P #612

A Novel Scalable Electroporation Platform for the Manufacturing of Gene Modified Hematopoietic Stem and Progenitor Cell Therapies

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INTRODUCTION

- Optimization of electroporation conditions and scalability from R&D to development and cGMP manufacturing environment requires significant time and resources, and it can be challenging.
- Platform scalability and process closure enables better and more predictive translation from R&D to Clinical environment.
- CTS Xenon Electroporation System was launched in Q4 2021; its design is based off the R&D Neon Transfection System but suited for clinical scale in regulated environment.
- The Xenon/Neon systems are opened programmable electroporation platforms, where parameter such as voltage (V), pulse width (ms), number of pulses, and pulse interval (last one being specific to Xenon) can be explicitly controlled by end user.
- Efficiency of Neon Transfection System has been previously demonstrated in blood and immune derived cells ($CD34^+$ and T cells)[1,2], providing promise in suitably of Xenon to be used for scale-up.

OBJECTIVES

- Scalability from R&D (Neon) to Clinical (Xenon) was assessed in HSCs.
- Comparability in cell viability and gene editing between Neon Transfection System and Xenon Electroporation System was evaluated.

METHODS

- Electroporation program and payload were optimized using Neon 100 uL tip format.
- Cell viability was determined by AO and DAPI staining, while gene editing efficiency was assessed by the presence of indels (insertions or deletions) and analyzed with Vor's internal bioinformatics tool.
- Scalability was evaluated from Neon to Xenon on a β-version of Xenon Electroporation System and prototype consumables, prior to launch.

RESULTS

Fig. 1. Illustration of Thermo Electroporation Instruments

- **Neon Transfection System**: is a Research Use Only (RUO) Instrument able to transfect up to 5 x 10^6 cells per reaction, using 10 μL or 100 μL format tip. Neon is pre-programmed with optimization protocols, where parameters are disclosed.
- **Xenon Electroporation System**: a translational Clinical CTS device, suitable for 20 x 10^6 to 2.5 x 10^9 cells. Software is 21 CRF part 11 compliant. Its Multi-Shot consumable is a closed system, reducing aseptic risks.

Neon
RUO
10 μL, 100 μL

Xenon
Clinical, GMP
1 mL, 5-25 mL

RESULTS (CONT'D)

Fig. 2. Illustration of Thermo Electroporation Consumables

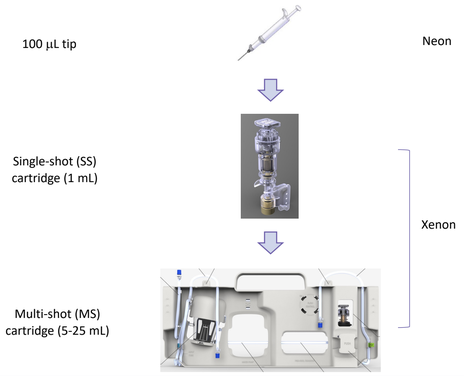
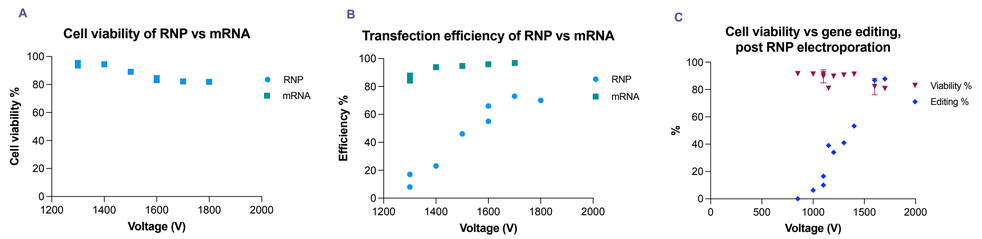


Fig. 3. Optimization of Electroporation Conditions with Final Cargo Increases Success of Electroporation Application

