OncoImmunology 4:12, e1054599; December 2015; Published with license by Taylor & Francis Group, LLC

Immunomodulation of the tumor microenvironment by neutralization of Semaphorin 4D

Elizabeth E Evans, Mark Paris, Ernest S Smith, and Maurice Zauderer*

Vaccinex, Inc.; Rochester, NY USA

Keywords: CTL, immune checkpoint blockade, invasive margin, Plexin, semaphorin, tumor rejection, tumor associated macrophage, tumor stroma.

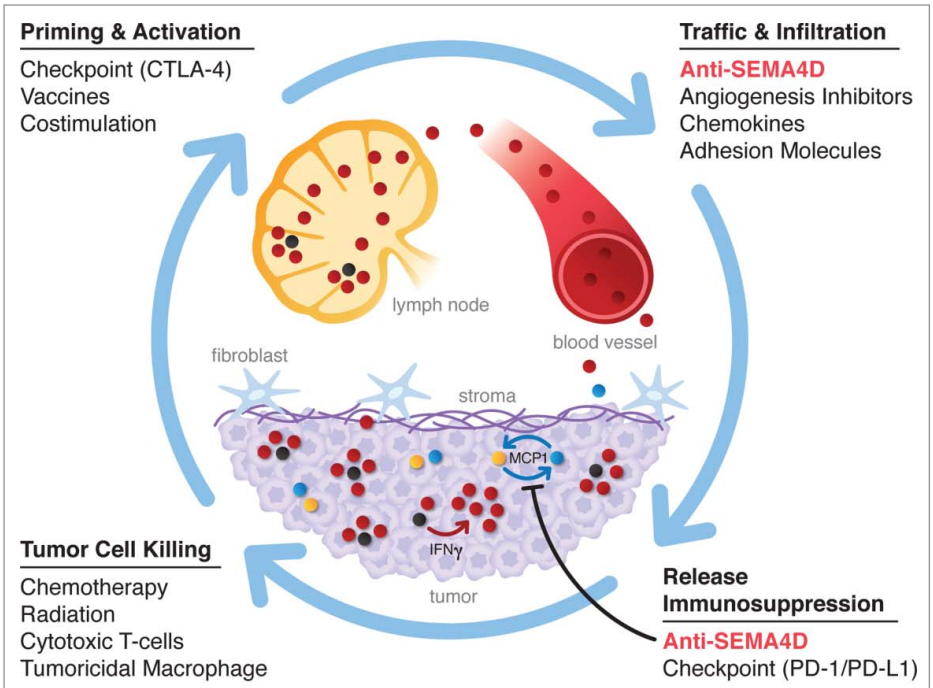
Semaphorin 4D is highly expressed at the invasive tumor margin and acts as a guidance molecule, restricting movement of tumoricidal immune cells into the tumor microenvironment. We recently showed that antibody neutralization of SEMA4D augmented activated monocyte and anticancer T-cell tumor penetration and that anti-SEMA4D antibody potentiated other immunomodulatory therapies in murine tumor models.

Semaphorin 4D/CD100 (SEMA4D) belongs to a class of immune semaphorins that regulates movement and differentiation of cells expressing cognate Plexin receptors. The expression of semaphorins has been associated with poor prognosis in a variety of human cancers.[1,2] The engagement of SEMA4D with its high affinity receptor PlexinB1 (PLXNB1) can affect vascular stabilization[3,4] and transactivate the oncogenes ERBB2 and Met.[5] Additionally, we have recently reported a novel mechanism of action whereby antibody blockade of SEMA4D promotes immune infiltration into tumor.[6] These findings have important ramifications for combination immunotherapy with other agents that increase the magnitude and duration of tumor-specific immune responses.

Immunohistochemical staining of murine syngeneic tumors has revealed a striking gradient of SEMA4D expression at the invasive tumor margin, (*i.e.,* the tumor-stroma interface). We recently confirmed prior reports that significant sources of SEMA4D in the tumor stroma include dendritic cells (DCs) and tumor-associated macrophages (TAMs), the latter of which can be regulated by hypoxia and contribute to aggressive tumor growth.[3] We determined that high levels of SEMA4D positively correlate with the presence of immunosuppressive M2-polarized TAMs and myeloid-derived suppressor cells (MDSCs), with concomitant exclusion of activated antigen presenting cells (APCs) and $CD8^+$ cytotoxic T lymphocytes (CTLs) from the tumor. Considering that SEMA4D has been previously reported to inhibit the movement of immune cells,[7] we hypothesized that the SEMA4D gradient restricts leukocyte penetration into the tumor.

