Optimized mRNA Design and Delivery for Non-Viral Generation of Chimeric Antigen Receptor (CAR) T Cells

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Introduction

CAR T cell therapy is successfully being applied to combat selected hematological cancers, but there are safety concerns and manufacturing challenges associated with the use of viral vectors for CAR T cell generation. Due to the non-integrating and non-permanent genetic modification, *in vitro* transcribed (IVT) CAR-encoding mRNA offers an efficient and safe alternative to viral vectors, but is still facing challenges for clinical translation due to mRNA's immunogenicity, poor stability, and low cellular uptake efficiency.

This study aims to improve mRNA technology for non-viral CAR T cell generation by investigating selected IVT-mRNA designs and delivery strategies into T cells in order to optimize CAR expression, mRNA stability and immunogenicity.
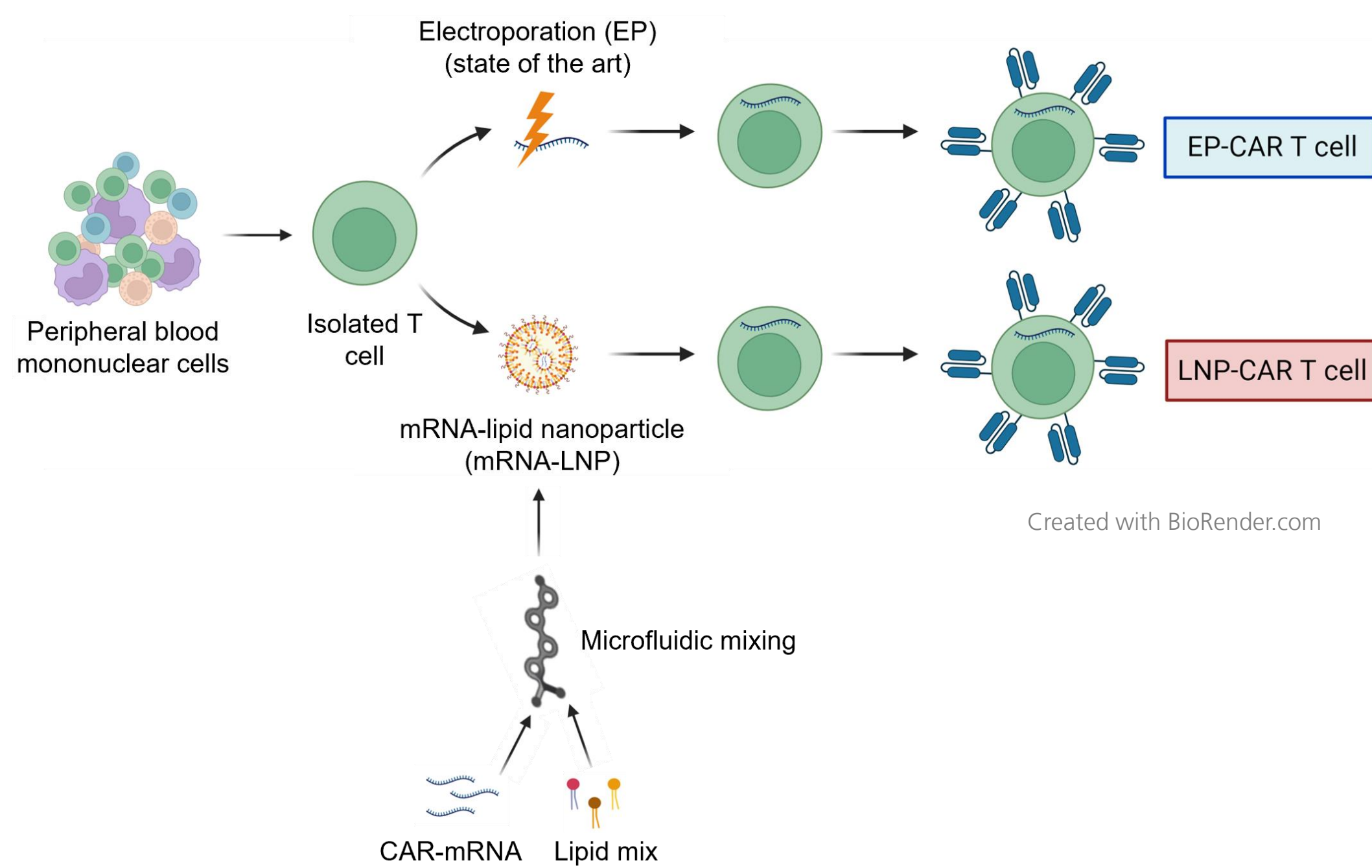
CAR expression
mRNA stability
mRNA immunogenicity

Optimization of mRNA Delivery[1]

Study design
Primary T cells derived from peripheral blood mononuclear cells from healthy donors were transfected with 6 µg CAR-mRNA/10^6 cells either by electroporation (state of the art) or by lipid nanoparticles.

