Diverse CD28-binding antibodies for costimulatory T-cell engager development

AUTHORS

Katherine Lam, Shirley Zhi, Lucas Kraft, Elena Vigano, Wei Wei, Cristina Faralla, Esther Odekunle, Melissa Cid, Stefi Carrara, Creagh Briercliffe, Kelly Bullock, Ingrid Knarston, Gesa Volkers, Michael Kennedy, Grace Leung, Bryan C. Barnhart, **Lindsay DeVorkin***, Sherie Duncan

**presenter*

AUTHOR AFFILIATION

AbCellera, Vancouver, Canada

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BACKGROUND

Optimal T-cell activation in the tumor microenvironment requires costimulatory signals

The anti-tumor activity of T cells, initiated via the T-cell receptor (TCR), can be enhanced through costimulatory engagement of the CD28 co-receptor.[1] This costimulation can be leveraged therapeutically using CD28-binding antibodies to improve the efficacy of T-cell activating strategies, such as CD3 T-cell engagers (TCEs), in the tumor microenvironment (Fig. 1).[2,3]

Costimulatory strategies

MHC-dependent bispecific | MHC-independent multispecifics

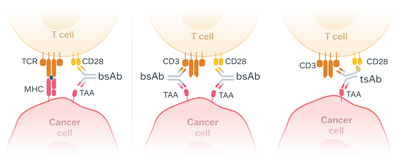

Figure 1. CD28-binding antibodies can enhance anti-tumor activity in combination with T-cell activating therapeutics.

AIM

Generate costimulatory molecules with potential for improved safety and efficacy profiles

CD28-costimulatory antibodies are a promising class of immunotherapies for diverse tumor targets, yet clinical challenges remain.[4,5]

To address these challenges, our aim was to develop a diverse set of CD28-binding IgG and heavy-chain only antibodies (HCAbs) that:

- do not show superagonist activity — a property associated with CD28-mediated toxicities
- have diverse binding properties for the development of immunotherapies across tumor targets and modalities

OUTCOME

Functionally diverse CD28-binding antibodies for T-cell engager development

We generated a panel of CD28-binding HCAbs and IgG antibodies. These antibodies bind a broad range of epitopes, leading to diverse functional activity that is clearly differentiated from benchmarks with known toxicity issues.

Combining these molecules with other T-cell activating strategies represents an opportunity to fine-tune novel costimulatory TCEs for diverse tumor targets.

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CD28-binding antibodies enhance T-cell activity without superagonism

Broad range of costimulatory T-cell activity with differentiation from clinical benchmarks

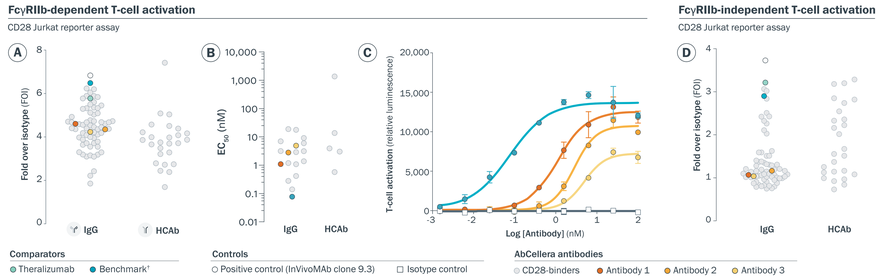


Figure 2. CD28 activation in the presence or absence of FcγRIIb confirms that antibodies are conditional CD28 agonists, with the majority showing lower crosslinking-independent activation of effector cells than superagonist benchmarks.

