# STREAMLINING T CELL ENGAGER DEVELOPMENT WITH A DIVERSE PANEL OF FULLY HUMAN CD3-BINDING ANTIBODIES, BISPECIFIC ENGINEERING TECHNOLOGY, AND AN INTEGRATED DISCOVERY ENGINE

Bryan C Barnhart, AbCellera
bo.barnhart@abcellera.com
Lindsay DeVorkin, AbCellera
Juntao (Matt) Mai, AbCellera
Kate Caldwell, AbCellera
Tim M Jacobs, AbCellera
Raffi Tonikian, AbCellera
Karine Herve, AbCellera
Yuri Hwang, AbCellera
Cristina Faralla, AbCellera
Wei Wei, AbCellera
Emma Lathouwers, AbCellera
Sherie Duncan, AbCellera
Aaron Yamniuk, AbCellera
Kush Dalal, AbCellera

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CD3 T cell engagers have the potential to be a cornerstone of immuno-oncology. However, a limited pool of CD3-binding antibodies and technological challenges in engineering bispecifics have hindered development. Discovering effective T cell engagers requires two target-binding arms — a CD3 arm that fine-tunes T cell activation and a tumor arm with high specificity for cancer cells — optimized as a whole to work in concert with each other. Beginning with diverse panels of antibodies increases the probability of finding appropriately potent and developable T cell engagers and reduces the need for downstream engineering.

We used microfluidic technology to screen more than 3.5 million single cells from humanized mice and identified >200 CD3-specific antibodies. Using high-throughput assays, we determined affinity for CD3εδ and CD3εγ, cross-reactivity to human and cyno primary T cells, CD3 binding kinetics, and epitope bins. We assessed T cell activation by measuring CD25 and CD69 expression by flow cytometry. We then used our bispecific engineering platform, OrthoMabTM, to generate a proof-of-concept panel of CD3 x EGFR bispecific antibodies. Developability properties were assessed, including hydrophobicity (aHIC), self-association (AC-SINS), polyspecificity (BVP-ELISA), stability (nanoDSF), and aggregation (aSEC). CD3 T cell engager potencies were measured using an NFAT reporter T cell activation assay and an xCELLigence tumor cell killing assay, and cytokine release was assessed by FLEXMAP CD.

We identified hundreds of fully human CD3-specific antibodies that are diverse, developable, and validated. The antibodies displayed a wide range of CD3 binding affinities (KD ~1 nM to 1 μM), binding kinetics, and T cell activation potencies (EC50 ~6 to 190 nM). Data on this novel panel includes epitope binning, which revealed human and cyno cross-reactive CD3-binders that are distinct from previously described cross-reactive antibodies. The antibodies were assessed using a range of biophysical assays and have favorable developability properties. In a proof-of-concept study, we used OrthoMabTM to generate a panel of CD3 x EGFR bispecific antibodies. The resulting bispecifics have favorable developability properties, and displayed a wide range of antigen-dependent T cell activation (EC50 ~2 pM to 2 nM) and tumor cell killing potencies (EC50 ~0.01 to 1 nM). From this panel, we expanded our analysis to identify potent T cell engagers that achieved >90% tumor cell killing with low levels of cytokine release.

By integrating our diverse panel of CD3-binding antibodies with our bispecific engineering and high-throughput antibody assessment capabilities, we identified developable CD3 T cell engagers with potent tumor cell-killing activity and minimal cytokine release.