mRNA sequence parameters and formulation optimization increase mRNA therapeutics performance

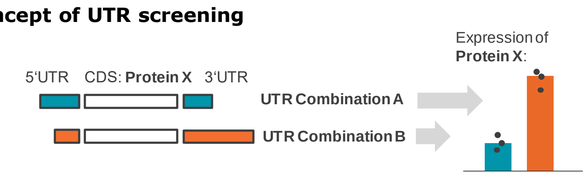
Dipankar Bhandari[1], Jörg Braun[1], Manuel Göpferich[1], Elena Khazina[1], Moritz Thran[1], Roland Böttger[1], Livia Palmerston Mendes[1], Dominik Vahrenhorst[1], Gemma Navarro[1], Patrick Baumhof[1] & Andreas Thess[1]

[1]CureVac SE, Tübingen, Germany

In vitro transcribed mRNA medicines enable cells to produce themselves proteins to fight disease. For efficient delivery to target cells, mRNA is often formulated as lipid nanoparticles (mRNA-LNP). mRNA medicines show promise in various clinical applications, such as preventing infectious diseases, as cancer immunotherapies, and as molecular therapies. Most prominently, mRNA-LNP have been validated as COVID vaccines; however, further improvement of both mRNA and formulation is required to further optimize potency and safety of mRNA medicines. We followed multiple optimization strategies to improve mRNA medicines regarding different aspects, such as boosting and fine-tuning protein expression and reducing innate immune activation. Machine learning models identified combinations of untranslated regions (UTRs) specifically increasing protein expression from either uridine- or N1mΨ-containing mRNA across various cell types. Introduction of miRNA binding sites enabled tissue-specific protein expression. Different RNA technologies, such as circular mRNAs, saRNAs or 3' sealed mRNAs enabled prolonged protein expression. Co-administration of immune suppressors reduced innate immune activation elicited by mRNA-LNP. Optimizing lipids and formulation can further improve efficacy and safety of mRNA-LNP. CureVac designed LNP formulations for i.m. vaccination that reduce systemic biodistribution while inducing strong adaptive immune responses. Taken together, various improvements in mRNA design and optimization of formulation each increased mRNA medicine performance.

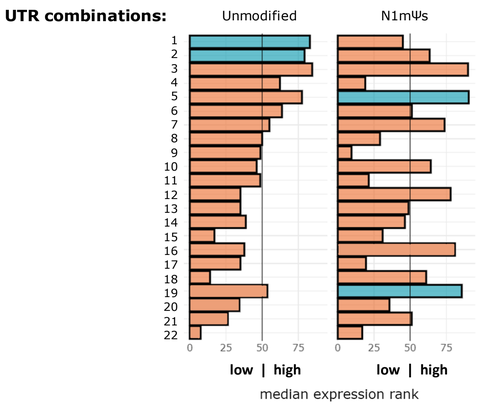
1. UTRs perform differently when uridines (unmodified RNA) are replaced by N1mΨs (modified RNA)

A Concept of UTR screening



- UTR screening of selected UTR combinations for both unmodified RNA (Us) and modified RNA (N1mΨs)
- Some of the UTR combinations were selected using machine learning
- Expression of 4 reporter proteins measured in HeLa & HepG2 cells
- Reporters: Rabies virus glycoprotein G, Firefly luciferase, EPO & HGF
- Statistics of expression rankings across 8 expression conditions

B UTR ranking is different between unmodified & modified RNAs



UTR performance

- Universal high performance
- Cell- / protein-specific high performance

Ranking of UTRs: UTR combinations were ranked based on highest expression as either unmodified (left panel) or N1mΨ RNAs (right panel). Four proteins were encoded by mRNAs containing various UTR combinations and were tested in two cell lines.

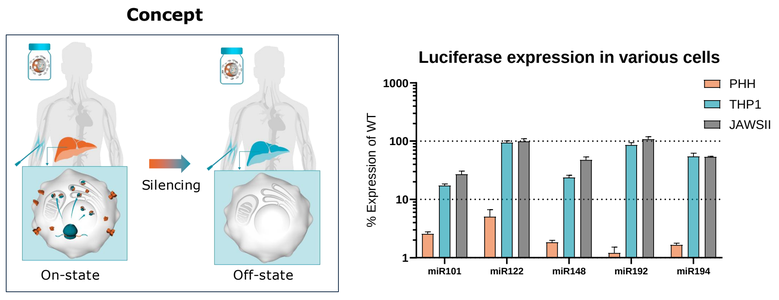
Blue: Highest consistency in performance across encoded proteins and cell types.

Orange: Medium / low consistency in performance across encoded proteins and cell types.

