Reduction of In Vivo Tumor Growth and Angiogenesis by a Humanized IgG4 Monoclonal Antibody to SEMA4D

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Abstract

Semaphorin 4D (SEMA4D; CD100) has been implicated in several key mechanisms of tumor progression, including neovascularization, tumor invasion, and metastasis. SEMA4D binding to its receptor plexin-B1 (PLXNB1) on endothelial cells transduces NET and promotes formation of new blood vessels and tumor growth in vivo. SEMA4D is over-expressed in a wide array of tumor types, and is also produced by recruited inflammatory cells present in the tumor microenvironment. Several recent papers have shown that in an environment lacking SEMA4D, the ability of mouse cancer cells to originate tumors and metastasize is severely impaired. Furthermore, SEMA4D binding by tumor-associated macrophages has been shown to support tumor angiogenesis and growth. In addition to its effects on endothelial cells, SEMA4D has a direct effect on tumor invasive growth and migration. A recent clinical study in soft tissue sarcoma correlates strong SEMA4D expression in tumors with a higher mitotic count and poor prognosis. SEMA4D binding to PLXNB1 on tumor cells results in NET transcription and migration of tumor cells. It has been further reported that overexpression of PLXNB1 and MET in breast and ovarian cancers is a highly prognostic factor. Tumors co-expressing PLXNB1 and MET were characterized as having a higher grade and an increased frequency of metastases. Collectively, these results suggest that expression of SEMA4D, either by tumor cells or by tumor associated inflammatory cells, functions as a crucial factor in tumor neovascularization, and that expression of the SEMA4D and or its high affinity receptor in tumors may further induce tumor growth rate and metastatic potential. Antibody neutralization of SEMA4D thus may represent a new therapeutic strategy for cancer treatment. We selected a humanized Ig4 antibody that binds with high affinity to rat, mouse, primate, and human SEMA4D, and utilized several in vitro functional assays to demonstrate that this antibody blocks SEMA4D – PLXNB1 interactions. Using syngeneic, xenograft and orthotopic tumor models we demonstrated that antibody mediated neutralization of SEMA4D in vivo inhibits tumor growth and tumor angiogenesis. This humanized antibody has successfully completed IND-enabling toxicology testing and is currently undergoing clinical trials.

Introduction

- SEMA4D
  - Binds PLXNB1 with 1 nM affinity and CD37 with 300 nM affinity
  - Is expressed abundantly on the surface of resting T cells and less strongly on B cells and APCs; it is overexpressed in a variety of human tumors including head and neck, prostate, colon, and lung
  - Use of anti-SEMA4D to inhibit the expression of SEMA4D reduced tumor growth and vessel formation in mouse xenograft studies
  - Therapeutic Rat anti-SEMA4D Antibody Neutralization of SEMA4D using a monoclonal antibody could inhibit tumor growth and invasion
- VX15/2503 binds with 5 nM affinity to cellular and soluble SEMA4D and was selected for clinical development to treat patients with advanced solid malignancies

Generation of anti-SEMA4D MAbs

- A panel of mouse hybridomas specific for human, monkey, and mouse SEMA4D or only human and monkey SEMA4D were generated in SEMA4D mice. Several hybridomas were selected for further analysis.
- In vitro biochemical and functional characterization was carried out with purified antibody from independent hybridomas
- Affinity measurement
- Functional Activity
- Based on this data a lead mouse antibody was selected (67-2).
- A humanized version of this Mab was created (VX15/2503)

Mouse and Human MAbs With High Affinity for Mouse and Human SEMA4D Have Been Selected

<table>
<thead>
<tr>
<th>MAbs</th>
<th>Human SEMA4D</th>
<th>Human VEGF</th>
<th>Human Angiopoietin 2</th>
<th>Human Angiopoietin 4</th>
<th>Human VEGF-Trap</th>
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<tr>
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<table>
<thead>
<tr>
<th>TaP 20 (mg/kg)</th>
<th>VX15/2503</th>
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<tr>
<td>5.4</td>
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</table>

Table shows Affinity (nM) Measured by Bcscnt

CT26 and EMT6 tumors exhibit slower growth rate in BALB/c MAbs versus WT mice

- Two syngeneic mouse tumor lines, CT26 and EMT6, were tested for their growth rate in BALB/c SEMA4D knock-out versus WT BALB/c mice. CT26 or EMT6 cells were injected into WT and SEMA4D–BALB/c mice and measured for their growth rate. The data shown demonstrate that both CT26 and EMT6 tumors grew slower in SEMA4D-deficient mice. Similar results were observed using BC243-p4 cells.

Treatment with anti-SEMA4D MAb 67-2 slows growth of CT26 tumors in BALB/c Mice

The humanized anti-SEMA4D antibody VX15/2503 was tested for its ability to block SEMA4D and reduce tumor growth and vascularization in NMSCG-6HTa mice. The data show high levels of HIF and SEMA4D. Treatment included VX15/2503, anti-VEGF (rAd A), and an isotype control. Mice were divided into 3 groups of 10 nude mice each, injected with 2 subcutaneous tumors and treated weekly with 1 mg of antibody by ip injection, starting day 2 post graft for 2 weeks duration. All mice were sacrificed on day 18 as a tumor growth inhibition (TGI) study. Treatment with VX15/2503 or anti-VEGF significantly (P<0.01) inhibited tumor growth and vascularization compared to Ig control.

Preclinical Evaluation

- Tissue cross reactivity analysis demonstrated similar binding of VX15/2503 to lymphoid tissues in human, cynomolgus, and rat tissues.
- Single and repeated dose intravenous infusion toxicity, PK, and PD studies with VX15/2503 in cynomolgus macaques and in rats with a recovery phase have been completed. Similar PK and PD profiles were observed for rats and cynomolgus monkeys.
- Anti-VX15/2503 antibodies were detected in both rat and cynomolgus macaques, typically within two weeks of injection. These data indicate the possibility of using VX15/2503 in cynomolgus monkeys.
- No adverse events were identified in any nonclinical study. Normal E4, FSH, and LH levels were observed in cynomolgus monkeys.
- Additional preclinical data for SEMA4D and VX15/2503 are presented as part of abstract A4578

Summary

- We have generated a highly affinity mouse Mab, 67-2, that blocks SEMA4D-PLXNB1 interactions and significantly slows the growth of both mouse and human tumors in vivo.
- MAb VX15/2503, a humanized antibody derived from Mab 67-2, inhibits tumor growth and vascularization, similar to that observed for anti-VEGF.
- VX15/2503 binds with high affinity to rat, cynomolgus macaque and human SEMA4D.
- VX15/2503 treatment reduced tumor growth rate compared to WT BALB/c mice.
- These studies, in conjunction with PK/PD, safety, and immunological studies in rat and cynomolgus monkeys, support the initiation of a phase 1 clinical study to evaluate the safety and tolerability of VX15/2503 in patients with advanced solid tumors.