PB001: Discovery Of Antibodies For Immunotherapy Of CRC Using A Proprietary Phage Display Platform

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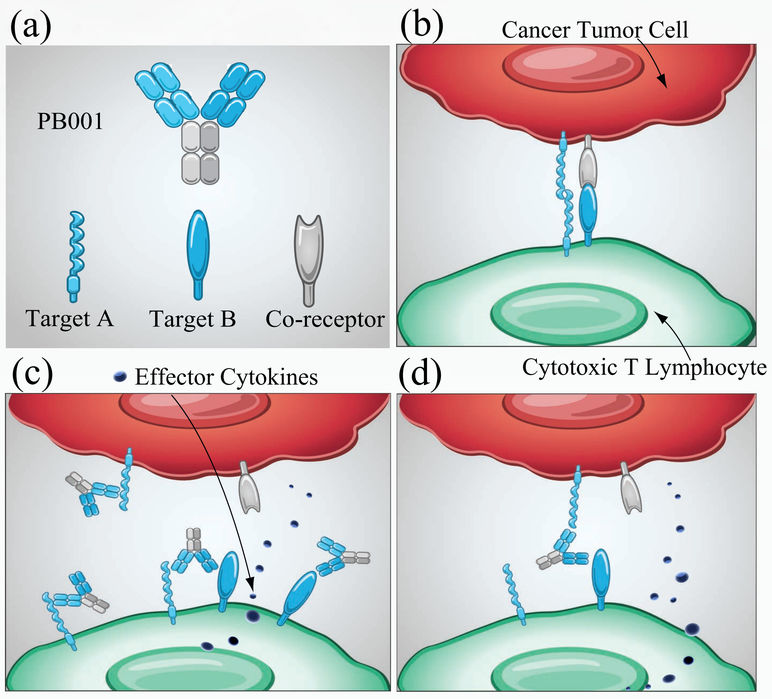
Summary

Discovery and development of a monoclonal antibody targeting a ***CRC-associated antigen (Target-A).***

>25 obtained unique clones were triaged based on their binding to antigen expressing cell lines, following developability assessment was performed. Selected characterization data are shown.

Identified candidates will be tested *in-vivo* which form part of a larger goal of the project ***PB001***: development of a ***bispecific antibody (targeting TA & TB) for immunotherapy of CRC. PB001 mechanism is presented.***

PB001: Mechanism of Action



-Targets are upregulated on activated T and NK-cells and are also expressed on various tumors.

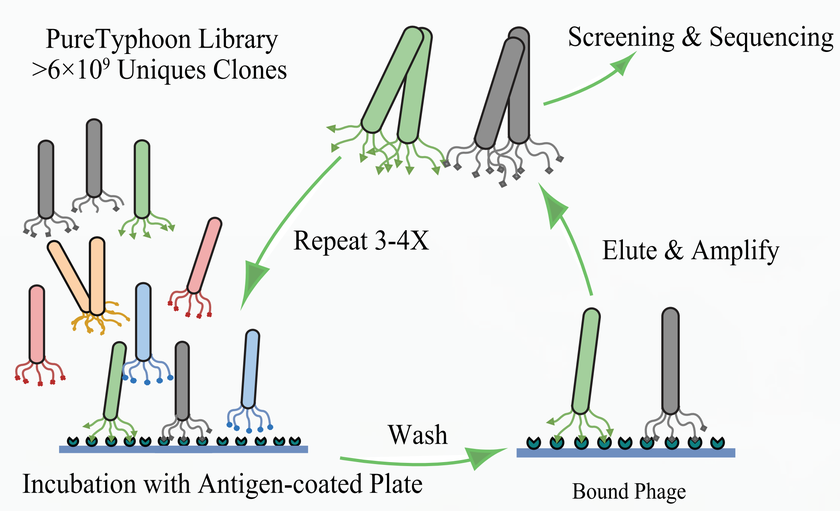
-Homophilic *trans* interaction of Target A (TA) prevents tumor cell lysis (b). Interaction with Target B (TB) leads to complete inactivation of T-lymphocytes.

