**Bolstering Therapeutic Efficacy and Speed to Clinic Using Non-viral T-cell Engineering.**

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**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 non-viral cell engineering can allow 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 and gene knockdown 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.

**Rapid Enablement of Clinical-scale TCR Engineering**

**Overcoming Clinical Challenges of Viral Delivery**

**Viral vectors challenges:**
- Low viral transduction rates (~15%)
- HBV antigens on non-tumor hepatocytes - T cells permanently engineered against HBV may cause liver damage
- Risk of insertional mutagenesis
- Complex & expensive to clinically implement

**MaxCyte mRNA electroporation:**
- Increased efficiency - high-light, transient TCR expression that confirmed in vivo anti-tumor activity
- Eliminated safety concerns – no insertional mutagenesis or chronic inflammatory issues
- Decreased complexity of manufacturing – clinical scale, regulatory compliant electroporation
- % the cost and significantly less time to manufacture

**Figure 1:** 80% of Hepatocellular Carcinoma (HCC) is caused by infection with Hepatitis B Virus (HBV) infection. T cells engineered to express an anti-HBV TCR represent a potential curative autologous cell therapy. The first therapeutic approach relied on a retroviral vector anti-TCR construct delivery and showed early promise, but clinical application was hindered due to viral delivery including chronic infection due to the long-term presence of the TCR-engineered T cells. The work published in Mol Ther. Nucleic Acids, 2, 2013 and depicted above demonstrated that electroporation of primary T cells with mRNA encoding the anti-HBV TCR produced high level, transient expression of the TCR which exhibited HBV-specific in vivo functionality and in vivo anti-HCC activity and could be produced at clinical-scale using the MaxCyte GT. See publication for detailed methods.

**Improving Efficacy of Tumor Infiltrating Lymphocyte (TIL) Therapies**

**Overcoming Immunosuppressive Tumor Environment Through PD-1 Gene Disruption**

**MaxCyte Flow Electroporation™ Technology for High-performance Cell Engineering**

- Designed to meet the stringent demands of cell therapy:
  - highly reproducible and transformation of difficult-to-transfected primary cells
  - clinical-scale, regulatory-compliant cell engineering
  - payload flexibility
- Non-viral technology that overcomes challenges associated with other delivery methods and provides:
  - increased efficiency
  - elimination of safety and toxicity concerns
  - decreased cost and complexity of manufacturing
  - reduced time to market
- Proven technology supported by numerous publications, clinical trials and 45+ partnered clinical development programs

**Figure 2:** 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 therefore whose ability may be suppressed by the tumor micro-environment. In Mol. Ther., 23(8), 1380-1390, 2015 and summarized above articles 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.

**Figure 3:** Gene editing is being used to improve cell-based therapies, including disruption of checkpoint protein genes to create engineered effector cells to tumor immunosuppression or knockdown of endogenous TCRs to reduce mispairing of endogenous and engineered TCRs and/or to create allogeneic donor cells. The work published in Mol Ther. 24(9), 1570-1583, 2016 and summarized above augmented their previous virus-based CRISPR-mediated delivery method with the MaxCyte GT for electroporation of mRNA(ACS) encoding the Ca9 protein and potential helper proteins. The high efficiency of mRNA delivery to primary T cells, high cell viability, and payload flexibility of MaxCyte Flow Electroporation™ enabling its rapid integration into the overall CRISPR gene editing workflow. A high level of PD-1 or Tim3 was seen upon viral delivery of single checkpoint-specific gRNAs. A dual gRNA targeting both PD-1 and Tim3 lead to ~60% knockdown of both proteins as assessed via FACS. See publication for detailed methods.

**Figure 4:** Researchers at Sangamo developed a CRISPR-targeted zinc finger nucleases 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 adenosviruses to deliver the ZFN constructs. In phase 1/2 trials of ZFNs shown that CD4 cells with the ZFN constructs could be engrafted, were safe and preserved. Toxicity related to the adenoviral vector precluded the intended trials from progressing. To rescue the therapy, the company turned to mRNA delivery of the ZFNs using the MaxCyte GT. The work published in Mol. Ther. Methods Clin. Dev., 3, 2016 and summarized above demonstrate the rapid progression from proof of principle development of ZFN delivery, through manufacturing qualification runs, preclinical toxicity studies and initiation of clinical trial NCT02506849 using the MaxCyte GT. See publication for detailed methods.

**References**


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MaxCyte GT

**Multi-gene Knockdown for Improved Therapeutic Potency**

MaxCyte Payload Flexibility Extends Functionality of CRISPR Machinery Delivery