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Results
LNPs outperformed EP due to prolonged CAR expression, intracellular CAR-mRNA persistence, and *in vitro* CAR T cell efficacy.
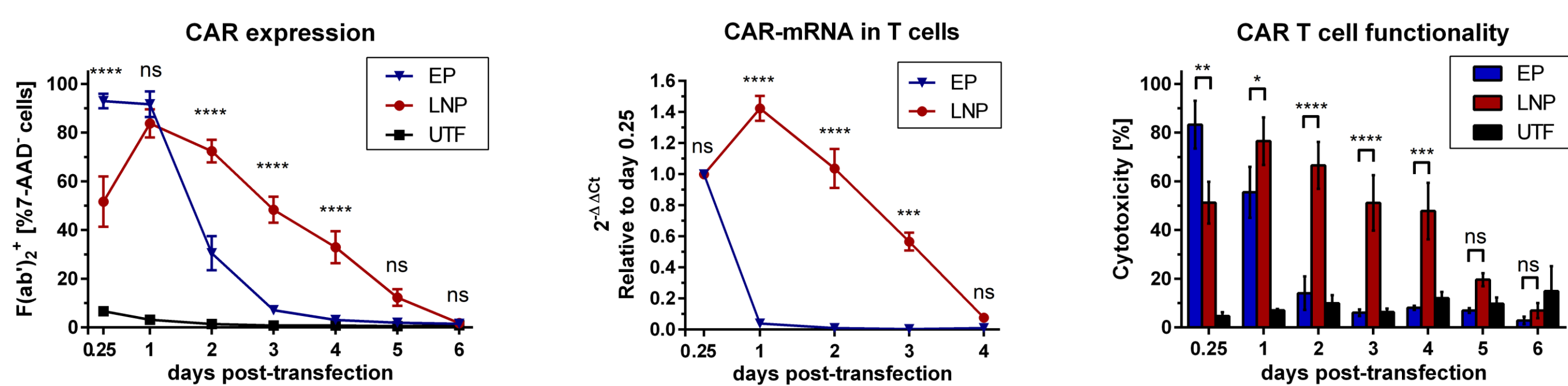

UTF: untransfected T cells; mean ± SEM of n=4 independent experiments/different donors
ns: not significant; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001, ANOVA with Holm-Sidak multiple comparison test

[1]Kitte *et al.*, Lipid Nanoparticles outperform Electroporation in mRNA-based CAR T Cell Engineering, 2023, Mol Ther Methods Clin Dev 31:101139.

Optimization of mRNA Design

Selection of a 5' cap analog
Incorporation of CleanCap AG (3' OMe) into IVT-mRNA led to a higher transcription efficiency and CAR expression intensity compared to anti-reverse cap analog (ARCA).