- UTR screening results point to promising new UTR combinations to further increase protein expression of CureVac-mRNAs

2. Incorporation of miRNA binding sites strongly reduces mRNA expression in mouse liver

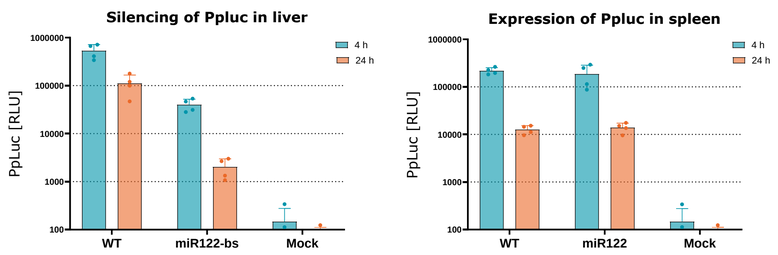
A Identification of miRNA binding sites to silence expression exclusively in the liver



- Various miRNA-bs allow silencing of mRNA in primary human hepatocytes (PHH)

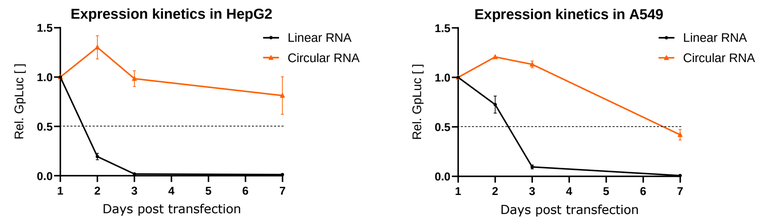
B miRNA122 strongly reduces expression in mouse liver

- Balb/c mice
- 5 µg mRNA-LNP encoding Ppluc (intravenous)
- Luciferase activity from lysates

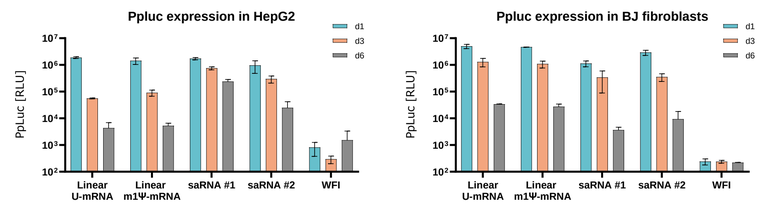


3. Different RNA technologies enable prolongation of protein expression in vitro

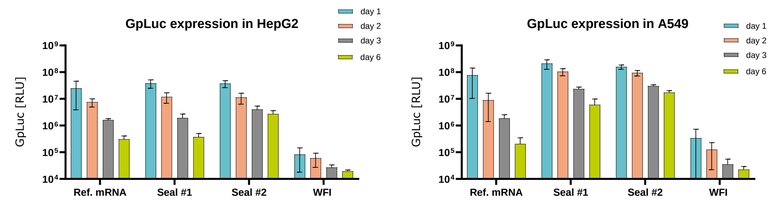
A CircRNA extends protein expression in cell lines



B saRNA extends protein expression cell type-specifically

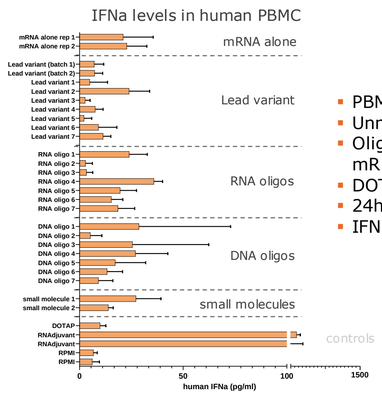


C 3' sealing extends protein expression in cell lines



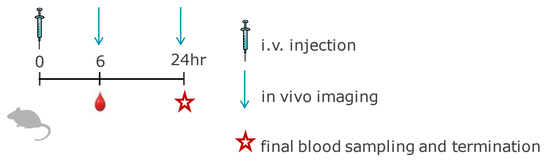
4. Trans immune suppression by TLR7/8 antagonists reduces mRNA reactogenicity in human PBMC and in vivo

A Various oligonucleotides reduce IFNa levels in human PBMC



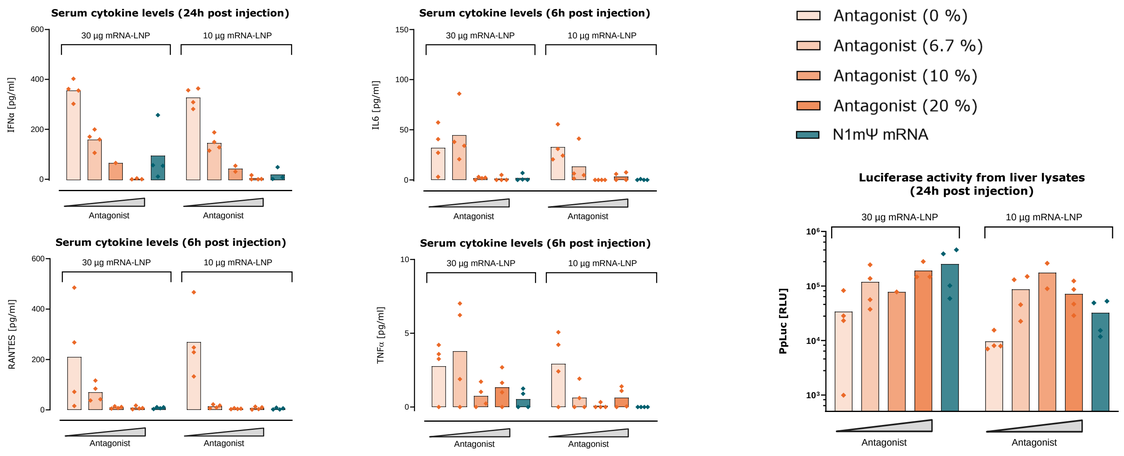
- PBMC stimulation
- Unmodified mRNA
- Oligos pre-mixed with mRNA at 1:5 mass ratio
- DOTAP transfection
- 24h incubation on cells
- IFNa ELISA

