

# Safety, Pharmacokinetics, and Pharmacodynamics of a Humanized Anti-Semaphorin 4D Antibody, in a First-In-Human Study of Patients with Advanced Solid Tumors

Amita Patnaik<sup>1</sup>, Glen J. Weiss<sup>2</sup>, John E. Leonard<sup>3</sup>, Drew Warren Rasco<sup>1</sup>, Jasjit C. Sachdev<sup>4</sup>, Terrence L. Fisher<sup>3</sup>, Laurie A. Winter<sup>3</sup>, Christine Reilly<sup>3</sup>, Robert B. Parker<sup>3</sup>, Danielle Mutz<sup>1</sup>, Lisa Blaydorn<sup>2</sup>, Anthony W. Tolcher<sup>1</sup>, Maurice Zauderer<sup>3</sup>, and Ramesh K. Ramanathan<sup>4</sup>

## Abstract

**Purpose:** Study objectives included evaluating the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD), and antitumor activity of VX15/2503 in advanced solid tumor patients.

**Experimental Design:** Weekly i.v. doses were administered on a 28-day cycle. Safety, immunogenicity, PK, efficacy, T-cell membrane-associated SEMA4D (cSEMA4D) expression and saturation, soluble SEMA4D (sSEMA4D) serum levels, and serum biomarker levels were evaluated.

**Results:** Forty-two patients were enrolled into seven sequential cohorts and an expansion cohort (20 mg/kg). VX15/2503 was well tolerated. Treatment-related adverse events were primarily grade 1 or 2 and included nausea (14.3%) and fatigue (11.9%); arthralgia, decreased appetite, infusion-related reaction, and pyrexia were each 7.3%. One pancreatic cancer patient (15 mg/kg) experienced a Grade 3 dose-limiting toxicity; elevated  $\gamma$ -glutamyl transferase.

Complete cSEMA4D saturation was generally observed at serum antibody concentrations  $\geq 0.3$   $\mu\text{g/mL}$ , resulting in decreased cSEMA4D expression. Soluble SEMA4D levels increased with dose and infusion number. Neutralizing anti-VX15/2503 antibodies led to treatment discontinuation for 1 patient. VX15/2503  $C_{\text{max}}$  and AUC generally increased with dose and dose number. One patient (20 mg/kg) experienced a partial response, 19 patients (45.2%) exhibited SD for  $\geq 8$  weeks, and 8 (19%) had SD for  $\geq 16$  weeks. Subjects with elevated B/T lymphocytes exhibited longer progression-free survival.

**Conclusions:** VX15/2503 was well tolerated and produced expected PD effects. The correlation between immune cell levels at baseline and progression-free survival is consistent with an immune-mediated mechanism of action. Future investigations will be in combination with immunomodulatory agents. *Clin Cancer Res*; 1–10. ©2015 AACR.

## Introduction

The tumor microenvironment (TME), comprised of interactions between proliferating neoplastic cells and stromal components, including endothelial cells, leukocytes, fibroblasts, and extracellular matrix (ECM) proteins, is critical for tumor growth (1, 2). The antitumorigenic effects of the immune system, promoted by infiltrating Th1 and cytotoxic T lymphocytes, is often

inhibited due to restricted entry of effectors into the TME (3). Recently developed immunotherapies that target regulatory pathways of these effectors have demonstrated durable clinical responses; examples include ipilimumab, an antibody targeting the CTLA-4/B7 pathway, and pembrolizumab and nivolumab, antibodies targeting the PD1/PDL1 pathway, which modulates T-cell activity (4). The combination of such immunotherapies or other immunomodulatory agents with a complementary therapy allowing increased infiltration of immune effectors into the TME may significantly increase response rates.

VX15/2503 is a humanized IgG4 mAb that binds specifically to semaphorin 4D (SEMA4D; CD100), which is widely expressed on leukocytes infiltrating into the tumor stroma as well as on many tumors, and regulates the inflammatory milieu within the TME (5, 6). Originally defined as an axonal-guidance factor, SEMA4D is a member of the semaphorin family of proteins that play important roles in physiologic processes affecting tumor progression, immune cell regulation, and vascular growth (5, 7, 8). Activation of PLEXIN-B1, the high-affinity SEMA4D receptor, induces tumor cell proliferation and migration, and activation and migration of endothelial cells (9–11). Stimulation of two other known SEMA4D receptors, PLEXIN B-2 (intermediate affinity; expressed on keratinocytes and other cells) and CD72 (low-affinity; expressed in lymphoid tissue), appears to be involved in

<sup>1</sup>South Texas Accelerated Research Therapeutics (START) Center for Cancer Care, San Antonio, Texas. <sup>2</sup>Cancer Treatment Centers of America, Goodyear, Arizona. <sup>3</sup>Vaccinex, Inc., Rochester, New York. <sup>4</sup>Virginia G. Piper Cancer Center at Scottsdale Healthcare/TGen, Scottsdale, Arizona.

**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Current address for Ramesh K. Ramanathan: Mayo Clinic, 13400 E Shea Blvd, Scottsdale, AZ; and current address for Glen J. Weiss and Lisa Blaydorn: Cancer Treatment Centers of America, 14200 W Celebrate Way, Goodyear, AZ.

**Corresponding Author:** John E. Leonard, Vaccinex, Inc., 1895 Mt Hope Ave, Rochester, NY 14620. Phone: 585-271-2700; Fax: 585-271-2765; E-mail: jleonard@vaccinex.com

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## Translational Relevance

Tumor growth depends on dynamic interactions within the tumor microenvironment (TME), and antitumorigenic immune activity is often inhibited due to restricted entry of effectors into the TME. SEMA4D is expressed by tumor cells and leukocytes infiltrating the tumor stroma, promotes tumor proliferation and metastasis, and regulates cellular adhesion and motility of cells of the immune and vascular systems. The humanized IgG4 anti-SEMA4D antibody (VX15/2503) was well tolerated in a first-in-human study enrolling advanced solid tumor patients, with 19% (8/42) of patients exhibiting stable disease for  $\geq 16$  weeks. Preclinical studies suggest that combining VX15/2503 with immune-modulating agents, such as CTLA4 or PD1/PDL1, may improve the clinical activity of immune checkpoint blockade inhibitors and other immune-enhancing therapies. Thus, combination therapy studies enrolling subjects with select tumor types are planned.

epithelial wound repair (12) and regulation of B-cell responses (13), respectively.

SEMA4D has both a cellular, membrane-bound form (cSEMA4D) and a biologically active soluble ligand (sSEMA4D) generated by cleavage of the cellular form (13). Immunohistochemical analysis of SEMA4D demonstrated that both the cellular and soluble forms are overexpressed on several tumor types, including breast, pancreatic, colon, ovarian, urogenital, and head, and neck (14), with overexpression correlating with poor prognosis in sarcomas and pancreatic cancer (6, 15, 16).

