Rapid and efficient generation of format-diverse co-stimulatory CD28 and CD3 multispecific antibody panels for T cell engagement via complementary technologies

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ADIMAB

BACKGROUND

Bispecific and multispecific antibodies are a rapidly growing segment of the therapeutic biologics landscape.

Adimab has developed multiple technologies that enable the assembly and production of multispecific antibodies. Many of these technologies are particularly applicable to the subset of multispecific antibodies known as T cell engagers (TCE):

- Mutations that direct desired HC-HC and HC-LC pairing[1]
- High-throughput production of multispecifics via Chain Exchange (ChEx)
- A broad affinity panel of anti-CD3 antibodies[2]
- Format-diverse anti-CD28 costimulatory antibodies
- Redirected T cell cytotoxicity (RTCC) assays to functionally evaluate TCE molecules[2]
- Fc-silencing solutions[3]

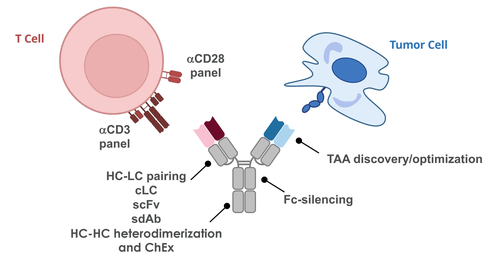

Figure 1. Representation of Adimab's toolkit in the context of TCEs

ADIMAB HC-HC HETERODIMERIZATION AND HC-LC PAIRING TECHNOLOGY FACILITATES PRODUCTION OF MULTISPECIFICS

Yeast-based and computational approaches were used to discover novel HC-LC and HC-HC mutations to specifically assemble IgG-like multispecific antibodies[1]. Pairing mutations are in the CH1, Ck, and CH3 domains respectively. A therapeutic multispecific antibody containing these mutations is currently in clinical trials[4]. An additional CH1-only mutation set drives preferential pairing with wild-type Cλ vs. Ck (not shown).

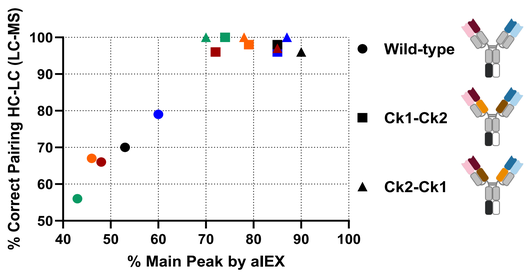

Figure 2. Analytical Ion Exchange (aIEX) and Fab LC-MS analysis for transient CHO transfections of VH and VL regions from 5 pairs (by color) of marketed antibodies. Adimab HC-HC heterodimerization mutations are present in all samples; transfections utilized equimolar (1:1:1:1) plasmid concentrations encoding each IgG subunit.

EFFICIENT, HIGH-THROUGHPUT GENERATION OF MULTISPECIFIC ANTIBODY PANELS

Adimab Chain Exchange (ChEx) has been applied to generate high quality panels of bispecific and multispecific antibodies..

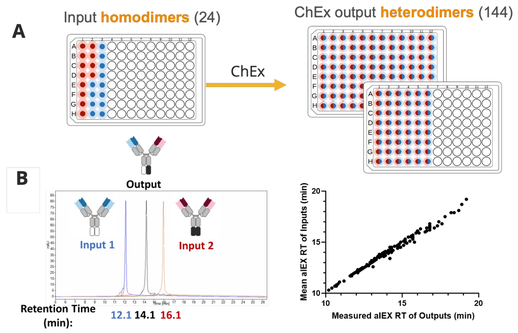

Figure 3. A. ChEx is used to generate up to 144 multispecific antibodies from 24 monospecific inputs. B. The measured analytical ion exchange (aIEX) retention time (RT) of output multispecifics are confirmed to be the mean retention time of the input molecules.

ADIMAB'S TOOLKIT ENABLES EVALUATION OF MULTISPECIFICS WITH INCREASING COMPLEXITY

Adimab's chain pairing technologies have been used to create antibodies of varying geometries and valencies, including asymmetrical molecules.

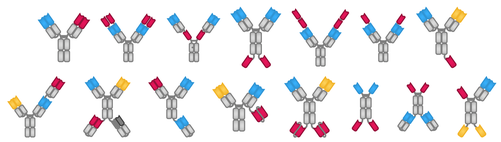

Figure 4. Example molecules made using Adimab technology

ADIMAB anti-CD3 PANEL OFFERS CYNO CROSS-REACTIVITY AND TUNABLE POTENCY

In vivo discovery followed by humanization and yeast-based optimization generated a broad affinity a-CD3 panel with excellent developability properties[2].

