**ABSTRACT**

Both Notch-Delta-like 4 (DLL4) and vascular endothelial growth factor (VEGF) pathways play a critical role in angiogenesis and tumor growth. Due to differential regulatory effects of VEGF and DLL4 on the vasculature, blockade of DLL4 or VEGF signaling inhibits tumor growth by distinct mechanisms: anti-DLL4 treatment induces an abnormal increase of poorly perfused blood vessels, which results in nonproductive angiogenesis unable to support tumor growth, whereas anti-VEGF therapy significantly decreases vasculature reducing the blood supply to tumors. In addition, DLL4 Notch signaling plays a key role in the maintenance of cancer stem cells. We have recently developed a bispecific monoclonal antibody (mAb) targeting DLL4 and VEGF. In vitro, this antibody exhibited low nanomolar binding affinity to VEGF and DLL4, and reduced human endothelial cell proliferation induced by VEGF. The bispecific antibody demonstrated significant in vivo anti-tumor efficacy in various solid tumors, induced tumor regression, decreased the frequency of tumor initiating cells, and delayed tumor recurrence following termination of chemotherapy. Analysis of tumor vasculature after treatment with anti-DLL4/anti-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 produce a marked anti-tumor effect, dual targeting resulted in additive tumor growth inhibition. The combination of anti-DLL4 and anti-VEGF resulted in broad spectrum efficacy in many different solid tumor types including breast, colon, ovarian and pancreatic tumors. Notably, serial transplantation studies indicated that the anti-cancer stem cell activity of anti-DLL4 was retained with the bispecific. In safety studies, OMP 35B83 demonstrated an acceptable safety profile in cynomolgus monkeys compared to controls with reduction of endothelial hyperplasia and suppression of vascular-related gene upregulation in the heart. These results indicate that our bispecific anti-DLL4/VEGF is broadly efficacious and may be useful for treatment of a variety of tumor types. We are currently enrolling patients with advanced refractory solid tumors in a Phase 1/2a clinical trial.

**RESULTS**

**Background**

Both DLL4 and VEGF play important roles in tumor angiogenesis. Blockade of DLL4 or VEGF inhibits angiogenesis through distinct mechanisms – DLL4 inhibits functional tumor vessel formation – VEGF inhibition blocks endothelial proliferation – DLL4 Notch signaling is part of a negative feedback loop in the angiogenic process. We previously demonstrated that targeting DLL4 Notch signaling in the tumors reduces tumorigenic potential of cancer stem cells in patient-derived xenograft tumor models (Hoey et al. Cell Stem Cell 5:166-177, 2009).

DLL4 blockade leads to upregulation of VEGF and unregulated endothelial hyperplasia. Anti-DLL4/VEGF retards the anti-cancer stem cells impact of anti-DLL4 and has potentially increased anti-angiogenic activity relative to current anti-VEGF therapeutics.

**Materials and Methods**

In vitro binding assay: Binding affinities for the anti-DLL4 and anti-VEGF arms of the bispecific mAb were determined by surface plasmon resonance (SPR) using a Biacore 3000 instrument. In vitro, the mAb was coupled to SPR chip either streptavidin (VEGF) or streptavidin-coupling to a CMS chip with NHS chemistry. Association and dissociation curves were examined for each antigen at multiple concentrations and the affinity was measured by globally fitting the data using a 1-1 binding model.

In vitro HEV permeation assay: Human endothelial cells were harvested from in vitro cultures and incubated for 24h with 1 ng/mL VEGF165, then washed and placed into chambers for 4 hours. The cell permeability was measured by detecting the VEGF165 concentration in the chamber fluid using a fluorescence plate reader.

**Implications of the Results**

The anti-DLL4 and anti-VEGF bispecific antibody demonstrated an improved cardiac profile in cynomolgus monkeys compared to both anti-DLL4 or anti-VEGF alone.

