Growing Portfolio of Modular Binders for the Rapid Generation of Bispecific Antibodies

Tran, H., White, I., Prokopowitz, C.M., Pham, H., Moussa, F.M., and Swoboda, R.K.
Abzyme Therapeutics, 321 Jones Boulevard, Suite 300, Royersford, PA 19468, www.abzymetx.com
Corresponding author: tran@abzymetx.com

Abstract

Antibodies are important research tools for detection, localization, and quantification of cellular proteins and are widely used for diagnosis and treatment of various human diseases. Clinical data indicate that Bispecific T-cell Engaging antibodies or BiTE will be the next generation of therapeutics for immune-oncology. In comparison with traditional antibodies, BiTE antibodies offer a significant improvement in therapeutic index at a lower antibody dosing. For example, the administered effective dose of bi-specific CD19/CD3 BiTe antibody (Blinatumomab, 34µg/dose) is five orders of magnitude lower than the reported effective dose of the standard-of-care, Rituximab. However, the use of bispecific antibodies has been hindered by manufacture difficulties. Abzyme's goal is it to overcome these problems by using **camelid VHHs as modular antibodies** for the rapid generation of bispecific antibodies. To enable to isolate modular antibodies with desired attributes, we are using a combined yeast-based display library/ FACS sorting approach. To ensure that the modular antibodies remain functional once fused to a C-terminus of any existing antibody, the antibodies are screened from yeast surface C-terminal display libraries.

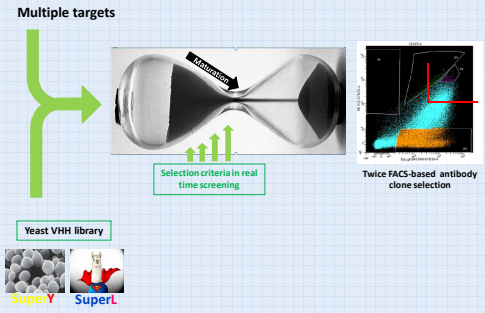
Through our "plug-and-play" process, an existing IgG traditional antibody or antibody fragments can be rapidly transformed into well-expressing and easy-to-produce Abz2 bispecific molecules. This creates endless opportunities for developing novel biologics with improved diagnostic or therapeutic indices. An IgG-like Abz2 bispecific antibody retains the benefits of a traditional antibody such as excellent binding characteristics (affinity and specificity), favorable pharmacokinetics, Fc effector function, protein stability, and low immunogenic potential.

We have successfully isolated a numbers of modular antibody clones against four important targets - CD3, transferrin receptor, albumin and digoxigenin. We have tested these antibodies extensively *in vitro* for their functionality. Results of the first productions of bispecific antibodies have shown that the Abz2 bispecifics can be efficiently produced. *In vitro* assays have confirmed the activity of the bispecific antibodies and *in vivo* assays are in progress. Obtained modular antibodies can be used for various applications such as anti-CD3 for BiTE generation; anti-TfR as a payload to deliver biologics across the blood brain barrier, anti-albumin to extend protein serum half-life of the antibody and anti-Digoxigenin for pre-targeted immunotheranostics.

We are in the process to isolate modular antibodies against another **60 targets** for different therapy indications. We present our novel plug and play model for rapid generation of bispecific antibodies, and data for the first group of modular antibodies generated by Abzyme as well as the first bispecific antibody generated with this approach.

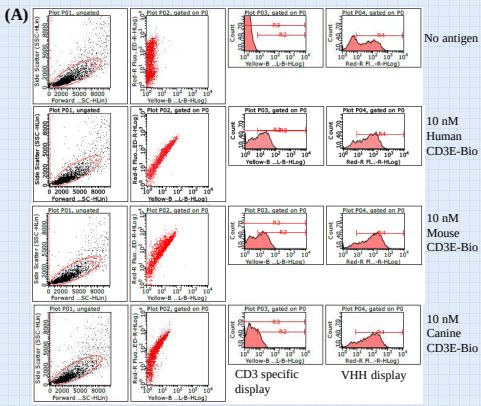
Experimental approach

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.



