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CAR-T cells harboring a camelid single domain antibody as a targeting agent to kill tumors expressing VEGFR2

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INTRODUCTION

Modulation of the immune system is showing tremendous promise in the treatment of malignancies. In addition to checkpoint inhibitors that re-activate T cells present in the tumor microenvironment, exogenously transduced chimeric antigen receptor (CAR) T cells are providing excellent responses in clinical trials for the treatment of leukemias. In this study, we describe the generation and characterization of novel anti-VEGFR2 antibodies for use in CAR-T cells that target VEGFR2-expressing tumors.

Angiogenesis is the process of new blood vessel formation and is essential for a tumor to grow beyond a certain size. Tumors secrete the pro-angiogenic vascular endothelial growth factor (VEGF), which acts upon local endothelial cells by binding to vascular endothelial growth factor receptors (VEGFR). Of the three VEGF receptors, VEGFR-2 is the primary regulator of endothelial cell proliferation and migration. As VEGFR2 is often overexpressed by malignant solid tumors, we are investigating the utility of anti-VEGFR2 CAR-T cells as a method to treat VEGFR2-expressing tumors.

Both camelid and human single chain antibodies were generated by screening phage display libraries. Two camelid and two human antibodies were characterized, and the top candidate selected for CAR-T studies.

CAR-T cells were engineered to express a camelid anti-VEGFR2 antibody in combination with the CD28 and 4-1BB costimulatory molecules and the CD3 zeta chain. The chimeric receptor was expressed well by transduced T cells and cytotoxicity studies are ongoing.

We previously showed the utility of camelid antibodies in CAR-T constructs as anti-CEACAM6 CAR-T cells show both *in vitro* and *in vivo* efficacy against the pancreatic tumor Bx-PC3. The use of a camelid antibody to target VEGFR2-expressing tumors should provide further evidence to support the concept that camelid single domain antibodies can be easily developed for CAR-T therapies.

ACKNOWLEDGMENTS

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RESULTS

Antibody binding to human VEGFR2/Fc by SPR

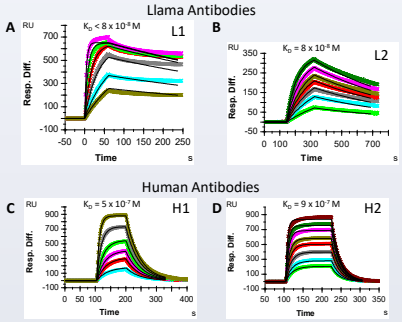

Figure 1: The binding kinetics for the interactions of antibodies to immobilized human and mouse VEGFR2/Fc were determined by SPR. None of antibodies showed binding to immobilized mouse VEGFR2/Fc at concentrations of 150~200 nM (data not shown). Sensorgram overlays show the binding of antibodies to immobilized human VEGFR2/Fc at concentrations of (A) 0.15 - 4 µM, (B) 75 - 750 nM, (C) 0.1 - 2 µM and (D) 0.2 - 3 µM, respectively. Calculated K_D values are shown.

Antibody binding to human VEGFR1/Fc, VEGFR2/Fc and VEGFR3/Fc by ELISA

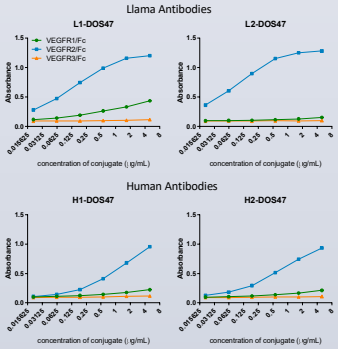

Figure 2: Each antibody was conjugated to the enzyme urease, and tested for binding to recombinant proteins by ELISA. All four antibody conjugates bind to recombinant VEGFR2/Fc, with the strongest binding observed with the llama antibody conjugates (consistent with K_D values determined in Figure 1). All antibodies show some cross-reactivity to VEGFR1/Fc. There was no detectable binding by any of the antibodies to VEGFR3/Fc.

