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A new electroporation system to achieve consistent performance from process development to clinical manufacturing for nonviral gene modified cell therapies

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ABSTRACT

Cell-based immunotherapy utilizing nonviral gene editing, such as Crispr-Cas9, is showing immense promise in the field as greater than 60% of gene therapy trials are utilizing ex-vivo gene modification (Pharmaprojects, Informa, May2021). However, the performance benefit and specificity of the Cas-9 protein can only be realized with an efficient electroporation platform that can safely deliver the ribonucleoprotein complex (RNP) to the target cells. The CTS™ Xenon™ Electroporation System has been developed to scale directly from the existing research grade Neon Transfection system without significant re-optimization. Furthermore, it is a stand-alone unit of operation that can also be integrated into an automated workflow with a closed, single-use, large volume consumable.

The Xenon Electroporation system has been shown to achieve efficient gene editing in activated human primary t-cells. Using the CRISPR-Cas9 protein system and sgRNA targeting multiple genes, knock-out of the target loci was achieved reproducibly and in a scalable manner from small scale to large scale. In addition, multiplexing was also shown to be efficient to develop cell therapies with numerous genes knocked out. Newly developed knock-in enhancers are also able to achieve >30% knock-in efficiency with a single electroporation delivering both the RNP and double-stranded donor DNA all at once.

The advancement of CRISPR-Cas9, in combination with efficient delivery can provide researchers and drug developers the ability to quickly and seamlessly scale their therapeutic platforms from early research and development to clinical manufacturing. Speed, efficiency and proper quality control are critical to ensure that more patients can benefit from the promising cell and gene therapies being developed.

INTRODUCTION

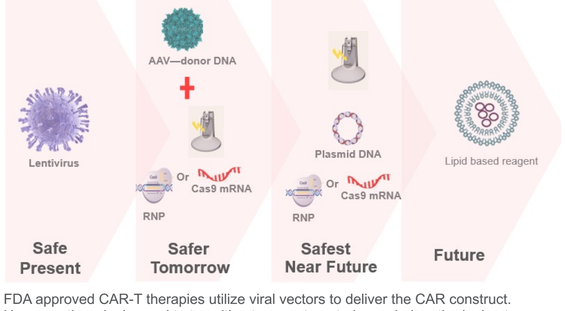
Figure 1. Xenon Electroporation System

- System based on core Neon (high voltage) technology
- Open platform that allows tailoring of electroporation parameters
- Modular design
- Connectivity and Tracking
- Cloud, OPC-UA* & DeltaV connectivity
- Software upgrade to enable compliance with 21 CFR part 11
- Consistent performance across various scales (100 uL – 1 mL – 5-25 mL)

1mL SingleShot (SS)

5-25mL MultiShot (MS)

Figure 2. Gene Transfer Methods



FDA approved CAR-T therapies utilize viral vectors to deliver the CAR construct. However, there is demand to transition to more targeted, nonviral methods due to random genomic integration, viral back bone clearance and viral production costs.

MATERIALS AND METHODS

Cell Source and Culture conditions

Immortalized jurkat cells cultured in RPMI+Glutamax supplemented with 10% FBS or PBMC were isolated from healthy donor leukopaks using standard Ficoll-Paque methods and cryopreserved. Upon thawing, PBMC were activated with CD3/CD28 Dynabeads™, cultured with OpTmizer™ media containing either 2% human serum or 5% Immune Cell Serum Replacement. Both cell models were maintained at 37C, 5% CO_2.

Electroporation

Three-days post activation, cells were prepared for electroporation by centrifugation and resuspended in standard electroporation buffer or newly developed genome editing buffer. Electroporation optimization (Table 1) was performed to determine the best condition for the cell model. Ribonucleoprotein (RNP) was formed by combining CTS TrueCut™ Cas9 Protein and Invitrogen™ TrueGuide™ Synthetic gRNA (Figure 3). Prepared cells and RNP were combined, incubated for five minutes, donor DNA template added and then electroporated using either the Neon or Xenon electroporation platforms. Cells were returned to complete media immediately after electroporation and cultured for 48-72hrs. Analysis was performed with Invitrogen™ Attune™ NxT Flow Cytometer with locus specific antibody targets.