(A) 92 antibodies, generated with human IgG1 Fc, were tested for CD28 FcγRIIb-dependent activation. Antibodies were incubated with effector cells endogenously expressing TCR, CD3, and CD28, and CHO-K1 cells engineered to express FcγRIIb and a TCR-engaging protein (Promega JA9331). Relative luminescence for each antibody was normalized to the isotype control (fold over isotype ⩾ 1 considered positive). Three example antibodies (1-3) with diverse functional responses are highlighted. **(B)** 22 antibodies were selected for a 1-in-4, nine-point titration series starting at 100 nM, EC_{50} values are shown. **(C)** Relative luminescence for example antibodies, benchmark, and a human IgG isotype control are shown. **(D)** FcγRIIb-independent agonist activities were similarly assessed using CHO-K1 cells engineered to express a TCR-engaging protein, but not FcγRIIb. *†Bivalent monoclonal CD28 benchmark antibody generated using sequences from REGN5678 patent.[6]*

CD28-binding antibodies do not show superagonist activity

Cytokine release profiles

PBMCs cultured with CD28-binding antibodies

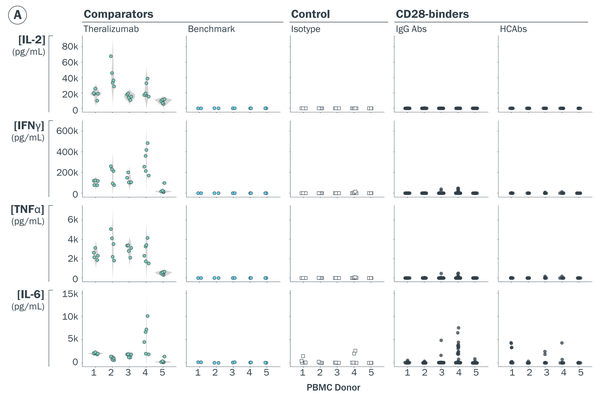
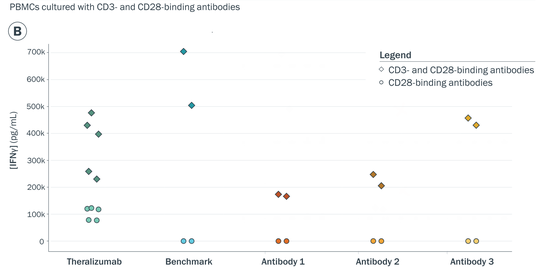


Figure 3. CD28-binding antibodies do not show superagonist activity when mixed with peripheral blood mononuclear cells (PBMCs) from healthy donors. (A) Cytokine release profiles are shown for five PBMC donors that were cultured with wet-coated antibodies (five replicates/antibody, 22 total AbCellera CD28-binders), as previously described.[7] Values below the lower limit of detection are shown at y=0. Theralizumab is a known CD28 superagonist molecule. Antibodies were generated as a human IgG1 with a N297A mutation.

IFNγ release profiles

PBMCs cultured with CD3- and CD28-binding antibodies



(B) In a proof-of-concept study for MHC-independent costimulation (Fig. 1), PBMC donors were cultured with a combination of plate-bound CD28- and soluble CD3-binding (OKT3) antibodies. A single PBMC donor and representative cytokine, IFNγ, is shown. The costimulatory effect of CD28-binding antibodies was within the range of clinical benchmarks, as shown for three example antibodies.