*In vitro*, VX15/2503 neutralized both cellular and soluble forms of SEMA4D and blocked its binding to its receptors. In murine tumor models, the murine anti-SEMA4D progenitor of VX15/2503, MAb 67-2, reduced the growth of syngeneic tumors, resulting in responses that were durable and promoted immunologic memory (17). Furthermore, these studies demonstrated that MAb 67-2 neutralized tumor-expressed SEMA4D, shifted the balance of M1 and M2 macrophage toward a proinflammatory, antitumorigenic M1 response, and increased the recruitment and activation of cytotoxic T lymphocytes into the tumor (17); the observed correlation between immune cell levels and progression-free survival (PFS) in the present is consistent with these immune-mediated mechanisms. Nonclinical toxicology studies of VX15/2503 performed in rats and cynomolgus macaques demonstrated that the antibody produced no toxicities of clinical consequence, was not immunosuppressive, and that its half-life increased with dose level (18, 19). The phase I study reported here is a first-in-human, open-label, multiple-dose, dose-escalation study that evaluated the safety, tolerability, PK, and PD of VX15/2503 in patients with refractory solid tumors.

## Materials and Methods

### Study drug

VX15/2503 is a humanized IgG4 mAb generated from the murine anti-SEMA4D antibody MAb 67-2 (18); it contains a hinge mutation to prevent *in vivo* Fab arm recombination (20). VX15/2503 was produced in culture using a proprietary CHO cell line constructed as previously described (18). The sequences of the inserted retrovector protein coding regions were verified by

analysis, and the production cells were tested according to Good Manufacturing Practice guidelines. The expressed antibody was purified using standard techniques and formulated at approximately 20 mg/mL in a sodium acetate buffer, pH 5.4. The vialled antibody was stable for  $>36$  months when stored at  $5 \pm 3^\circ\text{C}$  (18).

### Study design

This phase I study was a two-center, nonrandomized, open-label, multiple-dose, dose-escalation, and dose-expansion study of VX15/2503 in adult patients with advanced solid tumors, relapsed or refractory to standard treatment, for which no curative therapy was available. The primary objectives were to evaluate the safety and tolerability of VX15/2503 (including the MTD); secondary and exploratory objectives included assessments of immunogenicity, pharmacokinetics (PK), pharmacodynamics (PD), antitumor activity, and serum biomarker levels. VX15/2503 was administered intravenously, weekly (i.e., days 1, 8, 15, and 22) on a 28-day cycle. Seven dose levels were evaluated: 0.3, 1, 3, 6, 9, 15, and 20 mg/kg. The starting dose of 0.3 mg/kg was derived by determining the human equivalent dose from the rat (most sensitive species) no observed adverse effect level (100 mg/kg; ref. 18) and then applying a cumulative safety factor of 100. Dose escalation proceeded using a standard 3+3 scheme with the stipulation that patients in the enrolled population must have completed at least 1 cycle of treatment. The MTD was defined as the highest dose level with dose-limiting toxicities (DLT) in  $<33\%$  of the patients in the cohort. A 20 mg/kg expansion cohort was planned if no MTD was reached during dose escalation.

The study initially enrolled subjects under a sentinel dose strategy. The first 7 patients (all patients in cohort 1 and the first 3 in cohort 2) were treated with an initial dose on day 14 and followed for 14 days; the patients then advanced to weekly dosing on day 1. As no safety signals were observed, this regimen was eliminated by protocol amendment commencing with patient 8 (cohort 2).

Delays in patient treatment in cycle 1 due to toxicity were allowed at the discretion of the Investigator and Sponsor. The patient was allowed to continue treatment at the next lower dose level after the toxicity recovered to baseline or grade 1. No inpatient dose escalation was allowed.

Following completion of cycle 1, patients who did not experience a DLT, had stable disease and continued to meet all eligibility criteria could receive additional cycles of VX15/2503 at the same dose level, at the discretion of the Investigator and Sponsor. Additional cycles were repeated without interruption, except as necessary for hematologic or nonhematologic toxicity due to any reason (up to 14 days), or until disease progression or other unacceptable toxicity occurred.

Institutional Review Board approvals for the study protocol, amendments, and informed consent documents were obtained prior to study initiation; study procedures were conducted in accordance with the Declaration of Helsinki. The ClinicalTrials.gov identifier was NCT01313065.

### Inclusion criteria

Men and women  $\geq 18$  years old with histologically or cytologically confirmed advanced solid tumors, relapsed or refractory to standard treatment, and who demonstrated progressive disease prior to entry were eligible if they had the following: measurable disease as defined by RECIST 1.1: a life expectancy of  $\geq 3$  months

per Investigator assessment; an Eastern Cooperative Oncology Group (ECOG) performance status of  $\leq 2$ ; and adequate renal and hepatic function. Patients of reproductive potential must have been willing to use a medically acceptable method of contraception throughout the study period and for at least 4 weeks after the last dose of VX15/2503. Patients in the expansion cohort (20 mg/kg) additionally must have had one of the following characteristics: a diagnosis of a pancreatic neuroendocrine tumor or soft-tissue sarcoma, bone metastasis, or an advanced solid tumor with a T-cell count  $\geq 1,500$  cells/ $\mu\text{L}$  or a B-cell count  $\geq 250$  cells/ $\mu\text{L}$  at screening.

#### Exclusion criteria

Exclusion criteria included the following: received treatment with antineoplastic agents within 3 weeks of the start of therapy; received treatment with an investigational agent, hematopoietic growth factor support, or oral or parenteral corticosteroids at  $>10$  mg/day of prednisolone or equivalent within 4 weeks of the start of therapy; was on concurrent antineoplastic therapy with the exception of continuing luteinizing hormone-releasing hormone agonist/antagonist therapy for patients with castrate-resistant prostate cancer; required systemic immunosuppressive therapy; had untreated brain metastases, central nervous system tumor involvement, a previous diagnosis of autoimmune disease, an infection requiring parenteral antibiotic therapy or causing fever within 1 week of the start of therapy; a hepatitis B or C or human immunodeficiency virus (HIV) infection; clinically significant cardiac disease; sensitivity to VX15/2503 or the ingredients or excipients of VX15/2503; or other intercurrent illness or condition, including alcohol or drug abuse, which could impact the patient's compliance with or ability to complete the study. Women may not have been breastfeeding or pregnant and must have had a negative pregnancy test within 3 days of the start of therapy.

#### Safety assessments

Safety evaluations were performed throughout the study for all patients who received VX15/2503 (Safety Population). Evaluations included periodic physical examination as well as vital sign measurements, clinical laboratory testing [hematology, prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), serum chemistry, and complete urinalysis], and monitoring for adverse events. Cycle 1 assessments were generally performed at each weekly infusion, with adverse event monitoring also conducted between days 1 and 8; 12-lead electrocardiograms (ECG) were performed less frequently. Assessments conducted for cycle 2 and beyond were performed on the day of infusion.

