Bolstering Therapeutic Efficacy and Speed to Clinic Using Non-viral T- & NK-cell Engineering

Payal Roychoudhury, Krista Steger, Jessica Carmen, Rama Shivakumar, James Brady. MaxCyte, Gaithersburg, MD, USA

Abstract

Non-viral methods of engineering CAR T-cells and delivering gene editing tools have advanced to the clinic, establishing the feasibility of reducing or eliminating the reliance on recombinant viruses. This poster highlights how MaxCyte's enabling technology for cell engineering can skew therapeutic indexes and speed the path to the clinic while de-risking development. Specifically, we demonstrate low toxicity, high efficiency delivery of mRNA and/or gene editing machinery for the expression of CARs, TCRs, and gene knock out in T- and NK-cells using MaxCyte's clinically-validated, non-viral platform. Additionally, we highlight strategies for how this approach can augment your current CART programs or rapidly drive the development of your next-generation therapy.

Advancing CAR Therapy using NK Cell Engineering

High Performance, Clinical-scale Anti-CD19 CAR Expression

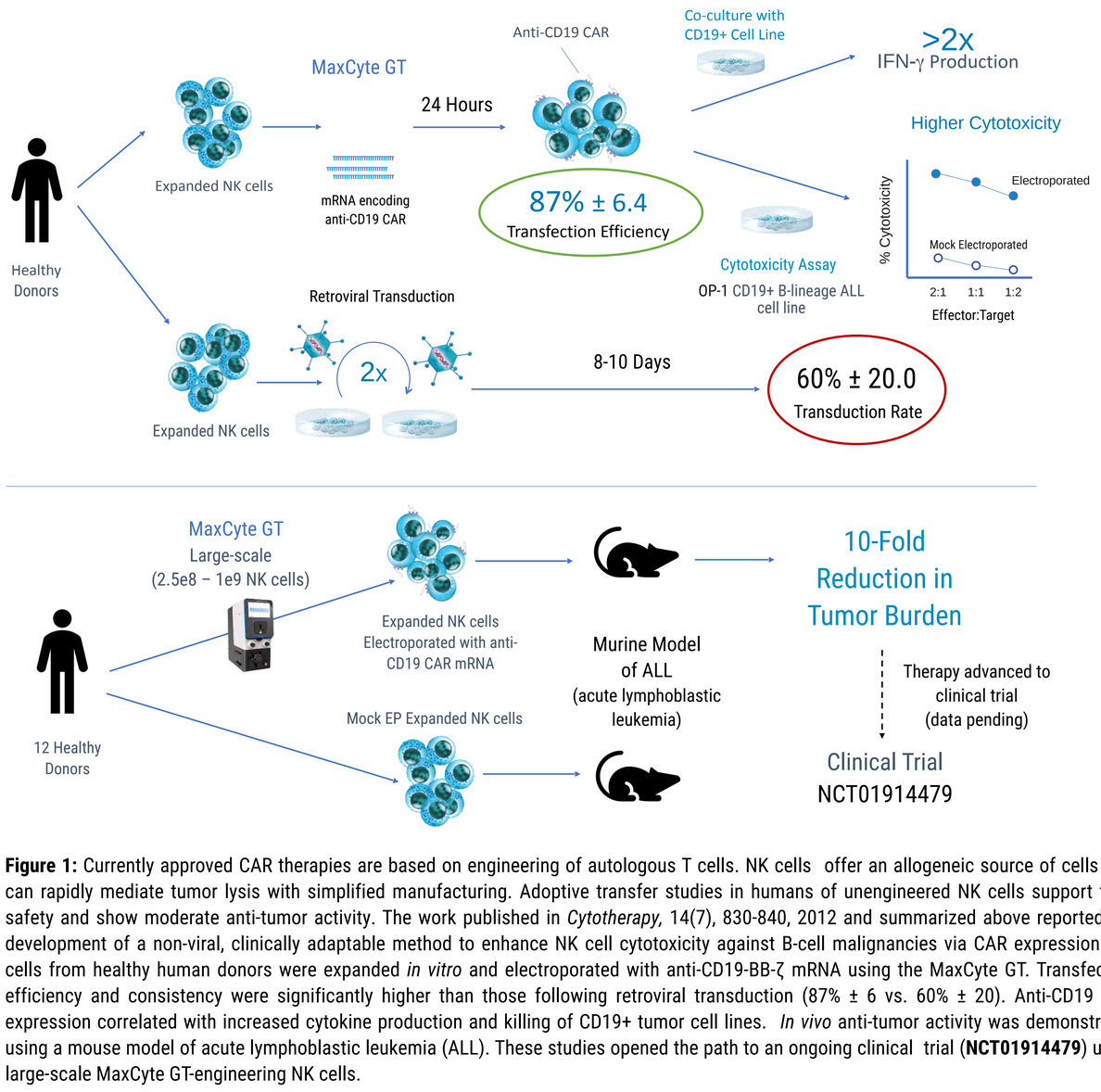
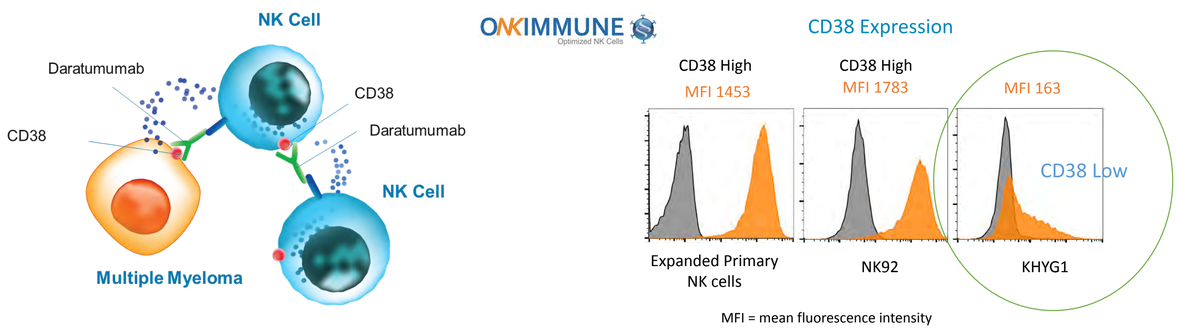