Novel HCAb and IgG antibodies with diverse binding properties

479 HCAb and IgG antibodies with high sequence diversity

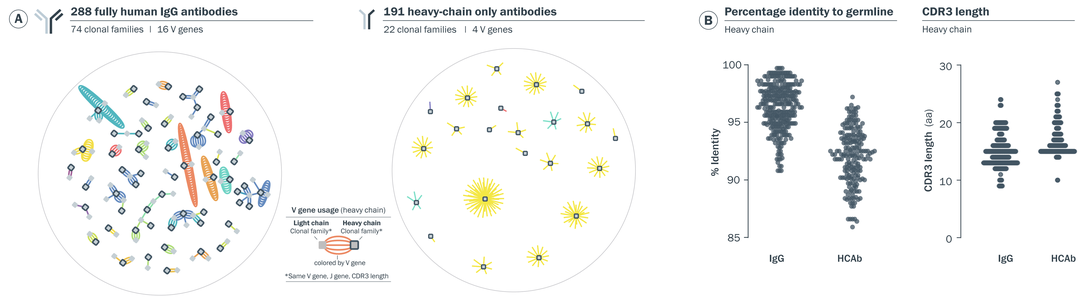
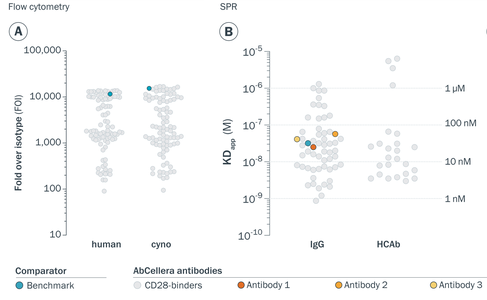


Figure 4. 479 CD28-specific HCAb and IgG antibodies were identified from immunization and deep single-cell screening. (A) To discover the functional antibodies in the above figures, we immunized humanized mice and camelids to identify IgG and HCAbs, respectively, that bind to both human and cyno CD28. Antibody sequence diversity was visualized using Celium™. **(B)** Antibodies showed a broad range of percentage identity to germline and CDR3 length, highlighting diversity in sequences obtained.

CD28-binding antibodies with epitopic diversity and a range of avidities

Cell binding

Flow cytometry



Avidity

SPR

Epitope community analysis

Compared to benchmark CD28 antibody

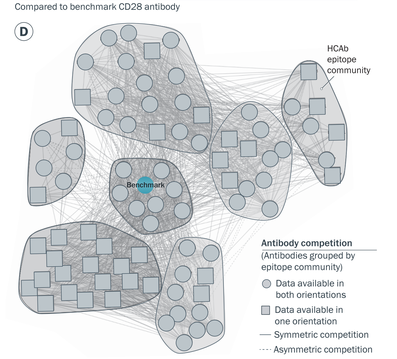


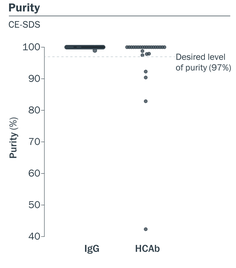
Figure 5. Antibodies showed diversity in epitopes and binding avidities. (A) A subset of cross-reactive antibodies (65 IgGs and 27 HCAbs, at 25 nM) were selected for expression and assessed for binding to human and cyno CD28-expressing cells. The median fluorescence intensity for each antibody was normalized to the isotype control, with positive binding (fold over isotype ⩾ 10) confirmed in 90 of the 92 antibodies. **(B)** Avidity was measured using the CD28 extracellular domain by surface plasmon resonance. Antibodies showed diverse binding avidities, ranging from 870 pM to 6.40 μM. **(C)** Sensorgrams show three example IgG antibodies (1-3) with diversity in on and off rate.

(D) Epitope binning was performed, with communities determined by ward.D hierarchical clustering based on competition events. 81 antibodies clustered in seven epitope communities and the majority of antibodies compete with the benchmark for binding to CD28, consistent with the limited extracellular space.

Biophysical characterization shows antibodies are of high quality

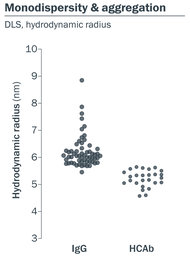
Purity

CE-SDS



Monodispersity & aggregation

DLS, hydrodynamic radius



Thermal stability

nDSF

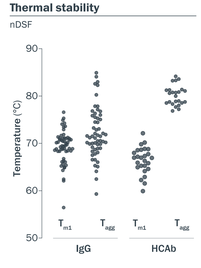


Figure 6. Preliminary analytical and biophysical characterization reveals favorable early developability properties. The majority of antibodies showed a high level of purity (> 97%), a hydrodynamic radius within the expected range (IgGs: 5.3-6.5 nm; HCAbs: 4.7-6.3 nm), and typical thermal stability profiles.