Competition between VEGF and antibodies for binding to VEGFR2/Fc

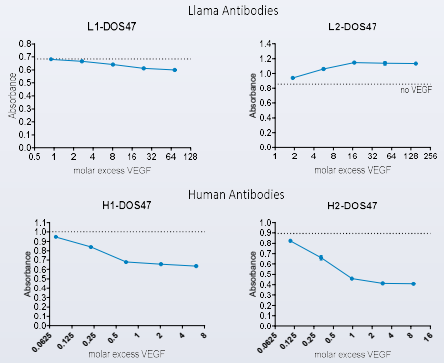

Figure 3: Antibody-urease conjugates were mixed with VEGF at a variety of different molar ratios, and then tested for binding to VEGFR2/Fc captured on ELISA plates. The binding of the two human antibody conjugates to VEGFR2 was inhibited by VEGF, suggesting these antibodies and VEGF bind to overlapping sites. The binding of L1-DOS47 was only minimally affected by VEGF, suggesting that the L1 antibody and VEGF bind to distinct sites. Interestingly, the binding of L2-DOS47 to VEGFR2 was enhanced by the presence of VEGF, suggesting that the L2 antibody binds better to the VEGF/VEGFR2 complex than to VEGFR2 alone.

Competitive ELISAs to study the binding epitopes of the L1 and H2 antibodies

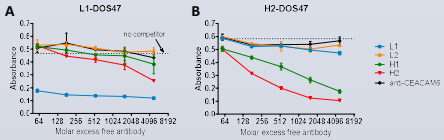

Figure 4: L1-DOS47 (A) and H2-DOS47 (B) antibody-urease conjugates were mixed with each of the four uncoupled antibodies (or anti-CEACAM6 as a negative control) at a variety of different molar ratios, and then tested for binding to VEGFR2/Fc coated on ELISA plates. As expected, binding of each antibody-urease conjugate was inhibited by the corresponding uncoupled antibody. In addition, the H2-urease conjugate was inhibited by uncoupled H1 antibody, suggesting that the two human antibodies share at least partially overlapping epitopes. The uncoupled H2 antibody also partially inhibited the binding of L1-DOS47, although only at very high molar ratios.

Antibody binding to VEGFR2 expressed on 293/KDR cells

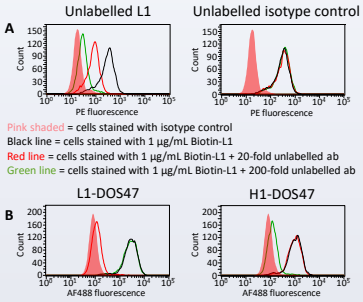

Figure 5: 293/KDR cells are HEK293 cells that have been transfected to stably express VEGFR2 (KDR). 293/KDR cells were stained with antibodies as described above. Antibodies L1 and H1 bind to VEGFR2 expressed on 293/KDR cells.

Generation of CAR-T cells expressing antibody L1

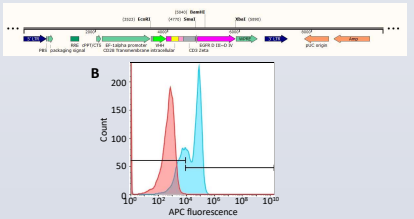

Figure 6: (A) Design of the CAR-T construct. (B) Expression of the CAR-T construct by transduced T cells. Red = CAR-T cells stained with secondary antibody-APC only. Blue = CAR-T cells stained with primary and secondary antibodies. 68.5% of CAR-T cells express the transduced construct.

CONCLUSIONS

- The two camelid anti-VEGFR2 antibodies have approximately 10-fold higher affinity for VEGFR2 than the two human anti-VEGFR2 antibodies
- Antibody L1 does not bind to the VEGF-binding site of VEGFR2
- Antibody L1 binds to VEGFR2 expressed on 293/KDR cells
- Antibody L1 was selected for further studies. A CAR-T vector has been constructed and transduced into T cells. Cytotoxicity studies are ongoing.