Generation of Agonist and Antagonist Human Monoclonal Antibodies Against an Immune Checkpoint Target from the H2L2 Mouse

Joshua Lowitz, Glen Lin, Rick Chang, Jennifer Somera, Leonel Santibanez-Vargas, Julia Teplyuk, Catherine Vo, Emmeline Truong, Billy Nguyen, Gang Deng^, Yiping Rong^, Jiuqiao Zhao^, and John S. Kenney
Antibody Solutions, Sunnyvale, CA, USA & ^Harbour BioMed, Newton, MA, USA

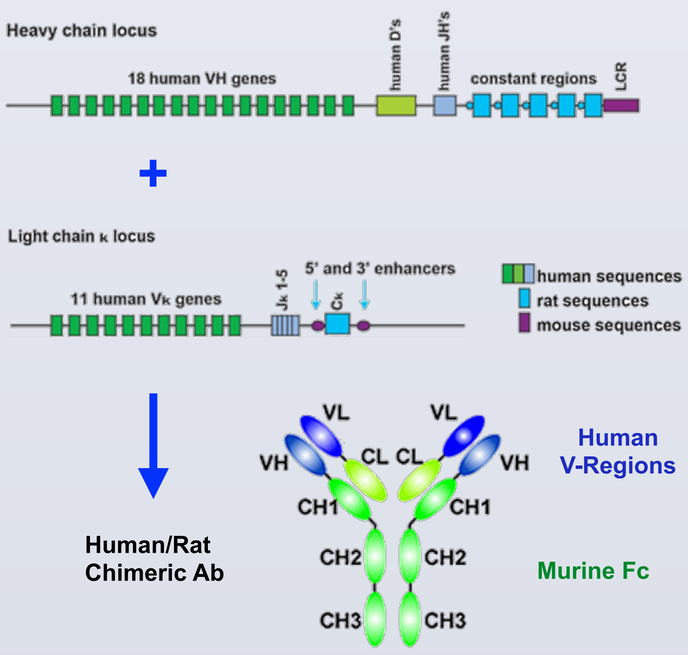
Introduction

Immunization of transgenic animals producing human antibodies is by far the most successful approach to obtaining "fully human" therapeutic antibodies. They allow for the rapid generation of candidate antibodies and the ability to transition lead candidates to clinical development without undergoing time consuming steps such as humanization or affinity maturation of antibodies from structural display libraries. In collaboration with Harbour Biomed and utilizing transgenic Harbour H2L2™ mice, Antibody Solutions has generated therapeutic human monoclonal antibody candidates against an immune checkpoint membrane protein target, designated "HBM-1".

The H2L2™ Mouse

The H2L2™ mouse is a "second generation" transgenic mouse engineered by Harbour Antibodies to produce antibodies with fully human V-region heavy and light chains and rodent constant regions to allow endogenous affinity maturation and immune effector function.

Harbour's H2L2™ mouse features an immune response comparable to normal mice and offers diverse human V-gene usage.



The H2L2™ mouse produces antibodies with human kappa light chains and rat IgG1, IgG2b, IgG2c, and IgM heavy chain isotype subclasses.

Hybridoma Library™ Platform

Antibody Solutions has adapted its Hybridoma Library™ antibody discovery platform for the Harbour H2L2™ Mouse.

The Antibody Solutions platform includes:

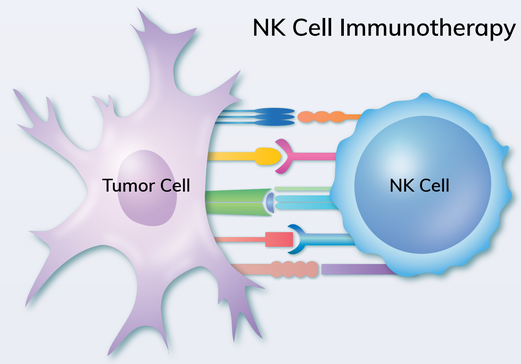
- Optimized immunization protocols (e.g., antigen design and selection, route, schedule and adjuvants).
- A robust hybridoma culture system.
- Single Cell FACS-cloning for true clones with no sub-cloning required.
- High-throughput cell-based screening for membrane protein targets.
- Ability to produce hundreds of clones within 3 months.

