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NONVIRAL *EX VIVO* MODIFICATION OF CELL THERAPY-RELEVANT CELL TYPES

Transfection is the process of introducing foreign material - generally nucleic acids (DNA or RNA) - into cells. Introduction of foreign nucleic acids using various chemical, biological, or physical methods can result in a change of the properties of the cell, allowing for the study of gene function and protein expression in the context of the cell, or transformation of the cell into a potential cell therapy product such as a CAR T cell.

A TYPICAL CELL THERAPY PRODUCTION WORKFLOW

Source › Selection & activation › Cell engineering › Cell culture › Characterization & infusion

METHODS OF TRANSFECTION

Viral

- Additional safety concerns
- Increased testing burden
- Payload limitations
- Expensive to produce

Viral engineering of T cells can lead to poor and inconsistent regulation of CAR expression

Nonviral

- Deliver DNA, RNA and/or protein
- Introduce sequence-specific nucleic acid modifications into the genome

Nonviral engineering approaches will allow more specific and controllable engineering and potentially a better therapy

ELECTROPORATION IS AN ALTERNATIVE METHOD FOR NONVIRAL CELL ENGINEERING THAT CAN ACHIEVE EQUIVALENT EFFICIENCY TO VIRAL METHODS

WHAT IS ELECTROPORATION?

Electroporation is a physical transfection method that uses an electrical pulse to create temporary pores in cell membranes through which substances like nucleic acids can pass into cells. It is a highly efficient strategy for the introduction of foreign nucleic acids into many cell types, including bacteria and mammalian cells.

STEPS

1. Prepare cells by suspending in electroporation buffer and add payload
2. Apply electrical pulse to sample
3. Return cells to growth conditions and allow them to recover
4. Assay cells for transfection efficiency and viability

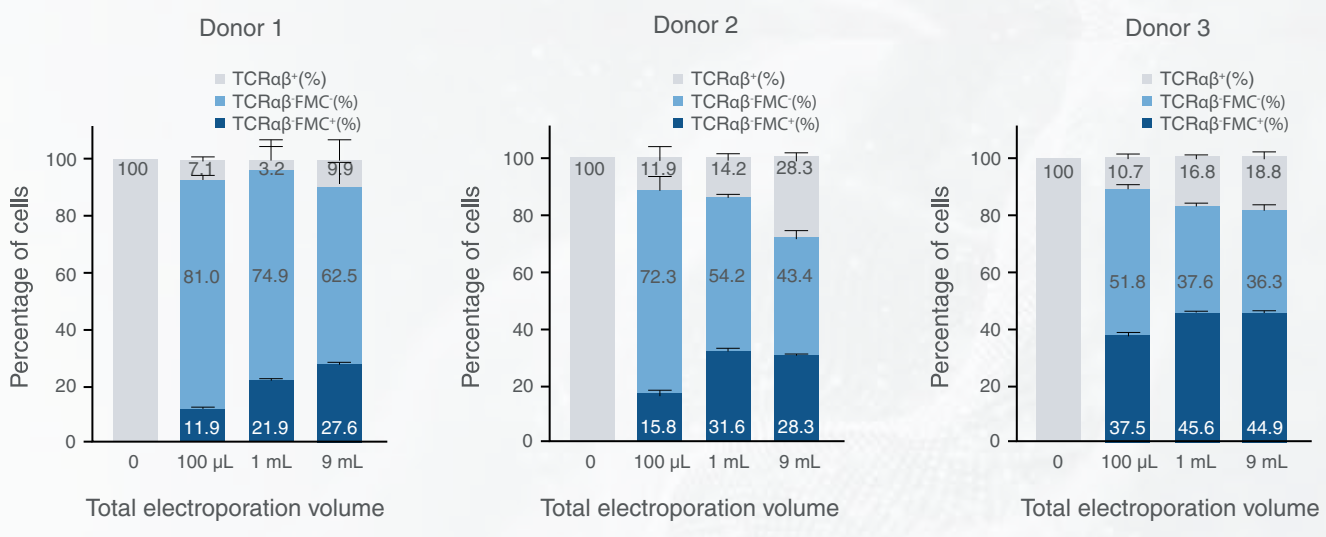
OPTIMIZE YOUR ELECTROPORATION PROTOCOLS BY CONTROLLING KEY FACTORS THAT DIRECTLY AFFECT PERFORMANCE:

Voltage · Pulse width · Pulse number · Pulse interval delay · Buffer conductance/ resistance

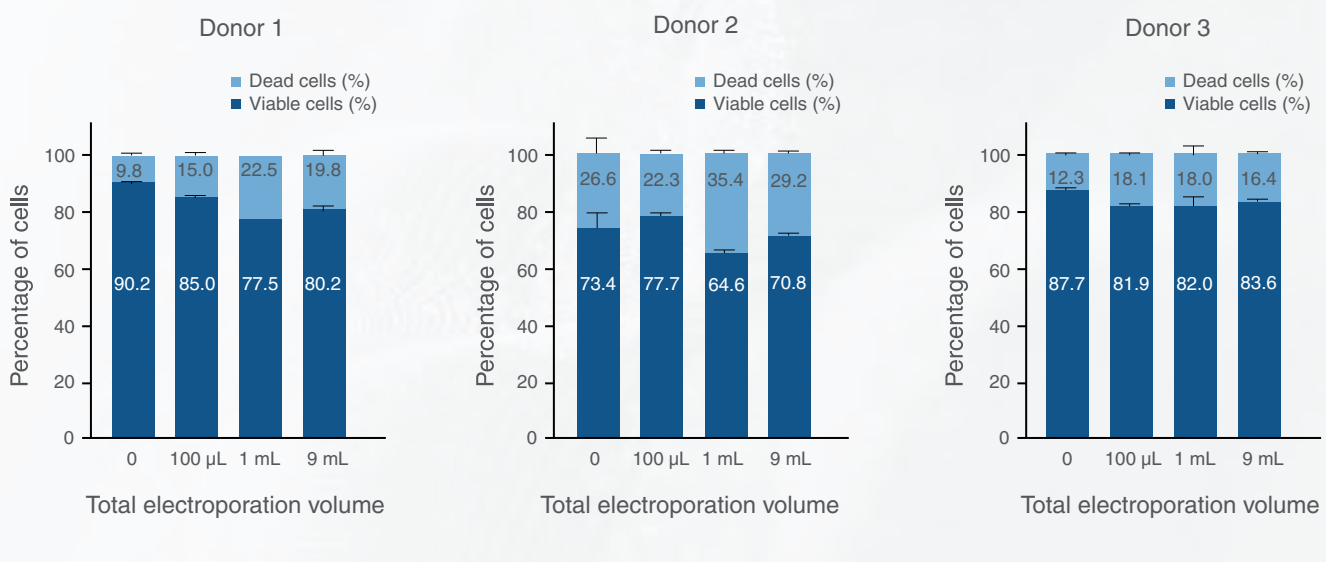
VOLTAGE IS THE MOST SIGNIFICANT FACTOR TO IMPACT FUNCTIONAL PERFORMANCE

Analysis of CAR T cell transfection with three leukapheresis-derived T cell donors, using Cas9/gRNA to knock out the endogenous T cell receptor (TCR) and knock in a double-stranded, linear DNA expressing a second-generation CAR construct. 5 x 107 cells/mL transfected on the Invitrogen™ Neon™ Transfection System (100 µL), or the CTS Xenon instrument, with a Gibco™ Xenon™ SingleShot chamber (1 mL) or a Gibco™ Xenon™ MultiShot cartridge (9 mL), or were left untransfected. Cells were assessed through flow cytometry four days after transfection for gene expression of CD19 on the CAR T cells. Cells were also assessed for viability with a cell counter utilizing Gibco™ Trypan Blue solution.

% Total Edited Cells (%KO + %KI)



% Viability (Trypan Blue exclusion)



OTHER IMPORTANT ATTRIBUTES OF A CELL THERAPY ELECTROPORATION SYSTEM:

- Scalable, flexible electroporation protocols
- GMP manufactured consumables
- Closed system processing
- Equivalent performance at small and large scale
- Software that enables compliance with 21 CFR pt 11