The detection of human anti-VX15/2503 antibodies (HAHA) in sera was performed using a validated ELISA based on the method published by Bourdage and colleagues (21); assays were performed by Covance Laboratories. Blood samples were collected at predose on days 1 and 15 of all cycles (and at day -14 for the sentinel patients only), at end of treatment (EOT), and on days 8 and 28 during follow-up. High-titer responses were defined empirically as those serum samples requiring dilution of 1:100 or greater.

Samples for immunophenotypic analysis of T cells (total, helper, or cytotoxic), B cells, and natural killer (NK) cells were also collected on day 1 (predose, 4 hours after the start of the infusion, and 24 hours after the start of the infusion), days 8, 15, and 22 of cycle 1; predose on day 1 of subsequent cycles; the EOT; and follow-up days 15 and 28. Cell levels were measured by flow

cytometric analysis (Covance Laboratories) using Multitest TBNK reagents [Becton Dickinson (BD)].

#### Adverse events and definition of dose-limiting toxicity

All adverse events were recorded and designated a grade according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.03; events were classified as unrelated or possibly, probably, or definitely related to treatment. DLTs were defined as any  $\geq$  grade 3 hematologic, nonhematologic, or laboratory toxicity that was not definitely related to the underlying disease reported during cycle 1.

#### Pharmacokinetics

All patients who received at least one dose of VX15/2503 and who provided at least one end-of-infusion (EOI) and postinfusion blood sample were evaluated for PK. In cycle 1, for the first and last infusions (days 1 and 22, or days -14 and 22 for the sentinel patients only), samples were collected before dose and the following times after the start of infusion: 1 hour, EOI, and 4, 8, 24, 48, and 96 hours; samples were also collected at 168 and 240 hours for cohort 1 and 2 patients, respectively. For study days 8 and 15 in cycle 1 and dosing days in subsequent cycles, blood samples were collected at predose and EOI. Additional samples were to be collected at the EOT visit and on days 8, 15, and 28 post-EOT. A validated ELISA was used to measure VX15/2503 serum concentrations (cf Supplementary Materials and Methods).

The serum concentration-time data for VX15/2503 were analyzed by noncompartmental analysis using WinNonlin software (Pharsight Corporation). Standard descriptive statistics were used to summarize serum VX15/2503 concentration data; standard PK parameters ( $C_{\text{max}}$ ,  $C_L$ ,  $T_{\text{max}}$ , and effective half-life, etc.) were determined by cohort and time point. Analyses of AUC and  $C_{\text{max}}$  to assess dose proportionality were performed as previously described (22).

#### Pharmacodynamics and biomarkers

PD evaluations were performed on all patients who received at least one dose of VX15/2503 and who provided at least one postinfusion blood sample. Validated assessments included cSEMA4D saturation and expression levels on circulating T lymphocytes, and serum sSEMA4D levels, as described (18); refer also to Supplementary Materials and Methods. Blood samples were collected before dose and EOI on days 1 and 15 of all cycles (and at day -14 of cycle 1 for sentinel patients only) for cSEMA4D analysis and on the same schedule as PK sample collection (except for no collection on day 3 of cycle 1) for sSEMA4D analysis. Assessment of changes in tumor immunohistology was planned, but acquisition of primary tumor samples was voluntary and none of the enrolled subjects consented for tumor biopsy.

Validated assays to determine serum levels of the soluble growth factors VEGF, HGF, placental growth factor (PLGF), and soluble MET, and the tumor biomarkers chromogranin A (23), bone-specific alkaline phosphatase, and urine N-telopeptide (24) were performed by Covance Laboratories for samples from expansion cohort patients with pancreatic neuroendocrine tumors or bone metastases, respectively. Samples were collected at screening, and for cycle 1 and even-numbered cycles at predose on day 1 (and on day -14 of cycle 1 for the sentinel patients only) and the end of cycle (days 25 to 28), as well as at EOT, and on day 28 during follow-up.

## Evaluation of tumor response

CT or MRI was used to assess antitumor activity according to RECIST 1.1; assessments were performed at screening and at the end (days 25 to 28) of every even-numbered cycle during treatment, and at end of treatment. For patients with bone metastases,  $^{18}\text{F}$ -NaF or  $^{18}\text{F}$ -FDG PET scans were also performed.

Objective response rate (confirmed complete response, confirmed partial response) and PFS were determined, as was duration of stable disease, defined as the time between the date of first dose of study drug and the earliest of the date of assessment of disease progression, withdrawal from the study, death, or censoring.

The determination of the number of normalized leukocytes and the assessment of correlations with PFS are described in Supplementary Materials and Methods.

## Statistical analysis

Demographic, safety, PK, PD, efficacy, and analytical data were summarized using standard methods [i.e., *n*, mean, geometric mean, SE, SD, coefficient of variation (CV), median, minimum, maximum, 95% confidence interval (CI), and the 25th and 75th percentiles] for continuous variables. Categorical variables were summarized using frequency counts and percentages. Some analyses were performed by cohort and time point; patients in cohorts 7 and 8 were grouped together as both cohorts received 20 mg/kg VX15/2503. Duration of stable disease and PFS was summarized using Kaplan–Meier methods, including quartiles of duration and probability of maintenance of stable disease at selected time points.

## Results

### Patient demographics and baseline characteristics

A total of 42 patients with advanced refractory solid tumors were enrolled at two study sites from May 17, 2011, to October 10, 2013; the last study visit was on January 7, 2014. Thirty-four patients were enrolled during the dose-escalation phase (cohorts 1 to 7) and 8 during the expansion phase (cohort 8). All patients received at least 1 weekly infusion of the intended dose of VX15/2503: 0.3 mg/kg (*N* = 4, cohort 1), 1 mg/kg (*N* = 4, cohort 2), 3 mg/kg (*N* = 3, cohort 3), 6 mg/kg (*N* = 4, cohort 4), 9 mg/kg (*N* = 4, cohort 5), 15 mg/kg (*N* = 8, cohort 6), and 20 mg/kg (*N* = 15, cohort 7/8). Study discontinuation was primarily due to disease progression (69.0%). The longest duration on study, including follow-up, was 385 days (breast carcinoma; 15 mg/kg; cohort 6).

Patient demographics and baseline characteristics are summarized in Table 1 and were similar across cohorts. Most enrolled patients were white, female, and had an ECOG status of 1 or 2 at study entry. The most common primary tumor sites were colon (33.3%), breast (11.9%), and pancreas (11.9%); lung (11.9%), liver, and lymph node (7.1%) were the most common sites of metastatic disease. All patients had undergone prior antineoplastic therapy with a mean of 5.2 treatments (range, 1–20); most had prior radiotherapy (61.9%) and surgery (73.8%). The mean time since last treatment with antineoplastic agents was 3.0 months (range, 1–31 months).

### Safety and tolerability

VX15/2503 treatment was well tolerated with the highest dose administered being 20 mg/kg; no MTD was determined. No dose-related safety trends regarding incidence or severity were observed.

