Long-acting circular RNA CAR drives potent and durable tumor elimination in vivo

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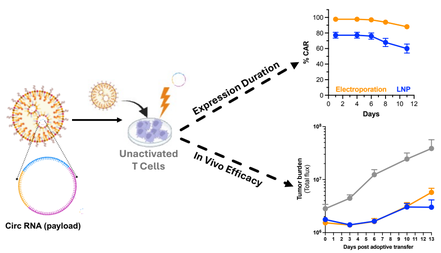
Abstract

In vivo engineered mRNA-based cell therapies can overcome many of the limitations of current stably integrated cell therapies such as Chimeric antigen receptor (CAR) T cell therapy. Broader patient access, ease of manufacture, significantly lower cost, immediate availability of drug product, ease of repeat dosing and improved safety profile with the absence of stable integration and debilitating lymphodepletion regimens are some of the key advantages of an in vivo engineered mRNA cell therapy approach. However, due to the short-lived nature of RNA expression, levels of CAR protein expression and duration must be improved to match the effectiveness observed with stable integration-based cell therapy.

We previously demonstrated the development of circular RNA (circRNA) IRES sequences that enable 10x higher levels than the current state of the art IRES and up to 25 days of CAR expression when transfected into non-activated primary human T cells. These circRNA CAR-T cells drove multiple rounds of robust repeat tumor cell killing *in vitro* over 15 days in culture. When adoptively transferred into a tumor bearing mouse, circRNA CAR-T cells effectively controlled tumor burden up to 7 days post transfer.

Building on this work, we show that our circRNAs can transfect T cells with up to 80% efficiency within the mononuclear cell (MNC) population in fresh leukopaks using lipid nanoparticle (LNP) based delivery. These transfected cells, when adoptively transferred into tumor bearing mice, showed complete tumor elimination on par with lentivirus transduced T cells, with sustained tumor control lasting 21 days post transfer. We also demonstrate here, the ability of miRNA-based genetic circuits within circRNA to be highly effective in de-targeting expression in non-target cells while preserving expression in target cells. By integrating Strand's proprietary programmable genetic circuitry for highly specific payload expression, advanced circRNA production processes, and enhancements in expression duration and levels, we are developing an in vivo engineered circRNA CAR platform for potent and long-lasting CAR therapy.

Background Data



We have previously demonstrated

1. Novel IRES's, in circRNA, with >5-10x improved payload expression in resting 'activated' primary T cells, over current state of the art IRES's.
2. Strands IRES allow for superior tumor clearance in a repeat tumor challenge and long-term cultures than current start of the art circRNAs.
3. circRNAs encapsulated in our optimized LNPs drive robust transfections and duration expression, >21 days, of CAR payloads in unactivated T cells in-vitro.
4. T cells transfected with LNPs encoding CAR driven from circRNAs can outperform T cells transfected via electroporation at tumor clearance.

Preferential transfection of T cells within fresh unactivated MNCs through passively targeting LNPs

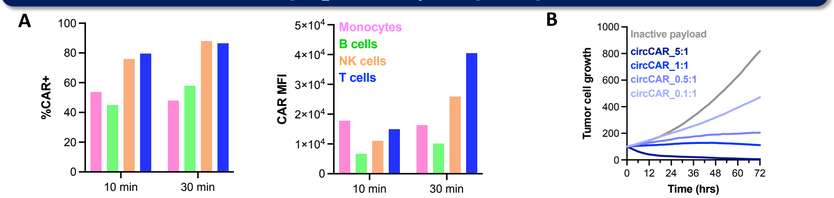


Fig 1: A: CAR expression in T cells, B cells, NK cells, and Monocytes after a 10- or 30-minute incubation of fresh unactivated Mono-nucleated cells (MNCs) with LNPs containing a circRNA encoded CAR. **B:** Transfected fresh unactivated MNCs show a robust and dose dependent anti-tumor cytotox against cancer cell lines in-vitro

LNP transfected unactivated MNCs exhibit potent cytotoxic activity against multiple cell lines

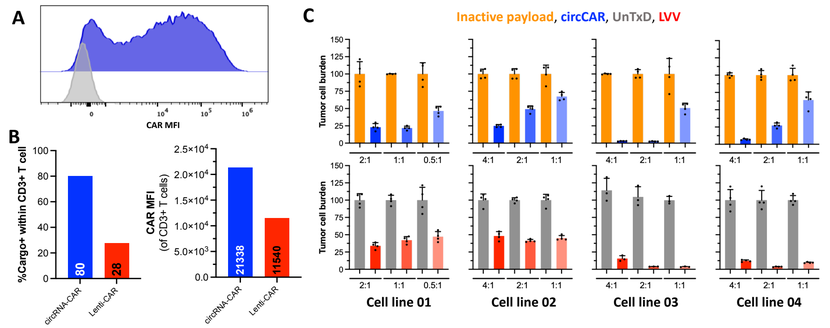


Fig 2: A: Unactivated MNCs transfected with an LNP encoding circRNA-CAR show a robust expression of the CAR payload. **B:** circRNA-CAR payloads are expressed at higher levels (both % and per cell MFIs) within Unactivated T cells compared to T cells that have been transduced with a Lentivirus encoding the same CAR and expanded using standard protocols (CD3+28 cocktail and 10-day expansion). **C:** In-vitro tumor cytox at different Effector : Tumor cell co-culture ratios of transfected MNCs or transduced T cells against four different cancer cell lines.