mean ± SEM of n=5
*p<0.05, paired t-test; ****p<0.0001, unpaired t-test
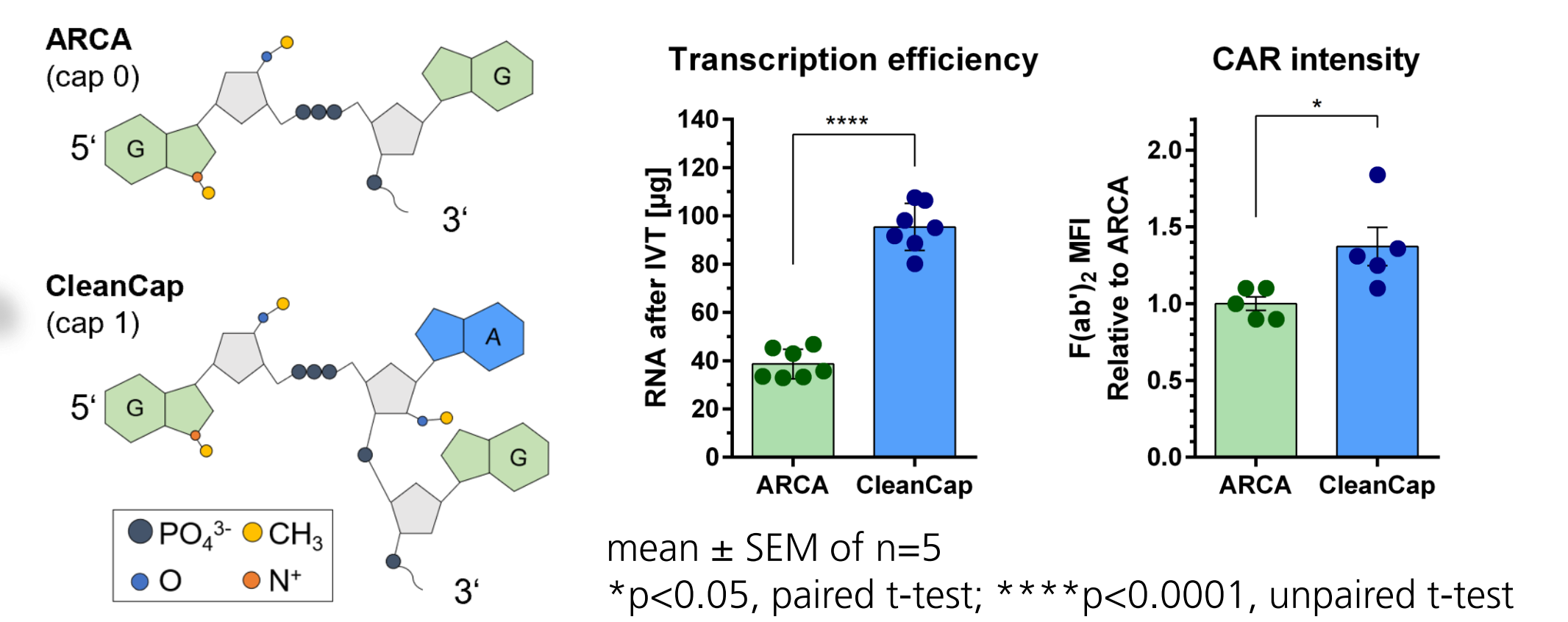
Impact of nucleotide modification
N^1-methylpseudouridine (m1ψ) resulted in reduced mRNA immunogenicity, but also CAR expression intensity is decreased compared to unmodified mRNA (uridine, U).

mean ± SEM of n=3
*p<0.05, paired t-test; **p<0.01; ***p<0.001, ANOVA with Holm-Sidak multiple comparison test
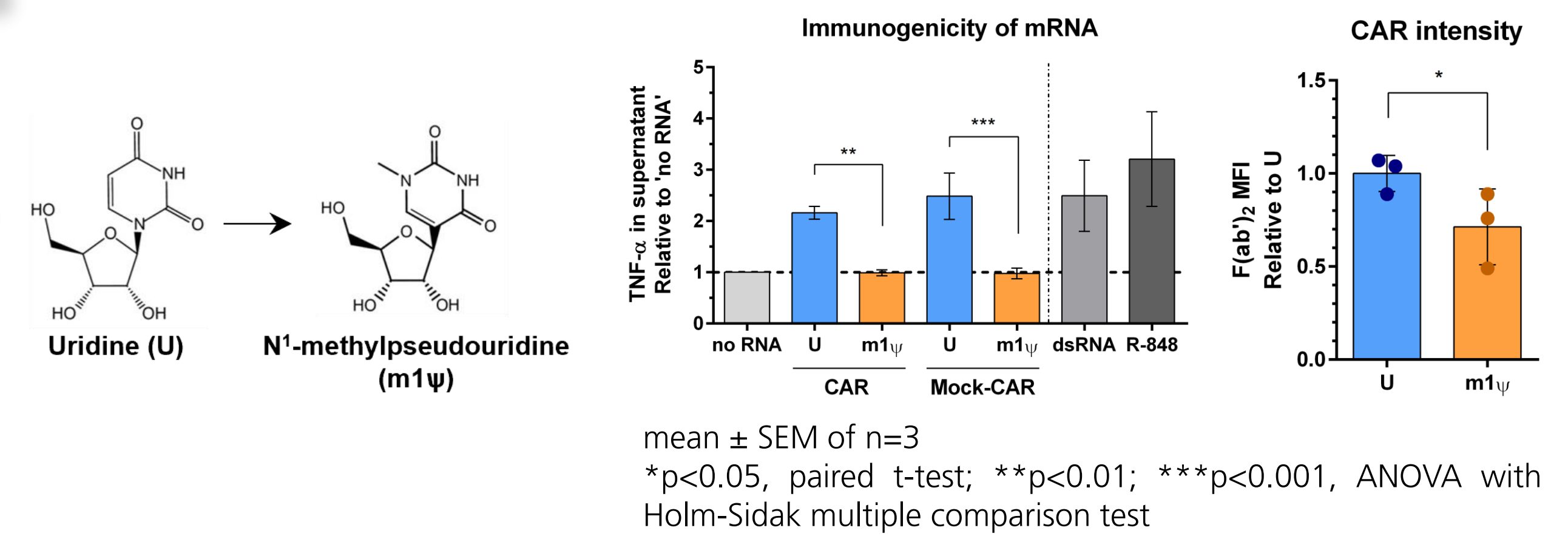
Impact of untranslated regions (UTRs)
CAR expression could be enhanced by incorporation of human alpha globin (hAG) 5' UTR and 3' UTRs deriving from amino-terminal enhancer of split (AES)-mRNA and mitochondrially encoded non-coding 12S rRNA (mtRNR1).