**Table 1.** Demographics and selected baseline characteristics

	Total (N = 42) <sup>a</sup>
Age (years)	
Mean (SD)	64.8 (11.69)
Median (minimum, maximum)	65.5 (34, 85)
Sex, n (%)	
Male	17 (40.5)
Female	25 (59.5)
Race, n (%)	
White	39 (92.9)
Black	0
Asian	0
American Indian or Alaska Native	1 (2.4)
Native Hawaiian or other Pacific Islander	0
Other	2 (4.8)
Primary tumor site, n (%)	
Colon	14 (33.3)
Breast	5 (11.9%)
Pancreas	5 (11.9)
Abdomen	3 (7.1)
Lung	3 (7.1)
Other <sup>b</sup>	12 (28.5)
Prior radiotherapy, n (%)	42 (100.0)
Prior antineoplastic therapy, n (%)	42 (100.0)
Number of prior regimens	
Mean (SD)	5.2 (3.29)
Median (minimum, maximum)	5.0 (1, 20)
Prior surgery, n (%)	31 (73.8)
ECOG performance status, n (%)	
0	12 (28.6)
1	29 (69.0)
2	1 (2.4)

<sup>a</sup>The safety population includes all patients who received one or more doses of VX15/2503.

<sup>b</sup>Includes the following primary sites: 2 patients each for thigh, uterus and/or cervix, and unknown, and 1 patient each with kidney and ureter, esophagus, prostate, ovarian, bile duct, or thyroid.

Twelve deaths, none treatment related, occurred during the study; 7 deaths were due to disease progression and 5 were due to unknown causes. Patients received a mean of 2.9 cycles (range, 1–14 cycles). The median duration of exposure was 73.4 days (range, 1–372 days) with a median number of doses and total dose administered of 8.0 doses (mean: 11; range, 1–54 doses) and 5,411.6 mg (mean: 9,987.7 mg; range, 44.0–64,800 mg), respectively.

Sixty-nine treatment-emergent adverse events (TEAE) were considered possibly, probably, or definitely related to VX15/2503; 11 of these reported by 6 patients (14.3%) were definitely related. As summarized in Table 2, 26 patients experienced treatment-related TEAEs, with the most common being nausea (6/42, 14.3%) and fatigue (5/42, 11.9%). Six patients (14.3%) experienced a TEAE that led to study discontinuation, with malignant neoplasm progression being the only event reported in >1 patient (2/42, 4.8%). All treatment-related events were grade 1 or 2 except a grade 3  $\gamma$ -glutamyltransferase (GGT) elevation which was the sole DLT; it was considered severe and possibly treatment related. This DLT was experienced at week three by a pancreatic cancer patient (cohort 6, 15 mg/kg) with progressive liver metastases. The patient presented with a grade 1 elevation at baseline; treatment was delayed at week 4 due to the DLT, and the patient was discontinued from the study in week 5 (progressive disease). This DLT resulted in enrollment of 5 additional patients into cohort 6; two discontinued in cycle 1 due to disease progression and no subsequent DLTs occurred in the

**Table 2.** Treatment-related adverse events in  $\geq 5\%$  of patients

Adverse event or DLT	Patients reporting, n (%)								Total N = 42
	Cohort 1 0.3 mg/kg n = 4	Cohort 2 1.0 mg/kg n = 4	Cohort 3 3.0 mg/kg n = 3	Cohort 4 6.0 mg/kg n = 4	Cohort 5 9.0 mg/kg n = 4	Cohort 6 15.0 mg/kg n = 8	Cohort 7/8 <sup>a</sup> 20.0 mg/kg n = 15		
Any treatment-emergent AE	4 (100.0)	4 (100.0)	3 (100.0)	4 (100.0)	4 (100.0)	8 (100.0)	15 (100.0)	42 (100.0)	
Any treatment-related AE	1 (25.0)	2 (50.0)	2 (67.0)	3 (75.0)	1 (25)	6 (75.0)	11 (73.3)	26 (61.9)	
Nausea	0	0	0	1 (25.0)	1 (25.0)	0	4 (26.7)	6 (14.3)	
Fatigue	1 (25.0)	0	0	1 (25.0)	1 (25.0)	0	2 (13.3)	5 (11.9)	
Arthralgia	0	0	0	0	1 (25.0)	2 (25.0)	0	3 (7.1)	
Decreased appetite	0	1 (25.0)	1 (33.3)	0	0	1 (12.5)	0	3 (7.1)	
Infusion-related reaction	0	1 (25.0)	0	0	0	1 (12.5)	1 (6.7)	3 (7.1)	
Pyrexia	0	0	0	1 (25.0)	0	0	2 (13.3)	3 (7.1)	
Myalgia	0	0	0	1 (25.0)	0	0	1 (6.7)	2 (4.8)	
Pruritus	0	0	0	1 (25.0)	0	1 (12.5)	0	2 (4.8)	
DLT: grade $\geq 3$ elevated $\gamma$ -glutamyltransferase <sup>b</sup>	0	0	0	0	0	1	0	1 (2.4)	

NOTE: The safety population includes all patients who received at least one dose of VX15/2503.

Abbreviation: AE, adverse event.

<sup>a</sup>Includes patients from dose-escalation cohort (cohort 7) as well as patients from the expansion cohort.

<sup>b</sup>This grade 3 event was a DLT. No other DLTs were reported. All other events were grade 1 or 2.

remaining 3 patients. Thus, dose escalation proceeded to cohort 7 (20 mg/kg).

A grade 2 infusion-related reaction was experienced by patient 202004 in cohort 2 (1.0 mg/kg) who exhibited increasing HAHA titers from postinfusion 1 through the fourth and final weekly infusion. Although this subject had received prior mAb therapy, no HAHA was evident at baseline. This patient was discontinued because the HAHA response was neutralizing, accelerating VX15/2503 clearance, and reducing antibody-mediated PD effects. A high titer response ( $>1:100$  dilution) was observed in 5 other patients (11.9%) and a low-titer response in 18 (42.9%); neutralizing antibodies effects were not observed in these patients. However, 1 of these 23 patients (cohort 6; 15 mg/kg) experienced transient infusion-related reactions during doses 2 and 3 that correlated temporally with the HAHA response. This subject received one additional VX15/2503 infusion at a reduced rate with no infusion-related reaction.

