Development of VHH antibodies to biomarkers of hematological malignancies for BiTE and CAR-T therapies

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Abstract

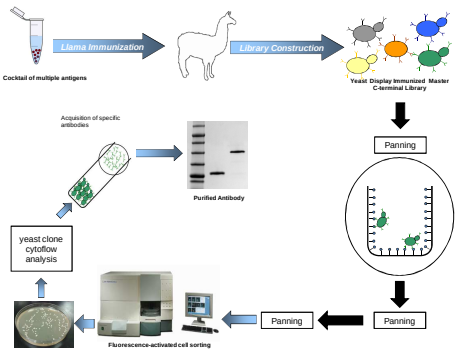
Clinical data indicate that Bispecific T-cell Engaging antibodies (BiTE) and Chimeric Antigen Receptor (CAR) T cell therapy will be the next generation of therapeutics for immune-oncology. As of May 2018, there are over 100 clinical trials underway that target over 25 different surface biomarkers in almost every human tissue. While the impressive success of BiTE and CAR-T therapies has been obtained mainly in clinical trials against various hematological malignancies, application of the technologies to solid tumors remains challenging. The most prominent target for BiTE and CAR-T therapies is CD19 that has shown impressive success in clinical settings to treat Acute Lymphoblastic Leukemia (ALL), Non-Hodgkin Lymphoma (NHL), and Chronic Lymphocytic Leukemia (CLL). Despite the high levels of complete response rates in patients, relapse from CD19-targeted therapy can occur via a suppressive tumor microenvironment or antigen escape. Thus, new targets are being identified and evaluated to treat hematological malignancies. Among these new targets are CD5, CD22, CD30, CD33, CD38, CD70, CD123, CD138, CD147, ROR1, and BCMA.

Almost all BiTE and CAR-T constructs rely on scFv - a fusion of heavy chain and light chain variable regions connected via a linker peptide. Unfortunately, however, scFv are known to be poorly expressed and subject to self-aggregation. Abzyme's goal is it to overcome these problems by using naturally occurring heavy chain-only camelid VHHs as modular antibodies for BiTE and CAR-T constructs. In contrast to scFv, camelid VHHs possess a number of advantages including smaller size, modularity, enhanced stability and ease-of-production.

Using Abzyme's proprietary SuperLlama/SuperYeast platform, we have successfully isolated a suite of VHH antibodies against various biomarkers of hematological malignancies. We have tested these antibodies extensively in vitro for specific function including binding to soluble and membrane-associated targets. We are currently testing the antibody-directed T cell cytotoxicity. We present our novel platform for rapid generation of functional VHH antibodies suitable for BiTE and CAR-T applications. We are looking for pharmaceutical collaborators to further develop and advance these antibodies into clinical trials.

Our state-of-the art SuperY/SuperL approach allows rapid generation of high-affinity antibodies to multiple targets. The SuperY technology utilizes yeast triple-mode system consisting of *in vivo* antibody maturation, cell surface presentation and secretion. SuperL is a novel approach to generate natural camelid VHH using small amount of antigens in microgram ranges. The screening allows isolation of VHH with desired attributes e.g. like cross-reactivity in real-time.

Experimental approach



RESULTS

Interspecies reactive VHH antibodies (anti-CD3 VHH) to streamline pre-clinical studies in animal models

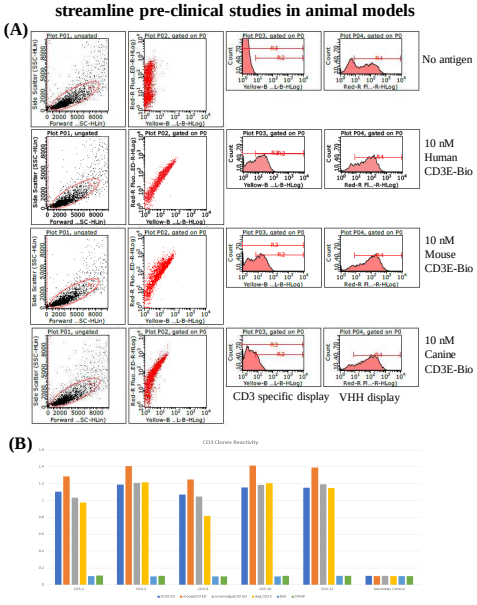


Figure 1. Single cell analysis shows high level of surface display of anti-CD3 VHH and interspecies reactivity of anti-CD3 VHH with CD3-Epsilon in human, mouse, and canine **(A)**. ELISA data reflect the interspecies reactivity of anti-CD3 VHH with various CD3 antigens **(B)**.

VHH antibodies to EMMPRIN/CD147

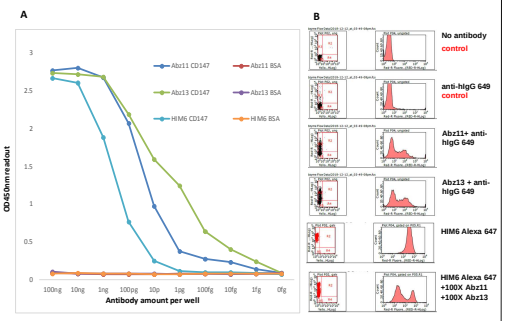


Figure 2. Binding of Abz11, Abz13 and HIM6 to EMMPRIN/CD147 measured by ELISA **(A)** and cytoflow analysis using Jurkat cells **(B)**.

