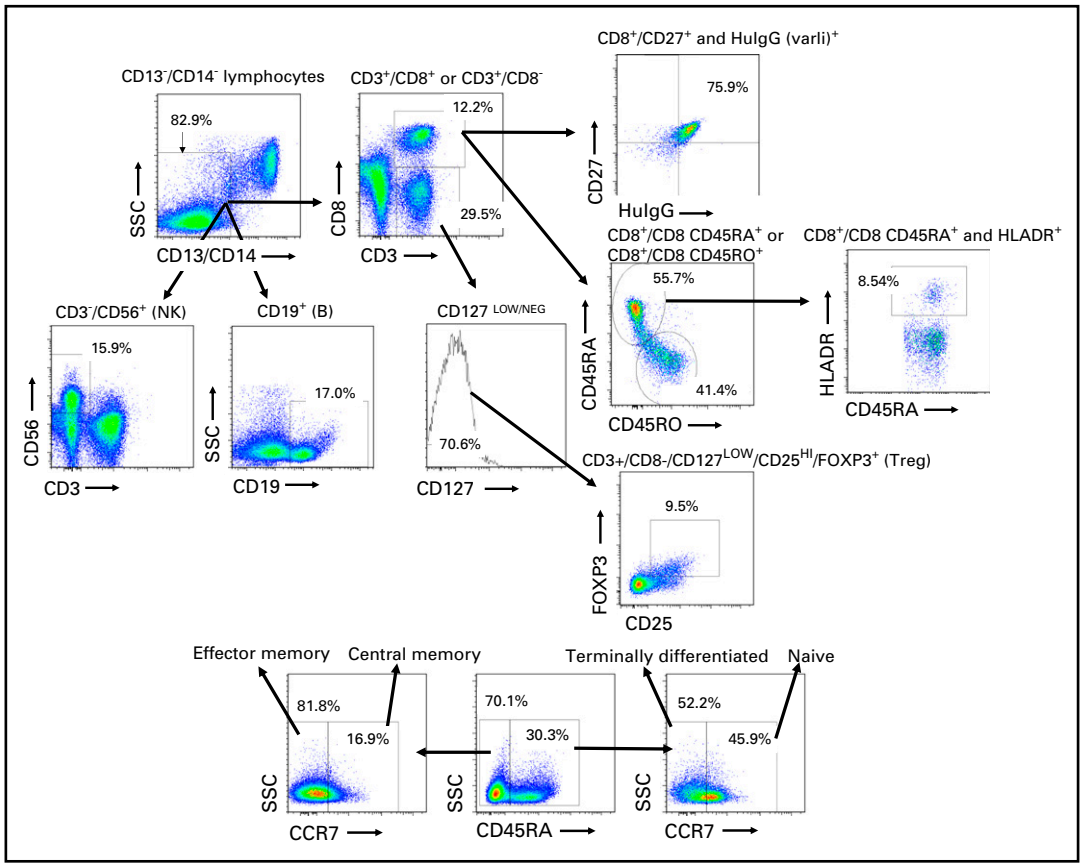

Fig A3. Flow cytometry gating strategy and methods. For surface staining, peripheral blood mononuclear cells or TIL were thawed at 37°C, washed, and plated in 96-well V-bottom plates. Thawed cells were stained according to the manufacturer's directions (ThermoFisher). Briefly, cells were washed in PBS and set at 1e6/ml in PBS with 1:1000 dilution of fixable live/dead Aqua for 30 minutes on ice in the dark. Cells were washed twice with PBS prior to further staining with a cocktail of fluorescently labeled antibodies that were specific for the following: CD13 and CD14 (exclusion gate); CD19, CD3, CD8, CD56, CD45RO,CD45RA, CD27, CD25, and HLA-DR (antibodies from BD, eBioscience, and BioLegend). After incubation, cells were washed, incubated in Fix/Lyse (Becton Dickenson) for 10 minutes, and resuspended in fluorescent-activated cell sorting buffer for flow cytometry. For detection of FoxP3, cells were surface stained as above, fixed, and permeabilized in FoxP3 staining buffer (eBioscience), then incubated with anti-human FoxP3. $CD3^+$ cells were also separately stained for differentiation status using CD8, CD45RA, and CCR7 as shown. Labeled cells were washed with Permwash buffer before final fixation. Stained samples were acquired using a LSR Fortessa running DIVA 6.0 (Becton Dickenson) and analyzed using FlowJo (Treestar, Ashland, OR). NK, natural killer; SSC, side scatter; Treg, T regulatory cell.