-Cells exposed to a bispecific antibody inhibit homophilic and heterophilic interaction in *cis & trans* thereby activating T-cells (c, d).

Phage Display Platform

- Selections using proprietary ***PureTyphoon*** library on:
a) Antigen-expressing stable cell lines
b) In-house prepared recombinant proteins & domains thereof.

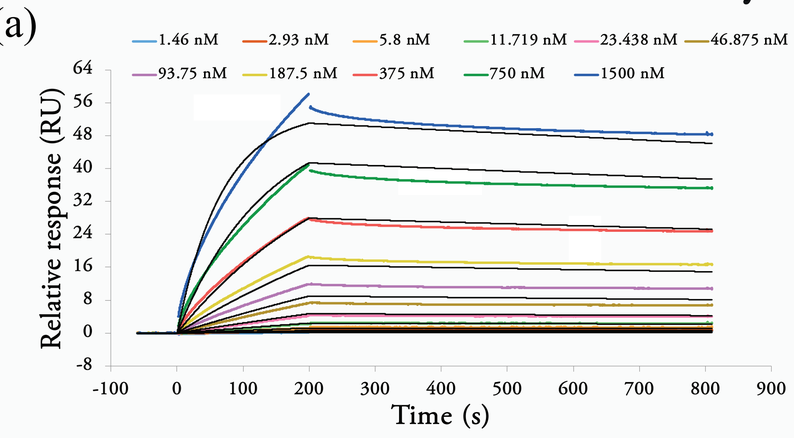
- Library specifics:
a) Display format: scFv displayed on P-IX or PIII phage coat protein
b) Scaffold selected for stability and developability
c) Diversity to mimic natural human antibody repertoire
d) Length and amino acid polymorphism



Characterization of Human mAbs

Interactions by SPR Analysis

(a)



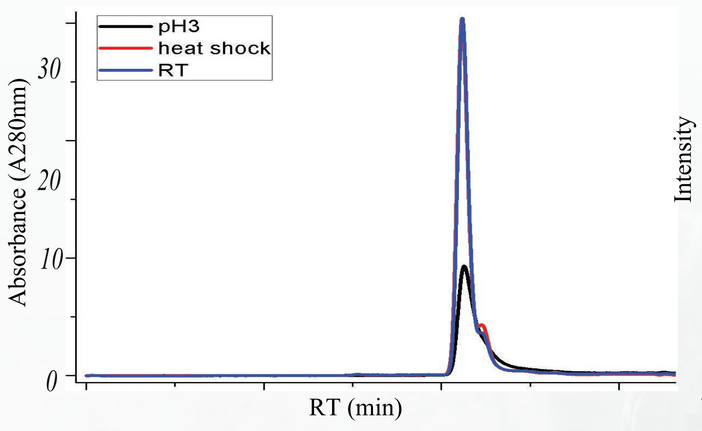
(b) Affinities calculated from a 1:1 binding model

Antigen	Anti-TA clone ID	K_D app
Target A (TA-NTD-FC)	TA2	17.0±5 nM

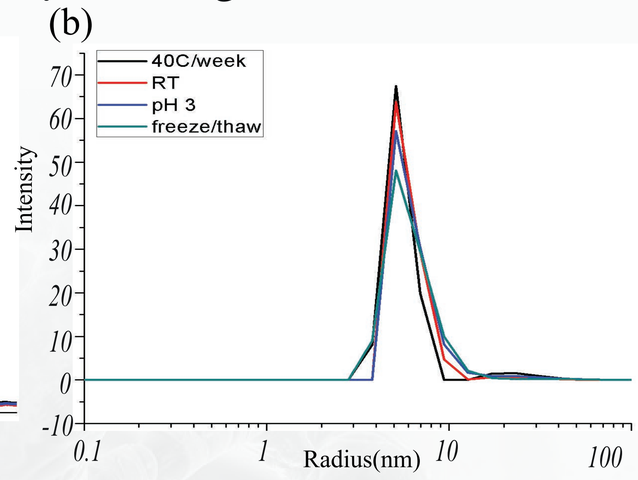
a) Multi-cycle kinetic titration of representative antibody clone ID TA2, amine-coupled as ligand, with target TA-NTD-Fc (1.5 nM-1500.0 nM). Colored lines indicate data measured in response to analyte injected at the concentrations given. **Black line:** best fits of measured data to the 1:1 binding model using default fitting parameters.