mean ± SEM of n=3
*p<0.05; ****p<0.0001, ANOVA with Holm-Sidak multiple comparison test
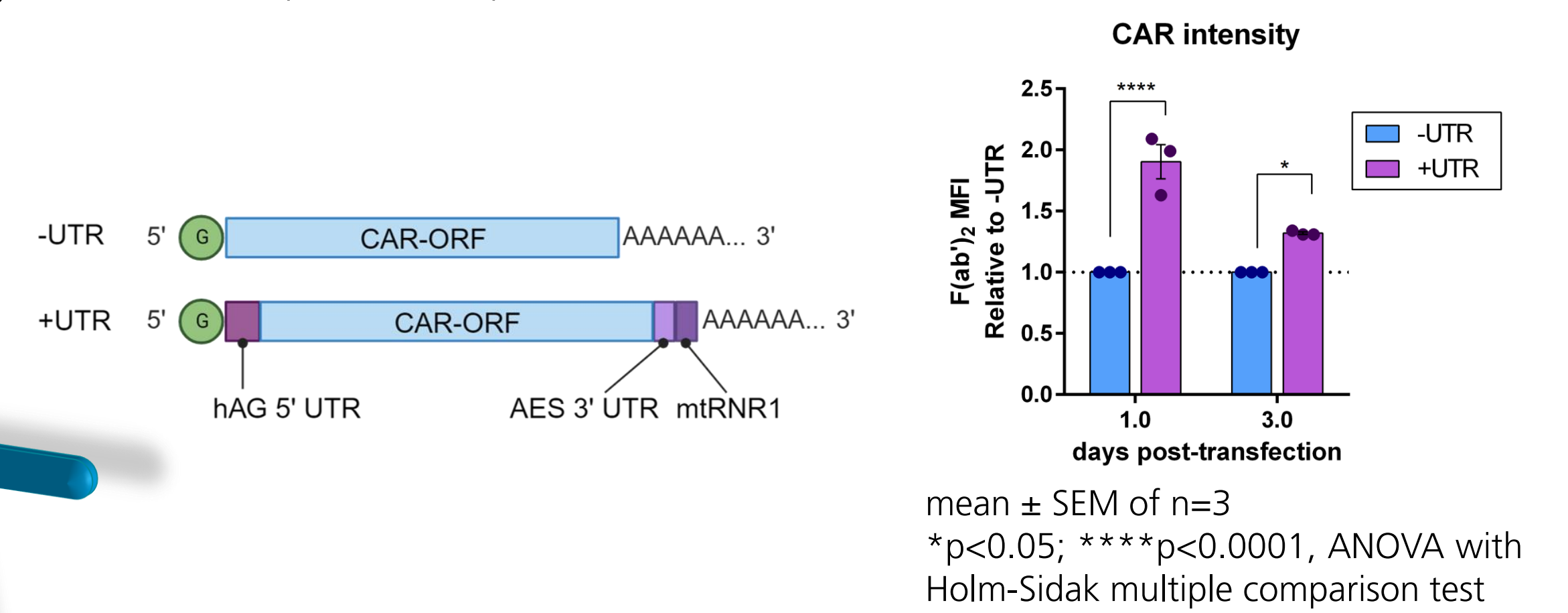
mRNA-CAR T cell generation from cancer patients

Results
mRNA-LNPs allowed generation of functional mRNA-CAR T cells from cancer patients.