VHHs against CD147 disrupt tubule formation in HUVEC cells

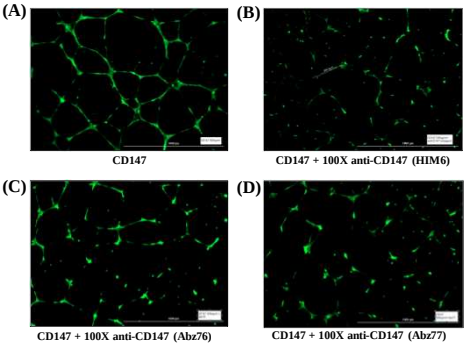


Figure 3. HUVEC cells were incubated with either CD147 alone (A), or in the presence of HIM6 (B), or Abz76 (C), or Abzy77 (D) at 100X molar excess of CD147. The tubule formation was detected by a fluorescent cell permeable dye, Calcein AM, using a microscope.

VHHs antibodies to CD19, CD22, CD33, ROR1, and BCMA

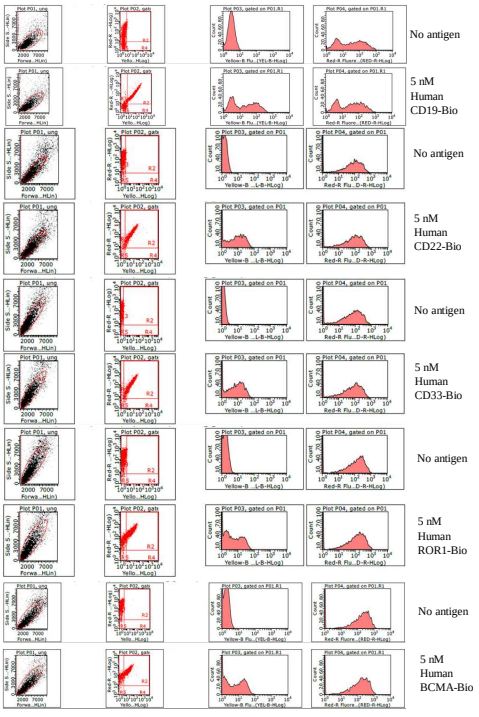


Figure 4. The single cell yeast clones obtained after Fluorescence-Activated Cell Sorting (FACS) were induced and expressed followed by their analysis using cytoflow. The analysis shows high level of surafce display of the indicated VHH antibodies.

VHH antibodies bind to their respective antigens in ELISA

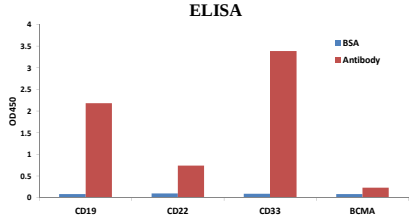


Figure 5. The well of the ELISA plate were coated with the indicated antigens followed by addition of the TES extract of the bacterial cells expressing respective VHH antibodies having FLAG tag at the C-terminus. The bound VHHs were detected by anti-FLAG HRP.

VHHs against CD19 and CD22 bind to their cell surface receptors on Raji cells

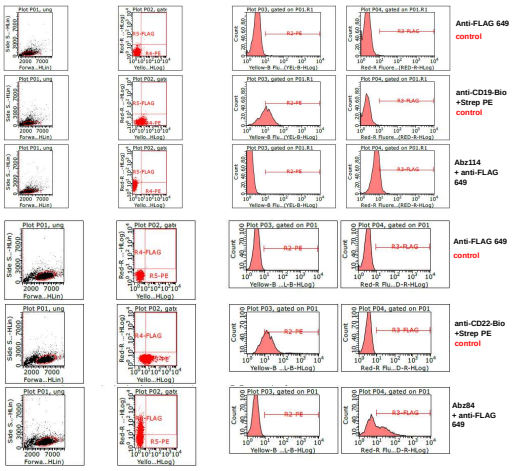


Figure 6. Raji cells were incubated with the indicated antibodies for 1 hr at +4°C. The cells were washed with 1X PBS containing 0.1% BSA. The washed cells were analyzed by Guava flow cytometer.

Next Step

The VHH antibodies will be further tested for their ability to bind to their respective receptors on mammalian cell surface. The antibodies that bind to their receptors will be tested for antibody directed T-cell cytotoxicity using BiTE.

Abzyme's Active programs to generate single domain modular antibodies

Target	Program	Target	Program	Target	Program	Target	Program
Digoxigenin	Program 1	FGF2/bFGF	Program 2	ANGPTL4	Program 2	LAG3	Program 3
Albumin	Program 1	mPDGF	Program 2	LepR	Program 2	GITR	Program 3
TfR	Program 1	CCL20	Program 2	BCMA	Program 3	IL-21R	Program 3
CD3E	Program 1	TIGIT	Program 2	PD1	Program 3	IL-6	Program 3
VEGF	Program 1	PD-L1	Program 2	BAFFR	Program 3	OX40	Program 3
CD147	Program 2	B7-H4/B7x	Program 2	CD138	Program 3	MICA/MICB	Program 3
ANG2	Program 2	SOST	Program 2	CD22	Program 3	HER2/erbB2	Program 3
TNF-alpha	Program 2	EPHB4	Program 2	CD30	Program 3	EGFR/erbB1	Program 3
IL-21	Program 2	4-1BB	Program 2	CD33	Program 3	Hyaluronate	Program 3
IL-23/IL-12	Program 2	CD19	Program 2	ROR1	Program 3		
IL-4	Program 2	OX40L	Program 2	B7-H3	Program 3		

Summary and future research

Abzyme's SuperY/SuperL approach allows rapid generation of well-expressed, high-affinity modular antibodies. We have successfully generated VHH antibodies to both small molecules and large proteins with desired attributes. Produced antibodies are modular that can be used as a building block for multi-specific antibody engineering. Abzyme's SuperY/SuperL approach was used to generate VHH antibodies to approx 60 targets for therapeutic and diagnostic applications. We expect to test some of these targets in animal models as well as human clinical trials in near future.

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