

## Biophysical determinants of CAR T cell killing at cell-cell junctions

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Engineered T cells expressing chimeric antigen receptors (CARs) are tunable anti-tumor therapeutics that have been successful in treating some blood cancers but are minimally effective against solid tumors due, in part, to target antigen heterogeneity. The solid tumor microenvironment is physiochemically complex. We perform cell-cell killing assays to measure parameters that control elimination of cancer cells by CAR T cells. Target detection and cell-cell interactions are monitored for up to 16 hours under physiological conditions. Cell engagement and responses are captured in Diascopic, RICM (Reflection Interference Contrast Microscopy (RICM)), and fluorescent configurations to monitor cells, contact areas, and CAR expression, respectively. Using these data, we implement machine learning to measure the effective killing rate of cancer cells when interacting with the CARs. We observe that tumor cells interacting with CARs that spread on the substrate have a lower probability of survival when compared to the interactions with CARs in suspension, suggesting a mechanical component to activation and killing. We note that the CARs with lower expression of receptors tend to engage target cells for much longer when compared to the ones with a higher receptor density, consistent with spatial and temporal patterning of CAR triggering. In future we plan to probe the bidirectional interaction, tumor heterogeneity leading to a differential CAR response and modulated CAR response based on tumor heterogeneity.