Nonclinical Safety Assessment of VX15/2503 – A Humanized IgG4 Monoclonal Antibody to SEMA4D

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Abstract (Number 4578)

VX15/2503 is a humanized IgG4 monoclonal antibody that binds with approximately 3 nM affinity to Semaphorin 4D (SEMA4D, CD100). SEMA4D is an important mediator of axonal growth cone guidance, vascular development, angiogenesis, and T cell and B cell activation. SEMA4D is found predominantly as a 150 kDa transmembrane protein on lymphocytes and as a 240 kDa, homodimeric, soluble form of the protein. VX15/2503 or its murine progenitor (MAb 67-2) suppressed tumor growth and angiogenesis in syngeneic, xenograft and transgenic tumor models. Toxicology and pharmacology studies of VX15/2503 were performed in Sprague-Dawley rats and cynomolgus macaques using single or five weekly intravenous injections. Toxicology profiles were similar for both species in both studies. Single dose studies employed doses of between 0.01 and 100 mg/kg; repeat dose studies evaluated 10, 30 and 100 mg/kg. No adverse histopathologic or clinical effects were noted in either species injected with doses up to 100 mg/kg. Appetence, body weights, serum chemistry and ophthalmologic factors were also unaffected, as were primate ECG results. Thus the NOAEL was established at 100 mg/kg for both species. Partial to complete T cell associated SEMA4D saturation was observed in all animals across the dose ranges evaluated and SEMA4D saturation was dose dependent. Single dose animals that reached a VX15/2503 serum concentration of ≥2 µg/mL exhibited transient complete T cell saturation. Prolonged saturation occurred at the 100 mg/kg dose level. Repeat dose animals in the 100 mg/kg dose group were on average at least 20% saturated for 134 and 169 days in rats and cynomolgus macaques, respectively. Single dose VX15/2503 half-life values increased with dose and varied from 27 to 246 hours in rats; similar results were obtained from the primate single dose study. Repeat dose C_{max} and AUC (exposure) values were higher in both species than those from the single dose studies and steady-state was achieved after the fourth dose. Anti-VX15/2503 responses were detected in the sera of most animals in both studies. Human tissue arrays incubated with VX15/2503 showed diverse distribution of SEMA4D on resident or itinerant lymphocytes in lymphoid tissues as well as in sections of brain, lung and endometrium. Based on these results VX15/2503 was selected for clinical development in oncology.

Introduction

- SEMA4D
  - Binds PLXNB1 with 1 nM affinity and CD72 with 300 nM affinity
  - Exists in both cellular and soluble forms
  - Is expressed abundantly on the surface of resting T cells and less strongly on B cells and APCs; it is upregulated upon cellular activation
  - Activates B lymphocytes and induces dendritic cell maturation for antigen presentation to T lymphocytes
  - Binding to PLXNB1 transactivates MET promoting angiogenesis and stimulating invasive growth of tumors
  - Is overexpressed in a variety of human tumors including head and neck, prostate, colon, and lung
- Use of shRNA to knockdown the expression of SEMA4D reduced tumor growth and vascularization in mice
- Therapeutic Rationale for anti-SEMA4D Antibody: Neutralization of SEMA4D using a monoclonal antibody could inhibit tumor growth and invasion
- VX15/2503 binds with 3 to 5 nM affinity to cellular and soluble SEMA4D and was selected for clinical development to treat patients with advanced solid malignancies
- Additional research characterization data for SEMA4D and VX15/2503 are presented as part of abstract #3667

Species Selection

- Biacore analyses demonstrated that VX15/2503 exhibits 3 to 5 nM affinity for mouse, rat, marmoset, cynomolgus macaque, and human SEMA4D
- In vitro, VX15/2503 blocked functional binding of SEMA4D to PLXNB1 expressed on human and mouse cells
- Immunohistochemical analyses demonstrated reactivity of VX15/2503 with lymphocytes present in human, cynomolgus macaque, and rat lymphoid tissues
- A flow cytometric assay demonstrated dose dependent VX15/2503 binding to T cell associated SEMA4D on lymphocytes from rat, cynomolgus macaque, and human
- VX15/2503 binds with 3 to 5 nM affinity to soluble SEMA4D present in the sera of rats, cynomolgus macaques, and humans
- Normal healthy Sprague-Dawley rats and purpose bred cynomolgus macaques of Chinese origin were used in nonclinical safety evaluation studies.