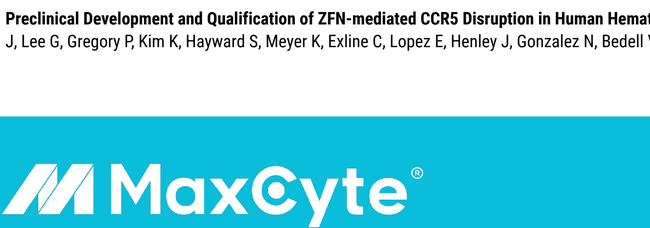
Figure 1: Currently approved CAR therapies are based on engineering of autologous T cells. NK cells offer an allogeneic source of cells that can rapidly mediate tumor lysis with simplified manufacturing. Adoptive transfer studies in humans of unengineered NK cells support their safety and show moderate 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-ζ mRNA using the MaxCyte GT. 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 GT-engineering NK cells.

NK Cell Therapy Augments Cytotoxic Activity of Approved Biotherapeutic Antibody

Expression of High Affinity CD16 in NK CD38 Low Cells as Treatment for Multiple Myeloma



A. Strong CD16-158V Expression in KHYG1 Cell & >90% Viability for 5 Days Post Electroporation



B. Improved Daratumumab Cytolytic Activity Against Primary Multiple Myeloma Cells

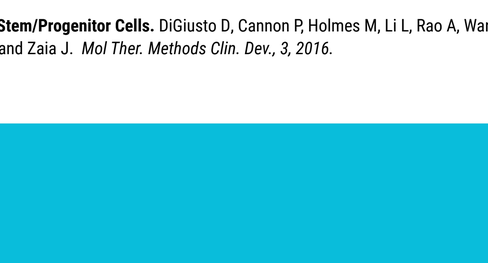


Figure 2: Daratumumab is an FDA approved antibody that recognizes the CD38 surface receptor on multiple myeloma cells. NK cells also express CD38 which precludes their use as a cell therapy for use in combination with Daratumumab. KHYG1 cells displayed 10-fold lower expression of CD38 than expanded primary NK cells or NK92 cells (evaluated via FACS) and chosen as the NK cell population for further therapeutic development. **A.** KHYG1 cells were electroporated with mRNA encoding the high affinity CD16-158V (HA-CD16) Fc receptor using the MaxCyte GT. Expression levels and viability were evaluated for 5 days. Representative of 3 experiments. **B.** Primary, CD38+ multiple myeloma were incubated with Daratumumab (Dara) alone, with Dara and mock electroporation KHYG1 cells, or Dara and HA-CD16 electroporated cells at various effector to target cell ratios. Expression of HA-CD16 was observed for over 5 days with >90% viability. Daratumumab cytolysis against primary multiple myeloma cells was significantly increased in the presence of HA-CD16-expressing KHYG1 cells. This represents a potential adoptive cell therapy to augment the efficacy of an already approved biotherapeutic antibody. *Data courtesy of Onkimmune, Ltd.*