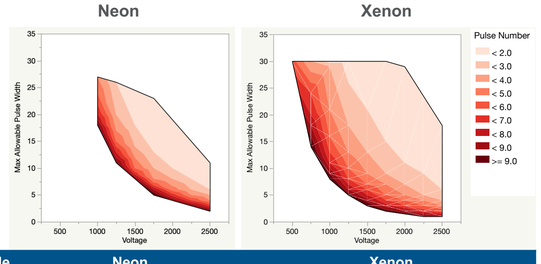
Table 1. Electroporation Protocols for Optimization

A	1700V/20ms/1-pulse
B	1400V/30ms/1-pulse
C	1400V/20ms/2-pulses
D	1150V/30ms/2-pulses
E	1600V/10ms/3-pulses
F	2300V/3ms/4-pulses

Figure 3. CRISPR-Cas9 Ribonucleoprotein (RNP) complex

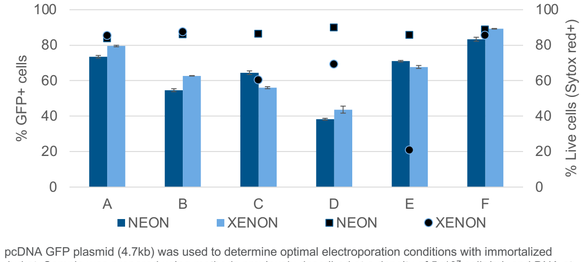
RESULTS

Figure 4. Neon vs Xenon Electroporation operating range



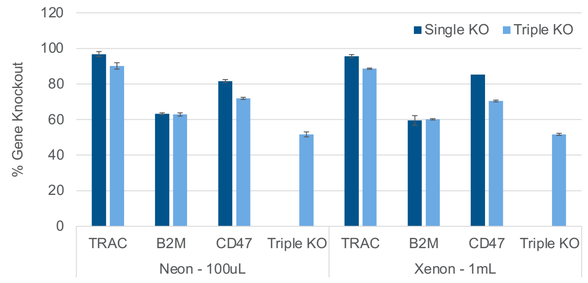
EP variable	Neon	Xenon
Voltage	500 – 2500 volts	500 – 2500 volts
Width	1 – 100 ms	1 – 30 ms
Number	1 - 10	1 - 10

Figure 5. Neon to Xenon translation of Electroporation Protocols



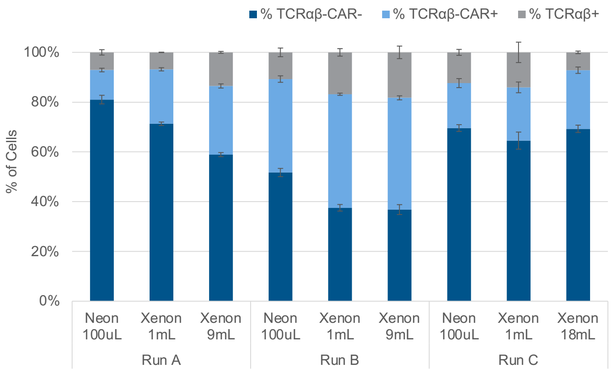
pcDNA GFP plasmid (4.7kb) was used to determine optimal electroporation conditions with immortalized Jurkat. Sample was prepared using methods previously described at a density of $5x10^7$ cells/mL and DNA at 80ug/mL. Transfection performance analyzed 24-hours post-electroporation via flow cytometry.