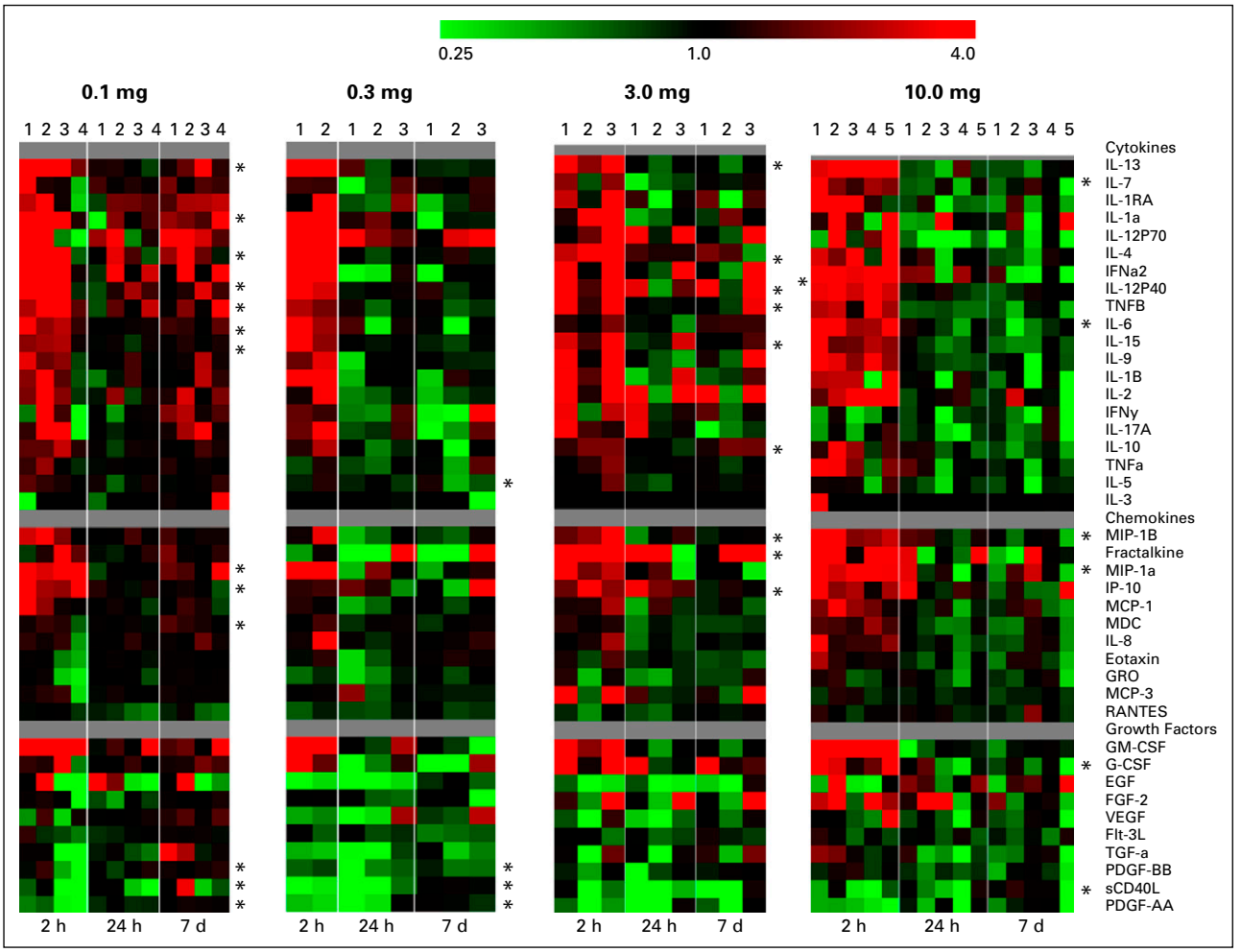

Fig A4. Changes in serum factors after varlilumab administration is consistent across dose levels. (A) Heatmap illustrations of changes in cytokines, chemokines, and growth factors in serum from patients who were treated with varlilumab at different dose levels. *P* values for changes at the 2-hour time point versus baseline by two-tailed paired *t* test are indicated. $*P \leq .05$.

