Chimeric Antigen Receptor (CAR) messenger RNA (mRNA) loaded freshly isolated peripheral blood lymphocytes as a point-of-care targeted tumor immunotherapy

Linhong Li, Cornell Allen, Pachai Natarajan, and Madhusudan V. Peshwa*

MaxCyte Inc, 22 Firstfield Road, Suite 110, Gaithersburg, MD, USA
*Corresponding Author Contact: mvpeshwa@maxcyte.com / +1 301 273-5033 (cell)

Abstract:
CAR is a validated approach to enhance specific anti-tumor activity. Initial clinical trials using lentiviral vectors encoding anti-CD19 CAR demonstrated durable clinical responses. However, the ability to translate these findings to target other antigens in solid tumors has been limited because of clinical trials due to “off-target” activity of virus-vector modified CAR-T cells. Using mRNA-loaded CAR (mCAR) encoding CAR genes under control of toxicity to normal tissue and allowing translation of CAR-T cell immunotherapies to solid tumors. In preliminary human studies, mCAR encoding anti-CD19 CAR was reported to be safe and resulted in immunologic reduction in tumor burden in two patients. In this published study, transfected CAR-T cells were evaluated in an expanded T-cell protocol developed with MaxCyte’s mRNA delivery system and utility of existing therapeutic approaches. Our results indicate that this can effectively load mCAR in vitro into isolated peripheral blood lymphocytes obtained from therapeutic approaches with high cell viability and clinical efficiency. In a xenograft-specific cytotoxicity assay, mCAR-PBL exhibit dose-dependent neutrophil-based CAR-T cell killing and equivalent CAR-T cell killing from the same donor. In several studies using isolated and expanded lymphocytes, mCAR-PBL demonstrated antigen-specific and antigen-independent activity.

We have scaled-up mRNA-PBL manufacturing to process an entire Therapeutic Apheresis product in 20 minutes and are working to translate into human clinical trials.

mRNA CAR-PBL Manufacturing

MaxCyte Platform enables ex vivo Cellular Engineering resulting in Enhanced Potency products with Rapid, Robust, Scalable, Automated, Cost-effective Manufacturing

MaxCyte Platform Overview
- High viability and loading efficiency
- Consistent product quality and function
- Scalable: [10^6 - 10^7] cells per run
- Minimal cell disturbance / Toxicity
- Closed, automated platform
- Applications: Autologous Cell, Allogeneic Cell, Abdominal Cell

Manufacturing process uses sterile pre-packaged, single-use, closed system for aseptic processing

α-CAR delivery as mRNA using MaxCyte result in Overall Survival kinetics similar to LV

Treatment of Advanced Leukemia in Mice with mCAR Engineered T Cells

α-CAR delivery as mRNA using MaxCyte result in Overall Survival kinetics similar to LV

Treatment of Advanced Leukemia in Mice with mCAR Engineered T Cells

Summary of Human POC Data
Cancer Immunology Research (Feb, 2014)

Anti-Mesothelin CAR-mRNA loaded ex vivo expanded T-cells for treatment of Mesothelioma and Pancreatic Cancer patient

α-Mesothelin CAR-mRNA loaded in expanded T-cells using MaxCyte GM-CSF Electroporation System for manufacture of all product lots

Product resulted in expression of "Hi CAR" T-cells, and exhibited 60% cell viability and 85% cells expression of CAR reductase in mRNA loaded expanded T-cells

Clinical Observations: Patients infused were reported to be safe without any event evidence of a human factor toxicity agent against normal tissues
- α-Mesothelin T-cells permeated transiently in peripheral blood after iv administration; were observed traffic to primary & metastatic tumor sites - α-Mesothelin T-cells elicited an anti-tumor immune response revealed by (1) anti-tumor activity in vivo (2) role of cytokines in vivo
- Clinical benefits (anatomical unloading) (1) clinical benefit imaging (2) patients

Summary:
- mRNA CAR appears to have potential!!
  - In vitro & animal data demonstrates utility of using mRNA CAR to replace viral-vector CAR
  - Multiple human clinical trials ongoing of mRNA CAR loaded expanded T-cells to demonstrate safety & biological activity
- Can the process of mRNA-CAR loading be adapted to process that does not require ex vivo CAR-gene insertion
  - In vitro & preliminary animal results confirm that mRNA CAR can be effectively loaded into isolated peripheral blood lymphocytes with 100% viability, efficiency, CAR expression, and anti-tumor activity
- Increases product-specific manufacturing time from 12-24 hours to 10 hours (without requiring any ex vivo cell expansion)