

TUNING THE CELL-CELL DISTANCE VIA THE NON-SIGNALING EXTRACELLULAR SPACER DOMAIN OF CHIMERIC ANTIGEN RECEPTORS IS CRITICAL FOR OPTIMAL ACTIVITY

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Background Chimeric antigen receptors (CARs) are synthetic receptors that target engineered immune cell effector functions against target cells expressing specific antigens. CAR activity can be significantly improved by optimization of multiple parameters, including geometry of the immunologic synapse, biophysical properties of the extracellular domains, and signaling properties of the intracellular domains. Native T cell activation is driven by adhesion molecules and T cell Receptors (TCRs) binding to peptides displayed on the Major Histocompatibility Complex (MHC) of target cells, with a well-defined cell-cell distance of 14–15 nm in synaptic contact areas. However, the optimal synaptic distance, and the importance of cell-cell synaptic distance for CAR activity has not been systematically determined. Here we investigate the role of spacer length on the recognition of clinically relevant tumor antigens using a panel of 4 previously-published spacers and 41 novel spacers derived from human extracellular proteins and ranging in length from 3.6–30.6 nm.

Methods We tested all of these spacers with five known CAR targeting domains that bind epitopes at varying distances from the cell membrane on the target cells, including three ROR1 CARs (R11, R12, 2A2), one CD19 CAR (FMC63) and one HER2 CAR (herceptin). Additionally, we developed a model system in which a single linear epitope could be systematically presented at different distances from the target cell membrane using our spacer sequences. In this model, an anti-HA scFv (clone 2E2) was used as the CAR binding domain and an HA peptide (YPYDVPDYA) was used as a model epitope. We tested all possible CAR constructs in vitro by evaluating cytokine production and target cell killing kinetics (primary and serial restimulation). Additionally, we tested whether our in vitro observations are predictive of in vivo performance by choosing five spacers that cover a wide range of performance for two CARs with different predicted optimal spacer lengths in the in vitro study (R12, FMC63) and tested them in mouse xenograft models.

Results We demonstrate that both in vitro and in vivo CAR activity is dependent on spacer length, with optimal activity observed at a synaptic distance of about 20 nm, substantially longer than the 14–15 nm TCR:MHC complex. Furthermore, the optimal range of synaptic distances is far narrower than previously appreciated.

Conclusions We identified spacers that led to improved activity over the current state-of-the-art CAR sequences, suggesting that our biophysically-optimized spacers can be used to rapidly create optimal CAR constructs for arbitrary binder-epitope pairs.

Ethics Approval All animal procedures and housing were conducted in accordance with the Lyell/Explora umbrella IACUC protocol ID EB17–010–117.

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