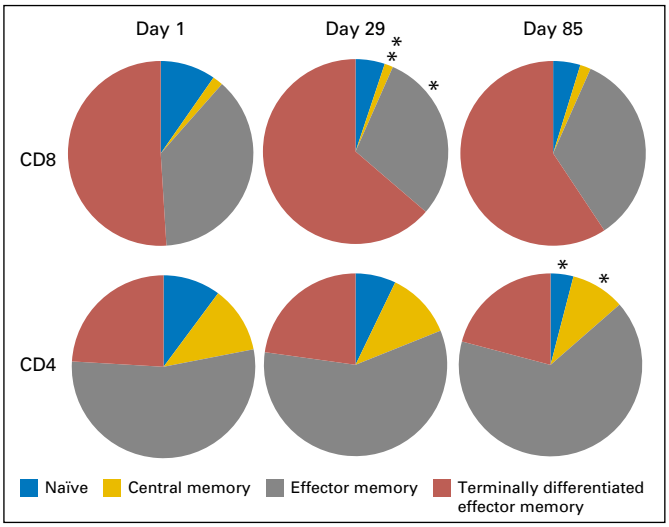

Fig A5. Varlilumab impacts the ratio of naïve and memory T-cell subsets. Peripheral blood mononuclear cells from nine patients across all dose levels purified from whole blood were stored frozen and analyzed together by flow cytometry for CD3, CD8, CD45RA, and CCR7. Naïve, $CD45RA^{+}/CCR7^{+}$; central memory, $CD45RA^{-}/CCR7^{+}$; effector memory, $CD45RA^{-}/CCR7^{-}$; terminally differentiated effector memory, $CD45RA^{+}/CCR7^{-}$. Changes that are statistically significant from baseline values using two-tailed paired *t* test are indicated. $*P \leq .05$; $** P \leq .01$.

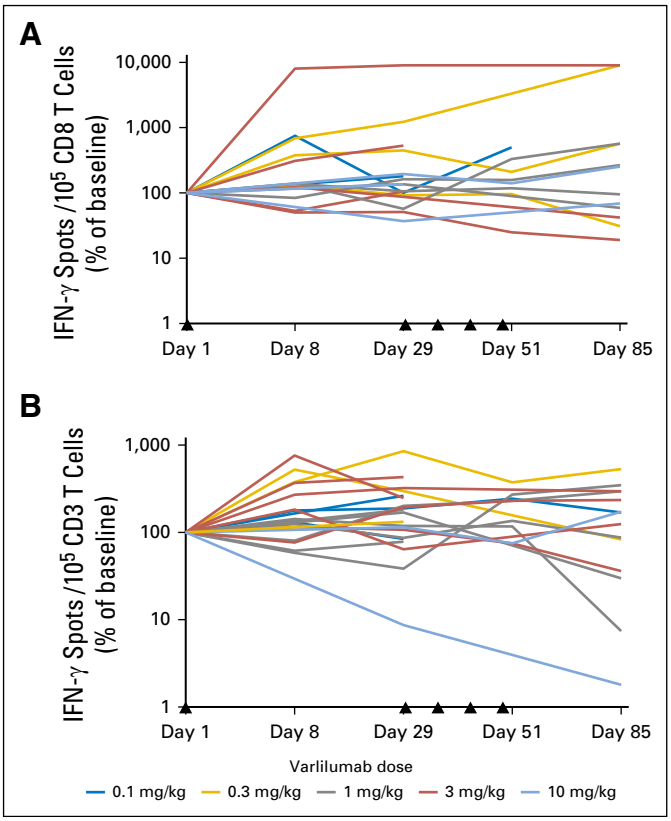

Fig A6. Effect of varlilumab on T-cell response to recall antigens or nonspecific activation with PHA. Peripheral blood mononuclear cells purified from whole blood were stored frozen and analyzed together for interferon-gamma (IFN-γ) production in response to peptides from (A) CMV, EBV, and Flu or to (B) PHA stimulation by ELISpot. Results represent the percentage of IFN-γ–producing cells relative to pretreatment sample for individual patients who were treated at different dose levels. Black triangles (▲) represent varlilumab dosing.