Tissue Cross Reactivity

- A total of 36 human tissues were assessed for VX15/2503 reactivity (a subset is shown below)
- Human and cynomolgus macaque tissue reactivity profiles were similar
- Residential or infiltrating lymphocytes were reactive in many tissues, as expected
- Spleen was the only rat tissue reactive with VX15/2503

Species	Brain	Gut	Heart	Kidney	Liver
Human	-	+	-	-	-
Cynomolgus Macaque	-	+	-	-	-
Species	**Skin**	**Spleen**	**Tonsil**	**Endometrium**	**Blood Cells**
Human	-	+	+	+	+
Cynomolgus Macaque	-	+	+	+	+

Toxicology Study Designs

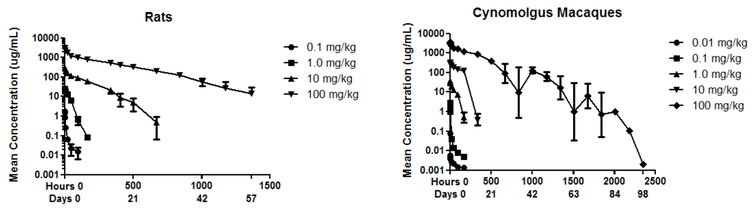
- Appropriate acclimation and quarantine periods preceded each study
- VX15/2503 was manufactured identically to cGMP processes except for the scale of production
- VX15/2503 is formulated at 20 mg/mL in 20 mM sodium acetate buffer, pH 5.4, containing 130 mM NaCl and 0.02% polysorbate 80
- Single dose cynomolgus macaque, and rat and primate repeat dose study analyses were GLP compliant

Parameter	Single Dose Rat	Single Dose Primate	Repeat Dose Rat	Repeat Dose Primate
No./sex/group	3	2 to 4	10 to 20 (main) 9 (satellite)	3 to 7
Dose Levels (mg/kg/dose)	0, 0.01, 0.1, 1.0, 10, 100	0, 0.01, 0.1, 1.0, 10, 100	0, 10, 30, 100	0, 10, 30, 100
PK/PD/Immunogenicity	All	All	Satellite	All
Clinical Observations	Obs Daily; BW, Weekly	Obs Daily; BW/Food Consumption, Weekly	Obs Daily; BW/Food Consumption, Weekly	Obs Daily; BW/Food Consumption, Weekly
Clinical Pathology	Serum Chemistry	Serum Chemistry Hematology, Coagulation, Urinalysis	Serum Chemistry, Hematology, Coagulation, Urinalysis	Serum Chemistry, Hematology, Coagulation, Urinalysis
Safety Pharmacology	--	ECG, Vitals	--	ECG, Vitals
Immunophenotype Analyses	--	IPT	IPT	IPT
Histopathology	--	All Groups	Control, High Dose	All Groups

Single and Repeat Dose Pharmacokinetics

Single Dose PK Parameters

- Antibody serum concentrations of VX15/2503 were analyzed after a single IV injection of the antibody
- PK parameters for rat and cynomolgus macaque were similar (see below)
- Serum antibody concentrations determined using a sandwich ELISA utilizing recombinant (marmoset) soluble SEMA4D as the capture protein.



Dose (mg/kg)	Rat C_{max} (µg/mL)	Rat AUC_{0-t} (µg•hr/mL)	Rat Half-life (days)	Cynomolgus Macaque C_{max} (µg/mL)	Cynomolgus Macaque AUC_{0-t} (µg•hr/mL)	Cynomolgus Macaque Half-life (days)
0.01	NC	NC	NC	0.11	0.36	1.8
0.1	1.99	11.40	0.1	3.36	23.09	0.2
1.0	34.14	834.7	1.2	39.35	1,974	1.9
10	317.7	27,431	4.4	327.1	38,741	3.9
100	3,305	488,194	10.3	3,681	477,154	10.0

Repeat Dose PK Parameters

- Five weekly doses of 100 mg/kg VX15/2503 produced similar high antibody exposure values in rats and primates