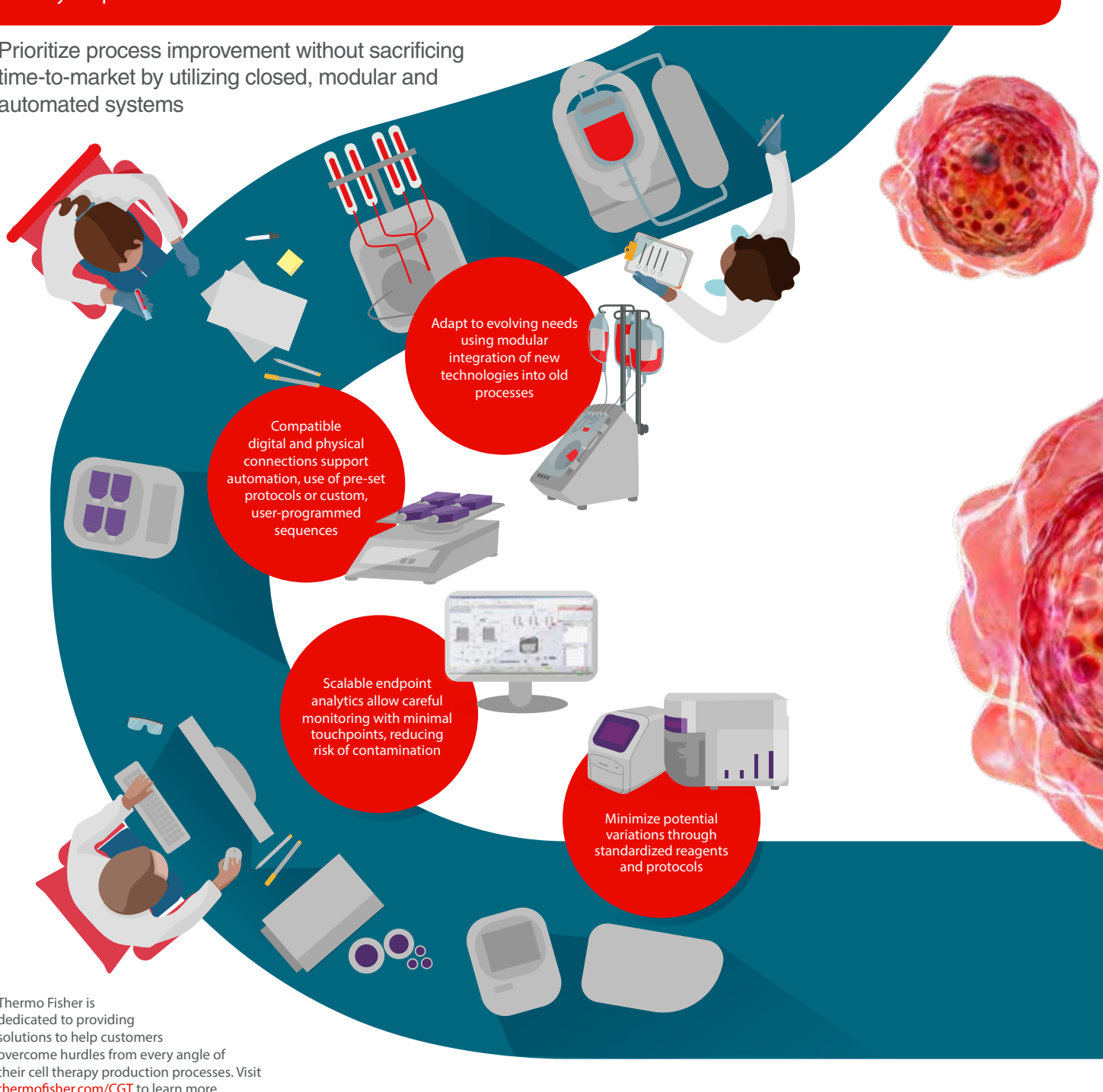
THE GIBCO CTS XENON ELECTROPORATION SYSTEM:

- High speed, large volume—transfect up to 2.5 x 10^9 T cells in less than 25 minutes
- Proven performance and viability—up to 90% gene knockout and 80% viability
- Process flexibility—user-programmable system helps enable you to create and optimize electroporation protocols for various cell types and payloads from process development through commercial manufacturing
- Nonviral transfection—can be used to deliver DNA, RNA, and protein payloads
- Closed-system processing—MultiShot (MS) consumable enables sterile welding to PVC or C-Flex™ tubing

Putting solutions into practice:

evolve your process from research to commercial scale

Prioritize process improvement without sacrificing time-to-market by utilizing closed, modular and automated systems



Thermo Fisher is dedicated to providing solutions to help customers overcome hurdles from every angle of their cell therapy production processes. Visit thermofisher.com/CGT to learn more.

Find out more and request a demo at thermofisher.com/xenon

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