NORMOTHERMIC MACHINE PERFUSION OF EX VIVO PORCINE LIVER: DEMONSTRATOR STUDIES FOR PRECLINICAL TESTING OF AN RNA THERAPEUTIC AND MULTI-DAY PERFUSION FEASIBILITY

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INTRODUCTION

The prediction of efficacy, pharmacokinetics, and hepatotoxicity of novel preclinical RNA therapeutics and other biological therapies is of high importance but remains a challenge due to the lack of translational models. *In vitro* models, often do not fully recapitulate the *in vivo* cellular diversity, complex organ architecture and gene/protein expression.

AIM

1) **To explore the feasibility of *ex vivo* liver normothermic machine perfusion (NMP) of whole porcine liver to study the spatiotemporal mRNA editing efficacy of a novel preclinical LNP-encapsulated Oligo X in a disease-associated gene.**
2) **To assess the feasibility of sustaining liver viability during perfusion over multiple days**

METHODS AND EXPERIMENTAL SETUP

1. Porcine livers were procured from the slaughterhouse or the Utrecht animal center. The portal vein and hepatic artery were cannulated and flushed with saline and HTK solution. Bile duct was cannulated, and liver was connected to the LiverAssist™ device.
2. The liver was perfused with autologous blood under oxygenated and normothermic conditions (38°C, 40% oxygen).
3. Liver function was monitored by perfusate blood gas and electrolyte analysis (i-STAT), and ICG clearance assay.

1) Spatiotemporal mRNA editing efficacy

i. The liver was perfused with autologous blood under oxygenated and normothermic conditions and a bolus injection of LNP-encapsulated Oligo X was administered to the hepatic artery at 1 mL/min for 10 min according to the scheme in figure 1A.
ii. Biopsies were taken from the different liver lobes (figure 1B) at pre-specified time points, mRNA extracted and analyzed for editing efficiency.

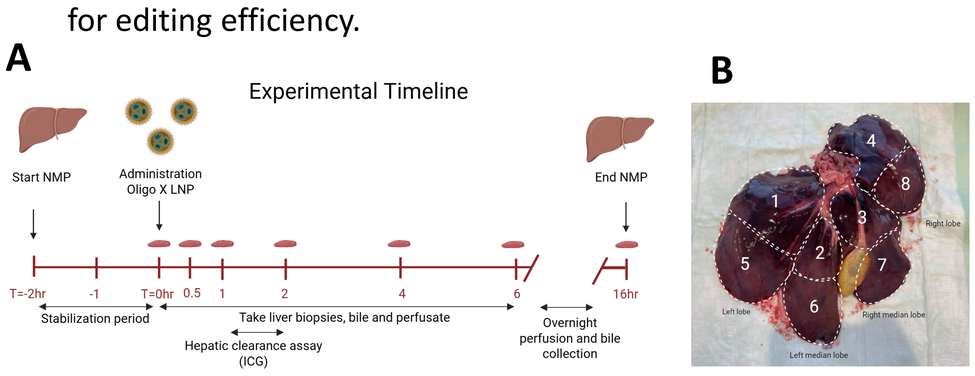

Figure 1. *(A) Schematic representation of the experimental timeline and (B) biopsy locations to measure spatial mRNA editing efficiency.*

1) Multi-day perfusion

i. A pediatric hemoconcentrator was integrated into the perfusion circuit to remove waste products from the perfusate into an ultrafiltrate (figure 2).
ii. Electrolytes were replenished by infusing substitution fluid.

