COMPARISON OF TCBUSTER™ NON-VIRAL TRANSPONSON SYSTEM TO LENTIVIRAL TRANSDUCTION IN PRIMARY HUMAN T CELLS

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Background TcBuster™ is a commercially available hyperactive transposase that efficiently integrates poly-cistronic CAR constructs into primary human immune cells, overcoming many of the challenges associated with viral delivery. The use of viral vectors to generate these therapeutics has been the primary mechanism for payload delivery allowing relatively high efficiency of target editing with limited adverse effects. However, these viral vectors suffer from limited cargo size, risk of immunogenicity, and are both costly and difficult to produce. Non-viral transposase systems now rival viral gene editing, delivering equivalent efficiency with a much more cost-effective workflow.

Methods A range of different plasmid sizes, all containing a CD19 CAR, were inserted into primary human T cells from 3 healthy donors using either TcBuster™ or the equivalent lentiviral comparison. Flow cytometry was used to evaluate the efficiency of cargo insertion and resulting T cell phenotypes. The activity and anti-cancer efficacy of the engineered CAR T cells was evaluated following co-culture with Nalm-6 - luciferase target cells by luciferase assay and supernatants were collected for cytokine quantification on the Ella, an automated multi-analyte ELISA system.

Results TcBuster™ achieved similar integration efficiencies for smaller cargo sizes into primary human T cells to that of lentivirus; however, the transposase outperformed lentivirus when inserting larger poly-cistronic CAR constructs. The viability and fold expansion of the modified T cells was similar between the two technologies; however, the TcBuster™ transposed cells generated a larger population of memory T cells (stem cell memory and central memory) compared to their transduced counterparts. CD19 CAR T cells generated with TcBuster™ were similarly cytotoxic against Nalm-6 target cells to that of lentivirus transduced T cells but distinct differences were observed in the cytokine secretion profiles from each system.

Conclusions Here, we compared the generation, activity, and efficacy of CAR T cells produced using either TcBuster™, a non-viral transposon system, or lentivirus. While this work shows that there are many similarities between transposed and lentivirus modified CAR T cells, we highlight some key strengths when engineering with the TcBuster™ system, such as the ability to integrate larger cargos and more robust memory cell populations.

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