Targeting Notch/Delta-like ligand 4 (DLL4) and vascular endothelial growth factor (VEGF) pathways by an anti-DLL4/anti-VEGF bispecific monoclonal antibody inhibits tumor growth and reduces cancer stem cell frequency in solid tumors

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ABSTRACT

Both Notch/Delta-like ligand 4 (DLL4) and vascular endothelial growth factor (VEGF) pathways play a critical role in angiogenesis and tumor growth. Due to differential regulatory roles, DLL4 and VEGF on the vasculature, blockade of DLL4 or VEGF inhibits tumor growth by distinct mechanisms: anti-DLL4 treatment induces an abnormal increase of poorly perfused blood vessels, which results in a non-tumorigenic phenotype of tumor vessels. In vitro, this antibody bound with low nanomolar affinity to hVEGF and hDLL4, and reduced HUVEC proliferation induced by VEGF. The bi-specific antibody demonstrated significant in vivo anti-tumor efficacy in various solid tumors, delayed tumor recurrence following termination of the frequency of tumor initiating cells. Analysis of tumor vasculature after treatment with anti-DLL4/VEGF revealed inhibition of vascular gene expression and endothelial cell proliferation, indicating that the anti-VEGF effect on the vasculature is dominant over the anti-DLL4 effect. Notably, at doses where both anti-DLL4 and anti-VEGF alone produces suboptimal anti-tumor effect, dual targeting resulted in an enhanced anti-tumor growth inhibition. These results indicate that anti-DLL4/anti-VEGF is active in ovarian, gastric xenograft models and may be useful for treatment of a variety of solid tumors.

BACKGROUND

• Both DLL4 and VEGF play important roles in tumor angiogenesis.
• Blockade of DLL4 or VEGF inhibits angiogenesis through distinct mechanisms.
• DLL4 blockade inhibits functional tumor vessel formation.
• VEGF blockade inhibits endothelial proliferation.
• DLL4-Notch signaling is part of a negative feedback loop in tumor angiogenesis.
• We previously demonstrated that targeting DLL4/Notch signaling in tumors reduces tumorigenic potential of cancer stem cells in patient-derived xenograft tumor models (Hoey et al. Cell Stem Cell 5:168-177, 2009).
• DLL4 blockade leads to upregulation of VEGF, and unregulated endothelial hyperproliferation.
• Anti-DLL4/VEGF retains the anti-cancer stem cell impact of anti-DLL4 and has potentiated anti-angiogenic activity relative to current anti-VEGF therapies.
• OncMed’s anti-DLL4/anti-VEGF bispecific design:

MATERIALS AND METHODS

In vitro binding assay: Binding affinities for the anti-DLL4 and anti-VEGF arms of the bispecific molecule were determined by surface Plasmon resonance using a BIACore X. Each antibody was coupled to the SPR chip by either streptavidin-biotin (VEGF) or direct coupling to a CMS chip with NHS chemistry. Association and dissociation curves were determined for each antigen at multiple concentrations and the affinity was measured by globally fitting the data using a 1-1 binding model.

In vitro HUVEC proliferation assay: Human endothelial cells were harvested from in vitro cultures and distributed in the number of 5,000 into a 96 well plate, then placed into starvation [low serum] medium overnight before being incubated with the mixture of hVEGF (10 ng/mL) and various concentrations of antibodies. From the highest 20 ug/mL to the lowest 0.0512 ug/mL. After seven days, the cells were incubated with Alamar Blue at 37°C before reading the plate with Molecular Device Micro-plate Reader SpectraMax M5.

In vivo mouse xenograft efficacy studies: NOD/SCID mice were implanted subcutaneously with patient-derived tumor xenografts of human ovarian tumor OMP-OV40, colon tumor OMP-C8 or gastric tumors STM1. Tumors were treated with control mAb, anti-DLL4, anti-VEGF or the bi-specific antibody when the tumors reached the size of 150 mm

Immunofluorescence analysis: Histologic analysis was performed on tumors. Slides were imaged with an Olympus BX51 microscope using IPLabs v4.0.

RESULTS

In vivo tumor xenograft studies: NOD/SCID mice bearing subcutaneous OMP-C8 tumors were treated with anti-DLL4, anti-VEGF or the bi-specific antibody in combination with in situ 4T1 tumors. Following termination of chemotherapy, tumors were harvested from 4-5 mice of each treatment group and dissociated into single cells, which were depleted of mouse cells, and re-plated in a high density of mice. For the incidence and size were recorded up to 90 days after implantation of tumor cells. The remaining mice continued to be treated with antibodies single agents for additional several weeks.

CONCLUSIONS

• Anti-DLL4/anti-VEGF bi-specific mAb has significant activity in xenograft tumor models.
• Simultaneous inhibition of DLL4 and VEGF produces anti-tumor effect superior to anti-DLL4 alone.
• Simultaneous inhibition of DLL4 and VEGF decreases tumorigenic potential of cancer stem cells.
• The bi-specific antibody has superior effect over anti-DLL4 alone on delaying tumor recurrence and reducing cancer stem cell frequency in tumors.
• We have developed a clinical candidate and working toward an IND filing in 2014.