Developing an Adoptive Cell Therapy Against AML

Sophisticated Gene Editing & mRNA-based CAR Expression Enable Novel NK Cell-based Therapy

Scientific Brief

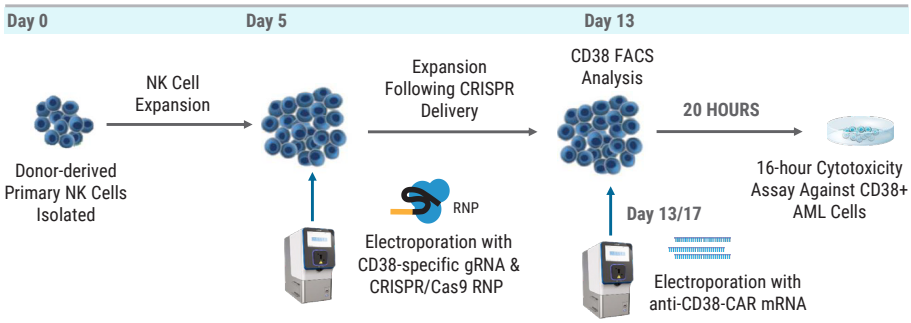
Abstract — Advancing Adoptive Cellular Therapies Using Non-viral-based Engineering

Excitement over recent promising breakthroughs in autologous cellular therapies has been tempered by the manufacturing costs associated with viral-based gene delivery methods and by safety concerns associated with random integration of viral vectors. As an alternative to viral-based gene delivery, we describe the use of the clinically validated and scalable MaxCyte Flow Electroporation® Technology for delivering chimeric antigen receptor (CAR) mRNA and CRISPR/Cas9 ribonucleoprotein (RNP) to natural killer (NK) cells. We also show efficient knockout of CD38 and expression of anti-CD38 CAR via mRNA delivery into NK cells with low cytotoxic effects.

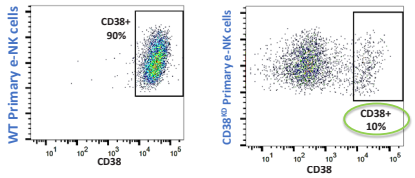
Case Study — ONK Therapeutics

Optimized NK Cells

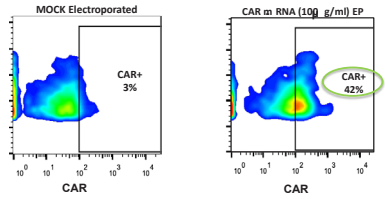
Experiment Workflow



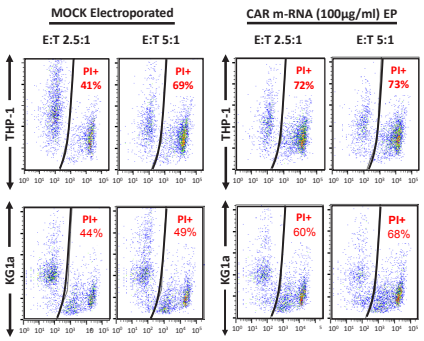
A. CD38-specific, CRISPR-mediated Knockout



B. Expression of anti-CD38 CAR via mRNA Electroporation



C. Cytotoxicity of CAR-expressing, CD38^{KD} NK Cells



Background

CD38 is a validated target for CAR immunotherapy in myeloma and is expressed on a majority of AML blast cells. Unfortunately CD38 is also expressed on primary NK cells and is upregulated upon *ex vivo* expansion precluding their use in anti-CD38 CAR immunotherapies. CRISPR/Cas9 editing was used to knockout CD38 expression & mRNA delivery of anti-CD38 CAR used to engineering CAR expression.

CRISPR Engineering of Primary Expanded NK Cells Followed by Expression of anti-CD38 CAR via mRNA Delivery.

Donor-derived primary NK cells were isolated and expanded, followed by CD38 knockdown using CRISPR-mediated Cas9 RNP-based gene editing using MaxCyte electroporation. Cells were expanded followed CRISPR editing and assessed via FACS on Day 13. Anti-CD38 CAR mRNA was delivered via electroporation on Day 13. 20 hours post electroporation cells were co-cultured in a 16-18 hour cytotoxicity assay against CD38+ AML cells. **(A)** Dot Plots represent CD38 expression on primary donor-derived expanded NK cells (+/- electroporation) 8 days post CRISPR delivery. **(B)** Dot Plots represent CD38 CAR expression 20 hours post CAR mRNA electroporation. **(C)** Dot plots represent results of cytotoxicity assay with mock- or CAR mRNA-electroporated CD38^{KD} cells. *Data courtesy of ONK Therapeutics.*

Summary

- Non-viral engineering enables rapid development of next-generation therapies with simplified, more cost-effective manufacturing.
- The high efficiency and low toxicity of MaxCyte Flow Electroporation provides for strong expression of exogeneous genes such as CARs and/or high knock-in/knockdown frequencies.
- MaxCyte Flow Electroporation® Technology (co)delivers of a diversity of payloads including mRNA, sgRNA, RNPs, and plasmid & minicircle DNA providing flexibility for sophisticated, non-viral engineering including:
  - transient mRNA expression
  - nuclease-mediated gene editing (CRISPR, TALEN, ZFN)
  - transposon insertion (Sleeping Beauty, piggyBac)
- MaxCyte clinical scalability and regulatory compliance provide for streamlined clinical translation of new therapies.

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