Antibody neutralization of SEMA4D resulted in a marked redistribution of immune cells at the tumor invasive margin in multiple tumor models. Specifically, we documented an increased frequency of activated tumor-infiltrating macrophages, a significant increase in intratumoral $CD3^+$ T cells and dispersion of M2 TAMs and MDSCs. The cytokine milieu within anti-SEMA4D neutralizing antibody-treated tumors also reflected a pro-inflammatory profile, with increased levels of interferon γ (IFNγ) and tumor necrosis factor α (TNFα), as well as reduction in MCP-1, an immunosuppressive chemokine that acts as a MDSC chemoattractant and modulator of Teffector (Teff) to regulatory T (Treg) cell ratios.[8] Further characterization revealed that anti-SEMA4D antibody treatment shifted the balance of suppressive and activated effector T cells, resulting in increased Teff:Treg cell ratios within the tumor. Importantly, tumor-specific cytotoxic T-cell activity significantly increased following anti-SEMA4D antibody treatment, an immunologic response localized to the tumor, with minimal T-cell and cytokine activity in the peripheral lymphoid organs such as the spleen. These activated T cells were required for tumor growth inhibition, as selective T-cell depletion abrogated the effects of anti-SEMA4D antibody treatment. It has been reported that efficient entry of functional tumor-specific T cells into the tumor correlates with improved survival and response to immunotherapy in the clinic.[9] Consistent with these observations, anti-SEMA4D treatment of Tubo.A5 syngeneic tumors resulted in complete tumor regressions and immunologic memory, as demonstrated by resistance of regressor mice to subsequent tumor challenge. In other tumor models, similar dramatic effects were obtained through treatment with anti-SEMA4D in combination with other immunotherapies, as described below.

*Correspondence to: Maurice Zauderer; Email: mzauderer@va.com
Submitted: 05/18/2015; Accepted: 05/20/2015
http://dx.doi.org/10.1080/2162402X.2015.1054599



	No treatment	Anti-SEMA4D
APC	Suppressive M2, MDSC	Pro-inflammatory
Treg	Abundant	Reduced
CD8	Excluded	Increased & Activated
Cytokines	High MCP1, IL10	High TNFα, IFNγ
SEMA4D	Gradient at Invasive Margin	Neutralized, No Barrier

Figure 1. Effects of anti-SEMA4D therapy on the cycle of cancer immunity. Key steps in the cycle of cancer immunity are shown, along with examples of therapies that can induce and/or amplify the immune response to cancer (adapted from Chen and Mellman review, Immunity 2013, 39:1). Rational combination therapies can drive the cycle toward productive anti-tumor immunologic effects. Based on our pre-clinical studies, anti-SEMA4D is included as a novel mechanism to regulate immune cell migration and reduce immunosuppression within the tumor microenvironment. The specific immunomodulatory effects of anti-SEMA4D treatment on various cell types and immune regulators are outlined in the table below.

Of particular relevance to the promise of immunotherapy, we hypothesized that agents capable of increasing peripheral immune responses (such as immune checkpoint blockade and vaccination) may benefit from the enhanced penetration of T cells into the tumor in response to anti-SEMA4D antibody blockade. Therefore, we tested this hypothesis using anti-SEMA4D antibody in combination with other immunotherapies against tumor models that showed partial responses to each single agent. The combination of anti-SEMA4D with anti-CTLA-4 or anti-PD-1 antibodies improved survival and the complete regression frequency of Colon26 tumor-bearing mice. Specifically, anti-SEMA4D and anti-CTLA-4 single agent therapies resulted in 8% and 23% complete tumor regression, respectively, whereas the combination significantly increased the frequency to 78% (67/86); all regressions were durable and regressor animals rejected subsequent homologous tumor challenge. Furthermore, combinations with immunomodulatory chemotherapy, such as cyclophosphamide, also enhanced the response to the monotherapy.

Our understanding of the mechanism of action of SEMA4D within the complex tumor ecosystem is evolving. Semaphorins are pleotropic molecules, with a wide variety of reported activities in neural, immune, and vascular[9] systems. While embryonic deletion of SEMA4D has been implicated in modulating immune responses, our data suggest that antibody blockade of SEMA4D neither enhances nor suppresses systemic immune response, but rather regulates the infiltration of immune cells into the tumor environment (TME). We have confirmed the direct effects of SEMA4D on APC migration [7] and have documented the redistribution of immune cells and resultant immune-mediated effects in the TME. Further investigations into the precise mechanisms of SEMA4D-mediated leukocyte trafficking are thus warranted.

