Applying Immune Repertoire Capture® Antibody Discovery to Engineer Safe and Effective Tumor-Targeted 4-1BB Bispecifics

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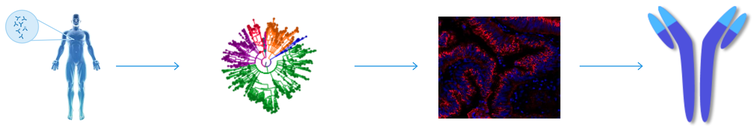
Background

- 4-1BB is a costimulatory receptor expressed on multiple immune cell types
- 4-1BB activation can induce anti-tumor responses, including increased T cell proliferation, release of granzyme and IFNγ, formation of immunological memory, and dendritic cell maturation
- 4-1BB can be activated by native 4-1BBL trimer or engineered anti-4-1BB IgG
- Early 4-1BB IgGs in clinical trials have led to severe hepatotoxicity or poor efficacy
- 4-1BBxTAA bispecific antibodies could be used to selectively activate 4-1BB in TME

Immune Repertoire Capture® for discovery of unique tumor-targeting antibodies



Plasmablast B cells collected from a patient with an anti-tumor immune response

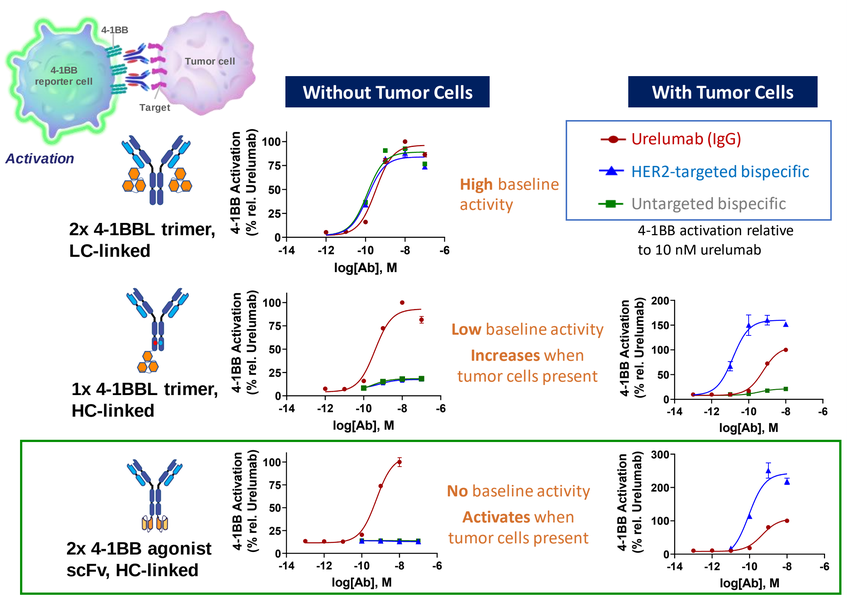
Antibody chains expressed by single plasmablasts were sequenced

Identified antibodies selectively binding to non-autologous human tumor tissues

Hit antibodies (target agnostic)

4-1BB weaponization formats to direct tumor dependent activation

FIGURE 1 – Tumor-targeted and untargeted 4-1BB bispecific molecules of different formats were tested for ability to activate 4-1BB reporter cells without and with tumor target cells



- scFv-based 4-1BB bispecific format exhibited better tumor-selective 4-1BB activation profile than 4-1BBL trimer designs; chosen as optimal format for future molecule design

Weaponized secondary method to rapidly assess 4-1BB bispecific competency from hit antibody library

FIGURE 2 – Anti-4-1BB weaponized anti-Fc molecule in combination with different primary IgGs were tested for activation of 4-1BB reporter cells with CT26 tumor target cells

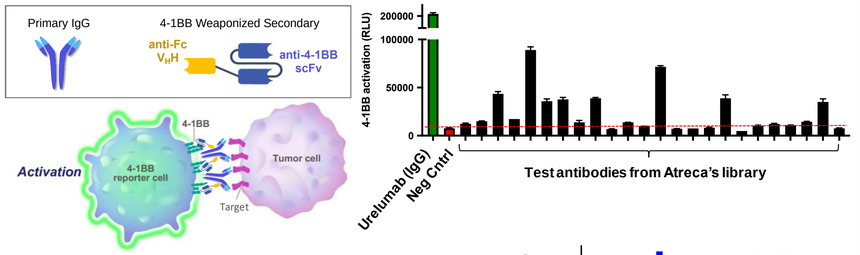


FIGURE 3 – Activation of 4-1BB reporter cells by primary IgG with anti-4-1BB weaponized secondary molecule with and without CT26 tumor target cells

- One antibody (X) had high level of 4-1BB activation without tumor target cells and was excluded from further development