Figure 6. Scalable performance for multiplex targeted knock-out



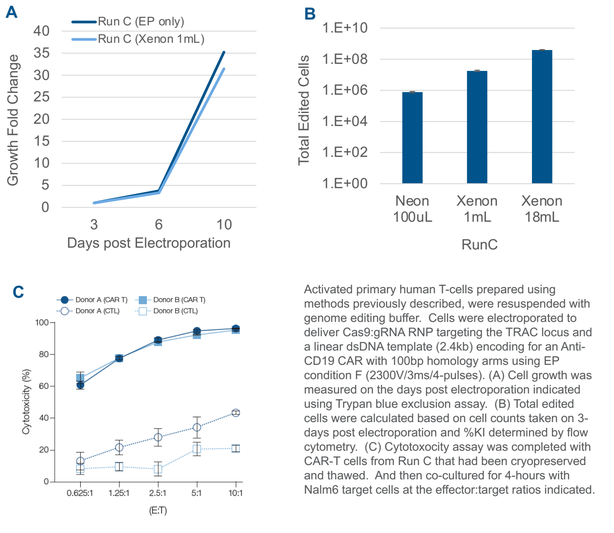
Activated primary human T-cells prepared using methods previously described, were resuspended with newly developed genome editing buffer. Cells were immediately electroporated to deliver either a single RNP or a pool of three RNPs targeting either TRAC, B2M or CD47. Cells were electroporated at $5x10^7$ cells/mL, Cas9 amount was 120ug/mL per target and gRNA was 28ug/mL. Gene knockout and viability was assessed 72-hrs post-electroporation via flow cytometry.

Figure 7. Scalable Transfection Performance with multiple cell donors to produce nonviral CAR-T cells



Activated primary human T-cells from three healthy donors prepared using methods previously described, were resuspended with genome editing buffer. Cells were electroporated to deliver Cas9:gRNA RNP targeting the TRAC locus and a linear dsDNA template (2.4kb) encoding for a second generation anti-CD19 CAR with 100bp homology arms using EP condition F (2300V/3ms/4-pulses). Knock-in efficiency and cell viability was analyzed 72-hours post-electroporation via flow cytometry.

Figure 8. Functional performance of CAR-T cells generated with Xenon Platform



Activated primary human T-cells prepared using methods previously described, were resuspended with genome editing buffer. Cells were electroporated to deliver Cas9:gRNA RNP targeting the TRAC locus and a linear dsDNA template (2.4kb) encoding for an Anti-CD19 CAR with 100bp homology arms using EP condition F (2300V/3ms/4-pulses). (A) Cell growth was measured on the days post electroporation indicated using Trypan blue exclusion assay. (B) Total edited cells were calculated based on cell counts taken on 3-days post electroporation and %KI determined by flow cytometry. (C) Cytotoxicity assay was completed with CAR-T cells from Run C that had been cryopreserved and thawed. And then co-cultured for 4-hours with Nalm6 target cells at the effector:target ratios indicated.

CONCLUSIONS

- The CTS Xenon Electroporation Platform with the new Genome Editing buffer achieves efficient performance for both knockout and nonviral knock-in.
- Xenon systems enables the development of nonviral gene modified T-cell therapy and can ultimately be incorporated into closed, automated workflows.
- Transfection performance is consistent and reproducible from Xenon SingleShot consumable to Xenon MultiShot consumable.
- CAR-T cells produced with the Xenon system are functionally cytotoxic and able to successfully kill CD19 expressing cancer cells.
- Large scale Xenon platform allows for electroporation optimization with provided protocols and 1mL SingleShot consumable, but also allows for customization of electroporation parameters.

REFERENCES

1. Roth TL, Puig-Saus C, Yu R, Shifrut E, Carnevale J, Li PJ, Hiatt J, Saco J, Krystofinski P, Li H, Tobin V, Nguyen DN, Lee MR, Putnam AL, Ferris AL, Chen JW, Schickel J, Pellerin L, Carmody D, Alkorta-Aranburu G, del Gaudio D, Matsumoto H, Morell M, Mao Y, Cho M, Quadros RM, Gurumurthy CB, Smith B, Haugwitz M, Hughes SH, Weissman JS, Schumann K, Esensten JH, May AP, Ashworth A, Kupfer GM, Greeley SAW, Bacchetta R, Meffre E, Roncarolo MG, Romberg N, Herold KC, Ribas A, Leonetti MD, Marson A. (2018) Reprogramming human T cell function and specificity with non-viral genome targeting. Nature. Jul;559(7714):405-409.
2. Eyquem J, Mansilla-Soto J, Giavridis T, van der Stegen SJ, Hamieh M, Cunanan KM, Odak A, Gönen M, Sadelain M. (2017) Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature. 543(7643):113-117.

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TRADEMARKS/LICENSING

For more information about collaboration opportunities contact: donpaul.kovarcik@thermofisher.com

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