Combining Harbour's H2L2™ mouse with the Antibody Solutions Hybridoma Library™ process provides a superior platform for the generation of human therapeutic antibodies.

Presented at:

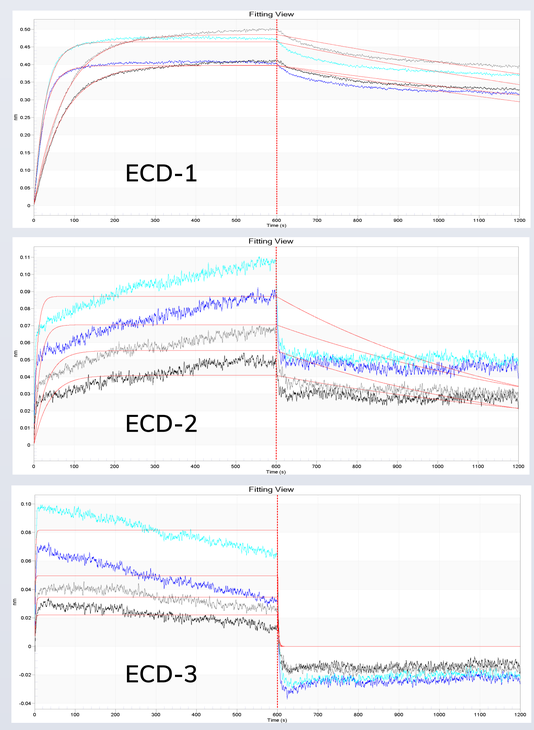
Cell Surface Antigen "HBM-1"

To evaluate the H2L2™ mouse for antibody drug discovery, an immune checkpoint molecule designated "HBM-1" was selected. "HBM-1" is a glycoprotein expressed on the surface of NK Cells. It has been shown to play a role in immune cell activation and is a therapeutic target.



Adapted from Cavanagh, M., et al. British Society for Immunology, 2018.

Three forms of the recombinant extracellular domain of HBM-1 were evaluated by Antibody Solutions in a ligand:receptor binding assays to confirm bio-activity.



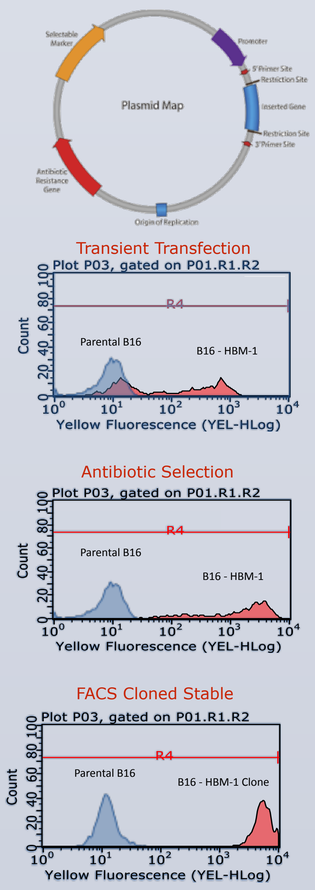
Label-free binding kinetics to evaluate HBM-1 antigen bio-activity via ForteBio Octet Red96

ECD-1 and ECD-2 both exhibited binding to receptor and thus were validated as suitable immunogens.

Membrane Antigen Cell Expression

The generation of cells expressing the HBM-1 target protein was a key element to a successful therapeutic antibody discovery campaign.

Design and Synthesis of Plasmid with Codon Optimization
↓
Transfection of Murine B16 cells
↓
Antibiotic-selection
↓
Cell surface Ag detection
Stain with HBM-1 Ctrl mAb
↓
FACS sorting High HBM-1 expressing clones
↓
Isolation of Stable clone
↓
Production of Cell Banks for screening (or immunization)



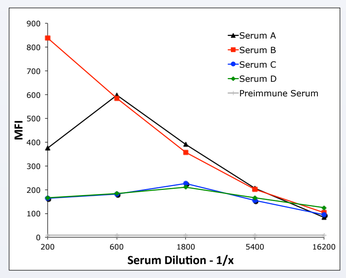
Results for Target "HBM-1"