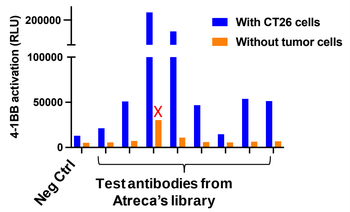
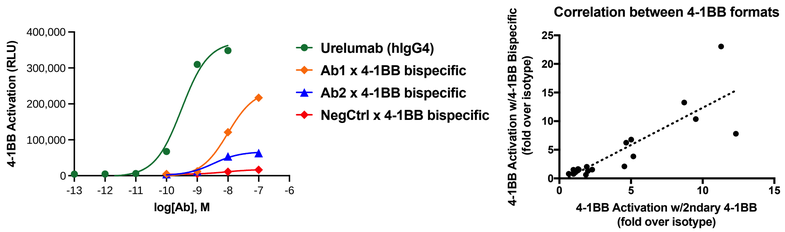


FIGURE 4 – Validation of 4-1BB activation by 4-1BB bispecific antibodies that had been identified using weaponized secondary molecule screening approach

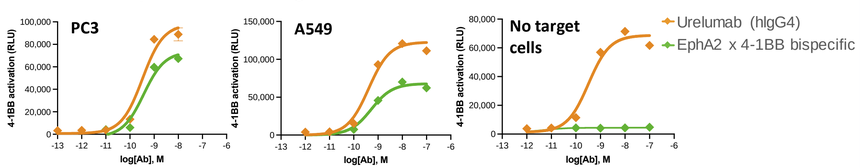


- Unmodified IgGs in combination with 4-1BB weaponized secondary molecules successfully predict ability to stimulate 4-1BB activation with 4-1BB bispecific molecules
- A library of IgG antibodies can be screened for 4-1BB activity and target-specific activity prior to making bispecific molecules

Discovery of novel EphA2 x 4-1BB bispecific antibody with target-dependent 4-1BB activation

- A hit antibody with activity in secondary 4-1BB assay found to target EphA2, a well-known cancer antigen overexpressed in many patients, determined by membrane protein array
- Antibody binds novel conformational epitope, identified by yeast display and x-ray crystallography

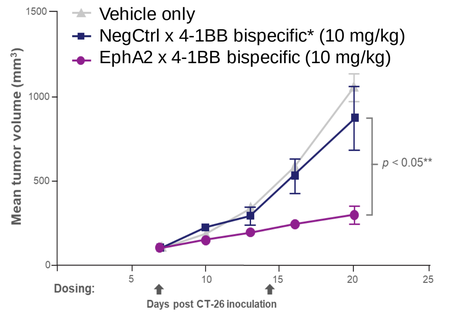
FIGURE 5 – *In vitro* activation of 4-1BB reporter cells by EphA2 x 4-1BB bispecific with and without EphA2+ tumor target cells



EphA2 x 4-1BB bispecific antibody has potent anti-tumor activity without safety signals

FIGURE 6 – CT26 syngeneic tumor growth in response to EphA2 or untargeted 4-1BB bispecifics

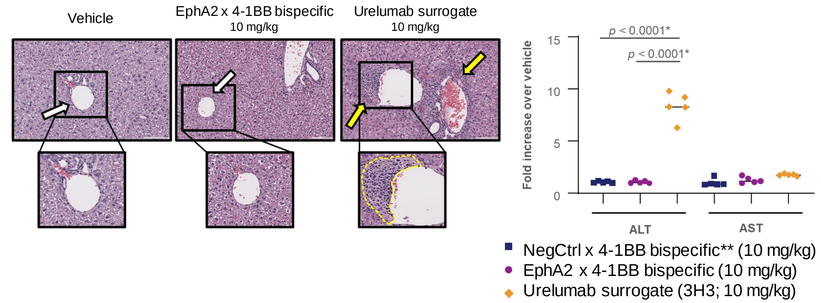
- Treatment of established tumors
- Robust effect of EphA2 x 4-1BB on tumor growth compared to untargeted 4-1BB
- No body weight loss or other adverse clinical signs noted



*Anti-hen egg lysozyme "targeting" 4-1BB bispecific, control for EphA2 targeting of 4-1BB.
**One-sided Wilcoxon rank sum test for normalized area above the curve at end of study.

FIGURE 7 – Liver toxicity in animals treated with EphA2 x 4-1BB bispecific

- No signs of liver inflammation observed with EphA2 x 4-1BB by histological analysis
- EphA2 x 4-1BB did not increase ALT *in vivo* like the Urelumab surrogate



*One-way ANOVA. **Anti-hen egg lysozyme "targeting" 4-1BB bispecific, control for EphA2 targeting of 4-1BB.

Conclusions

- Atreca's Immune Repertoire Capture® technology allows a target agnostic way of identifying novel antibodies for targeting cancer cells
- *In vitro* 4-1BB activation found to be more tumor-selective by 4-1BB bispecifics containing bivalent agonistic scFvs than trimeric 4-1BBL fusion designs
- A 4-1BB weaponized secondary method can be used to identify potential new antibodies that can induce 4-1BB activation before generating bispecific molecules
- A unique EphA2 targeting antibody was identified and was engineered into a 4-1BB bispecific with tumor-specific 4-1BB activation and *in vivo* efficacy without liver toxicity

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; EphA2, erythropoietin-producing hepatocellular receptor A2; Fc, fragment crystallizable region; HC, heavy chain; IgG, immunoglobulin G; IFNγ, interferon gamma; IP, intraperitoneal; LC, light chain; RLU, relative light unit; scFv, single chain fragment variable; TAA, tumor-associated antigen; TME, tumor microenvironment; V, variable.