References

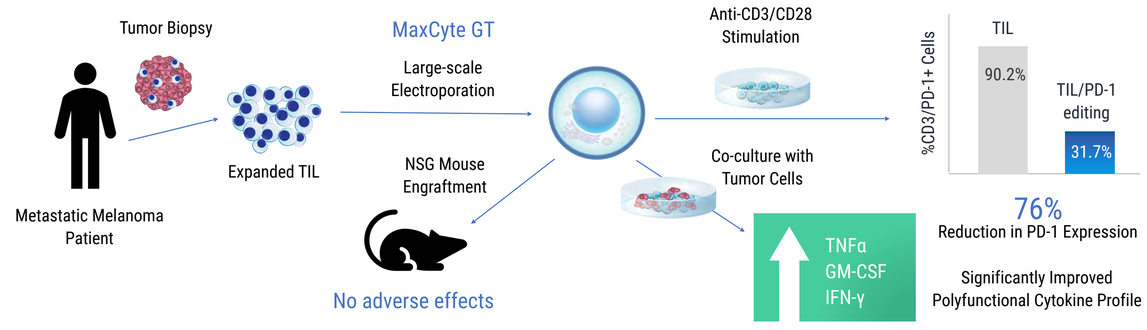
A Clinically Adaptable Method to Enhance the Cytotoxicity of Natural Killer Cells Against B-cell Malignancies. Shimasaki N, Fujisaki H, Cho D, Masselli M, Lockey T, Eldridge P, Leung W, and Campana D. *Cytotherapy, 14(7), 830-840, 2012.*

Clinical Scale Zinc Finger Nuclease-mediated Gene Editing of PD-1 in Tumor Infiltrating Lymphocytes for the Treatment of Metastatic Melanoma. Beane J, Lee G, Zheng Z, Mendel M, Abate-Daga D, Bharathan M, Black M, Gandhi N, Yu Z, Chandran S, Giedlin M, Ando D, Miller J, Paschon D, Guschin D, Rebar E, Reik A, Holmes R, Gregory P, Restifo N, Rosenberg S, Morgan R, and Feldman S. *Mol Ther., 23(8), 1380-1390, Aug 2015*

Preclinical Development and Qualification of ZFN-mediated CCR5 Disruption in Human Hematopoietic Stem/Progenitor Cells. DiGiusto D, Cannon P, Holmes M, Li L, Rao A, Wang J, Lee G, Gregory P, Kim K, Hayward S, Meyer K, Exline C, Lopez E, Henley J, Gonzalez N, Bedell V, Stan R, and Zaia J. *Mol Ther. Methods Clin. Dev., 3, 2016.*

Improving Efficacy of Tumor Infiltrating Lymphocyte (TIL) Therapies

Overcoming Immunosuppressive Tumor Environment Through PD-1 Gene Disruption



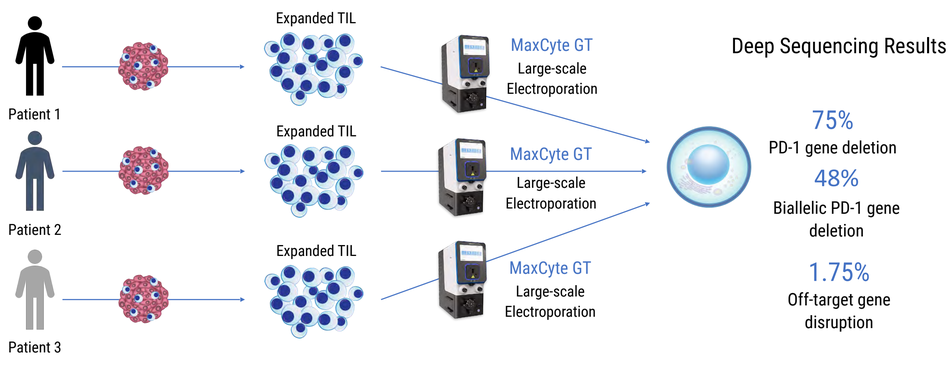
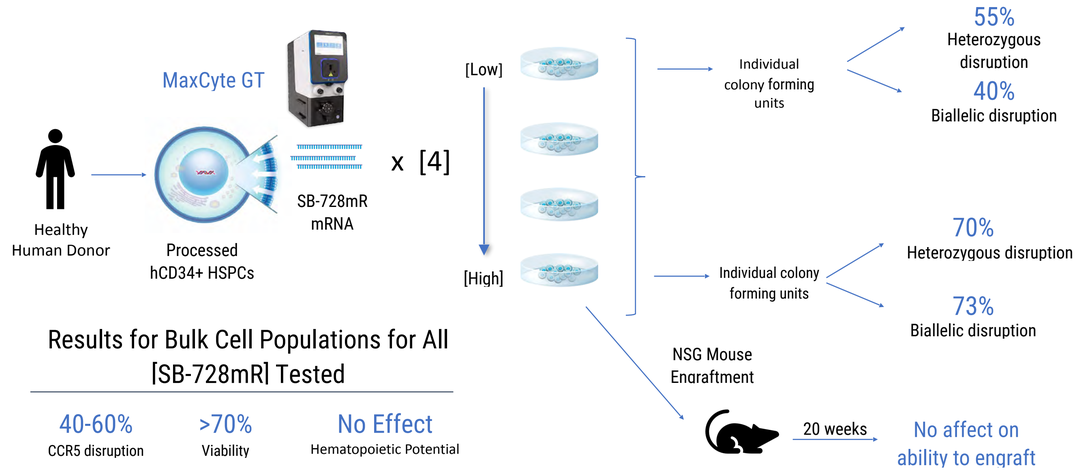