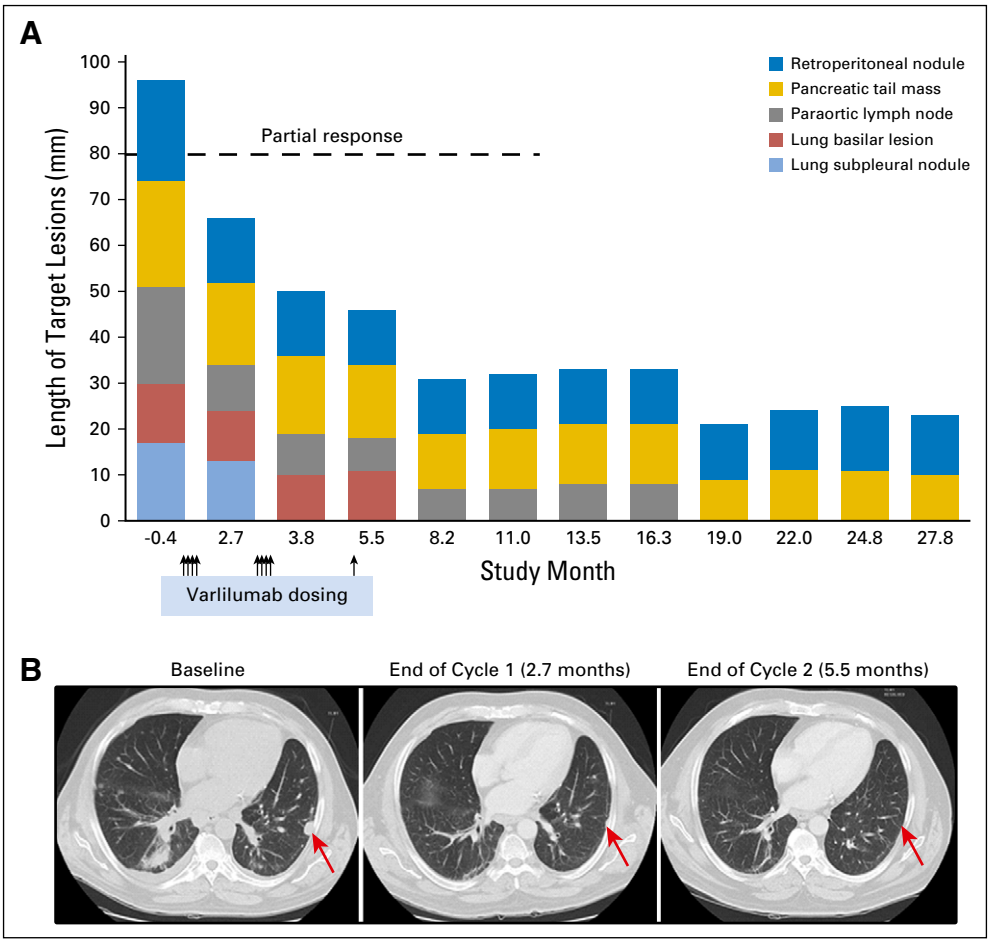

Fig A7. Durable partial response in a patient with renal cell carcinoma (RCC). The patient, with a 43-month history of stage IV RCC, had previously received three lines of treatment, including sunitinib/lenalomide and everolimus, and had most recently experienced progression of disease after 3 weeks of investigational therapy (sphingosine-1-phosphate–targeted monoclonal antibody). (A) Patient experienced a partial response (31% shrinkage of target lesions) after the first cycle of varlilumab. Although varlilumab was discontinued at 5.5 months, further reduction in tumor volume, including complete resolution of lung and paraortic lymph lesions, was observed, and 78% shrinkage of target lesions was achieved at 19 months. No additional anticancer therapies have been received and the patient continues to experience a partial response at 27.8 months. (B) Complete radiographic resolution of lung subpleural nodule (arrow).

