T CELL KILLING IS FACILITATED BY MULTIPLE CYTOTOXIC PATHWAYS

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Background Chimeric antigen receptor (CAR) T cell therapies show remarkable progress in treating liquid tumors, with a complete remission rate of over 57%.1 Translating the success of CAR T cells to solid tumors will need an understanding of the key mechanisms responsible for the cytotoxicity of CAR T cells. The primary factors contributing to tumor resistance against CAR T therapies are widely contested2, therefore, we seek to explore the impact of different CAR T cell killing mechanisms of tumors.

Methods We examine CAR T cell killing of a leukemic cell line, NALM6, and an ovarian cancer cell line, SkOV3-CD19, in the presence of Granzyme B inhibitors and a Fas ligand inhibitor. We develop a fluorescent membrane reporter that translocates to the nucleus upon specific proteolytic cleaving by Granzyme A and B.

Results • Overexpressing native Granzyme B inhibitor, Protease Inhibitor-9 (PI-9), in NALM6 and SkOV3-CD19 does not affect killing frequencies in CAR(19-41BB) and 19-28BB CAR T cell cytotoxicity assays.
• Treating 19-41BB with a small molecule inhibitor of Granzyme B does not impact killing frequencies in cytotoxicity assays against NALM6 and SkOV3-CD19.
• Overexpressing PI-9 in NALM6 and SkOV3-CD19 does not affect 19-41BB CAR T killing frequencies or killing kinetics in single cell time-lapse assays.
• Inhibition of Fas ligand on 19-41BB CAR T cells does not impact killing frequencies against NALM6 and SkOV3-CD19.

Conclusions Our findings suggest that suppressing Granzyme B activity with small molecules or native proteins does not impair killing frequencies of 19-41BB CAR T cells en masse or at the single cell level. We hypothesize that Granzyme A facilitates CAR T killing in the absence of Granzyme B, implying redundancy in granzyme expression. This study provides a comprehensive understanding of the main mechanisms associated with CAR T cell-mediated killing.

REFERENCES