Figure 3: Tumor infiltrating lymphocytes (TILs) demonstrate a 50-70% response rate following TIL infusion in metastatic melanoma patients. These TILs are known to express PD-1 and therefor whose activity may be suppressed by the tumor micro-environment. In *Mol. Ther.,* 23(8), 1380-1390, 2015 and summarized above are studies that show the use of the MaxCyte GT to reproducibly disrupt PD-1 at clinical scale and that PD-1 modified cells have improved functionality upon antigen stimulation and do not cause adverse effects *in vivo*. See publication for detailed methods.

Advancement of an HIV Clinical Program for CCR5 Gene Disruption

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



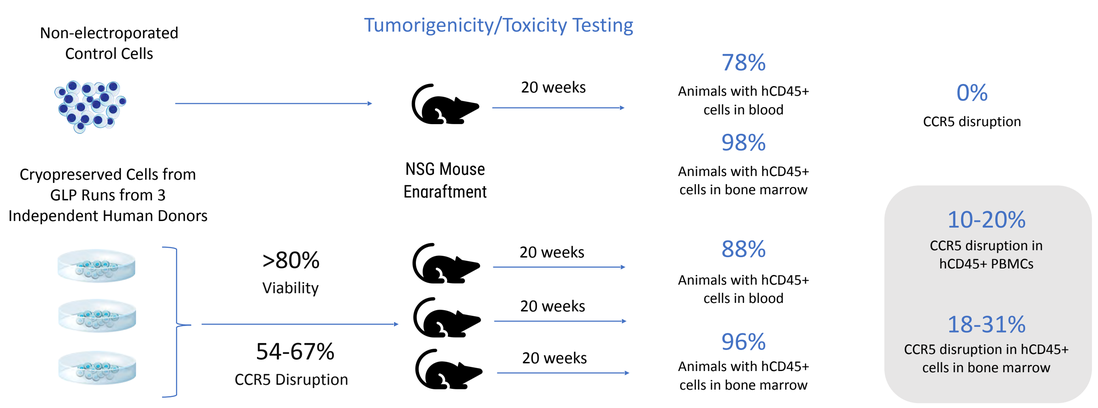


Figure 4: Researchers at Sangamo developed a CCR5-targeted zinc finger nuclease that they showed was active in a variety of CD4 T cells and HSPCs and that conferred resistance to HIV infection.This therapy was advanced to the clinic using adenoviruses to deliver the ZFN constructs. The phase 1/2 trials 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 GT. 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 GT. See publication for detailed methods.

Flow Electroporation® Technology: High-performance Cell Engineering

- Designed to meet the stringent demands of cell therapy:
  - highly efficient and reproducible transfection of difficult-to-transfect primary cells
  - non-toxic
  - clinical-scale, regulatory-compliant
- Non-viral technology that overcomes challenges associated with other delivery methods and provides:
  - ability to use novel cell populations, such as NK cells
  - increased efficiency
  - elimination of safety and toxicity concerns
  - decreased cost and complexity of manufacturing
  - reduced time to market
- Proven technology supported by numerous publications, 15+ clinical trials and 50+ partnered clinical development programs

Corresponding Author: James Brady; jamesb@maxcyte.com MaxCyte, Inc., Tel: (301) 944-1700 info@maxcyte.com, *www.maxcyte.com*

©2020 MaxCyte, Inc. All Rights Reserved. Flow Electroporation is a trademark and MaxCyte and MaxCyte STX are registered trademarks of MaxCyte, Inc.