B Evaluation of trans immune suppression by a TLR7/8 antagonist in vivo (i.v. injection)



- 129SV mice
- TLR7/8 lead antagonist mixed with mRNA at various ratios
- Unmodified mRNA encoding Firefly luciferase
- Antagonist – mRNA mixtures formulated as LNP
- Intravenous injection of 10 or 30 µg mRNA-LNP
- Serum from 6h and 24h post injection
- Organs from 24h post injection
- Cytokines are analyzed from serum
- Luciferase activity in organ lysates

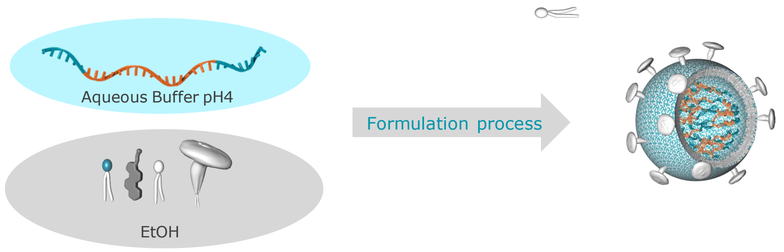
C TLR7/8 antagonist reduces cytokine levels in serum and improves expression in liver after i.v. injection



5. CV LNP is optimized for local biodistribution and enables significant immune response upon i.m. vaccination with very low expression in liver

A Lipid Nanoparticle composition

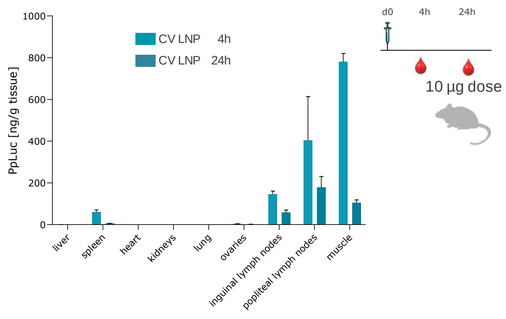
- Ionizable amino lipid is the key component that drives efficacy and tolerability
- PEG-lipid stabilizes the particle against aggregation
- Structural lipids are usually cholesterol and/or DSPC/DSPE



CureVac LNP Technology

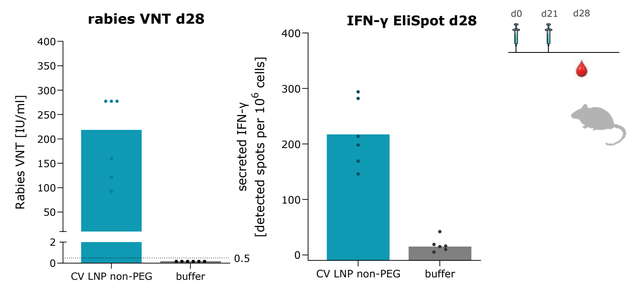
- ✓ Proprietary excipients
- ✓ Non-PEG LNP
- ✓ Competitive profile versus state of the art LNP
- ✓ Delivery to the muscle (vaccines)
- ✓ Local Mode-of-Action with very low unwanted systemic expression in liver
- ✓ Higher stability against dilution and freeze/thaw stress

B CV LNP is optimized for local biodistribution



- i.m. injection of 10 µg CV LNP results in expression in muscle and immune compartments (Lymph nodes & spleen)
- Levels in liver are below 1 ng/g tissue
- Formulation CV LNP results in Muscle/Liver ratio (4h) of 1693

C CV LNP enable significant immune response in Rabies Rav-G vaccination in mice



- Significant levels of VNT and T-Cells induced

Conclusions

- Potent UTRs differently improve expression of unmodified and N1mΨ mRNAs in various cell types
- Incorporation of miRNA binding sites enables spatial fine-tuning of protein expression
- Different RNA technologies can extend protein expression in cells
- Co-administration of TLR7/8 antagonists reduces the release of pro-inflammatory cytokines elicited by IVT-mRNA
- CureVac LNPs are optimized for local expression and high adaptive immune responses

Acknowledgements