The unique distribution of SEMA4D in the tumor invasive margin acts as a key spatial modulator, providing a protective barrier against immune cell penetration. This gradient of expression is not observed in normal tissues, as SEMA4D is normally expressed predominantly by immune cells. We believe the localized tumoral enhancement of immune activity may be critical to reducing off-target toxicities otherwise associated with systemic immune activation. We have not observed dose limiting toxicities in preclinical and toxicological studies,[10] and we have recently completed a Phase I safety trial for patients with advanced solid tumors in which anti-SEMA4D (VX15/2503) antibody was well tolerated (manuscript in preparation). Further, we suspect that peripheral immune activation induced by other immunotherapies may be redirected into the TME upon combination with anti-SEMA4D antibody treatment. As such, we believe that anti-SEMA4D antibody can be added to the arsenal of potent agents available for rational design of combinatorial immunotherapies, as described in **Figure 1**.

Disclosure of Potential Conflicts of Interest

Vaccinex, Inc., a private Delaware corporation, has patent rights based on inventions described in this publication and is developing a humanized anti-SEMA4D antibody for clinical use. All authors are employees of Vaccinex, Inc.

Funding

Financial support provided by Vaccinex, Inc.

References

1. Ch'ng ES, Kumanogoh A. Roles of Sema4D and Plexin-B1 in tumor progression. Mol Cancer 2010; 9:251; PMID:20858260; http://dx.doi.org/10.1186/1476-4598-9-251
2. Worzfeld T, Swiercz JM, Looso M, Straub BK, Sivaraj KK, Offermanns S. ErbB-2 signals through Plexin-B1 to promote breast cancer metastasis. J Clin Invest 2012 Apr 2; 122(4):1296-305; PMID:22378040; http://dx.doi.org/10.1172/JCI60568
3. Sierra J, Corso S, Caione L, Cepero V, Conrotto P, Cignetti A, Piacibello W, Kumanogoh A, Kikutani H, Comoglio PM, et al. Tumor Angiogenesis and progression are enhanced by Sema4D produced by tumor-associated macrophages. Journal of Experimental Medicine 2008 Jun 2; 205:1673-85; PMID:18559453; http://dx.doi.org/10.1084/jem.20072602
4. Smith ES, Jonason AJ, Reilly C, Veeraraghavan J, Fisher T, Doherty M, Klimatcheva E, Mallow C, Cornelius C, Leonard JE, et al. SEMA4D compromises blood-brain barrier, activates microglia, and inhibits remyelination in neurodegenerative disease. Neurobiol Dis 2014; 73:254-68; PMID:25461192; http://dx.doi.org/10.1016/j.nbd.2014.10.008
5. Giordano S, Corso S, Conrotto P, Artigiani S, Gilestro G, Barberis D, Tamagnone L, Comoglio PM. The semaphorin 4D receptor controls invasive growth by coupling with Met. Nat Cell Biol 2002 Sep; 4(9):720-4; PMID:12198496; http://dx.doi.org/10.1038/ncb843
6. Evans EE, Jonason AS, Jr., Bussler H, Torno S, Veeraraghavan J, Reilly C, Doherty MA, Seils J, Winter LA, Mallow C, et al. Antibody blockade of semaphorin 4D promotes immune infiltration into tumor and enhances response to other immunomodulatory therapies. Cancer Immunol Res 2015 Jan 22; 3(6):689-701; PMID:25614511
7. Delaire S, Billard C, Tordjman R, Chedotal A, Elhabazi A, Bensussan A, Boumsell L. Biological activity of soluble CD100. II. Soluble CD100, similarly to H-SemaIII, inhibits immune cell migration. J Immunol 2001 Apr 1; 166(7):4348-54; PMID:11254688; http://dx.doi.org/10.4049/jimmunol.166.7.4348
8. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res 2009 Jun; 29 (6):313-26; PMID:19441883; http://dx.doi.org/10.1089/jir.2008.0027
9. Galon J, Angell HK, Bedognetti D, Marincola FM. The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures. Immunity 2013 Jul 25; 39(1):11-26; PMID:23890060; http://dx.doi.org/10.1016/j.immuni.2013.07.008
10. Leonard JE, Fisher TL, Winter LA, Cornelius CA, Reilly C, Smith ES, Zauderer M. Nonclinical Safety Evaluation of VX15/2503, a Humanized IgG4 Anti-SEMA4D Antibody. Mol Cancer Ther 2015 Apr; 14 (4):964-72; PMID:25657333; http://dx.doi.org/10.1158/1535-7163.MCT-14-0924