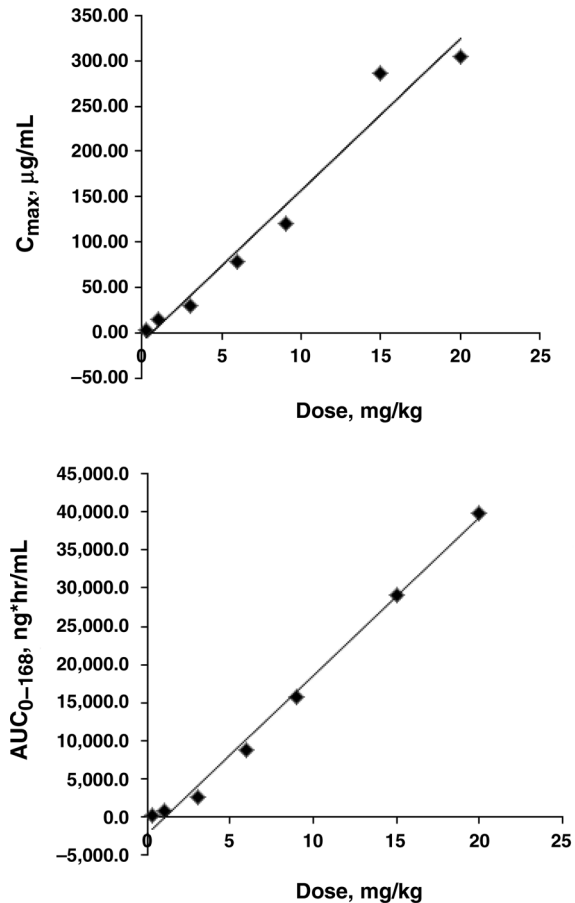
Flow cytometric analyses of lymphocyte levels and lymphocyte subsets, including T cells (total, helper, or cytotoxic), B and NK cells, showed no effects on these various populations following weekly administration of VX15/2503, regardless of dose level.

**Pharmacokinetics**

PK samples collected after the initial antibody dose for the first 7 patients covered the time period from 0 to 168 or 240 hours, whereas those for all other subjects were collected through 96 hours; day 22 samples for all patients treated with four weekly doses were collected through 96 hours following the fourth dose. Using these data the effective half-life ( $t_{1/2\text{ eff}}$ ) values for weekly doses of VX15/2503 administered to subjects at all dose levels ranged from 2.7 to 6.9 days;  $t_{1/2\text{ eff}}$  values at the three highest dose levels ranged between 4.3 and 5.2 days.

Following the first administration of VX15/2503, the  $C_{\text{max}}$  and total exposure ( $AUC_{0-168}$ ) ranged from 3  $\mu\text{g/mL}$  to 288  $\mu\text{g/mL}$  and 109  $\mu\text{g}\cdot\text{hr/mL}$  to 26,023  $\mu\text{g}\cdot\text{hr/mL}$  across escalating dose groups, respectively. Following 4 or 5 weekly doses of antibody, the  $C_{\text{max}}$  and  $AUC_{0-168}$  ranged from 3  $\mu\text{g/mL}$  to 411  $\mu\text{g/mL}$  and 131  $\mu\text{g}\cdot\text{hr/mL}$  to 38,351  $\mu\text{g}\cdot\text{hr/mL}$ , respectively. The  $C_{\text{max}}$  and AUC of VX15/2503 increased with dose level as shown in Fig. 1. Data analyses showed that increases in both  $C_{\text{max}}$  and AUC were slightly higher than dose proportional as the 95% CI values for the

slope terms were slightly greater than one (not shown). The median  $T_{\text{max}}$  values ranged from 2 to 4 hours across all dose levels. The accumulation ratio values ranged from 1.0 to 2.0.



**Figure 1.**

$C_{\text{max}}$  and AUC increase linearly with VX15/2503 dose level.  $C_{\text{max}}$  ( $\mu\text{g/mL}$ ) and AUC ( $\text{ng}\cdot\text{hr/mL}$ ) were plotted as a function of VX15/2503 dose level over the range of 0.3 to 20 mg/kg; a statistical analysis of dose proportionality was performed using the power model (22). The equation  $\ln(\text{parameter}) = a + b \cdot \ln(\text{dose}) + \text{error}$  was used to estimate the slope and corresponding 95% CI.

### Pharmacodynamics and clinical biomarkers

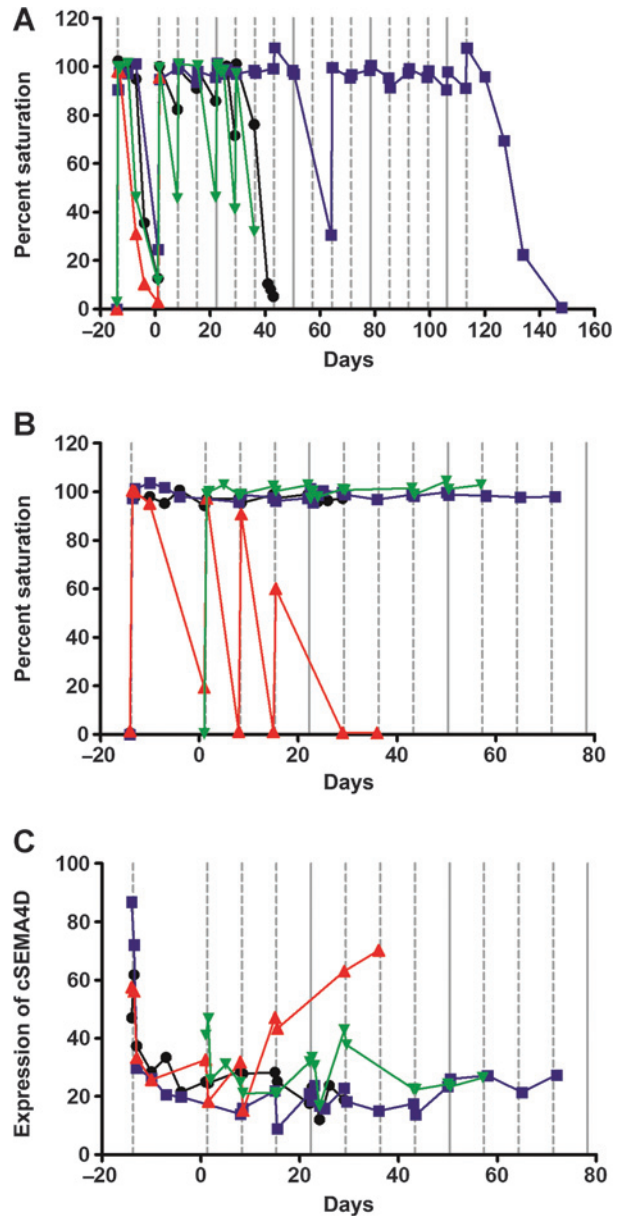
Expected PD effects associated with the binding of VX15/2503 to cellular and soluble SEMA4D were generally consistent with results from previous *in vitro* and *in vivo* studies (18). Weekly doses of VX15/2503 produced repeated transient cSEMA4D saturation for 2 of 4 patients in the 0.3 mg/kg cohort (Fig. 2A; subjects 101001 and 201002); the third subject (101002) exhibited continued maximal saturation. The fourth subject in this cohort (101003) received only two infusions before discontinuing (disease progression). Cellular SEMA4D saturation declined following cessation of treatment and antibody clearance from the periphery; saturation values returned to baseline when VX15/2503 serum concentrations fell below the saturation threshold of approximately  $\leq 0.3$   $\mu\text{g/mL}$  (cf Fig. 2A, subjects 101001 and 101002).

Figure 2B shows that administration of doses of  $\geq 1$  mg/kg VX15/2503 produced complete, sustained cSEMA4D saturation after infusion for patients 102004, 202003, and 102005; these cSEMA4D saturation data are representative of patients treated at higher VX15/2503 dose levels. However, patient 202004, who developed a neutralizing HAHA response (cf Safety and Tolerability discussion, above), exhibited declining cSEMA4D saturation values, attesting to the increasing immune response following each infusion.

