

CAR mRNA loaded freshly isolated peripheral blood lymphocytes as rapid, targeted tumor immunotherapy

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Abstract

CAR is a promising approach to enhance specific anti-tumor activity. Initial clinical trials using lentivirus vectors encoding anti-CD19 CAR demonstrated durable clinical responses. However, the ability to translate these findings to target other antigens in solid tumors has met with limited successes because of clinical toxicities due to 'on-target off-tumor' activity of viral-vector-modified CAR T-cells. Using messenger RNA (mRNA) encoding CAR permits control of toxicity to normal tissue and allows translation of CAR T-cell immunotherapies to solid tumors. In preliminary human studies, mRNA encoding anti-mesothelin CAR was reported to be safe and resulted in measurable reduction in tumor burden in two patients. In this published study, mRNA CAR loading into *ex vivo* expanded T-cells was achieved using the MaxCyte GT System; an automated, US FDA Master File-supported cGMP-compliant closed system for cell processing.

In this poster, we evaluated the anti-tumor potency of mRNA CAR loaded into freshly isolated peripheral blood lymphocytes (mRNA-CAR-PBL). We hypothesized that if anti-tumor activity of mRNA-CAR-PBL was similar to mRNA CAR loaded *ex vivo* expanded T-cells, it would permit commercial development of mRNA-CAR-PBL in a rapid format utilizing existing transfusion medicine infrastructure. In *in vitro* antigen-specific cytotoxicity assays, mRNA-CAR-PBL exhibited similar dose response to mRNA CAR loaded into *ex vivo* expanded T-cells from the same donor. In animal studies using localized and disseminated tumors, mRNA-CAR-PBL demonstrated anti-tumor activity and survival in dose-dependent manner. Our results indicate that we can effectively load mRNA CAR into freshly isolated peripheral blood lymphocytes obtained from therapeutic apheresis with ~90% cell viability and >90% efficiency.

We have scaled-up mRNA-CAR-PBL manufacture to process an entire Therapeutic Apheresis product (~1x 10¹⁰ cells) in ~20 minutes to allow for multiple doses from a single cell processing run and are working to translate into human clinical trials.

MaxCyte GT Transient Transfection Platform



- High viability and loading efficiency
- Consistent product quality and function
- Scalable [5 x 10⁵ → 2 x 10¹⁰ cells per run]
- Minimal cell disturbance / toxicity
- Customized biology [enhanced POTENCY]
- Regulatory Support
 - Master File with US FDA and Health Canada
 - CE mark
- ISO 9001:2008 certified Quality Systems
 - No human- or animal-derived materials employed
 - Rapid, automated platform utilizes single-use disposable technology
- Clinical / Commercial Validation
 - Used in multiple (10+) human clinical trials (US, Canada, Singapore, Japan)
 - Used in commercially marketed therapy (Japan)
- Broad IP (US & ROW patents / applications)

Summary

- ✓ mRNA CAR appears to have potential !!
 - ✓ *In vitro* & animal data demonstrates ability of using mRNA CAR to replace viral-vector CAR
 - ✓ Multiple human clinical trials ongoing of mRNA CAR loaded into expanded T-/NK-cells to demonstrate safety & biological activity
- Can the process of mRNA-CAR loading be adapted to process that does not require *ex vivo* T-cell selection and expansion?
 - *In vitro* and preliminary animal results confirm that mRNA CAR can be effectively loaded into freshly isolated lymphocytes with >95% viability, efficiency, CAR expression, and anti-tumor activity
 - reduces patient-specific manufacturing time from 10–12 days to ~5 hours (without requiring any *ex vivo* cell expansion)
 - Issued US Patent covers composition of matter, method of manufacturing and clinical utility for mRNA CAR PBL platform

Chimeric Antigen Receptor (CAR)

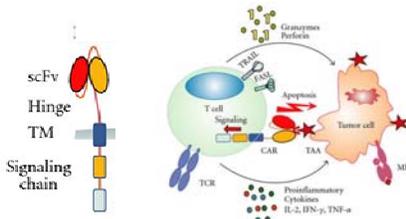


Figure 1. Schematic representation of different components of a chimeric antigen receptor (CAR) (left) and the mechanisms of the targeted tumor cell killing by the T cells expressing the CAR (right).

Retro-/Lenti-viral CAR delivery challenges

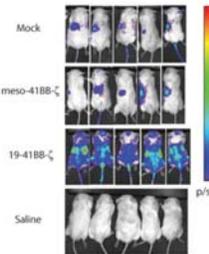


Figure 2. RV/LV-CAR is a gene therapy product. There are safety issues associated with it such as 'on-target off-tumor' toxicity against normal tissues, CAR expression is permanent and uncontrolled, the application is likely limited to treatment of B-cell malignancies, initial efforts to expand treatment to solid tumors has led to SAEs (patient death), and B-cell malignancy treatments have B-cell aplasia and require life-long IVIG infusion.

