Electroporation of a Non-Integrative DNA Nanovector for Efficient, Semi-Automated, GMP Manufacturing of CAR T Cell Therapies

Scientific Brief | Cell Therapy

Abstract

Chimeric Antigen Receptors (CARs) have shown extraordinary efficacy in numerous clinical trials as an adoptive cell therapy to treat hematological malignancies. Still, CAR T therapy faces significant challenges, ranging from long lead times and expensive manufacturing to complicated vector engineering. Here we describe nano-S/MARt (nS/MARt), a novel DNA vector platform for stable CAR expression with minimal disruption of T cell activity. This antibiotic-free, nanovector technology uses scaffold/matrix attachment regions (S/MARs) for DNA vector maintenance and replication, and transfects primary human T cells efficiently without toxicity. When combined with GMP-compliant MaxCyte® Flow Electroporation® and CliniMACS Prodigy® automated cell processing, nS/MARt enabled the production of recombinant T cells with stable CAR expression and enhanced anti-tumor activity in only five days. The result was a shortened manufacturing protocol, producing safer cell therapeutics for thousands of patients from a single batch.

GMP-Compatible, Large-Scale CAR T Cell Manufacturing with nS/MARt Vectors

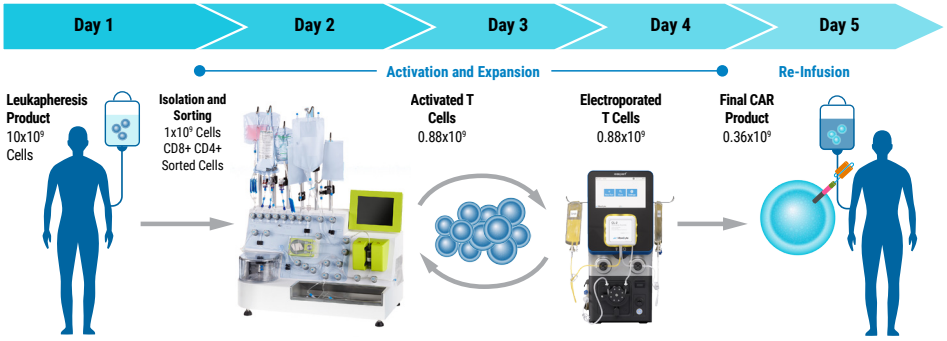

Figure 1. A manufacturing protocol was developed to allow the generation of clinical-grade recombinant T cells using nS/MARt vectors. For this, the CliniMACS Prodigy device (Miltenyi®), a fully automated and closed system for the isolation and culturing of primary human CD3+ cells was coupled with the ExPERT GTx™ large scale electroporation platform (MaxCyte).

Potent Tumor Cell Killing & IFNγ Production at Scale

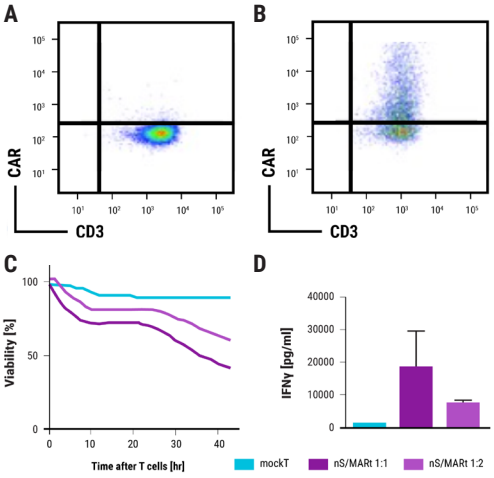

Figure 2. A) CD3+ cells were isolated from a leukapheresis product with the TCT process using the CliniMACS Prodigy. B) Sorted cells were activated for 3 days with T cell TransAct™, IL-7, and IL-15. On day three 1 x 10^8 cells were counted and electroporated with 125 µg/ml of DNA using the GTx™. Shortly after electroporation, the cells were returned to the CliniMACS Prodigy and fed with IL-7- and IL-15 supplemented medium for one day. On day 5, cells were harvested and analyzed by FACS for CAR expression. C) The capability for killing tumor cells was tested in an *in vitro* killing assay D) and INF-γ production measured.

MaxCyte has Minimal Impact on Cell Proliferation & CD4:CD8 Ratios

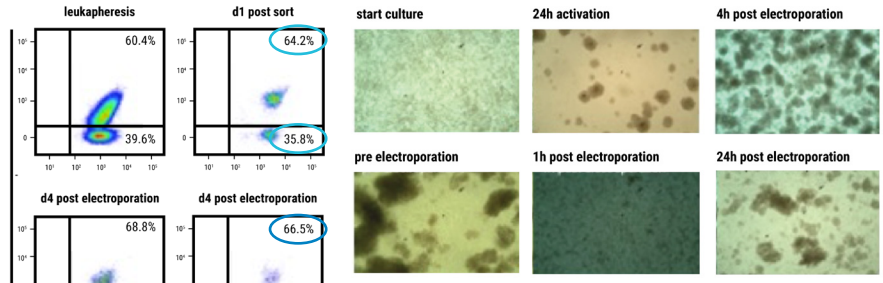

Figure 3. A) FACS plots show the same percentage of CD8+ and CD4+ cells within the CD45+/CD3+ subset before and after manufacturing. B) Samples taken from the CliniMACS Prodigy® at different time points pre and post electroporation show the T cells' proliferative capacity.

Summary

- MaxCyte Flow Electroporation provides efficient, scalable and GMP-compliant delivery of CARs.
- MaxCyte enabled efficient delivery of nS/MARt vectors to difficult-to-transfect primary T cells.
- T cell proliferation was not impaired and CD4:CD8 ratios were not altered.
- Active T cells demonstrated highly efficient killing capabilities *in vitro*.

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