Translation of a complex, 3x CRISPR edited, lentiviral vector engineered T cell process onto the Cellares Automated Cell Therapy Manufacturing Platform

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Background

The University of Pennsylvania (UPENN) has a longstanding history of administering autologous investigational cell therapy products into humans to demonstrate clinical proof of concept. Investigational cell therapy products implementing gene editing combined with viral integration of transgenes (TCR and CAR constructs) offer potential application in development of universal "off-the-shelf" cell therapy products. The **NY**-ESO-1–transduced **C**RISPR 3X-**e**dited cells (NYCE), developed at UPENN, have demonstrated enhanced tumor specificity, improved persistence, and a favorable safety profile in clinical trials (Stadtmauer et al., 2020). This is achieved by knocking out endogenous TRAC, TRBC, and PDCD1 genes while integrating a lentiviral vector encoding for the cancer-specific transgene NY-ESO-1 TCR (8F-TCR). Incorporating multiplex genome editing into a fully-closed clinical cell manufacturing process presents challenges for scalability and automation. The Cellares Cell Shuttle platform was evaluated as a potential solution to address these challenges. Initial feasibility studies generated 3X CRISPR edited transduced cells (Edited 8F-TCR) using the gene editing unit of the platform. Edited 8F-TCR cells generated on the Cell Shuttle instrumentation, demonstrated TCR-mediated, antigen-specific cytotoxicity. These preliminary results suggest potential for the complex manufacturing process of adoptive T cell therapies to be adapted onto a scalable, automated manufacturing platform.

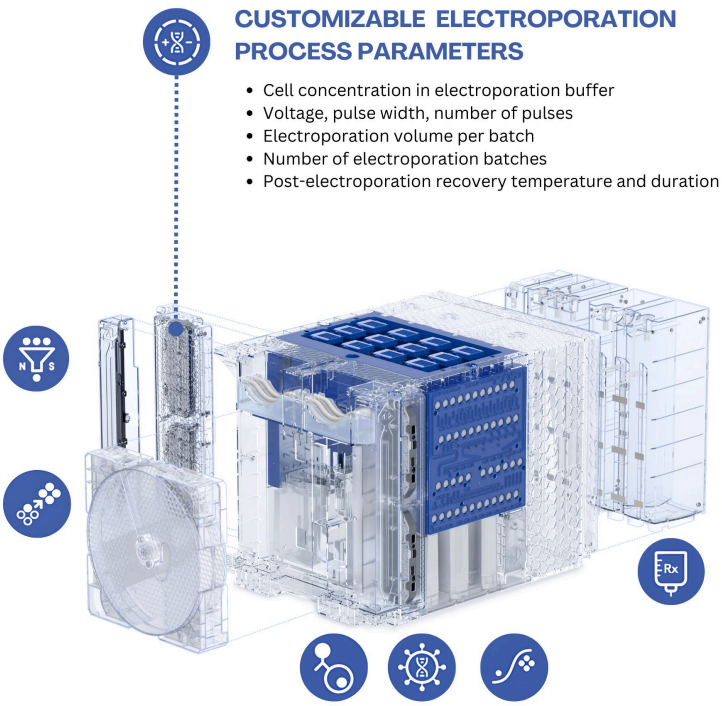
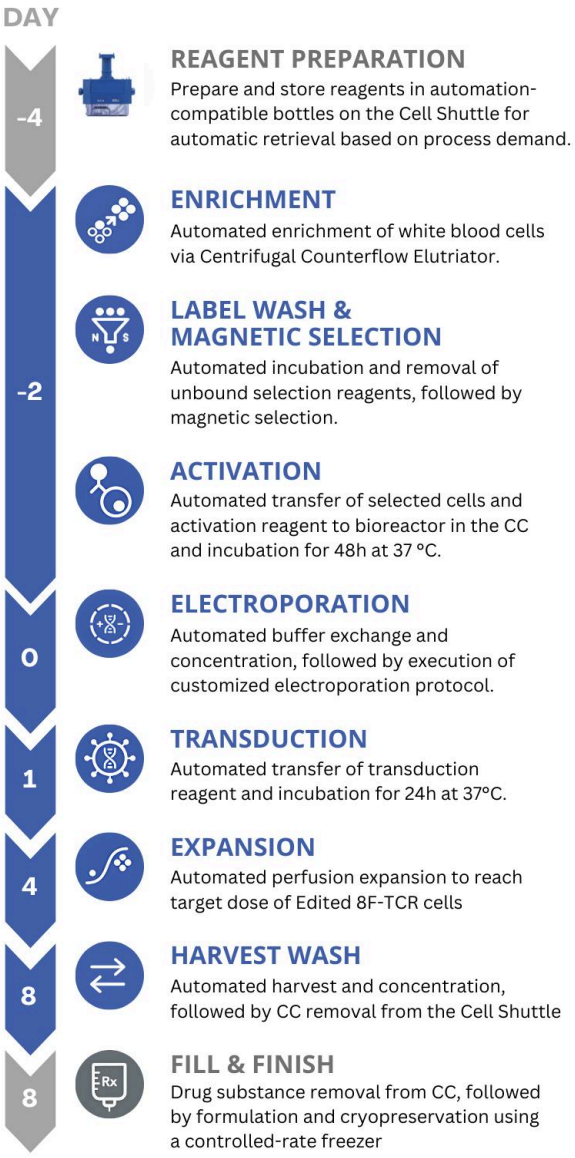


Figure 1. Schematic of the manufacturing process on the Cellares Cell Shuttle technology.