RESULTS

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



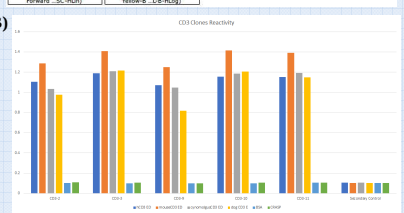


Figure 1. Single cell analysis show high level of surface display of anti-CD3 VHH and interspecies reactivity of anti-CD3 VHH with CD3-Epsilon in human, mouse, and canine **(A)**. ELISA data reflect the interspecies reactivity of anti-CD3 VHH with various CD3 antigens **(B)**.

VHH antibodies to small molecules - anti-Digoxigenin

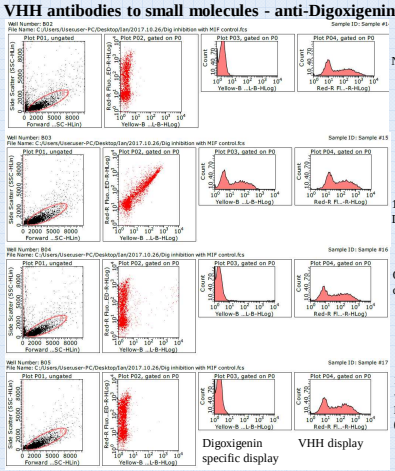


Figure 2. High surface expression of Digoxigenin VHH antibody (Red-R) and Digoxigenin antigen binding to VHH (Yellow-B). Low binding is shown when Dig-BSA is present for competition.

VHH to angiogenesis factors - anti-VEGF

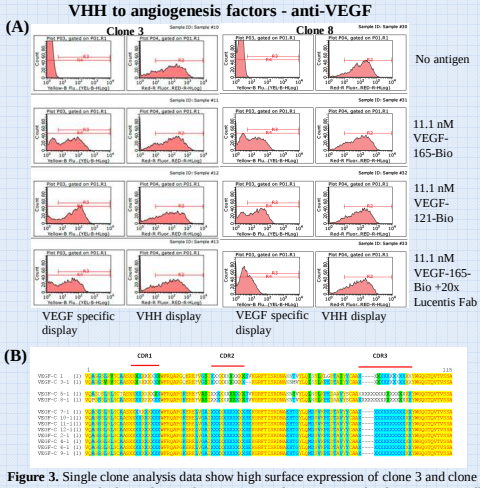


Figure 3. Single clone analysis data show high surface expression of clone 3 and clone 8 VEGF VHH antibody (Red-R) and VEGF (165 and 121) antigens binding to VHH (Yellow-B). VEGF antigen binding to clone 8 anti-VEGF VHH is reduced by 35% when antigen is blocked with Lucentis Fab, while there is only 4% reduction in clone 3 VHH binding antigen, reflecting that clone 8 and Lucentis bind to same epitope while clone 3 binds different epitope **(A)**. Different anti-VEGF VHH sequences and classes are generated to bind different epitopes **(B)**.

Anti-TfR VHH to engineer BBB-penetrating antibodies

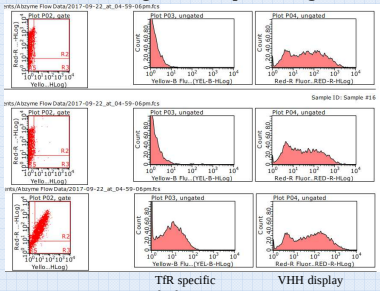
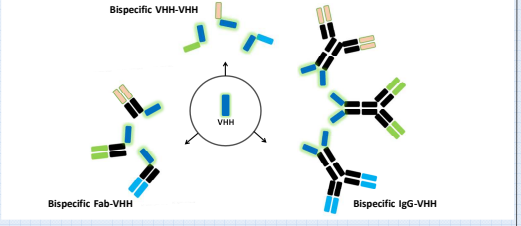
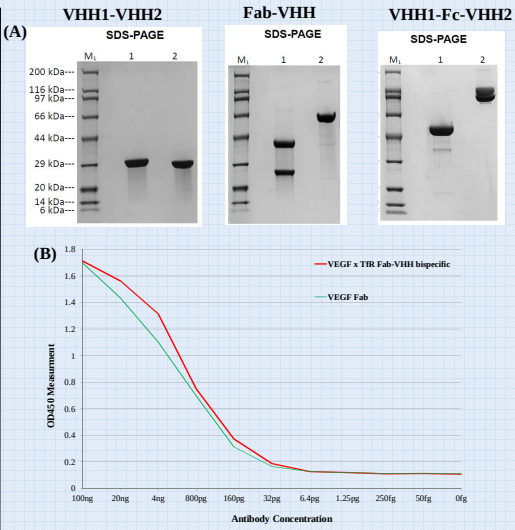