Figure 2C shows that peripheral T-cell cSEMA4D expression declined within 24 hours after first infusion due to internalization of the antibody/receptor complex; data shown are for cohort 2 patients 102004, 202003, 202004, and 102005 treated with 1 mg/kg of VX15/2503. Results at higher VX15/2503 dose levels were similar. A 60% reduction of cSEMA4D expression was generally sustained during treatment intervals of up to 1 year (not shown). Cellular SEMA4D expression remained suppressed until VX15/2503 serum levels declined following cessation of dosing, allowing cSEMA4D levels to return to baseline (not shown). Similar results were observed following a neutralizing anti-VX15/2503 immune response; see cohort 2 patient 202004 (Fig. 2C) whose cSEMA4D levels rose to baseline.

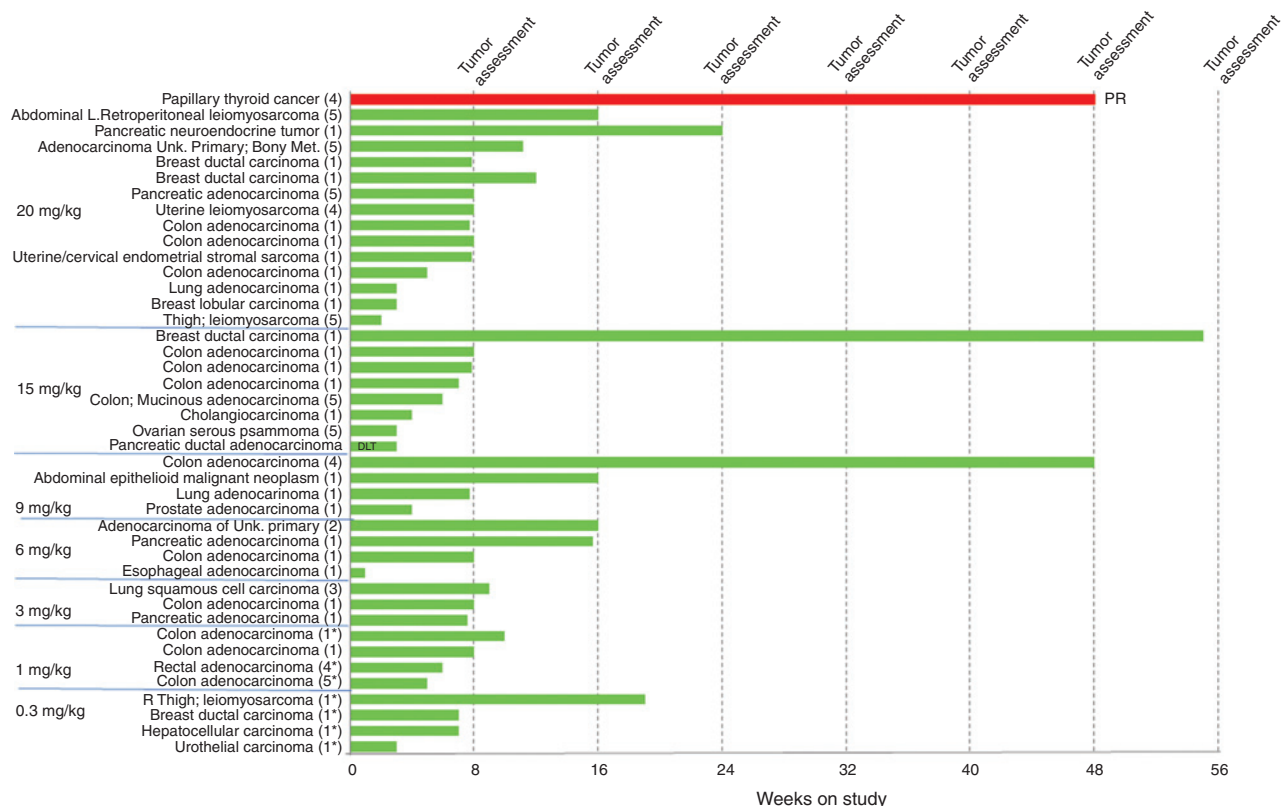
Levels of total sSEMA4D (free and antibody-complexed ligand) in sera increased with dose level and with infusion number at a given dose level, consistent with the expected increased half-life of the VX15/2503-soluble SEMA4D complex versus that of the free soluble receptor (cf 9.0 mg/kg patients; Supplementary Fig. S1). Levels appeared to reach steady state after 8 to 10 weekly doses (patients 105009 and 205011); patients dosed for shorter periods did not reach steady state. Total sSEMA4D levels declined after treatment cessation and antibody clearance (Supplementary Fig. S1), as illustrated by patients 105009, 205011, and 205012, who received their last dose on days 330, 170, and 50, respectively. These patients' SEMA4D levels were analyzed  $\geq 20$  days after EOT, and data show sSEMA4D levels approaching baseline for patient 105009. Similar results were obtained for other patients (not shown).

No pharmacologic effects of VX15/2503 administration were observed on the levels of serum VEGF, PLGF, MET, or HGF as their levels were unaffected by dose level or dose number (data not shown). Similarly, an absence of pharmacologic effects was noted for chromogranin A, bone-specific alkaline phosphatase, and urine N-telopeptide assessed for expansion cohort patients with pancreatic neuroendocrine tumors or bone metastases, respectively (not shown).



**Figure 2.**

T-cell-associated SEMA4D saturation and cellular expression and the impact of anti-VX15/2503 immune response. A, saturation analysis from cohort 1 (0.3 mg/kg). The percent saturation by VX15/2503 of cSEMA4D on peripheral blood CD3<sup>+</sup> T cells for subjects in the 0.3 mg/kg dose cohort was determined using a flow cytometric assay; values of  $\geq 20\%$  indicated saturation of cSEMA4D. The four subjects in this cohort all received a sentinel dose. The symbols represent: Patient No. 101001; Patient No. 101002; Patient No. 101003; Patient No. 201002. B, cSEMA4D saturation analysis from cohort 2 (1.0 mg/kg). The impact of a neutralizing anti-VX15/2503 immune response on T-cell SEMA4D saturation is illustrated by the data for cohort 2 patient 202004; all cohort 2 subjects except patient 102005 received a sentinel dose. The symbols represent: Patient No. 102004; Patient No. 202003; Patient No. 202004; Patient No. 102005. C, cellular SEMA4D expression analysis for cohort 2 (1.0 mg/kg). Expression of cSEMA4D on purified peripheral blood CD3<sup>+</sup> T cells was measured by flow cytometry for samples collected from patients in cohort 2. The symbols represent: Patient No. 102004; Patient No. 202003; Patient No. 202004; Patient No. 102005.