Table A1. Pretreatment Patient Demographic and Disease Characteristics

Characteristic	Dose Escalation Cohort (n = 25)	Melanoma Expansion Cohort (n = 16)	Renal Cell Carcinoma Expansion Cohort (n = 15)
Age, median (range), years	66 (42-83)	69 (29-83)	61 (45-68)
Age ≥ 65 years, No. (%)	16 (64)	11 (69)	5 (33)
Male, No. (%)	16 (64)	10 (63)	13 (87)
ECOG performance status			
0	11 (44)	7 (44)	8 (53)
1	14 (56)	9 (56)	6 (40)
2	0	0	1 (7)
Tumor type, No. (%)			
Colorectal cancer	10 (40)	0	0
Melanoma	7 (28)	16 (100)	0
Ovarian	3 (12)	0	0
Prostate	2 (8)	0	0
Renal cell carcinoma	2 (8)	0	15 (100)
Non–small-cell lung cancer	1 (4)	0	0
Stage at study entry, No. (%)			
IV	25 (100)	16 (100)	15 (100)
Duration of disease, median (range), years	4.8 (2.0-24.6)	3.8 (0.6-26.3)	4.5 (2.4-17.8)
Lines of treatment, median (range)			
Anticancer therapy	4 (0-8)	1 (0-5)	3 (1-5)
Cytotoxic chemotherapy	3 (0-8)	0 (0-1)	0 (0-1)
Prior treatments received, No. (%)			
Checkpoint blockade	5 (20)	13 (81)	1 (7)
Anti–CTLA-4	5 (20)	13 (81)	0
Anti–PD-1	2 (8)	1 (6)	1 (7)
Kinase inhibitor	8 (32)	3 (18)	15 (100)
Cytotoxic chemotherapy	22 (88)	6 (38)	4 (27)
Cytokine (IL-2 or interferon)	4 (16)	4 (25)	4 (27)
Other monoclonal antibodies	13 (52)	0	4 (27)
Other investigational	9 (36)	2 (13)	0
Radiotherapy	14 (56)	10 (63)	9 (60)

Abbreviations: CTLA-4, cytotoxic T-cell lymphocyte-4; ECOG, Eastern Cooperative Oncology Group; PD-1, programmed death-1.

Table A2. Pharmacokinetic Parameters of Varlilumab After Single and Multiple Dosing

	Dose	No.	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (μg/mL)	AUC 0-all (h·μg/mL)	AUC 0-inf_pred (h·μg/mL)	V_{ss} pred (mL/kg)	Cl pred (mL/h/kg)
0.1 mg/kg	1	4	35.7 (22.5)	9.8 (9.8)	3.6 (2.2)	142 (117)	189 (127)	24.3 (11.9)	1.03 (1.12)
	5	3	21.1 (21.5)	4.5 (1.7)	4.0 (1.3)	148 (183)	159 (189)	25.9 (5.4)	1.49 (1.12)
0.3 mg/kg	1	3	65.4 (27.8)	11.4 (10.3)	11.3 (3.7)	990 (410)	1077 (457)	19.4 (5.4)	0.33 (0.17)
	5	4*	116.3 (50.9)	6.0 (1.8)	14.9 (4.8)	2,495 (1,664)	2,615 (1,716)	20.2 (3.9)	0.18 (0.14)
1.0 mg/kg	1	9	115.1 (61.5)	7.4 (6.4)	36.7 (10.0)	4,268 (2,856)	4,696 (2,918)	34.9 (16.4)	0.34 (0.29)
	5	5	216.7 (119.3)	8.4 (10.0)	48.9 (9.8)	13,775 (8,118)	15,848 (10,518)	18.4 (6.6)	0.09 (0.05)
3.0 mg/kg	1	3	218.0 (167.8)	3.0 (0.1)	126.7 (72.0)	13,311 (6,745)	16,579 (9,244)	44.4 (22.9)	0.25 (0.19)
	5	3†	395.4 (150.1)	3.7 (1.8)	232.3 (79.4)	81,864 (24,066)	105,988 (41,191)	18.3 (7.5)	0.03 (0.01)
10 mg/kg	1	5	250.8 (156.6)	5.3 (2.0)	357.0 (55.8)	57,944 (21,602)	79,792 (33,740)	41.2 (18.4)	0.15 (0.07)
	5	3	461.9 (296.6)	1.9 (0.3)	399.2 (42.1)	162,166 (78,199)	181,205 (91,810)	28.2 (8.7)	0.07 (0.03)

NOTE. Data given as mean (standard deviation).
Abbreviation: AUC, area under the curve.
*Includes two patients who were dose reduced from 1.0 mg/kg.
†Includes two patients who were dose reduced from 10 mg/kg.