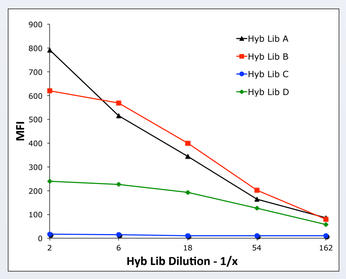
Immunization

- 2 forms of HBM-1 ECD
- 2 adjuvants
- 4 cohorts of H2L2™ mice

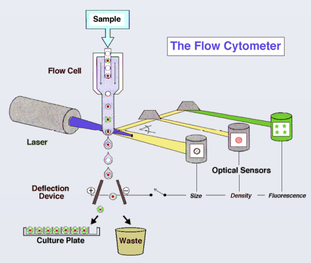
Generation of HBM-1 Hybridoma Libraries™ from each cohort

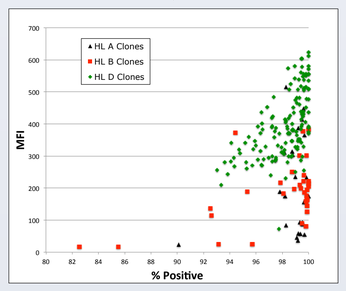
- All libraries exhibited binding to target by Flow and ELISA.
- Library A, B, and D had strongest cell binding and were selected to pursue clones.





Cloning of HBM-1 Hybridomas by FACS

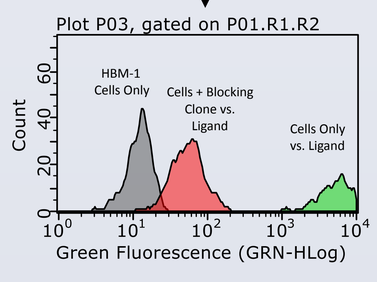




Clones screened by flow cytometry against HBM-1 expressing cells

Flow Cytometry Neutralizing Assay

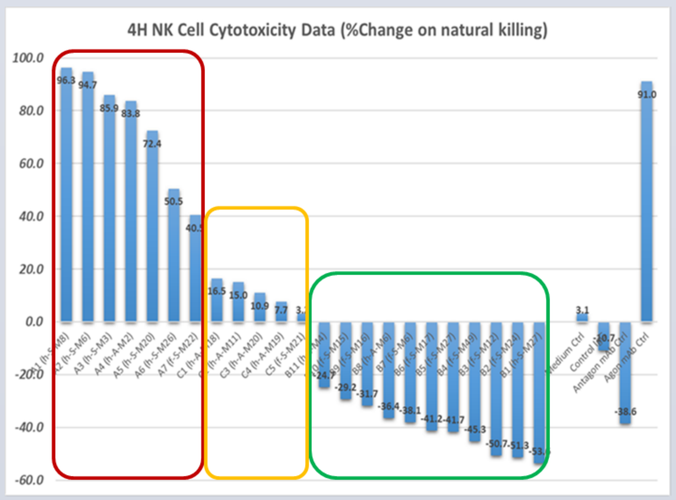
- HBM-1 binding clones were tested for ability to block ligand binding to HBM-1 expressing cells.
- Clones were also tested for cross-reactivity to cynomolgus monkey HBM-1.



H2L2™ Clones Against HBM-1

Hybridoma Library	Positive Clones Identified	Cross-reactive to Cyno. HBM-1	Expanded	Antagonists
Hyb Lib A	25	21	25	13
Hyb Lib B	64	57	30	16
Hyb Lib D	183	83	50	48
Total	272	161	105	77

Functional Cytotoxicity Assay for Agonist / Antagonist Activity



Human NK cells and fluorescently labeled target cells were incubated with purified HBM-1 H2L2™ mAbs and controls. The effect on NK cell killing activity was monitored by fluorescent cell imaging. Compared to baseline, increased cell killing (Group A) indicated putative agonist mAbs, while decreased cell killing (Group B) indicated putative antagonist mAbs.

Conclusions

Antibody Solutions has successfully used Harbour Antibodies' H2L2™ mouse in our Hybridoma Library™ platform to generate 272 monoclonal human antibodies to an immune checkpoint therapeutic target, "HBM-1". The panel of HBM-1 antibodies display a diverse range of target binding affinity by flow cytometry, cross-reactivity to cynomologus HBM-1, neutralization of ligand binding, and agonist/antagonist activity in a functional cell killing assay.