

Engineering MART-1 Antigen-Specific T Cells: Evaluating Transfection Efficiency and Functional Cytotoxicity

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Cancer immunotherapy seeks to harness the immune system to selectively target tumor cells. This study investigated whether human T cells could be engineered to recognize melanoma cells expressing the MART-1 antigen and evaluated their functional cytotoxicity in vitro. Donor-derived T cells were transfected with MART-1 RNA to induce transient expression of antigen-specific receptors. Receptor expression was quantified using flow cytometry, and cytotoxic activity against melanoma cells was assessed via live-cell imaging. Transfection efficiency varied across experimental batches, ranging from 7.4% to 36.8%, and corresponded with limited cytotoxic activity. These findings indicate that factors such as RNA integrity, post-thaw cell viability, and donor variability significantly influence the generation of functional antigen-specific T cells. While robust cytotoxic responses were not achieved, this study identifies critical technical constraints in T cell engineering workflows. By highlighting key limitations in transfection efficiency and functional activation, these results provide actionable insights for optimizing protocols and improving the reliability of antigen-specific T cell generation for immunological assays and future immunotherapy applications.

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