There are also issues with commercial implementation, scalability, and COGS issues. A two-week long *ex vivo* expansion process resulted in a high degree of variability in cell product, requirement for large-scale cGMP Lentivirus manufacturing (technical & COGS issue). The transduction efficiency of viral vectors is ~10–50% and only ~20% of final product expresses CAR. Expansion is inefficient and results in bystander cell growth. This means there is a need to manufacture 5X of effective CAR T-cell dose.

Safer, Non-Viral, & Commercial Approach for Delivery of CAR as messenger RNA (mRNA)

α-CD19 CAR Delivery as mRNA Using MaxCyte Result in Overall Survival Kinetics Similar to LV



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Figure 3. Specific trafficking and proliferation of RNA CARs in tumor-bearing mice. NOD/SCID/*gcr-1*^{-/-} (NSG) mice were injected IV with 10⁶ Nalm-6 cells followed 7 days later with 5x10⁶ T-cells 4 hours after electroporation with the indicated mRNA constructs. The T cells had been stably transfected with a lentiviral construct to express firefly luciferase, and mice were imaged for bioluminescence. CD19 RNA CARs exhibit increasing bioluminescence signal and anatomic distribution consistent with migration to sites of disease and CAR T-cell proliferation. Photon density heat maps on day 3 post injection suggest that mock T cells or T cells expressing RNA CARs with irrelevant specificity against mesothelin pool passively in the spleen (left flank on heat map) and do not increase in photon density, indicating a lack of proliferation.

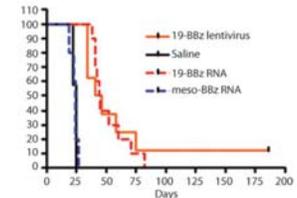


Figure 4. Survival with RNA CAR CTLs compares favorably with that with lentiviral generated CAR CTLs in the same model, although no long-term survivors are noted with a single infusion of RNA CAR CTLs, consistent with our observation that single injection does not entirely eliminate disease (n=12, summation of two independent experiments).

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Development of an α-Mesothelin CAR mRNA T-Cells for Solid Tumors

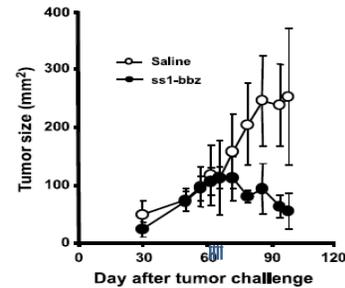


Figure 5. Regression of advanced vascularized tumors in mice treated with RNA CAR T cells. Flank tumors were established by M108 injection (s.c.) in NOD/scid/*ycl-1*^{-/-} (NSG) mice (n=6). Sixty-six days after tumor inoculation, mice were randomized to equalize tumor burden and treated with ss1-bbz RNA-electroporated T cells. The T cells (10–15x10⁶) were injected intratumorally every 4 d for a total of four injections; mice treated with saline served as controls (n=3). Tumor size was measured weekly.

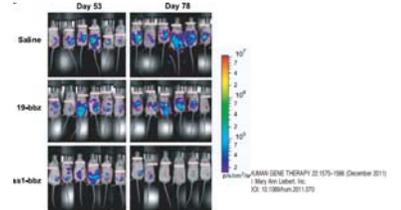


Figure 6. i.p. tumors were established in NSG mice (n=6 per group) by i.p. injection with 8 x 10⁶ M108-luc cells. Beginning on day 58, RNA CAR-electroporated T cells (1x 10⁷) expressing ss1-bbz were injected twice weekly for 2 wk. RNA CAR T cells expressing CD19-bbz RNA CAR or saline were injected as controls. On day 78, the luminescence signal was significantly decreased in the ss1-bbz mice compared with the CD19-bbz mice (P < 0.01).

Cancer Research

Multiple Injections of Electroporated Autologous T Cells Expressing a Chimeric Antigen Receptor Mediate Regression of Human Disseminated Tumors

MaxCyte GT Transfection System for Delivery of CAR as mRNA

mRNA CAR-PBL Manufacturing with MaxCyte GT

Process completed in ~2-3 hours v/s ~2 weeks

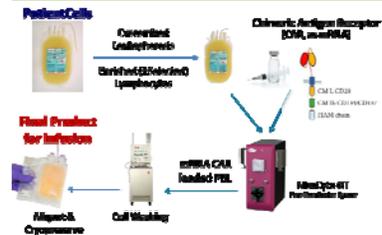


Figure 9. MaxCyte Platform enables *ex vivo* cellular engineering resulting in enhanced potency products with rapid, robust, scalable, automated, cost-effective manufacturing.



Figure 10. Potential applications of MaxCyte GT technology platform in enabling various approaches in therapeutics, protein, and virus manufacturing and cell-based drug screening with the use of different cell types, either autologous cells, allogeneic cells, and cell lines.