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 expanding portfolio technology for CAR engineering can skew therapeutic index 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.

Rapid Enablement of Clinical-scale TCR Engineering

Overcoming Clinical Challenges of Vital Delivery

Viral vectors challenges:
- Low viral transduction rates (~15%)
- HIV antigens from non-human transgenes - T-cells permanently engineered against HIV may cause liver damage
- Inconsistent transgene expression
- Complex & expensive to clinically implement

MaxCyte mRNA electroporation:
- Increased efficiency - high-level, transient TCR expression that conferred in vivo anti-tumor activity
- Eliminated safety concerns - no insertional mutagenesis or chronic inflammatory issues
- Decreased complexity of manufacturing - clinical scale, regulatory compliant electroporation
- ¼ 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-TOR construct delivery and showed early promise, but clinical application was hindered due to viral delivery including chronic inflammation due to the long-term presence of the TCR-engineered T cells. The work published in Mol Ther. Nucleic Acids, 2, 2013 and described 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 vitro functionality and in vivo anti-HCC activity and could be produced at clinical-scale using the MaxCyte GT. See publication for detailed methods.

Advancing CAR Therapy using NK Cell Engineering

High Performance, Clinical-scale Anti-C19 CAR Expression

Clinical Observations

- No adverse clinical events
- No difference in premature deaths
- No toxicity associated with modified cells

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
  - Decrease cost and complexity of manufacturing
  - Reduced time to market
- Proven technology supported by numerous publications, clinical trials and 45+ partnered clinical development programs

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 therefore whose activity may be suppressed by the tumor microenvironment. In Mol. Ther. 23(3), 1380-1390, 2015 and summarized above are studies that show the use of the MaxCyte GT to reportedly 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 vitro. 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

Results for Bulk Cell Populations for All [58-728mR T pp]

- 55% Immunodeficiency
- 50% Cytotoxicity

Clinical Evaluations

- No adverse clinical events
- No difference in premature deaths
- No toxicity associated with modified cells

Figure 4: Researchers at Sangamo developed a C19-targeted zinc finger nuclease that they showed was active in a variety of CD4 T cells and HSPOPcs that had 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 non-viral delivery of the CCR5-specific ZFN using the MaxCyte GT. The work published in Mol Ther. Methods Clin. Dev. 3, 2016 and summarized above demonstrated the rapid progression from process development of ZFN delivery, through manufacturing qualification runs, pre-clinical毒性 studies and initiation of clinical trial NTCT250049 using the MaxCyte GT. See publication for detailed methods.

References


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