LNP transfected unactivated MNCs expressing circRNA-CAR drive potent and durable tumor elimination in-vivo

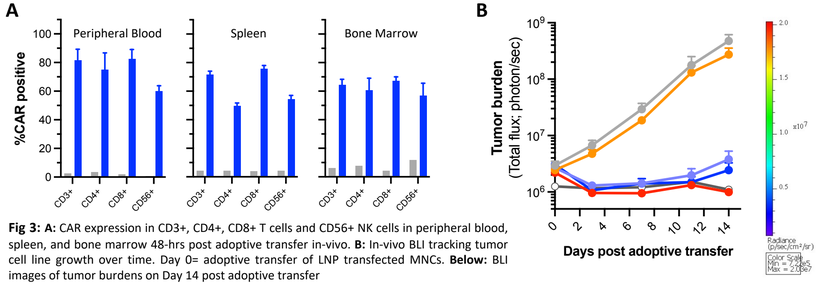
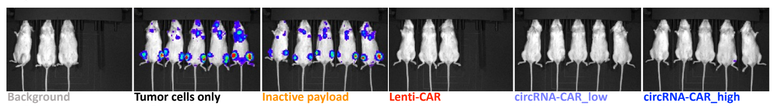


Fig 3: A: CAR expression in CD3+, CD4+, CD8+ T cells and CD56+ NK cells in peripheral blood, spleen, and bone marrow 48-hrs post adoptive transfer in-vivo. **B:** In-vivo BLI tracking tumor cell line growth over time. Day 0= adoptive transfer of LNP transfected MNCs. **Below:** BLI images of tumor burdens on Day 14 post adoptive transfer



Cell type specific miRNAs can be leveraged to create high fidelity genetic circuits enabling organ de-targeting

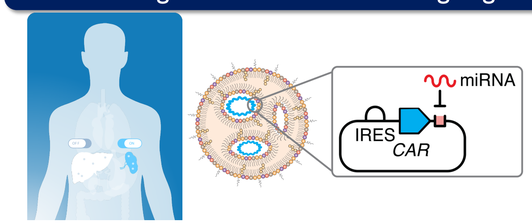


Fig 4: We have developed a platform in which we design programmable genetic "circuits" that detect molecular cues in a cell. These circuits are encoded within our RNA payloads to limit their expression only within cells that exhibit a desired molecular signature. Through in silico modeling and high-throughput in vitro screening, we can select miRNA candidates to de-target specific combinations of organs to drive robust expression of payloads only in the desired cell types with high fidelity.

Genetic circuits can be used to encode cell type specificity for payload expression from circRNAs

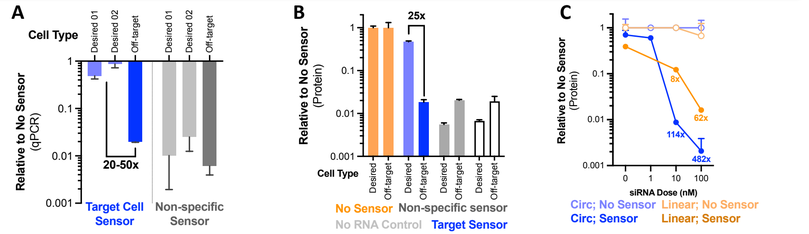


Fig 5: A and **B:** Encoding genetic circuits allows for highly specific and rapid degradation of circRNAs in off- target cells while driving robust expression in desired cell types. **A;** qPCR for circRNA and **B;** protein payload expression. The non-specific is a positive control miRNA expressed in all three cell types. **C:** circRNAs exhibit higher fidelity and allow for a larger on/off dynamic range of expression compared to linear RNAs.

Genetic circuits enable >90% KD in undesired organs while driving robust on-target expression from circRNAs in-vivo

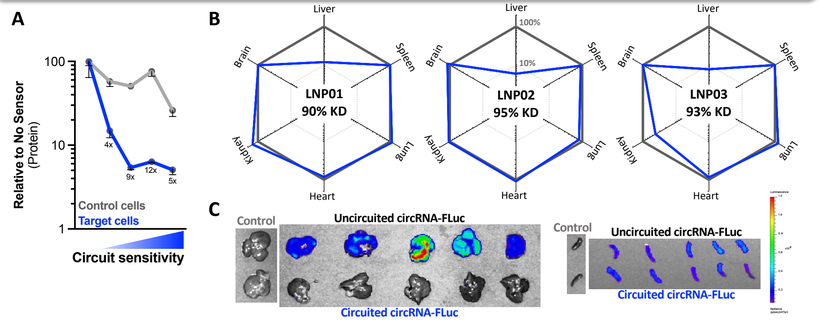


Fig 6: A: Genetic circuits on circRNAs can be fine tuned to achieve a range of desired payload expression knock-downs in off-target cells. **B:** Circuited circRNAs can robustly and reliably de-target payload expression in the Liver without affecting expression in other organ types. **C:** Representative images of Liver and Spleen for LNP01 from figure **B**.

Conclusions

- Strands LNPs can passively target T cells within fresh MNC pools and drive robust expression of optimized circRNA CAR payloads.
- Transfected fresh unactivated T cells exhibit higher expression of CAR payloads from circRNAs than Lentivirus delivered payloads. And show robust anti-tumor cytox activity against multiple cell lines in-vitro.
- Fresh unactivated MNCs transfected with LNPs encoding circRNA CARs show strong expression in-vivo post adoptive transfect.
- Single dose of MNCs transfected with LNPs encoding circRNA CARs show robust and durable tumor control in-vivo.
- Strands proprietary genetic circuits can be deployed in a circRNA context and outperform established linear modified RNA benchmarks enabling robust on-target expression while minimizing undesired off-target expression.
- Genetic circuits can be leveraged to create systemically delivered off-the-shelf in-vivo CAR-T therapies.

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