**Figure 3.** Duration of PFS for enrolled patients by dose cohort. Duration of time on study is plotted for each patient by dose of VX15/2503. The primary site and tumor type, as determined from the Cancer History listings, are provided for each patient. Study termination reasons are shown in parentheses; these were (1) disease progression; (2) patient withdrawal; (3) death; (4) physician decision; and (5) adverse event. Asterisks indicate a sentinel-dose patient. PFS data were taken from the Derived Efficacy Variables (Safety Population) listing, which lists duration of stable disease (in months). A value of 30.4 days per month was used to calculate PFS in weeks. Patients with 7.99-week duration were considered to have 8-week PFS. Two patients in cohort 6 [Patient 000106015 (ovarian carcinoma) and Patient 000206015 (colon carcinoma)] were excluded from the analysis because they were withdrawn from the study before the first postbaseline efficacy assessment. L, left; PR, partial response; R, right; Unk, unknown.

**Antitumor activity**

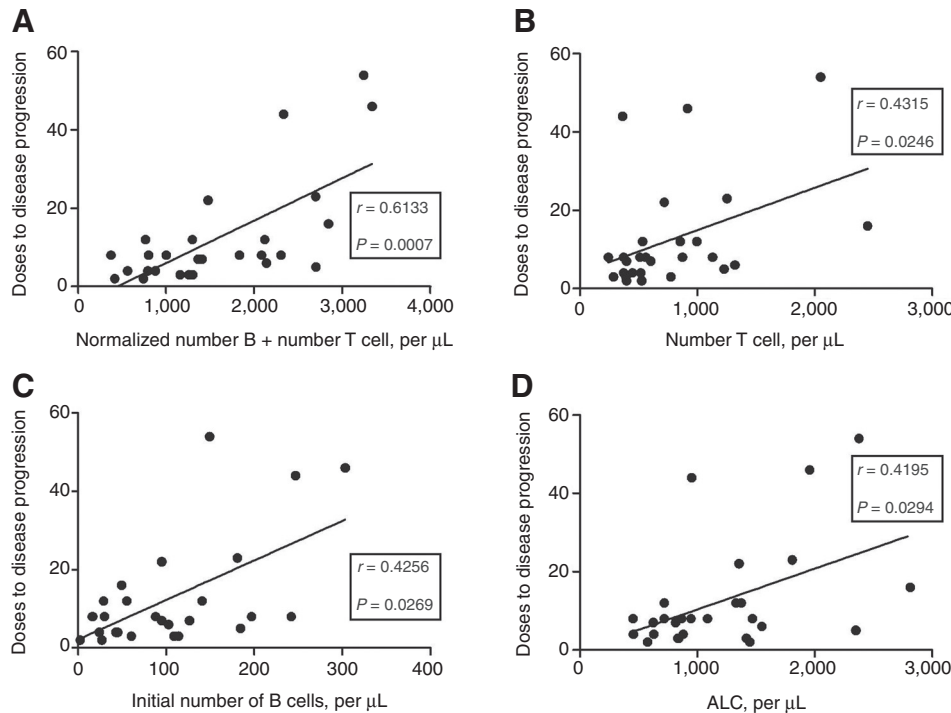
Patient PFS by cohort and by tumor type is shown in Fig. 3; tumor diagnosis and reasons for patient discontinuation are also shown. No complete responses were observed. The median duration of stable disease and the duration of PFS were 7.82 weeks (range, 0.57–54.8 weeks). One of 15 patients treated at 20 mg/kg (papillary thyroid cancer) experienced a partial clinical response of 334 days (47.7 weeks) that continued at study exit (physician decision; study day 337). A hilar lymph node in this subject decreased from 1.5 cm to 0.8 cm, representing a 53.3% reduction. This patient had received eight different prior therapies before entering this study. The longest period between prior treatment regimens was 37 months (between regimens one and two).

Nineteen patients (45.2%) exhibited no evidence of disease progression for at least 8 weeks (Fig. 3). Of these, 13 patients (31%) exhibited no evidence of disease progression for between 8 and 16 weeks, and 6 patients (14.3%), 5 of whom were in the ≥6 mg/kg cohorts, also exhibited no evidence of disease progression for ≥16 weeks. The longest durations of stable disease were observed for patients with colorectal (9 mg/kg; 48 weeks) and breast cancer (15 mg/kg; 55 weeks). These patients had received, respectively, three and six prior therapies; their respective longest prior periods of stable disease were 37 and 28 months.

Although assessed lymphocyte levels remained generally unchanged from baseline during treatment with VX15/2503, the normalized baseline number of B cells plus T cells for patients in the 9.0 to 20 mg/kg cohorts exhibited a significant correlation (Spearman rank coefficient  $r_s = 0.6133$ ;  $P \leq 0.001$ ) with their PFS duration (Fig. 4). Weaker but significant ( $P \leq 0.03$ ) correlations were observed for baseline values of absolute lymphocytes ( $r_s = 0.4195$ ), T cells ( $r_s = 0.4315$ ), and B cells ( $r_s = 0.4256$ ). Three patients with PFS of 48 to 55 weeks and 4 patients who exhibited the absence of disease progression for at least 16 weeks all had relatively high T- or B cells at baseline (T cells  $\geq 1,200/\mu\text{L}$ ; B cells  $\geq 250/\mu\text{L}$ ); these subjects were treated with VX15/2503 doses of 6 mg/kg or higher (1 patient in cohort 1 with SD of >18 weeks exhibited baseline lymphocyte levels below these values).

**Discussion**

VX15/2503 was well tolerated in advanced solid tumor patients when administered as a weekly i.v. infusion. The highest planned dose of 20 mg/kg was employed and no MTD was determined. No dose-related safety trends regarding adverse event incidence or severity were observed. The most common (>10% incidence) treatment-related adverse events were nausea and fatigue. All

**Figure 4.**

Spearman rank-order correlation between the normalized number of B cells plus T cells and PFS. The number of T cells and B cells at baseline was measured by flow cytometry, and absolute lymphocyte counts were determined by standard techniques. A, PFS (determined as the number of weekly VX15/2503 doses administered until disease progression) is plotted versus the number of normalized B cells plus T cells for subjects in the 9, 15, and 20 mg/kg dose cohorts ( $N = 27$ ). Panels B, C, and D show, respectively, PFS versus the number of T lymphocytes, B lymphocytes, or absolute lymphocyte counts at baseline. The respective Spearman rank-order correlation coefficients ( $r_s$ ) and  $P$  values for each analysis are provided.

treatment-related adverse events were grade 1 or 2 except the sole reported DLT, a grade 3 GGT elevation experienced in a 15 mg/kg cohort patient with progressive liver metastases who had elevated GGT levels at baseline. After further review, the DLT was considered only temporally related with VX15/2503 administration and more likely caused by underlying disease.

Twenty-three of the patients developed anti-VX15/2503 antibody responses, with a response titer  $>100$  detected in 6 patients (14.3%). Two patients [one with a high-titer response (cohort 2) and one with a low-titer response (cohort 5)] developed grade 2 infusion-related reactions that were temporally related with the HAHA response. Only the cohort 2 subject produced a neutralizing infusion reaction, which not only cleared administered antibody from the periphery but also neutralized VX15/2503-mediated effects on cSEMA4D saturation and expression, and reduced sSEMA4D levels. These findings resulted in treatment termination and study discontinuation for this patient.

