**Supplemental Methods for:**

**Nonclinical Safety Evaluation of VX15/2503; a Humanized IgG4 Anti-SEMA4D Antibody**

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**Supplemental Methods**

*SEMA4D Affinity Determinations*

Goat anti-Mouse IgG Fc (for MAb 67-2) or Goat anti-human IgG Fc (for VX15/2503) (Jackson ImmunoResearch) was immobilized on the chip surface by way of amine coupling. MAb 67-2 or VX15/2503 was captured and recombinant antigen was injected in a concentration range from 50 to 0 nM; samples were assayed in duplicate and the dataset was evaluated using BiaEvaluation software and globally fit to a 1:1 model. Sensorgrams were subjected to reference surface subtraction and subtraction of buffer response (zero analyte concentration).

## Animal Toxicology Studies

The single dose rat study employed three animals/sex/group; a similar single-dose study using cynomolgus macaques contained four/sex for control and two/sex for each test article dose group. . Rats were approximately 12 weeks of age at study initiation and weighed between 300 to 350 g. Cynomolgus macaques were three years old at study initiation and generally weighed between 2.4 and 3.8 kg each.

On all other non-dose days, animals were monitored once each day; animals were also closely examined during weekly bodyweight evaluations and prior to sacrifice for changes in food consumption and for changes in hematology (including platelet levels for macaques), clinical chemistry, coagulation (macaques) and urinalysis parameters.

Animals in the 0.01 through 10 mg/kg dose groups were sacrificed along with a cohort of control animals when animals dosed with 10 mg/kg no longer demonstrated any T cell SEMA4D saturation by VX15/2503. Once the 100 mg/kg group animals no longer exhibited cSEMA4D saturation, they too were sacrificed along with remaining control animals. Standard tissues were collected and stored in 10% neutral buffered formalin (NBF) prior to being processed for histopathologic examination. Eyes and testes were collected and stored in Davidson’s fixative in lieu of 10% NBF. Selected organs were weighed prior to fixation.

A GLP maximum feasible dose (MFD) study was also conducted using cynomolgus macaques. Previously described observations and study assessments were performed on these animals. At sacrifice an extensive panel of tissues was collected for analysis by histopathology. The maximum dose employed was determined by the antibody concentration in the existing clinical formulation as well as the maximum infusion volume for macaques.

One month toxicology studies followed the single dose studies and evaluated toxicity of five weekly doses of VX15/2503 to determine toxicity, PK, PD, and immunogenicity of the antibody in this repeat dose regimen. Twelve week old rats were split between a main toxicity subgroup and a subgroup utilized for PK/PD assessments. The main study group was comprised of 20 rats per sex in the control group, 15 per sex in the low and high dose group, and 10 per sex in the mid dose group. Satellite subgroups all contained 9 per sex per group. Cynomolgus macaques 3 to 4 years of age were divided as follows: seven per sex in the control group, five per sex in the low and high dose groups and three per sex in the mid dose group. At initiation of each study, rats weighed between 327 and 365 g and cynomolgus macaques generally weighed between 2.3 and 3.7 kg. VX15/2503 was administered intravenously each week for five weeks; the length of the infusions was roughly the same as that used for the single dose studies.

Prior to receiving the first dose, after the final dose and prior to sacrifice, animals in each of the one-month studies were observed for clinical changes. Physical examinations and clinical assessments (see above) were evaluated only in the cynomolgus macaque study. Rats and macaques were evaluated for changes in behavior after every dose and at least once daily on non-dose days. A portion of animals in each study were sacrificed three to four days after final dose, while the remaining animals entered a recovery phase. Additional animals were sacrificed along with control cohorts after cellular SEMA4D saturation levels returned to baseline. Standard tissue sets were collected at necropsy and tissues were placed in 10% NBF and processed for histopathologic examination. Eyes and testes were stored in Davidson’s fixative prior to evaluation. Selected organs were weighed prior to fixation.

*Pharmacokinetic Analyses*

Maximum serum concentration (Cmax) and time to maximum concentration (Tmax) were both noted. Total exposure was estimated by the linear trapezoidal rule when analyzing serum antibody concentration versus time area under the curve (AUC). Total antibody exposure was calculated over the time range of 0 to 168 hours (AUC0-168) or to the last measurable concentration (AUC0-t). Elimination half-life was determined, when appropriate, using the equation ln(2)/λΖ, where λΖ is the elimination constant. Systemic clearance was calculated using the quotient of dose and AUC0-168. Volume of distribution at steady state was calculated as CL•MRT. The single dose rat study was analyzed using Prism GraphPad version 10 (GraphPad Software Inc., La Jolla, CA). Cmax, Tmax, AUC, and half-lives were determined as above using a single compartment one or two phase model. VX15/2503 serum concentration data from the rat bioavailability study were analyzed with both nonlinear (biphasic and monophasic) and linear analyses.