Blue: Automated process steps
Gray: Manual process steps

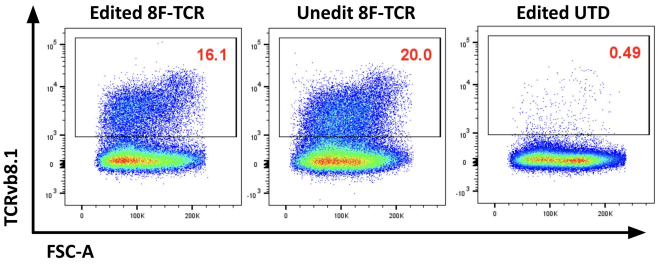


Results

1 High efficiency CRISPR-Cas9-mediated modifications and successful detection of the engineered TCR

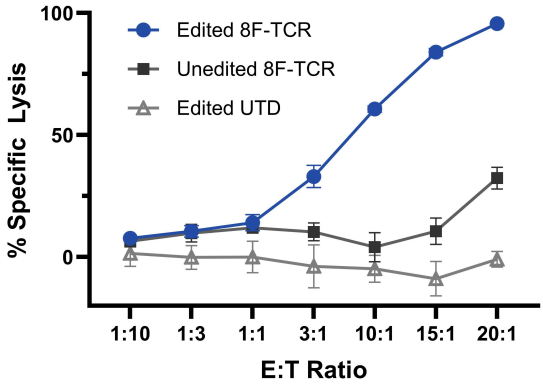
The frequencies of TRAC, TRBC, and PDCD1 gene disruptions were quantified using Inference of CRISPR Edits (ICE) from Sanger sequencing data. Edited 8F-TCR sample achieved high frequency of gene disruption (>84% across all genes), while no gene disruption was detected in the unedited control (Unedited 8F-TCR). The frequency of transgenic TCR (Vb8.1) was assessed via flow cytometry, achieving 16% in the thawed final product.

Sample	Description	Editing Efficiency (%)			Transduction Efficiency (%)
		TRAC	TRBC	PDCD1	
Edited 8F-TCR	Transduced (8F-TCR) and Electroporated (CRISPR 3X)	93	84	91	16.1
Unedited 8F-TCR	No Electroporation Control	0	0	0	20.0
Edited UTD	No Transduction Control	93	83	91	0.5



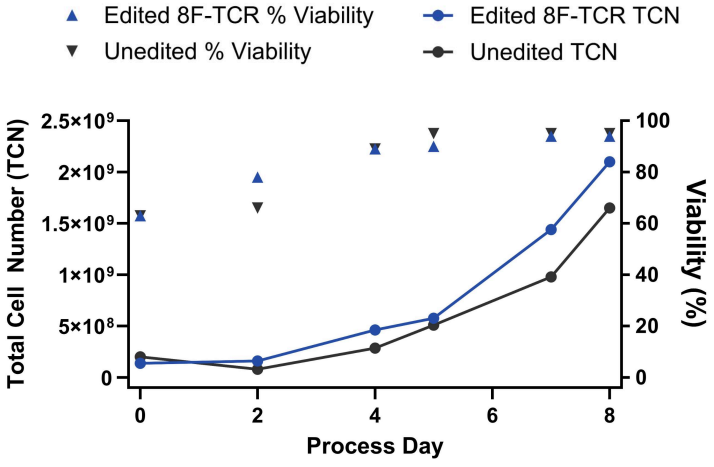
2 Antigen-specific cytotoxicity against NY-ESO-1-expressing cancer cells

Edited 8F-TCR effector cells (E) exhibited TCR-mediated dose-dependent lysis of NY-ESO-1 expressing Nalm6 target cancer cells (T). In contrast, unedited 8F-TCR cells exhibited less than 10% lysis of target cells with E:T ratios up to 15:1, while Edited UTD cells showed no lysis across all tested ratios.



3 In-process analytics demonstrate automation feasibility

Large-scale expansion of Edited 8F-TCR cells demonstrated high viability (>94%) and fold-change (15x) by day 8. Higher cell number is expected with further expansion. Memory phenotype of Edited 8F-TCR cells was comparable to small-scale controls (data not shown).



Conclusion

We successfully translated an engineered T cell process, incorporating multiplex genome editing (Edited 8F-TCR), onto the Cellares Cell Shuttle instrument, producing cellular material that exceeded potency, efficacy, and safety requirements.

The endogenous TCR was effectively replaced with the cancer antigen-targeting 8F-TCR, achieving stable expression, as confirmed by flow cytometry. The engineered T cells exhibited strong cancer-specific cytolytic activity and IFN-gamma release in a co-culture assay, highlighting their potential for targeted immunotherapy.

These results demonstrate the capability of the Cellares Cell Shuttle technology to manufacture engineered T cells while streamlining complex workflows to meet the growing patient demand for these life-saving cell therapies.