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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 are challenges that require a significant investment of time and resources.
- Platform scalability and process closure may increase translatability from R&D to a GMP environment.
- CTS™ Xenon™ Electroporation System, launched in Q4 2021; is an electroporation system intended for clinical manufacturing, its design is based off the R&D Neon™ Transfection System.
- The Xenon/Neon systems are open programmable electroporation platforms, where parameters such as voltage, pulse width, number of pulses, and pulse interval (last one being specific to Xenon) can be explicitly controlled in manufacturing 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) manufacturing was assessed in Hematopoietic Stem and Progenitor Cells (HSPCs).
- Cell viability and gene editing readouts were used to assess the comparability of Neon Transfection System and Xenon Electroporation System.

METHODS

- Electroporation program and payload were optimized using Neon 100 µL tip format.
- Cell viability was determined by Acridine Orange and DAPI staining.
- Gene editing efficiency was assessed by measuring the presence of indels (insertions or deletions), 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. Thermo Fisher Scientific Electroporation Instruments

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

RESULTS (CONT'D)

Fig. 2. Thermo Fisher Scientific Electroporation Consumables

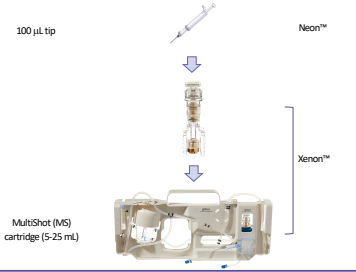
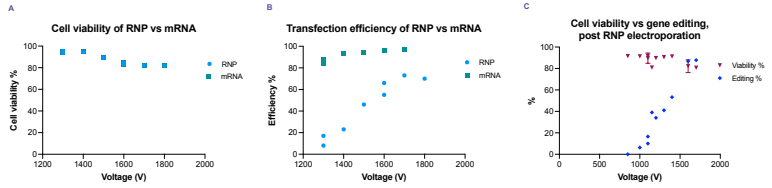


Fig. 3. Optimization of Electroporation Conditions with Final Cargo Increases Success of Electroporation Application

- HSPCs were electroporated either with GFP-mRNA or Ribonucleoprotein complex (RNP) using optimization protocols recommended by Thermo Fisher Scientific. 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 SingleShot 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**) up to 80%, when using RNP as payload. Average gene editing for 100 µL Neon tip was 81% ± 8, while SingleShot was 82% ± 9.
- Neon might be a predictor for scale-up of electroporation conditions.

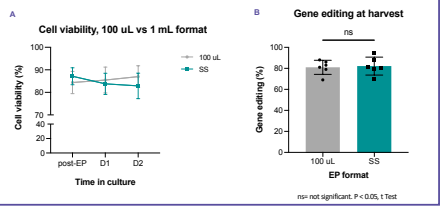
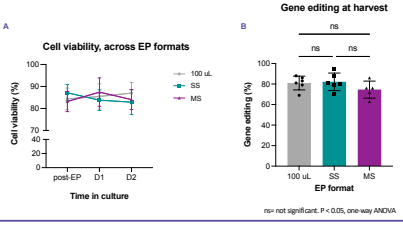


Fig. 5. Cell Viability and Editing is Maintained Across Thermo Fisher Scientific platforms

- Scalability was evaluated from 100 µL Neon to 1 mL SingleShot and MultiShot 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, SingleShot average was 82% ± 9 and an average of 75% ± 8 for 5 mL on MultiShot (**B**), while using prototype consumables, prior to their launch. The average on 100 µL tip was 81% ± 7.



CONCLUSIONS

- Comparable cell viability and gene editing between products from Neon 100 µL tip and 1 mL SingleShot cartridge on Xenon suggest scalability.
- Cell viability post-electroporation across formats was comparable.
- Gene editing efficiency of RNP was lower on MultiShot cartridge, with an average of 75% for 5 mL of input volume; compared to an average of 81% on 100 µL Neon tip.
- Xenon Electroporation System is a promising tool for the clinical scale manufacturing of gene modified HSPCs.

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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

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