Achieving Therapeutic Efficacy with a High Performance, Electroporation-based Cellular Engineering Technology.

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Abstract

Advances in cell-based therapies for treating cancer and other diseases have created a demand for rapid and flexible manufacturing processes. While virus-based delivery methods have provided the foundation for the cell therapy revolution, the costs of manufacturing viral vectors and related safety issues have created an urgent need for non-viral approaches to cellular engineering that can be implemented at scale in a GMP environment. In this poster, we present data on a GMP-compliant, scalable electroporation-based technology for engineering primary human cells, stem cells and cell lines with high efficiency while maintaining high cell viability. Examples include introducing precise genomic modifications into primary T cells and hematopoietic stem cells via transfection of mRNA and RNPs encoding zinc finger nucleases and CRISPR/Cas9 components. We also include data on redirecting the specificity of T cells and NK cells by expressing recombinant receptors. Finally, we provide examples of cellular engineering with induced pluripotent stem cells (iPSCs) and other types of progenitor cells.

Universal, High Performance Primary & Stem Cell Engineering

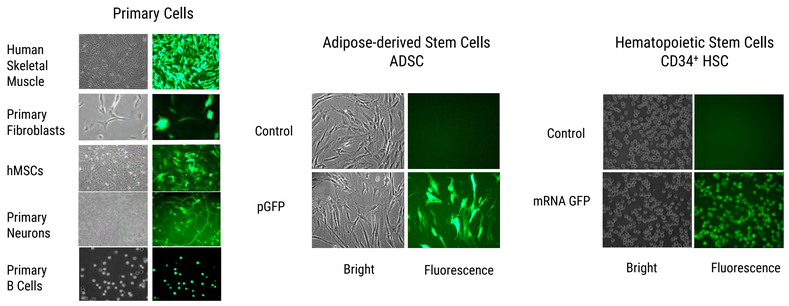
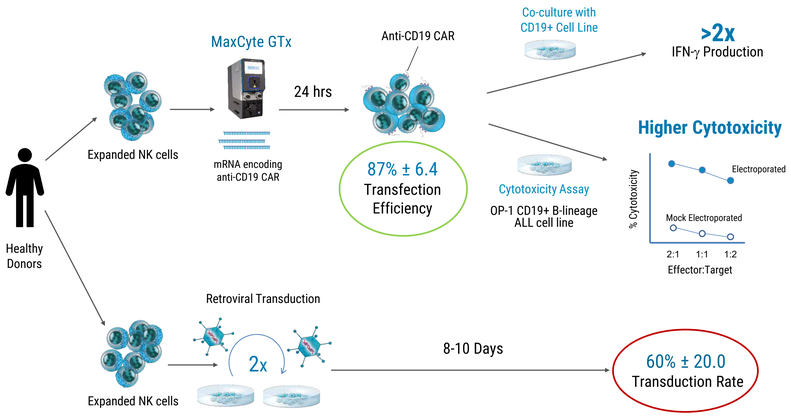


Figure 1: Human primary or stem cells were electroporated with pGFP (GFP mRNA for HSCs) using the MaxCyte STX. Transfection efficiency and viability were assessed at 24-48 hours post electroporation.

Advancing CAR Therapy using NK Cell Engineering

High Performance, Clinical-scale Anti-CD19 CAR Expression



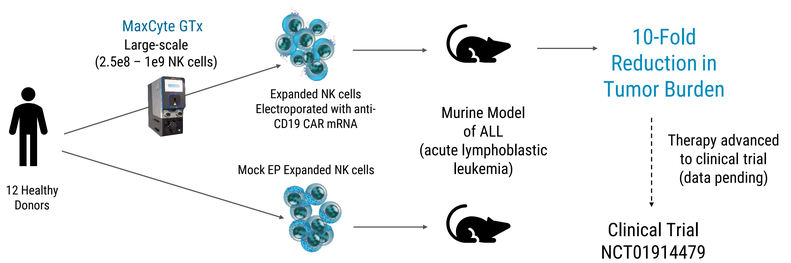


Figure 2: Currently approved CAR therapies are based on engineering of autologous T cells. NK cells offer an allogeneic source of cells that can rapidly mediate tumor lysis with simplified manufacturing. Adoptive transfer studies in humans of unengineered NK cells support their safety and show moderate anti-tumor activity. The work published in *Cytotherapy,* 14(7), 830-840, 2012 and summarized above reported the development of a non-viral, clinically adaptable method to enhance NK cell cytotoxicity against B-cell malignancies via CAR expression. NK cells from healthy human donors were expanded *in vitro* and electroporated with anti-CD19-BB-z mRNA using the MaxCyte GTx. Transfection efficiency and consistency were significantly higher than those following retroviral transduction (87% ± 6 vs. 60% ± 20). Anti-CD19 CAR expression correlated with increased cytokine production and killing of CD19+ tumor cell lines. *In vivo* anti-tumor activity was demonstrated using a mouse model of acute lymphoblastic leukemia (ALL). These studies opened the path to an ongoing **clinical trial NCT01914479** using large-scale MaxCyte GTX-engineering NK cells.