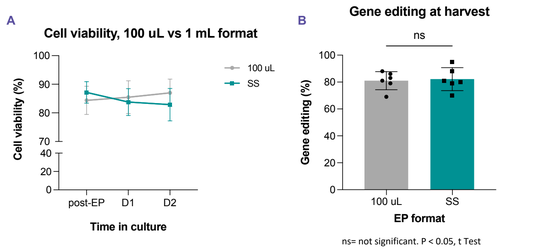
- HSPCs were electroporated either with GFP-mRNA or RNP using optimization protocols recommended by Thermo Cell viability was recorded 2 days post-electroporation, as well as transfection efficiency. For mRNA, GFP expression was determined by flow cytometry, while RNP efficiency was determined by indel %.



- GFP expression is a common tool to select electroporation conditions. Payload used during selection of electroporation conditions is relevant. Cell viability was comparable when transfecting RNP or GFP-mRNA (**A**); however, transfection efficiency behaves differently according to payload used, GFP-mRNA transfection is high in broader electroporation parameters, while RNP efficiency correlates with voltage applied (**B**).
- Using the suitable payload, programs selected should meet the criteria of high cell viability and transfection efficiency (**C**).

Fig. 4. 1 mL Single-shot Electroporation on Xenon is Translatable from 100 μL Neon Tip

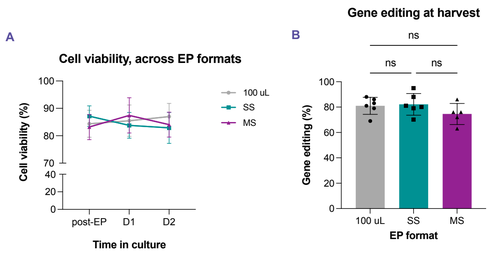
- 1 mL reaction in Xenon cartridge is an intermediate scale suitable for Process Development work; as well as technology transfer from R&D on Neon instrument.
- The program selected to evaluate scalability from Neon 100 μL tip, showed cell viability (**A**) and gene editing (**B**) higher to 80%, when using RNP as payload. Average gene editing for 100 μL Neon tip was 81% ± 8, while Single-shot was 82% ± 9.
- Neon might be a predictor for scale-up of electroporation conditions.



ns= not significant. P < 0.05, t Test

Fig. 5. Cell viability and editing is maintained across Thermo formats

- Scalability was evaluated from 100 μL Neon to 1 mL Single-shot and Multi-shot cartridge in a β-version of Xenon Electroporation System.
- Cell viability during cell culture post-electroporation is comparable across formats (**A**).
- Gene editing maintained high efficiency, Single-shot average was 82% ± 9 and an average of 75% ± 8 for 5 mL on Multi-shot (**B**), while using prototype consumables, prior to their launch. The average on 100 μL tip was 81% ± 7.



ns= non-significance. P < 0.05, one-way ANOVA

CONCLUSIONS

- Xenon Electroporation System is a promising tool for the clinical scale manufacturing of gene modified HSPCs.
- Scalability from Neon 100 μL tip to 1 mL Single-shot cartridge on Xenon was comparable.
- Neon Transfection System is reliable predictor to scale-up electroporation.
- Cell viability post-electroporation across formats was comparable.
- Gene editing efficiency of RNP was lower on Multi-shot cartridge, with an average of 75% ± 8 for 5 mL of input volume; compared to an average of 81% ± 7 on 100 μL Neon tip.

References

1. Modarai S. R. et al. *Molecular Therapy – Nucleic Acids.* 2018;11:116-29.
2. Gundry M. C, et al. *Cell Reports. 2016*;17(5):1453-1461.

Acknowledgments

We thank the Research, Technical Operations, and Lab Operations teams at Vor Biopharma. Instrument and consumable images were provided by Thermo Fisher.

Presented at

ASGCT 2022, May 16-19, 2022.