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 H2L2TM mice, Antibody Solutions has generated therapeutic human monoclonal antibody candidates against an immune checkpoint membrane protein target, designated "HBM-1".

The H2L2TM Mouse

The H2L2TM 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 H2L2TM mouse features an immune response comparable to normal mice and offers diverse human V-gene usage.

Hybridoma LibraryTM Platform

Antibody Solutions has adopted its Hybridoma LibraryTM antibody discovery platform for the Harbour H2L2TM Mouse.

- 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 H2L2TM mouse with the Antibody Solutions Hybridoma LibraryT process provides a superior platform for the generation of human therapeutic antibodies.

Presented at:

Cell Surface Antigen "HBM-1"

To evaluate the H2L2TM 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.

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.

Results for Target "HBM-1"

Immunization

- 2 forms of HBM-1 ECD
- 2 adjuvants
- 4 cohorts of H2L2TM mice

Generation of HBM-1 HybridomasTM from each cohort

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

Cloning of HBM-1 Hybridomas by FACS

- 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.

Flow Cytometry Neutralizing Assay

- HBM-1 binding clones were tested for cell killing and antagonsist activity.

Functional Cytotoxicity Assay for Agonist / Antagonist Activity

- Human NK cells and fluorescently labeled target cells were incubated with purified HBM-1 H2L2TM 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' H2L2TM mouse in our Hybridoma LibraryTM 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 cynomolgus HBM-1, neutralization of ligand binding, and agonist/antagonist activity in a functional cell killing assay.