# PB001: Development Of A Fully Human Antibody Against A Key Immune Checkpoint Modulator

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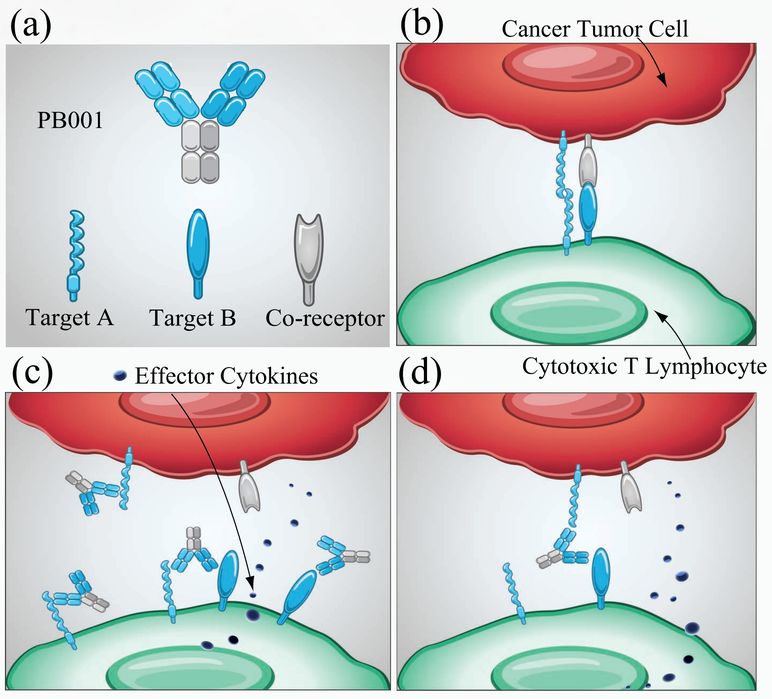
## Summary

Discovery and development of a monoclonal antibody targeting an immune checkpoint receptor (Target-B).

Several high affinity ($K_D$<60 nM) mAbs tested positive on stable lines by flow cytometry were characterized in-vitro. Selected characterization data for the mAbs are shown.

Identified candidates will be engineered with anti-Target A antibodies to make a ***bispecific antibody***, development of which forms a larger part of the ***project PB001;*** mechanism of action is presented.

## PB001: Mechanism of Action

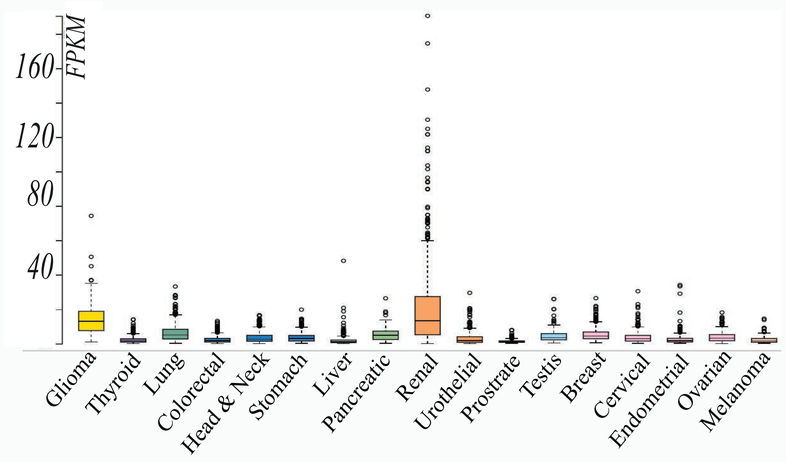



-Targets are upregulated on activated T and NK-cells and are also expressed on various tumors.

-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).

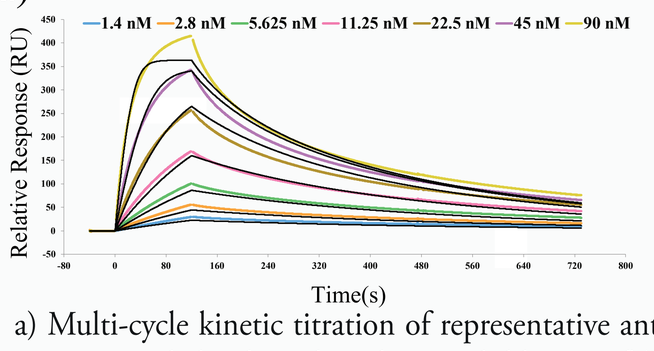
## Target-B Expression Profile




Target-B (TB) RNA-expression in cancer pathological state curated from TCGA database.

