Circular RNA Based Chimeric Antigen Receptor (CAR) Cell Therapy for Potent and Long-lasting Anti-Tumor Activity

Poster # 1825

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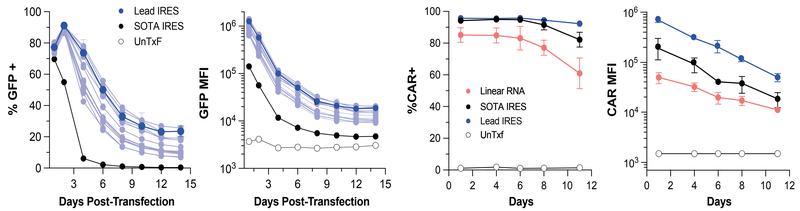
Introduction

Stably integrated ex vivo engineered Chimeric antigen receptor (CAR) T cell therapy approaches have been effective for the treatment of hematological malignancies. However, broader patient access is limited by extremely complex, time-consuming, and expensive manufacturing. Recently safety concerns have arisen from random vector integration leading to secondary malignancies. In vivo engineered, mRNA-based cell therapies can overcome these limitations of current cell therapies, but the approach needs to be optimized to drive complete and long-lasting tumor control. To overcome the transient nature of mRNA expression and improve efficacy, we developed optimized circular RNA (circRNA) sequences that drive long-lasting and therapeutically relevant levels of CAR expression in ***non-activated*** primary human T cells and ***freshly isolated*** human peripheral blood mononuclear cells (PBMCs). These circRNA-CAR cells drive potent cytotoxic responses against target tumor cells in short and long-term cultures and upon multiple rounds of target cell exposure. Additionally, we show that these circRNAs can be delivered to immune cells using LNPs and that these LNP transfected cells exhibit robust ***in-vivo efficacy***. In combination with Strand's synthetic circuits programming RNA for cell type specific expression, our platform *paves the way for the development of a truly off-the-shelf CAR cell therapy* with the capacity to rapidly iterate with multiple antigen targets at a much lower cost and accelerated pace.

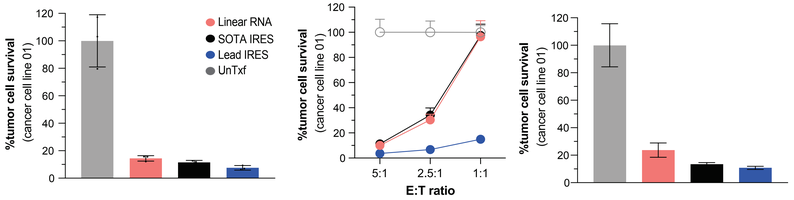
Novel IRES Screening identified circRNA IRES's with superior expression and persistence in T cells



A library of >400 unique IRES sequences was generated and tested in T cells. Multiple novel IRES sequences >10x better than current state of the art (SOTA) IRES's were identified.

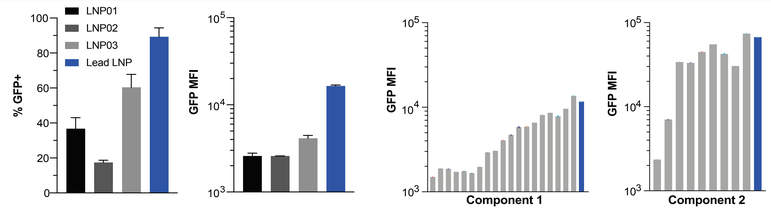
Strands novel IRES's drive a >10x higher expression of a CAR payload in Resting T cells, from circular RNA (circRNA)

Novel circRNA IRES drives enhanced tumor cell killing in repeat challenge assays



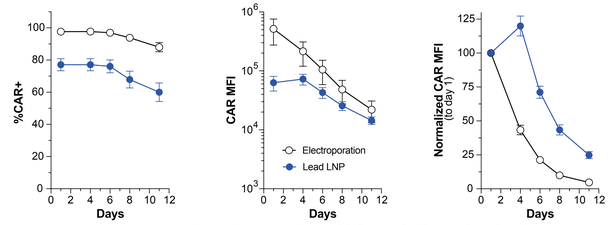
Left: Non-activated T cells electroporated with SOTA, lead circRNA IRES or linear RNA CAR show comparable tumor cytotoxicity at day 1 post transfection, co-cultured at 5:1 Effector:Tumor. ***Middle:*** Lead IRES CAR T cells show a markedly enhanced anti-tumor efficacy upon a *repeat tumor challenge* compared to linear RNA or SOTA IRES. ***Right:*** T cells expressing CAR from the lead IRES retain robust anti-tumor cytotoxic potential at 11 days post transfection.