Clinically Relevant Levels of Ex Vivo Gene Correction in X-linked Chronic Granulomatous Disease (X-CGD) Patient Stem Cells

Gene Editing Moves Towards the Clinic Using Non-viral, Fully Scalable Cell Engineering

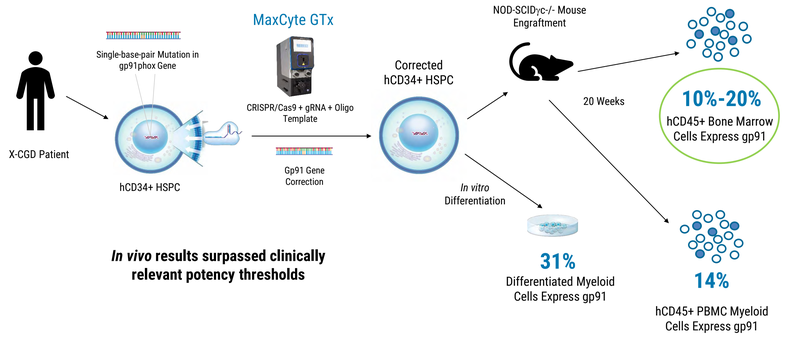


Figure 3: X-CGD is caused by a mutation in the CYBB gene which encodes a critical component (gp91-phox) of NADPH oxidase, an enzyme that is key for the anti-microbial activity of phagocytes. Correction of mutation within the faulty CYBB gene offers a new curative treatment for X-CGD patients. The patients' own cells are harvested, the mutated gene corrected using CRISPR-mediated gene editing, and the cells with the corrected gene returned to the patient. The engrafted cells multiply to create a new population of cells displaying 'normal' function and eliminating disease. In these studies, CD34+ hematopoietic stem cells (HSPCs) were isolated from X-CGD patients and electroporated with CRISPR/Cas9, guide RNA, and the gene correcting oligo template using the MaxCyte GTx. A portion of the cells were differentiated *in vitro* into myeloid cells and gene correction rates determined to be 31%. The other portion of corrected HSPCs were introduced into immunodeficient mice. After 20 weeks the engrafted human cells in the mouse peripheral blood expressed the corrected gp91 gene at 14%, while the engrafted cells in the bone marrow showed a 10% - 20% gene correction rate. These correction rates are within clinically beneficial potency thresholds. *Sci. Transl. Med.*, 9(372), Jan 2017.

Advancement of an HIV Clinical Program for CCR5 Gene Disruption

Rapid Development & IND-enabling Pre-clinical Studies Support Progression of Clinical Trial

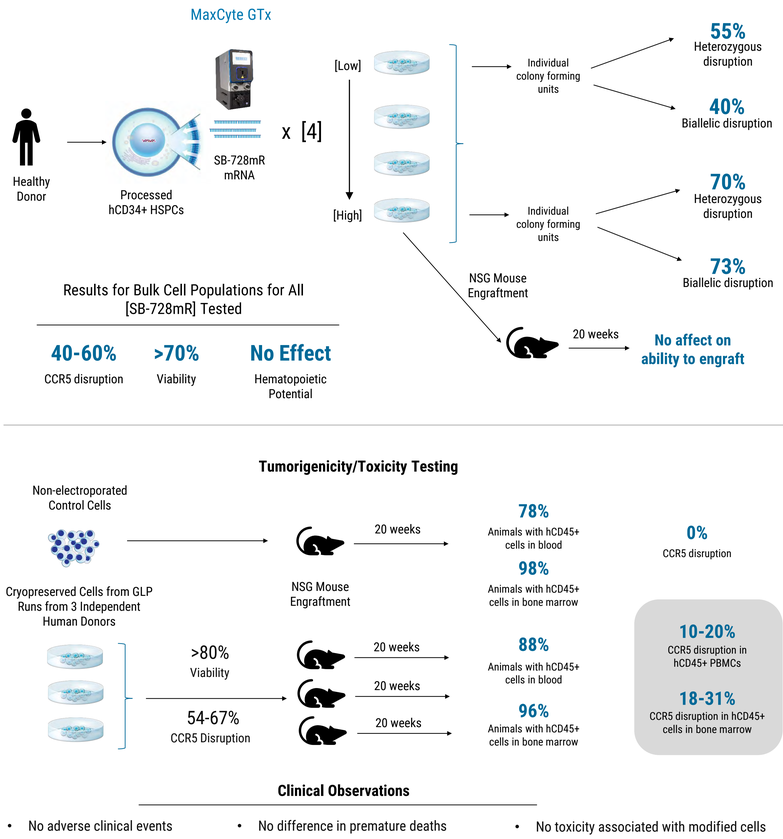
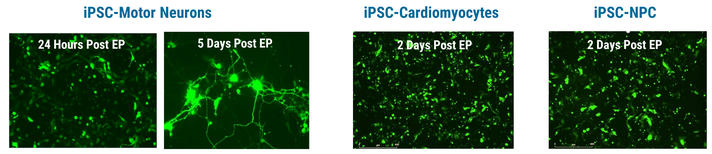