Developability Profiling

(a)

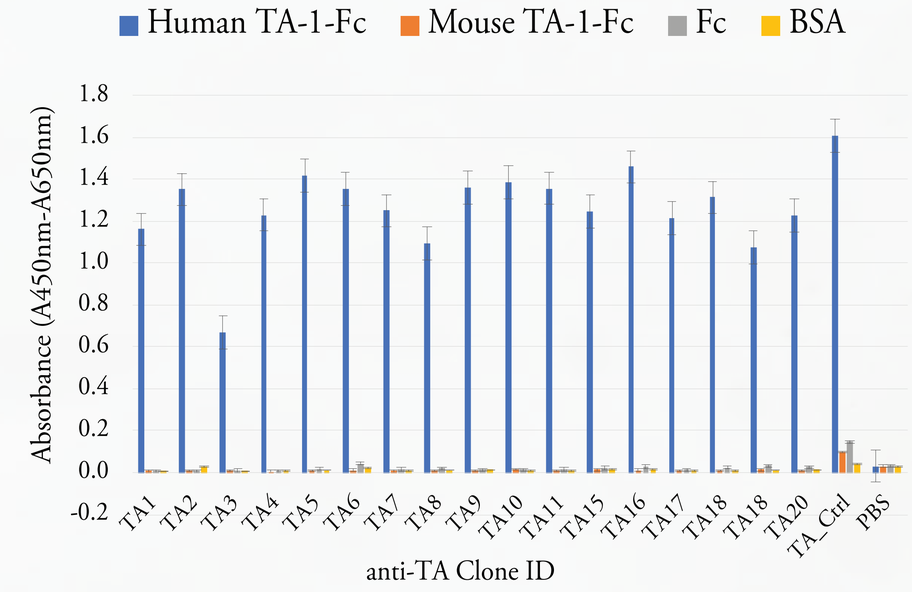


(b)



Analysis of forced degradation of IgG after acid and heat denaturation for a representative clone ID TA2. (a) SEC-HPLC chromatogram of pretreated sample conditions (n=3). **Black line:** Acid induced denaturation by sample incubation at pH3. **Red line:** Heat induced denaturation. **Blue line:** Sample without pretreatment. (b) DLS based characterization of IgG in stressed and native sample conditions.

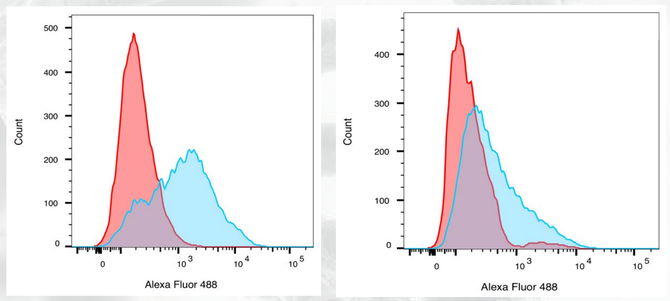
Anti-TA Fab-Phage Specificity Against Target-A (TA)



Binding Specificity by Flow Cytometry

(a) Staining: Jurkat Cells (b) Staining: CHO Cells

Flow cytometry staining of IgG TA2 on Jurkat and CHO lines.
Red histogram: wild type cell line.
Blue histogram: antibody binding to target A stable transfected cell line using a direct staining method.



Acknowledgement

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Conclusions

1)All tested antibodies recognize their cognate human antigen and do not recognize mouse ortholog. SEC & DLS analyses confirm monodispersity of these mAbs.
2)Antibodies are validated using flow cytometry for binding on cell surface.
3)Further characterization of IgGs is on-going (cell-based assays and in-vivo studies).

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