**Conclusion**

- Anti-DLL4/anti-VEGF bispecific mAb has significant activity in xenograft tumor models.
- Simultaneous inhibition of DLL4 and VEGF produces tumor effect superior to anti-DLL4 or anti-VEGF alone.
- Simultaneous inhibition of DLL4 and VEGF induces significant down-regulation of vascular-related genes and decreases vasculature density, suggesting a dominant anti-VEGF-mediated angiogenic effect over the anti-DLL4 effect on endothelial cell hyperplasia.
- The bispecific antibody has superior effect over anti-DLL4 alone on delaying tumor recurrence and reducing cardiac fibrosis in tumors.
- The bispecific antibody demonstrated an improved cardiac profile in cynomolgus monkeys compared to anti-DLL4 with reduction of endothelial hyperplasia and suppression of vascular-related gene upregulation in the heart.

- Ongoing phase 1a trial for anti-DLL4/anti-VEGF bispecific mAb (OMP-35B83)

**Figure 1**: Schematic representation of anti-DLL4/anti-VEGF bispecific mAb. The antibody contains heavy chains that drive heterodimer formation in the CSR domain that drive heterodimer formation. Each heavy chain is paired with a common light chain that supports binding of both the DLL4 and VEGF antigens.

**Figure 2**: Bi-specific antibody blockade in xenograft tumors. Monkeys were implanted with xenograft tumors STF1. Tumors were treated with a control mAb, anti-DLL4, anti-VEGF or the bi-specific antibody when the tumors reached the size of 150 mm³. Treatment was continued for 4 weeks. The tumor growth frequency and volume were measured. The results showed that the bi-specific antibody had the most significant effect on tumor growth in comparison to other treatments. The tumor growth frequency in the bi-specific antibody treatment group was significantly lower than in the control group. The tumor volume in the bi-specific antibody treatment group was also significantly lower than in the control group.

**Figure 3**: Anti-DLL4/VEGF bispecific antibody inhibits Colon XENOGRAFT Tumor Growth. Monkeys were treated with different combinations of anti-DLL4 and anti-VEGF. The tumor growth frequency and volume were measured. The results showed that the bi-specific antibody had the most significant effect on tumor growth in comparison to other treatments. The tumor growth frequency in the bi-specific antibody treatment group was significantly lower than in the control group. The tumor volume in the bi-specific antibody treatment group was also significantly lower than in the control group.

**Figure 4**: Anti-DLL4/VEGF Biochip Antibody Blocks Colon Tumor Growth. Using a mouse xenograft model, the biochip antibody was compared to a control mAb and anti-DLL4 alone. The results showed that the biochip antibody had the most significant effect on tumor growth in comparison to other treatments. The tumor growth frequency in the biochip antibody treatment group was significantly lower than in the control group. The tumor volume in the biochip antibody treatment group was also significantly lower than in the control group.

**Figure 5**: Anti-DLL4/VEGF biochip antibody blocks tumor growth. Using a mouse xenograft model, the biochip antibody was compared to a control mAb and anti-DLL4 alone. The results showed that the biochip antibody had the most significant effect on tumor growth in comparison to other treatments. The tumor growth frequency in the biochip antibody treatment group was significantly lower than in the control group. The tumor volume in the biochip antibody treatment group was also significantly lower than in the control group.

**Figure 6**: Anti-DLL4/VEGF biochip antibody blocks tumor growth. Using a mouse xenograft model, the biochip antibody was compared to a control mAb and anti-DLL4 alone. The results showed that the biochip antibody had the most significant effect on tumor growth in comparison to other treatments. The tumor growth frequency in the biochip antibody treatment group was significantly lower than in the control group. The tumor volume in the biochip antibody treatment group was also significantly lower than in the control group.

**Figure 7**: Bi-specific mAb Produces an Enhanced Anti-tumor Effect Compared to Either Agent Alone at Suboptimal Dose. Ovarian tumor OMP-CV40 and gastric tumor OMP-ST16 were treated with control mAb, 3 mg/kg of anti-DLL4 and anti-VEGF or the bi-specific mAb for once a week for 4 weeks. The treatment group showed significantly higher survival compared to the control group. The tumor volume in the treatment group was also significantly lower than in the control group.