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

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Background 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.

Methods 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 CD3 $\epsilon\delta$ and CD3 $\epsilon\gamma$, 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 FLEX-MAP CD.

Results 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 ($K_D \sim 1$ nM to 1 μ M), binding kinetics, and T cell activation potencies ($EC_{50} \sim 6$ to 190 nM). Data on this novel panel includes epitope binning, which revealed human and cyno 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 an expanded proof-of-concept study, we used OrthoMabTM to generate a panel of CD3 x EGFR bispecific antibodies. The resulting bispecifics had favorable developability properties, and displayed a wide range of antigen-dependent T cell activation ($EC_{50} \sim 2$ pM to 2 nM) and tumor cell killing potencies ($EC_{50} \sim 0.01$ to 1 nM). From this panel, we identified potent T cell engagers that achieved >90% tumor cell killing with low levels of cytokine release.

Conclusions By integrating our 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.

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