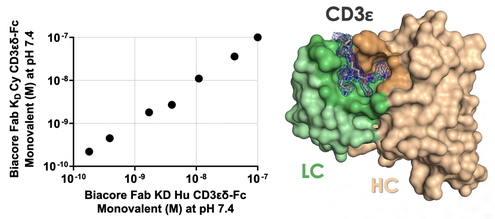

Figure 5. Adimab's anti-CD3 antibody lineage has human-cyno cross-reactivity across a broad range of affinities and binds to the N-terminal portion of CD3ε[2].

ADIMAB anti-CD28 PANEL OFFERS ENHANCED RTCC WITH FORMAT FLEXIBILITY

Anti-CD28 antibodies were isolated from diverse library types (Kappa, Lambda, CD3 cLC, and HCAb)

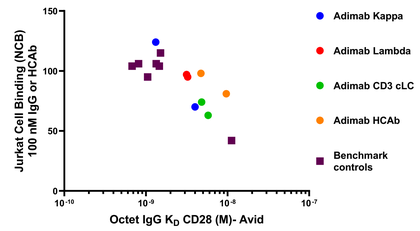

Figure 6. Normalized cell binding (NCB) to Jurkat cell line vs. CD28 affinity for lead antibodies isolated from indicated libraries.

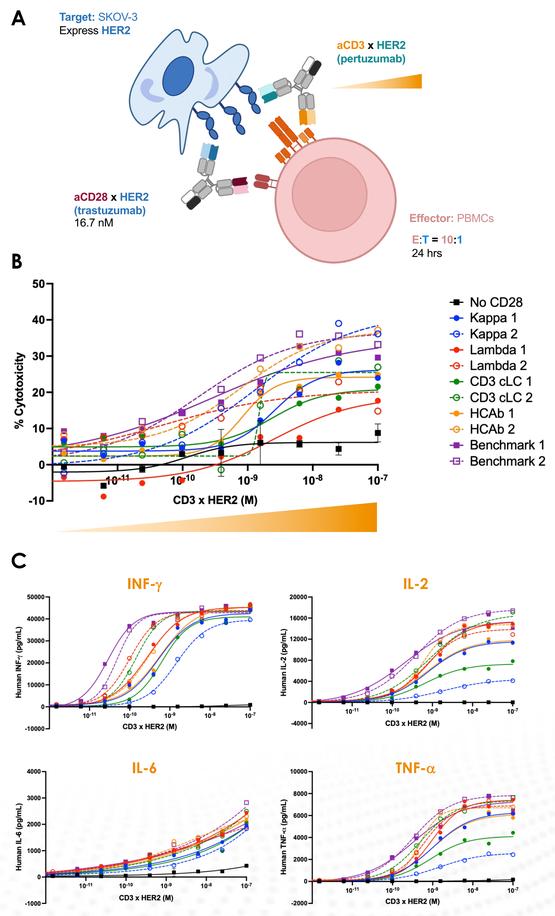

Figure 7. CD28 costimulation potentiates CD3 x HER2 RTCC A. Representation of RTCC assay set-up B. % cytotoxicity and C. secreted cytokines under RTCC assay conditions (E:T 10:1, 24 hrs) with ADI CD3 (K_D- 100 nM) with or without CD28 costimulation

ADIMAB anti-CD28 PANEL OPTIMIZATION YIELDS TUNABLE COSTIMULATION OPTIONS

Lead anti-CD28 antibody lineages (Figures 6,7) were optimized via CDRH1 and –H2 diversification. Variants with improved ("strong") and detuned ("weak") affinity were selected and characterized.

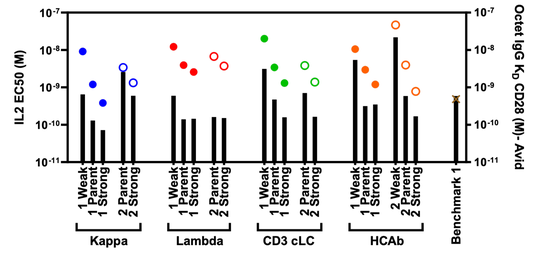

Figure 8. Affinity optimization of anti-CD28 lineages yields variants with variable costimulation. IL-2 EC50s (circles) determined for RTCC assay as described in Figure 7A and affinity (markers) was determined for each IgG.

SUMMARY

Multispecific antibodies offer promise in many therapeutic contexts. Adimab has developed an exemplary set of technologies that demonstrate the ability to direct desired antibody chain pairing (HC-HC and HC-LC), isolate and engineer single-domain antibodies, and generate large panels of multispecific antibodies with diverse topologies. Specifically, we illustrate these capabilities in the context of TCE multispecifics that leverage our affinity- and developability-optimized anti-CD3 and anti-CD28 antibody panels.

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