181 HIGH THROUGHPUT SCREENING STRATEGIES IN THE DEVELOPMENT OF LOGIC GATED CELL THERAPIES

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Background Generating potent clinical responses against solid tumors remains a challenge for CAR T cell therapy. This lack of efficacy is likely due in part to reduced on-tumor activity in the tumor microenvironment and the lack of appropriate target antigens that are expressed on tumor cells but not on critical healthy tissues. We addressed the second of these challenges by engineering T cells to target tumors only upon recognition of two antigens through AND Boolean logic.

Methods In our efforts to develop logic gated cell therapies we generated hundreds of binders for the priming receptor (PrimeR) and the CAR receptor. Here we demonstrate two strategies used for functional screening of PrimeR binders to identify binders with desired sensitivity and fidelity in driving the specific on-target expression of a fixed CAR.

In an arrayed strategy, we engineered T cells from 4 donors in multiwell plates using CRISPR-mediated, non-viral, site-specific integration of circuits bearing ~1000 PrimeR binders and receptor architectures with a fixed MSLN CAR. In addition, we employed a pooled screening strategy in which we engineered 2 donors of T-cells with a pool containing a subset of >300 unique PrimeR binders with a fixed MSLN CAR, using a similar engineering protocol.

Engineered T cells from both strategies were co-cultured with target cell lines to evaluate targeting fidelity and on-target functionality. In the arrayed setting, functional readouts (activation markers, cytokine secretion) were measured and reported directly. In the pooled setting, sorting based on functional markers was performed at end-point and sequencing was used to determine the enrichment of specific binders. Circuit fidelity was assessed by the lack of CAR expression and/or T-cell activation in response to target cell lines expressing the cytolytic antigen alone, or neither target antigens. On-target functionality was assessed by quantifying secretion of key cytokines in response to dual antigen stimulation.

Results We combined these metrics to filter out PrimeRs that allowed for CAR expression in the absence of logic gate activation, and to rank the remaining binders by their ability to drive CAR expression in the presence of both antigens. To account for multiple criteria when ranking binders, we used desirability functions, scaling each measurement to a (0–1) range, and using geometric means to combine desirabilities across different criteria.

Conclusions As each strategy comes with a different set of limitations and advantages, both serve as important tools for the effective selection of binders and receptors in the development of novel cell therapies.

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