Comparison of TcBuster™ Non-viral Transposon System to Lentiviral Transduction in Primary Human T Cells

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Introduction

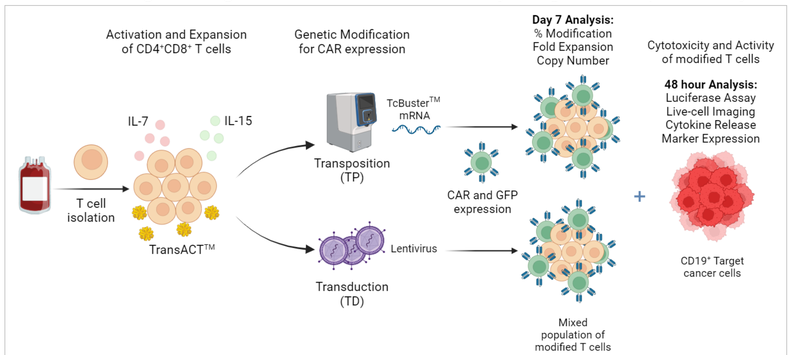
TcBuster™ is a commercially available hyperactive transposase that efficiently integrates multi-cistronic CAR constructs into primary human immune cells, overcoming many of the challenges associated with viral delivery. The use of viral vectors to generate these therapeutics has been the primary mechanism 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 costly to produce. Non-viral transposase systems now rival viral gene editing, delivering equivalent efficiency with a more cost-effective workflow. Here, we compared the generation, phenotype, 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 TcB (TcBuster) transposed and lentivirus transduced 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 targeted cytotoxicity.

Hypothesis

We hypothesize that the genetic editing of primary human T cells with the non-viral TcBuster transposase system will perform similarly to that of lentivirus at smaller cargo sizes but will significantly outperform viral methods when integrating larger cargos. We plan to analyze the differences in these two modification methods by comparing modified T cell phenotype, cytotoxic activity, and cytokine secretion.

Background/Methods

- *TcBuster* was first identified as an active *hAT* transposon within the red flour beetle, *Tribolium castaneum,* and is closely related to human *Buster* sequences.[1]
- A hyperactive mutant of *TcBuster* was engineered by screening a library of mutant proteins and testing the most active variants in HeLa cells[2]. The resultant TcBuster is used throughout this study.
- TcBuster modified CAR-T and CAR-NK cells have been shown to be specific and efficacious against target tumor cells[3] and are currently being reviewed preclinically.



Scheme 1: Typical workflow for genetic engineering of immune cells

Activation and Expansion: T cells from 3 healthy donors were isolated via CD4/CD8 magnetic selection and activated for 2 days with TransACT™ in the presence of IL-7 and IL-15 in Bio-Techne's GMP Human T cell Media.

Genetic modification: Using the MaxCyte® electroporation system, TcBuster was used to insert a range of different plasmid sizes, all containing a CD19 CAR, into the expanded T cells (**Figure 1**). Equivalent lentivirus payloads were used for transduction.

Day 7 Analysis: Flow cytometry was used to evaluate the efficiency of cargo insertion and resulting T cell phenotypes. Cell counts were used to track fold expansion and gDNA of each population was collected to determine copy number.

Cytotoxicity and Activity: Transposed and transduced CAR-T cells from 3 donors were separately co-cultured with CD19⁺ or CD19⁻ target cells after which supernatants for cytokine quantification on the Ella™ were collected and the anti-cancer efficacy from each modification method was evaluated by luciferase assay.

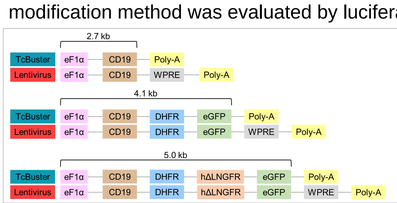


Figure 1: Depiction of the three plasmids inserted into expanded T cells. The plasmid insert size denoted in the following graphs is reported as the size of the relevant cargo integrated into the genome which is equal between the TcBuster transposon and the lentiviral vector.