LNP screening and optimization



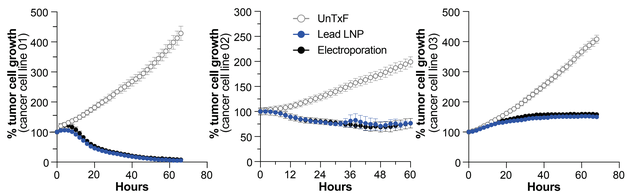
Multiple LNP formulations were screened for transfectability of primary human resting T cells, and a lead LNP formulation was exhaustively optimized through an iterative design of experiments (DOE) process.

Optimized LNPs lead to robust and stable expression of circRNA IRES encoded CAR cargo



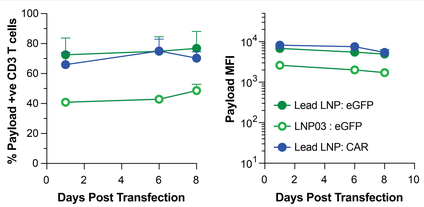
Resting T cells were transfected with circRNA-CAR via electroporation or LNP transfection and payload expression was quantified longitudinally. Strand's lead LNP exhibited significantly improved payload expression dynamics enabling a prolonged peak payload expression profile, unlike gold standard eletroporation methods.

LNP transfected T cells exhibit potent cytotoxic activity against multiple cancer cell lines



Lead LNP circRNA CAR transfected resting T cells exhibit potent tumor cytoxicity, benchmarked to electroporated cells, across 3 different tumor cell line models at 5:1 E:T ratio.

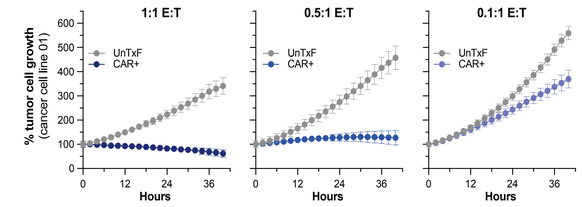
Optimized LNP drives>60% transfection of T cells within a freshly isolated PBMC pool



Freshly isolated primary PBMCs transfected with Strand's lead LNP leads to a >60% payload positive CD3+ T cell transfection, in a payload agnostic manner (eGFP and CAR). Furthermore, these CAR T cells display robust and stable payload expression for >1 week.

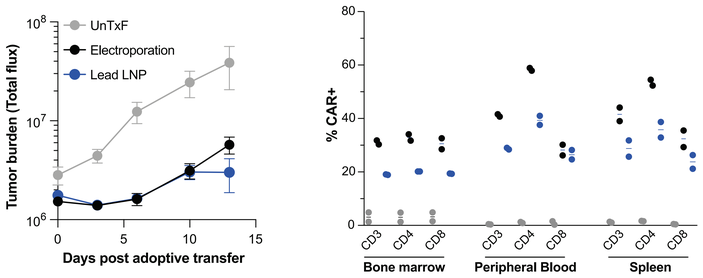
Additional workflow optimization has shown a reproducible 80-95% transfection rate with >80% cell recoveries 24 hrs post transfection (data not shown).

LNP transfected fresh PBMCs drive robust cytotoxic activity



Freshly isolated primary PBMCs were transfected with Strand's lead LNP circRNA CAR and co-cultured with cancer cells at multiple E:T ratios. Complete tumor control was seen at 1:1 E:T and 0.5:1 E:T, and some control even at a 0.1:1 E:T co-culture.

Strand LNP circRNA transfected resting T cells drive robust in-vivo tumor clearance



Left: Resting T cells transfected with circRNA CAR via electroporation or LNP transfection were adoptively transferred 24 hours post transfection into tumor bearing NSG MHC double KO mice (n=5 per group). LNP transfected groups show robust tumor control, comparable to the gold standard electroporated groups. ***Right:*** 3 days post adoptive transfer of transfected cells, significant levels of CAR+ T cells were observed across bone marrow, spleen, and peripheral blood, confirming persistent CAR expression in-vivo.

Conclusions

1. Novel circRNA IRES identified show 5-10x improvement in peak payload expression as well as duration of expression in resting primary human T cells over state of the art circRNA IRES.
2. Strand's lead circRNA IRES driven CAR shows superior tumor cell killing in repeat challenge assays and long-term cultures and outperforms linear modified and state of the art circRNA IRES.
3. Lead IRES circRNA CAR with optimized lead candidate LNP shows robust transfection and stable expression over long-term culture translating to comparable in vitro efficacy against multiple tumor cell models as electroporated T cells.
4. Strand's optimized lead LNP drives robust payload expression of T cells in freshly isolated PBMCs and robust cytotoxicity at low effector to target ratios.
5. Lead LNP IRES CAR transfected T cells show robust CAR expression in vivo and drive complete in vivo tumor control in an NSG tumor bearing mouse model.

These datasets demonstrate that Strand's platform of human immune cell engineering with our *novel circRNAs* in combination with our *optimized lead LNPs* allow for a *truly off-the-shelf RNA-based CAR cell therapy.*