Pharmacologic effects were similar to those observed in primates treated at similar VX15/2503 dose levels (18). Lymphocyte levels generally remained unchanged from baseline, regardless of antibody dose level or number. The threshold for cSEMA4D saturation of human T cells was estimated to be approximately 0.3  $\mu\text{g/mL}$ , similar to that determined for primate T cells (0.5  $\mu\text{g/mL}$ ; ref. 18). Furthermore, the expression of cSEMA4D on T lymphocytes declined for all patients during treatment; similar receptor internalization has been reported for CD20 on B lymphocytes (25). Cellular SEMA4D levels in fresh tumor specimens were not assessed as no patients consented to biopsies.

Levels of total sSEMA4D present in the sera of treated patients increased with dose level and with infusion number (Supplementary Fig. S1), consistent with the increased half-life of the VX15/2503-sSEMA4D complex versus that of the soluble ligand. Similar results were reported for secreted VEGF (26). Although

sSEMA4D levels declined following treatment cessation, study discontinuation after EOT generally precluded following patients' sSEMA4D levels return to baseline. Data from cohort 1 or 2 patients did show normalization of sSEMA4D levels, however (not shown). Finally, no serum biomarkers were identified as the levels of these proteins remained unchanged with VX15/2503 treatment.

This study was not designed to collect extensive PK data; nonetheless sufficient samples allowing for complete PK analyses were obtained from the first 7 patients (0.3 and 1.0 mg/kg dose cohorts), and PK parameters were estimated for the remaining patients using data derived from the limited samples available. Thus, the effective half-life estimates of 2.7 to 6.9 days for the dose range of 3 to 20 mg/kg VX15/2503 may not reflect the true half-life because these values were determined with samples collected over the more limited time period of 0 to 96 hours. The  $C_{\text{max}}$  and  $\text{AUC}_{0-168}$  values, determined following multiple weekly doses of VX15/2503, were reliably determined and exhibited a slightly greater than dose proportional increase with increasing dose level. Finally, little or no accumulation occurred after multiple weekly doses of VX15/2503 because of the low accumulation ratio values.

Exploratory evaluations of efficacy included antitumor activity and PFS. Nineteen patients (45.2%) exhibited no evidence of disease progression for at least 8 weeks and 8 (19%) showed a similar absence of disease progression for at least 16 weeks. One patient with papillary thyroid cancer (20 mg/kg cohort) achieved a partial response of 47.7 weeks that was ongoing at study exit. This patient along with the 2 other patients with the longest PFS (48–55 weeks; all in the 9 to 20 mg/kg cohorts) and 4 patients who had stable disease for  $\geq 16$  weeks (all in the 6 mg/kg cohorts and above) all had relatively high T- or B cells at baseline.

The observed strong correlation between the normalized baseline number of B cells plus T cells and PFS for patients in the 9.0 to

20 mg/kg cohorts suggested that these heavily pretreated patients entered the study with a more robust immune potential despite their prior therapy. Nevertheless, this elevated immune potential was not of itself sufficient to inhibit tumor growth as all patients had tumors that were progressing at the time they enrolled in the trial. Tumor stabilization and extended PFS in these patients were only induced following treatment with VX15/2503. This is consistent with preclinical studies in which striking immune-mediated antitumor effects were observed in tumor-bearing mice treated with the murine anti-SEMA4D antibody MAb 67-2 alone or in combination with checkpoint inhibitors (17). These preclinical studies demonstrated that neutralization by anti-SEMA4D antibody of SEMA4D residing at the tumor margin allowed enhanced trafficking of tumoricidal macrophage and activated CD8<sup>+</sup> lymphocytes into the TME. The neutralization of SEMA4D thus facilitated the infiltration of immune cells into the TME, thereby engendering an effective antitumor response. Although patients in the present study did not volunteer fresh biopsies to permit examination of SEMA4D expression and immune cell distribution in tumor, a recently published report characterized a similar gradient of SEMA4D expression in human colorectal carcinoma (27).

The findings from this first-in-human study support the further investigation of VX15/2503 for the treatment of advanced refractory solid tumors, as it was well tolerated at all doses evaluated, demonstrated expected PD effects, and 45% of patients exhibited the absence of disease progression for at least 8 weeks. Targeting SEMA4D represents a novel therapeutic strategy to promote immune infiltration into tumor that is complementary to the mechanism of actions of other immunomodulatory therapies. Administration of VX15/2503 in combination with immune-enhancing therapies such as checkpoint blockade inhibitors or vaccines that enhance overall immune response but do not affect ability to penetrate tumor may result in increased efficacy. Future studies in selected tumor types will utilize VX15/2503 in combination with other immunomodulatory agents.

### Disclosure of Potential Conflicts of Interest

G.J. Weiss reports receiving speakers bureau honoraria from Pfizer and is a consultant/advisory board member for Amgen and Pharmatech. J.E. Leonard,

T.L. Fisher, and M. Zauderer have ownership interest (including patents) in Vaccinex, Inc. A. W. Tolcher is a consultant/advisory board member for Abbvie, Akebia, AP Pharma, ArQule, Asana, Astex, Avid, BayerHealthcare, Bind, BioMed Valley Discoveries, Blend, Bristol-Myers Squibb, Celator, Clovis, Curis, Dicerna, Eisai, Endo, Genentech, Heron, Janssen, Lilly, MedImmune, Mersana, Merus, Nanobiotix, Nektar, Neumedicines, Novartis, Pfizer, Phamacyclics, Pierre Fabre, Sanofi-Aventis, Symphogen, Vaccinez, Valent, and Zynigenz. No potential conflicts of interest were disclosed by the other authors.

### Authors' Contributions

**Conception and design:** J.E. Leonard, T.L. Fisher, A.W. Tolcher, M. Zauderer  
**Development of methodology:** A. Patnaik, J.E. Leonard, J.C. Sachdev, T.L. Fisher, L.A. Winter

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** A. Patnaik, G.J. Weiss, D.W. Rasco, J.C. Sachdev, C. Reilly, D. Mutz, A.W. Tolcher, R.K. Ramanathan

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** A. Patnaik, J.E. Leonard, T.L. Fisher, L.A. Winter, R.B. Parker, R.K. Ramanathan

**Writing, review, and/or revision of the manuscript:** A. Patnaik, G.J. Weiss, J.E. Leonard, D.W. Rasco, J.C. Sachdev, T.L. Fisher, L.A. Winter, A.W. Tolcher, M. Zauderer, R.K. Ramanathan

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** J.E. Leonard, T.L. Fisher, C. Reilly

**Study supervision:** J.E. Leonard, J.C. Sachdev, T.L. Fisher, D. Mutz, A.W. Tolcher, M. Zauderer, R.K. Ramanathan

**Other (study research clinical coordinator):** L. Blydorn

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