Results

TcBuster outperforms lentivirus when inserting larger cargos

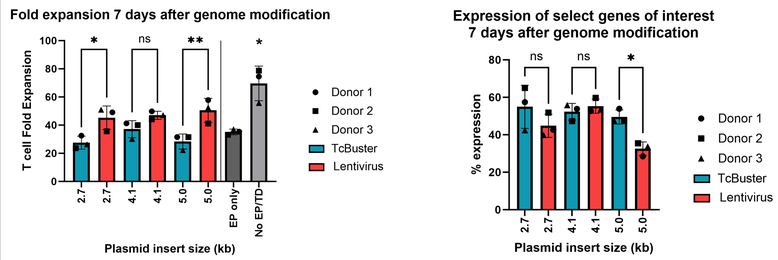


Figure 2: TcBuster efficiently integrates different cargo sizes into primary human T cells and allowed maximum cell growth within the G-Rex® vessel. TcBuster achieved similar integration efficiencies for smaller cargo sizes into primary human T cells to that of lentivirus; however, the transposase outperformed lentivirus when inserting larger CAR cassettes. The plasmid insert size is reported as the cargos integrated in the genome and is depicted in **Figure 1**. Cells were grown within a G-Rex® 6 well plate for 7 days following genetic modification at which point flow cytometry was used to evaluate the efficiency of cargo insertion. Both fold expansion and % expression were analyzed using an ordinary one-way ANOVA with comparisons to relevant means.

ISA suggests lower potential for gene disruption with TcBuster

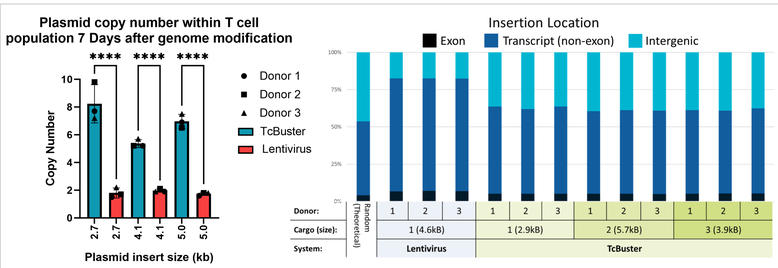


Figure 3: TcBuster inserts more plasmid copies in overall T cell population but has a safer insertion profile compared to lentivirus. T cells were collected 7 days after genome modification for Digital PCR analysis of the population's average copy number calculated as the difference between integrated copies and episomal copies normalized to RNAse P and corrected for % gene expression. Insertional Site Analysis revealed TcBuster preferentially inserted in less active, intergenic regions of the genome regardless of cargo size suggesting less potential for gene disruption. Copy number was analyzed using an ordinary one-way ANOVA with comparisons to relevant means.

Different modification methods yield different T cell phenotypes

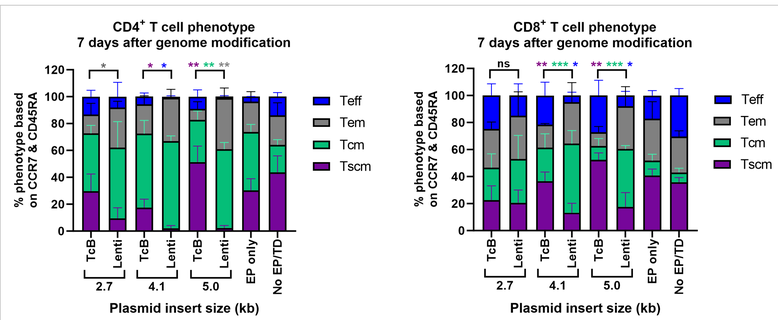


Figure 4: TcBuster modified cells yield higher percentage of stem cell memory (Tscm) CD4⁺CD8⁺ population. Modified cells were expanded for 7 days in Bio-Techne's GMP Human T cell Media and phenotype was determined by flow cytometric analysis of CCR7 and CD45RA expression. At larger cargo sizes, TcBuster produced significantly higher percentage of Tscm cells whereas lentivirus produced higher percentage of central memory (Tcm) cells. Significance was determined by multiple unpaired t tests between relevant means.