Dose (mg/kg)	Rat C_{max} (µg/mL)	Rat AUC_{0-t} (µg•hr/mL)	Rat Half-life (days)	Cynomolgus Macaque C_{max} (µg/mL)	Cynomolgus Macaque AUC_{0-t} (µg•hr/mL)	Cynomolgus Macaque Half-life (days)
10	226	23,653	NC	292	5,764	NC
30	1,647	350,738	11.6	1,767	54,774	NC
100	7,115	1,345,350	11.7	7,697	1,541,228	13.6

NC – Not Calculated

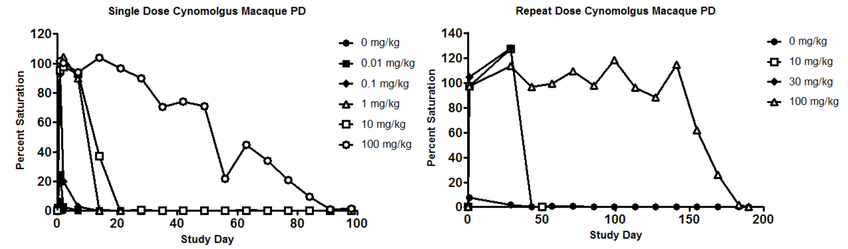
Immunogenicity

- Anti-VX15/2503 antibody levels were determined by a modified bridging (ACE-ELISA) assay using mild acid dissociation to increase drug tolerance
- Anti-VX15/2503 antibodies were typically detected within one to two weeks after injection
- Anti-VX15/2503 antibodies exerted only marginal effects on total exposure in low dose animals only

	ADA Frequency per Dose Group (mg/kg)					
Species	**0.01**	**0.1**	**1**	**10**	**30**	**100**
Rats	4/6	5/6	6/6	24/24	14/18	11/24
Primates	2/4	4/4	4/4	14/14	6/6	14/14

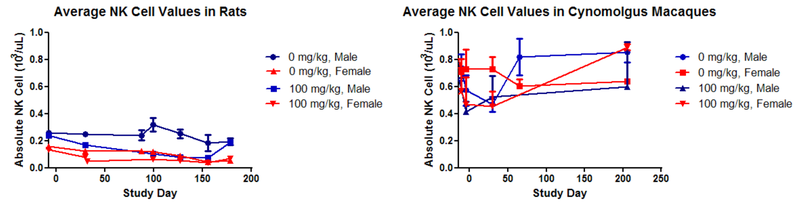
Pharmacodynamics

- Animals were monitored for primary PD using a flow cytometric assay measuring the percentage of T cell associated SEMA4D (cSEMA4D) occupied by VX15/2503
- PD profiles were similar for rats and cynomolgus macaques
- cSEMA4D in rats and cynomolgus macaques administered 100 mg/kg VX15/2503 was saturated for a total of 120 and 141 days, respectively
- As expected with secreted antigens, sSEMA4D increased in cynomolgus macaques dosed with at least 0.1 mg/kg of VX15/2503



Clinical Observations and Pathology

- No adverse effects were noted in any study; NOAEL = 100 mg/kg (highest dose tested)
- Sporadic changes in serum globulin ratios detected in both repeat dose studies; these changes were attributed to the high serum antibody levels
- All treated rats in the repeat dose study showed a statistically significant (40 to 70%) decline in circulating NK cells (below); a similar statistically significant finding was not observed in primates
- NK cell levels in treated rats were similar to controls by the end of the recovery period; finding not considered adverse due to absence of clinical correlates
- No test article related changes noted in clinical observations, physical or eye exams, bodyweights, ECG, vitals, other clinical pathology parameters, organ weights or histopathology
- Clinical pathology evaluations of study animals were performed using standard equipment and practices



Summary

- VX15/2503 binds with high affinity to rat, cynomolgus macaque and human SEMA4D
- VX15/2503 was safe when administered to rats and cynomolgus macaques as five weekly IV doses up to 100 mg/kg/dose
- A transient decline in absolute and relative NK cell values in rats after repeat doses of VX15/2503 was the only effect demonstrated; this observation not observed with primates and was not considered adverse due to absence of other clinical findings
- PK/PD profiles were dose dependent and similar between rats and primates; animals administered 5 weekly doses of VX15/2503 exhibited prolonged T cell SEMA4D saturation
- These studies supported the initiation of a phase 1 clinical study to evaluate the safety and tolerability of VX15/2503 in patients with advanced solid tumors
- The animal studies described here also support completion of chronic toxicology studies in rats