Figure 4: Researchers at Sangamo developed a CCR5-targeted zinc finger nuclease that they showed was active in a variety of CD4 T cells and HSPCs and that conferred resistance to HIV infection. This therapy was advanced to the clinic using adenoviruses to deliver the ZFN constructs. The phase 1/2 trials showed that CD4 cells with a disrupted CCR5 gene could be engrafted, were safe and persisted. Toxicity related to the adenoviral vector precluded the intended trials from progressing. To rescue the therapy, the company turned to mRNA delivery of the CCR5-specific ZFN using the MaxCyte GTx. The work published in *Mol Ther. Methods Clin. Dev., 3, 2016* and summarized above demonstrate the rapid progression from process development of ZFN delivery, through manufacturing qualification runs, pre-clinical toxicity studies and initiation of **clinical trial NCT02500849** using the MaxCyte GTx. See publication for detailed methods.

High Efficiency, High Viability iPSC Transfection

Expanding the Power of iPSCs

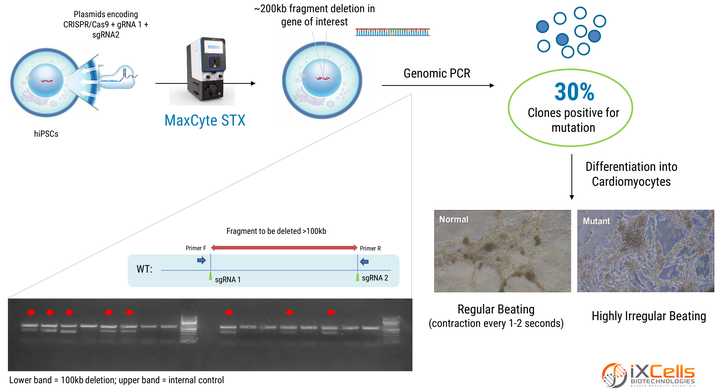


	Lipid-based Method		MaxCyte STX	
	Efficiency	Viability	Efficiency	Viability
Human iPSCs	< 10%	< 70%	94%	85%
iPSC-Motor Neurons	< 10%	< 60%	87%	89%
iPSC-Cardiomyocytes	< 10%	< 60%	60-80 %	86%
iPSC-NPCs	< 10%	< 70%	60-80%	90%

Figure 5: Human iPSCs (iXCells Biotechnologies) were treated with Accutase and dissociated into single cells before electroporation. Cells were electroporated with pGFP using the MaxCyte STX and replated on Matrigel-coated plates. Images were taken 24, 48 or 120 hours post electroporation. The transfection efficiency and viability were determined using a NovoCyte flow cytometer (ACEA). These data were compared to historic results using iXCells previously optimized lipid-based transfection method.

Cardiac "Disease-in-a-Dish" Using iPSCs-derived Cardiomyocytes

CRISPR-Mediated Gene Deletion Recreates Cardiac Arrhythmia



20 out of 60 hiPSC clones were positive for deletion using MaxCyte

Figure 6: A ~200kb fragment of a gene believed to be involved in cardiac arrhythmia was targeted for deletion. sgRNA 1 and 2 were sub-cloned into plasmids containing Cas9. The constructs were electroporated into hiPSCs using the MaxCyte STX. Electroporated cells were re-plated on Matrigel-coated plates and single cell colonies screened by genomic PCR (Primer F+R). 30% of the isolated clones had the desired deletion. Mutant and wild-type iPSCs were subsequently differentiated into cardiomyocytes using iXCells proprietary methods and beating monitored. Cardiomyocytes from wild-type iPSCs exhibited regular beating while those derived from mutant colonies beat irregularly mimicking cardiac arrhythmia.

Summary

- MaxCyte's ExPERT™ platform provides for engineering of primary and stem cells with **high efficiency** and **cell viability** enabling:
  - improved, more powerful disease modeling
  - high efficiency gene editing (correction, deletion or insertion)
  - development of high potency, highly efficacious human therapeutics
- Flow Electroporation® technology meets the stringent demands of cell & gene therapy:
  - highly efficient and reproducible transfection
  - non-toxic
  - payload flexibility
  - clinical-scale manufacturing
- Platform scalability and established regulatory-compliance supports rapid and seamless advancements from concept through clinic
- Supported by publications, clinical trials and 50+ partnered clinical development programs

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On Demand Webinar: High Transfection Efficiency of Human Induced Pluripotent Stem Cells and Their Derivatives. Presenter: Dr. Nianwei Lin, Ph.D.; Chief Operating Office, VP, and Co-Founder iXCells Biotechnologies. *November 2017*
https://www.maxcyte.com/webinars/ixcells-stemcells/

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