TcBuster & lentivirus modified CAR-T cells are similarly cytotoxic

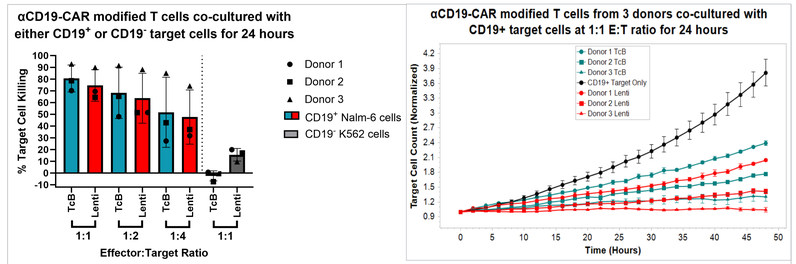


Figure 5: TcBuster modified T cells controlled target cell growth and were similarly efficacious to lentivirus modified T cells. T cells from 3 donors modified with the 4.1 kb plasmid were cryopreserved in CS10 following 7 days of culture after modification. These T cells were thawed and immediately added to either CD19⁺ or CD19⁻ target cells at different E:T ratios. Luciferase activity of remaining target cells was measured after 24 hours of co-culture. Target cell viability was monitored over a 48 hour period using an Incucyte SX5 at a set E:T ratio (1:1). Differences in target cell counts after 48 hours between TcBuster and lentivirus modified T cells in each donor were <1.3 fold.

T cell cytokine secretion correlates to targeted cell killing

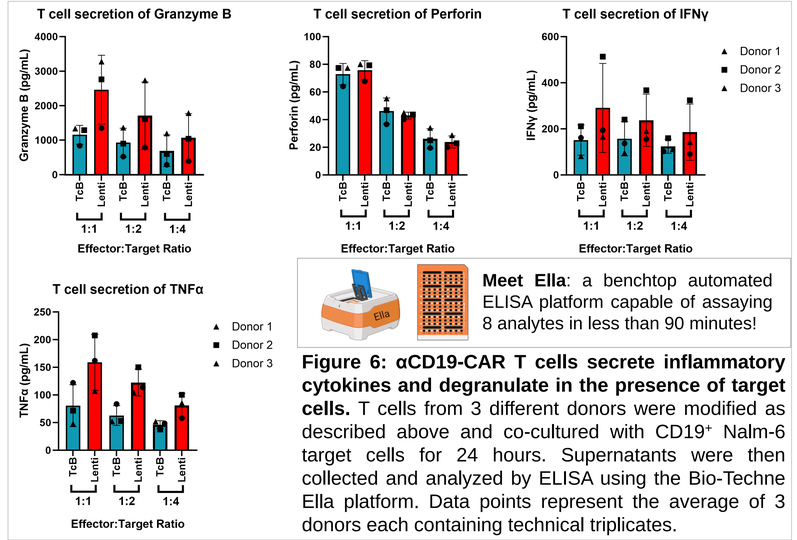


Figure 6: αCD19-CAR T cells secrete inflammatory cytokines and degranulate in the presence of target cells. T cells from 3 different donors were modified as described above and co-cultured with CD19⁺ Nalm-6 target cells for 24 hours. Supernatants were then collected and analyzed by ELISA using the Bio-Techne Ella platform. Data points represent the average of 3 donors each containing technical triplicates.

Conclusions

- The TcBuster transposase outperformed lentivirus when inserting larger constructs into primary human T cells.
- TcBuster modified T cells expanded to the maximum capacity within the G-Rex® vessel but had a lower overall fold expansion compared to the lentivirus transduced cells due to plating the cells at different densities at the start of the growth period.
- The TcBuster transposase tends to insert cargos within non-coding regions of the genome suggesting that this may be a safe genetic modification method.
- The population of TcBuster modified T cells contained a significantly higher percentage of stem cell memory T cells (Tscm) compared to lentiviral modified cells which had a larger population of central memory (Tcm) cells.
- αCD19-CAR T cells generated with either TcBuster or lentivirus were similarly efficacious against CD19 expressing target cells at as low as a 1:4 E:T ratio and over a 48 hour period.
- The cytokine secretion profiles corroborated the cytotoxicity results and confirmed degranulation increases as the E:T ratio increases.

Acknowledgements and References

Modified K562 cells were provided by Emily Hawkins and Hallie Hintz
Buster the Beetle was designed by Brianna Ettestad

1. Arensburger, P; *et al*. Genetics, **2011**.
2. Patrinostro, X; *et al.* Cell & Gene Therapy Insights, **2022**.
3. Pomeroy, EJ; *et al*. bioRxiv, **2021**.

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