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


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.

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.

Note: See text for additional information.

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 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-B2 (intermediate affinity; expressed on keratinocytes and other cells) and CD72 (low-affinity; expressed in lymphoid tissue), appears to be involved in...
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 ≥16 weeks. Preclinical studies suggest that combining VX15/2503 with immune-modulating agents, such as CTLA4 or PD1/PD-L1, 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.

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 vial antibody was stable for >36 months when stored at 5 ± 3 °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 intrapatient 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 ≥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 ≥3 months...
per Investigator assessment; an Eastern Cooperative Oncology Group (ECOG) performance status of ≤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 >1,500 cells/µL or a B-cell count ≥250 cells/µL 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.

PD evaluations were performed on 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 (Cmax, CL, tmax, and effective half-life, etc.) were determined by cohort and time point. Analyses of AUC and Cmax 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, 18F-NaF or 18F-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 week’s washout 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). 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 median 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.

Studies 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.

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 median 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.

Studies 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.

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 median 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.

Studies 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.
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 without further therapy. Because the HAHA response was neutralizing, accelerating VX15/2503 clearance, and reducing antibody-mediated PD effects. A grade 3 event was a DLT. No other DLTs were reported. All other events were grade 1 or 2.

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 were collected through 96 hours following the fourth dose. The accumulation ratio values ranged from 1.0 to 2.0.

Figure 1.

Cmax and AUC increase linearly with VX15/2503 dose level. Cmax (µg/mL) and AUC (ng·h/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

$$\ln(\text{parameter}) = a + b \times \ln(\text{dose}) + \text{error}$$

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 ≤0.3 µg/mL (cf Fig. 2A, subjects 101001 and 101002).

Figure 2B shows that administration of doses of ≥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 Tolerance 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 SEMA4D 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 ≥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).
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 to 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.

Although assessed lymphocyte levels remained generally unchanged from baseline during treatment with VX15/2503, no dose-related safety trends regarding adverse event incidence were observed except for nausea and fatigue. All adverse events were grade 1–2, except for one grade 3 nausea in cohort 6 (Patient 000106015; colon adenocarcinoma) who was withdrawn from the study because they were withdrawn from the study before the first postbaseline efficacy assessment.

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
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 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 5 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 µg/mL, similar to that determined for primate T cells (0.5 µg/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).

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 Cmax and AUCO–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 (>21 weeks; all in the 9, 15, and 20 mg/kg dose cohorts (N = 27). Panels A, 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) and P values for each analysis are provided.
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+ 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 tumor solids, 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-Meyers Squibb, Celator, Clovis, Cisnera, Essai, Endo, Genentech, Heron, Janssen, Lilly, MedImmune, Mersana, Merus, Nanobiotix, Nektar, Neumedicines, Novartis, Pfizer, Pharmacies, Pierre Fabre, Sanofi-Aventis, Symphogen, Vaccinez, Valent, and Zengenix. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions


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


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


Other (study research clinical coordinator): L. Blaydowen

Acknowledgments

The authors thank Dr. Gil Price, MD, for clinical support, Jennifer Seils for technical support, PPD, Inc., for data management, and Julie Deardorff, Ph.D, for assistance in writing this article. They also thank the research staff at South Texas Accelerated Research Therapeutics (START) Center for Cancer Care, San Antonio, TX, and The Virginia G. Piper Cancer Center at Scottsdale Healthcare/TGen, Scottsdale, AZ and the patients who participated in this study and made possible the clinical evaluation of VX15/2503.

Grant Support

This phase I study was funded solely by Vaccinex, Inc., resources. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 21, 2015; revised September 21, 2015; accepted September 22, 2015, published OnlineFirst October 7, 2015.

References


10. Campos M, Campos SG, Ribeiro GG, Eguchi FC, Da Silva SR, De Oliveira CZ, et al. Ki-67 and CD100 immunohistochemical expression is associated...
with local recurrence and poor prognosis in soft tissue sarcomas, respectively. Oncol Lett 2013;5:1527–35.
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, Glen J. Weiss, John E. Leonard, et al.


Updated version
Access the most recent version of this article at:

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2016/02/16/1078-0432.CCR-15-0431.DC1

Cited articles
This article cites 27 articles, 9 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/22/4/827.full.html#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.