# STX-003: A Novel Systemically-Delivered mRNA-Based Cancer Therapy Utilizing a Programmable Genetic Circuit Platform for Tumor-Specific Protein Expression and Reduced Toxicity

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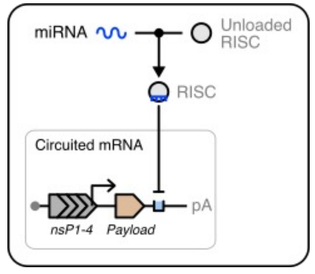
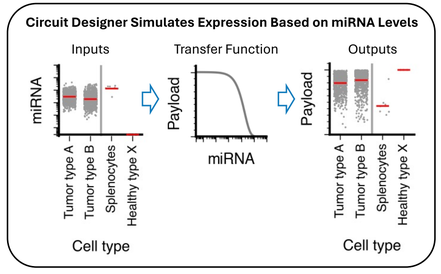
## INTRODUCTION

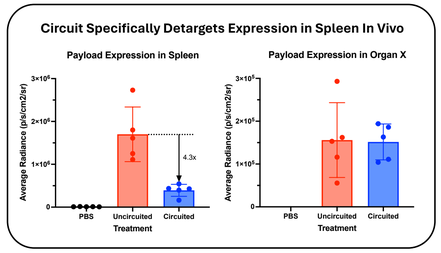
The advent of mRNA technology, coupled with advances in LNP-mediated delivery and in silico gene expression analysis, presents new opportunities for developing precision cancer therapies. We have developed a platform in which we design programmable genetic "circuits" that detect molecular cues in a cell. These circuits are encoded as RNA and incorporated into mRNA molecules to specifically express a payload protein in cells that exhibit a particular molecular signature. Through in silico modeling and high-throughput in vitro screening, we select candidate circuits and characterize their effectiveness for classifying a particular cell type. We then evaluate a circuit's effect on payload expression in vivo, first using a luciferase reporter payload, and then using the immunomodulatory cytokine IL-12 as an anti-tumor therapeutic payload. We applied this platform to the development of STX-003, a self-replicating mRNA bearing programmable genetic circuitry that selectively expresses IL-12 in tumors.

## IN SILICO MODELING INFORMS CIRCUIT SELECTION

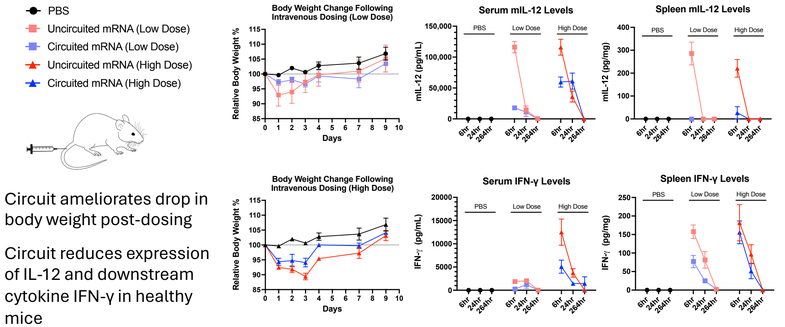



- RNA circuit senses and responds to miRNAs
- Circuit Designer simulates payload expression based on miRNA levels
- Candidate circuit was selected with the objective of detargeting the spleen
- Circuited mRNA was loaded into LNPs and administered systemically to mice
- Circuit specifically detargets the spleen

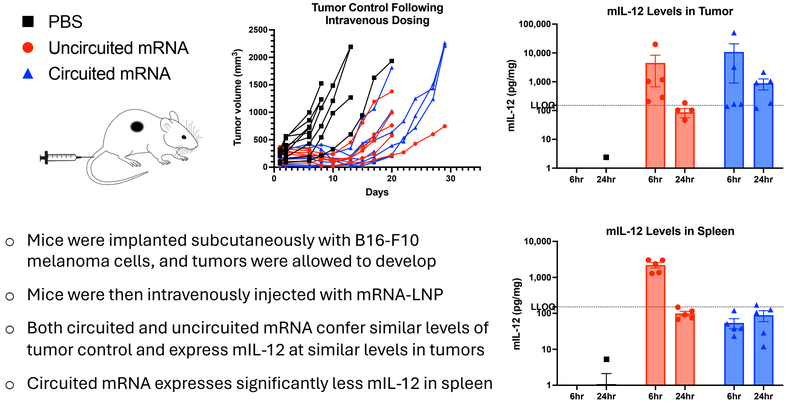



## CIRCUIT IMPROVES TOLERABILITY IN NAÏVE MICE



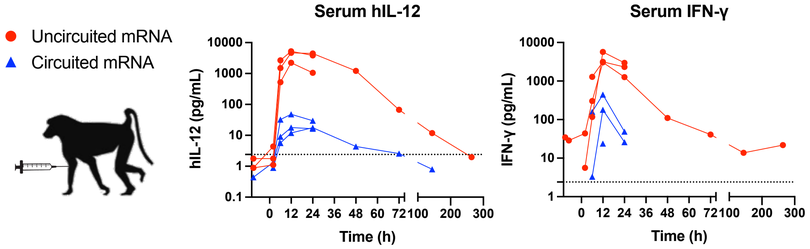

- Circuit ameliorates drop in body weight post-dosing
- Circuit reduces expression of IL-12 and downstream cytokine IFN-γ in healthy mice

## CIRCUIT TARGETS TUMORS AND DETARGETS SPECIFIC HEALTHY ORGANS IN B16-F10 ONCOLOGY MODEL

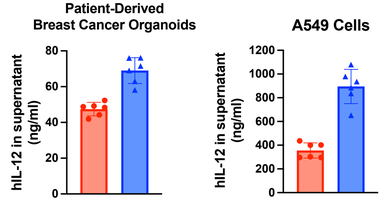



- Mice were implanted subcutaneously with B16-F10 melanoma cells, and tumors were allowed to develop
- Mice were then intravenously injected with mRNA-LNP
- Both circuited and uncircuited mRNA confer similar levels of tumor control and express mIL-12 at similar levels in tumors
- Circuited mRNA expresses significantly less mIL-12 in spleen

## CIRCUIT REPRESSES PAYLOAD IN NON-HUMAN PRIMATES WHILE PRESERVING EXPRESSION IN CANCER CELLS




- Cynomolgus monkeys were intravenously injected with mRNA-LNP
- Circuit confers ~99% reduction in circulating hIL-12 and ~95% reduction in circulating IFN-γ
- Transfection of circuited mRNA into cancer organoids or A549 cancer cells yields robust expression of hIL-12




## CONCLUSIONS

- **STX-003 expresses its IL-12 payload in tumors while selectively repressing its expression in spleen**
- **STX-003 controls tumors as effectively as an uncircuited mRNA upon systemic administration**
- **STX-003 circuit significantly reduces serum levels of IL-12 and IFN-γ in tumor-free mice and cynomolgus monkeys after intravenous dosing**
- **These data demonstrate that our programmable genetic circuit platform can enable tissue-specific expression of therapeutic proteins following systemic administration**