## Interactions by SPR Analysis

(a)




a) Multi-cycle kinetic titration of representative antibody TB3, amine–coupled as ligand, with a serial dilution of TB-ECD-Fc (1.4 nM – 90.0 nM). Colored lines indicate data measured in response to analyte injected at the concentrations given. Black lines indicate best fits of measured data to the 1:1 binding model using default fitting parameters.

(b)

| Antigen | Anti-TB clone ID | $K_D$ app |
|---|---|---|
| Target B (TB-ECD-FC) | TB1 | 59.0±5 nM |
| | TB3 | 5.27±5 nM |
| | TB4 | 6.84±5 nM |
| | TB5 | 10.3±5 nM |

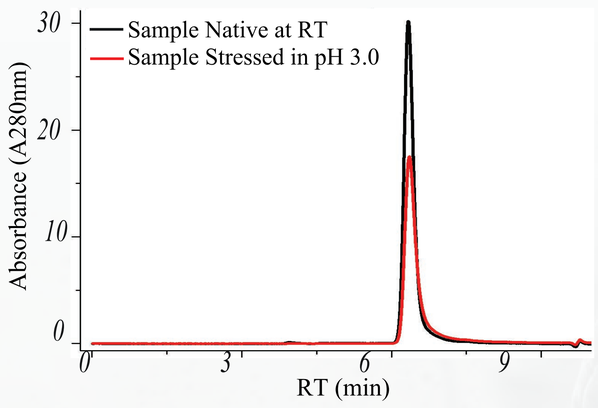
b) Affinities calculated from a 1:1 binding model for binding partners TB-ECD-Fc.

## Phage Display Campaigns

-Selection using proprietary scFv-displayed library on:
  a) Antigen-expressing stable cell lines
  b)Recombinant, in-house prepared full-length ECD proteins and domains thereof.
->*25* unique clones were obtained.
-Unique clones were triaged based on their binding to antigen-expressing tumor cell lines (flow cytometry).

## Biophysical Characterization

(a)




(b)

| Antigen | Anti-TB clone ID | Sample Treatment | MW (kDa) | Polydispersity % |
|---|---|---|---|---|
| Target B (TB) | TB1 | Native Sample at RT | 188 | 16.1 |
| | | Stressed at pH 3.0 | 176 | 12.0 |

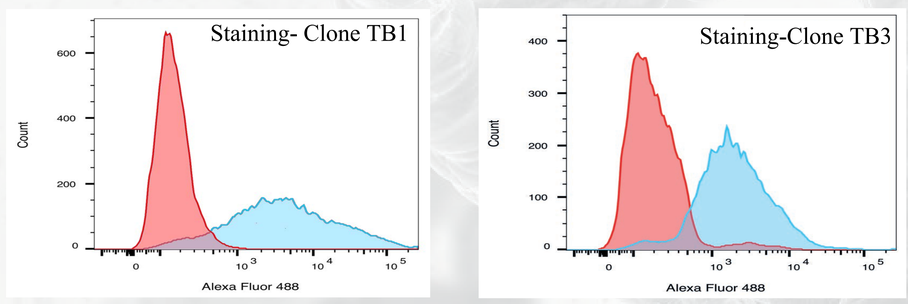
-SEC profiling of Clone ID TB1 (a).
-Orthogonal MW characterization & DLS polydispersity *(b)*.

## Anti-TB MAbs (IgG) Specificity Against Target-B (TB)




## Binding Specificity by Flow Cytometry

Flow cytometry staining of representative antibodies TB1 and TB3. Red histogram denotes wild type CHO cell lines. While the blue histogram denotes antibody binding to stable transfected CHO cell line with target antigen TB. Detection using a labeled secondary antibody as per direct flow staining approach.




## Acknowledgement

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## Conclusions

1)All MAbs recognize their cognate antigen with $K_D$s <60nM. SEC & DLS analyses confirm molecular mass and monodispersity of these MAbs.
2)All antibodies retain their specificity and binding properites on cells.
3)Further characterization of mAbs is on-going (cell-based assays and *in-vivo* studies).

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