Figure 4. Single cell analysis data show high surface expression of TfR VHH antibody (Red-R) and TfR antigen binding to VHH (Yellow-B)

VHHs as modular antibodies to engineer bispecifics

We create a series of bispecific antibodies by combining existing antibodies with VHH in plug-and-play mode. To increase the probability of a reactive VHH in the bispecific antibody we isolate antigen specific llama VHH from a C-terminal expression library. The llama VHH fragment can be easily shuffled to other existing antibodies converting them into a well-expressed and easy-to-purify bispecific antibody.





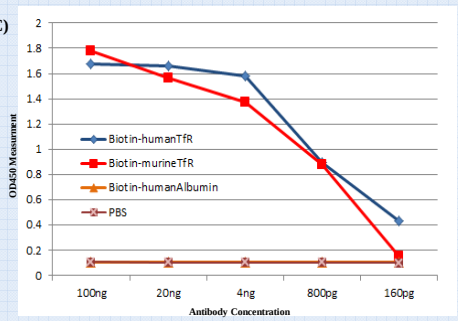


Figure 5. SDS-PAGE gels of purified bispecific antibodies in various formats **(A)**. ELISA data show similar binding activity of the monospecific anti-VEGF Fab and bispecific anti-VEGFxTfR Fab-VHH to VEGF antigen **(B)**. Bispecific antibodies in bispecific ELISA **(C)**.

The bispecific antibody recognizes both antigens simultaneously

To test the specificity of the bispecific antibodies, an ELISA plate was coated with one antigen, incubated with the bispecific antibody followed by the biotinylated 2nd antigen. The binding of the second antigen was detected with HRP coupled streptavidin. Our TFR antibody recognizes human and murine TFR.

1st Antigen	Antibody	2nd antigen	Detection agent	Signal
VEGF	VEGF x TFR	Bio-hTFR	Strep-HRP	2.85
VEGF	VEGF x TFR	Bio-mTFR	Strep-HRP	2.87
VEGF	anti-VEGF	Bio-hTFR	Strep-HRP	0.12
VEGF	anti-VEGF	Bio-mTFR	Strep-HRP	0.13
BSA	VEGF x TFR	Bio-hTFR	Strep-HRP	0.27
BSA	anti-VEGF	Bio-TFR	Strep-HRP	0.21

VEGF is comparably recognized by both antibodies

Antigen	Antibody	Detection agent	Signal
VEGF	Bispecific	Anti-Fab HRP	**3.43**
VEGF	Monospecific anti-VEGF	Anti-Fab HRP	**3.15**
BSA	Bispecific	Anti-Fab HRP	**0.11**
BSA	Monospecific anti-VEGF	Anti-Fab HRP	**0.09**

Abzyme's current pipeline

PROGRAMS	THERAPEUTIC INDICATIONS	DEVELOPMENT STAGE (DISCOVERY / OPTIMIZATION / PRECLINICAL / PHASE I / PHASE II)	COMMERCIAL RIGHTS/PARTNERS
NEURAMINIDASE	INFLUENZA		Abzyme, NIH
VIRAL GLYCOPROTEINS	VIRUS-INDUCED ENCEPHALITIS		Abzyme,
LYME/BISPECIFIC	LYME DISEASE		Abzyme, NIH
TFR-DIRECTED BISPECIFIC	CNS DISORDERS		
ANGIOGENESIS/BISPECIFIC INFLAMMATION/BISPECIFIC	OPHTHALMOLOGY		Abzyme
DIG-DIRECTED BISPECIFIC T CELL ENGAGER BISPECIFIC CHECKPOINT/BISPECIFIC ANGIOGENESIS/BISPECIFIC	IMMUNO-ONCOLOGY		Abzyme & UNDISCLOSED PARTNERS
UNDISCLOSED 6 TARGETS	IMMUNO-ONCOLOGY		VIBEX

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 is currently used to generate VHH antibodies to another 60 targets for therapeutic and diagnostic applications. We expect to conclude the antibody discovery programs for these 60 targets within 6-8 months.