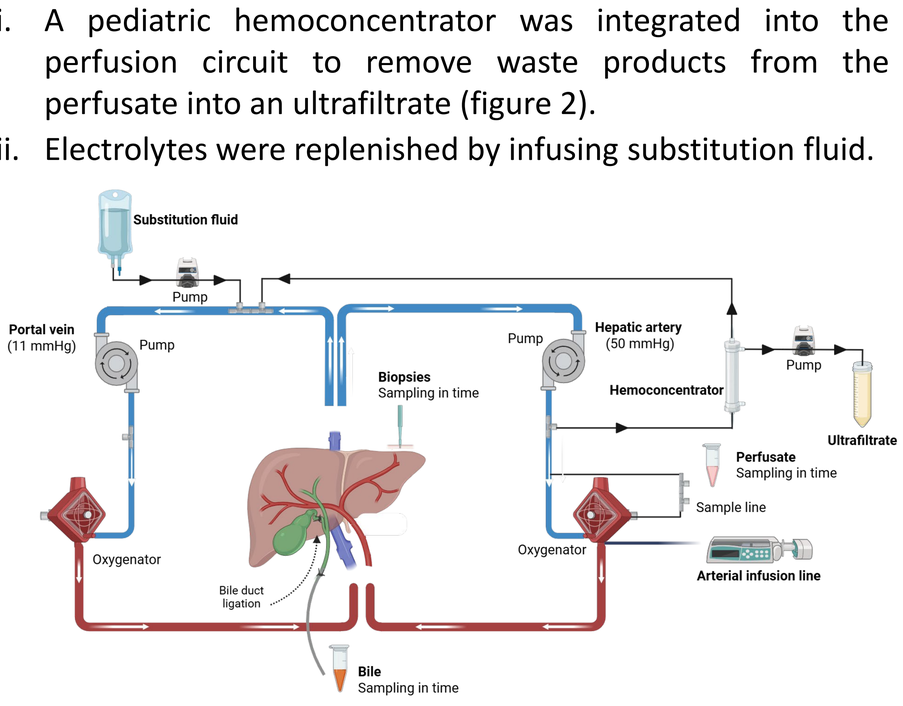

Figure 2. *Schematic of the modified perfusion setup with hemoconcentrator.*

REFERENCES

[1] Stevens LJ. et al., Clin Pharmacol Ther.(2023)114(1):137-147
[2] Stevens LJ et al., Drug Metab Dispos. (2021) 49(9): 780-789
[3] Stevens LJ et al., Drug Metab Rev. (2020) 52(3): 438-454

RESULTS

1) Spatiotemporal mRNA editing efficiency at gene target site 1 and site 2

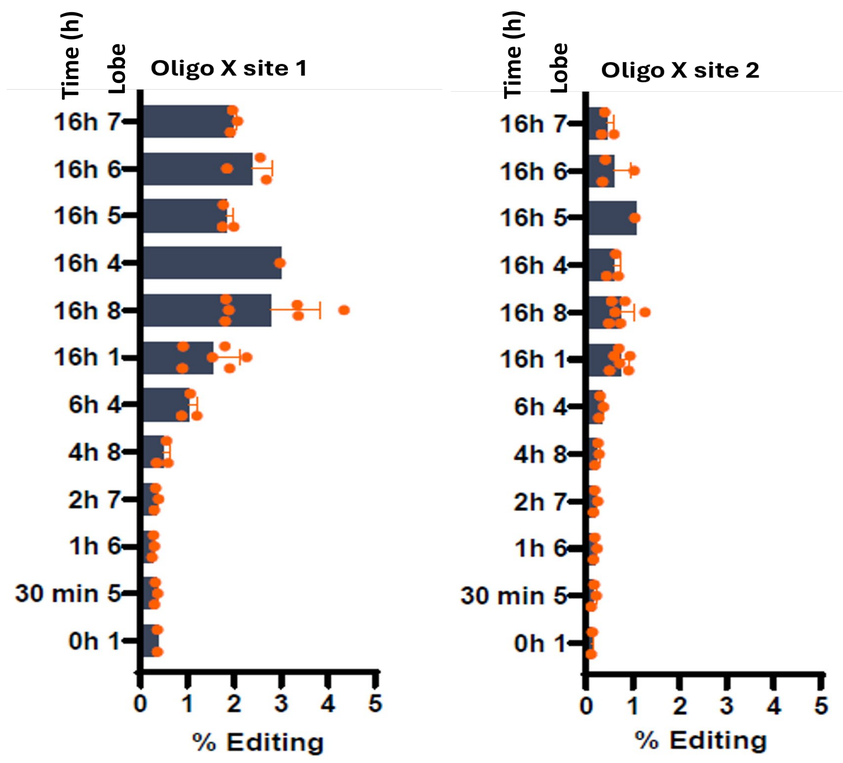

Figure 3 *Liver mRNA editing efficiency in the two therapeutically relevant gene target sites of interest. The left graph is of oligonucleotide target site 2 (main site) and the right graph is of oligonucleotide target site 2 (secondary target site).*

- Graphical overview of mRNA editing in disease-associated gene target site 1 and site 2.
- We observed an increase in mRNA editing of the liver gene target sites over the time of the perfusion, up to ~3.5% and ~1% in gene site 1 and 2 respectively which is in line with data from transfected human hepatocytes.
- At t=16h (end of perfusion), although all liver lobes were edited to a similar efficiency, so no spatial differences in efficiency, although gene target site 1 was more efficiently edited compared to site 2.

2) Long-term perfusion of *ex vivo* Liver – proof of concept

Figure 4. *Connection of the hemoconcentrator to the perfusion system.*

- A pediatric hemoconcentrator was successfully integrated into the perfusion system, with the inflow line directly after the arterial pump and the outflow line connected to the venous line (Figure 4).
- Flow to the hemoconcentrator could be turned off, permitting dosing of ICG (and test compounds) **without filtration from the system.**
- Arterial and venous flow remained stable during perfusion, and bile was actively produced for 48 hours (figure 5B-D)
- The hemoconcentrator efficiently filtered small (waste) molecules such as lactate, urea and creatinine from the perfusate into the ultrafiltrate (figure 5E-G).
- Up to 48h, the liver rapidly cleared ICG from the perfusate into the bile within 20 minutes of infusion, indicating good liver function throughout perfusion (figure 5h).
- Oxygen saturation remained at 100% except for the final 4h due to technical malfunction of the oxygenator on the portal side (figure 5D)

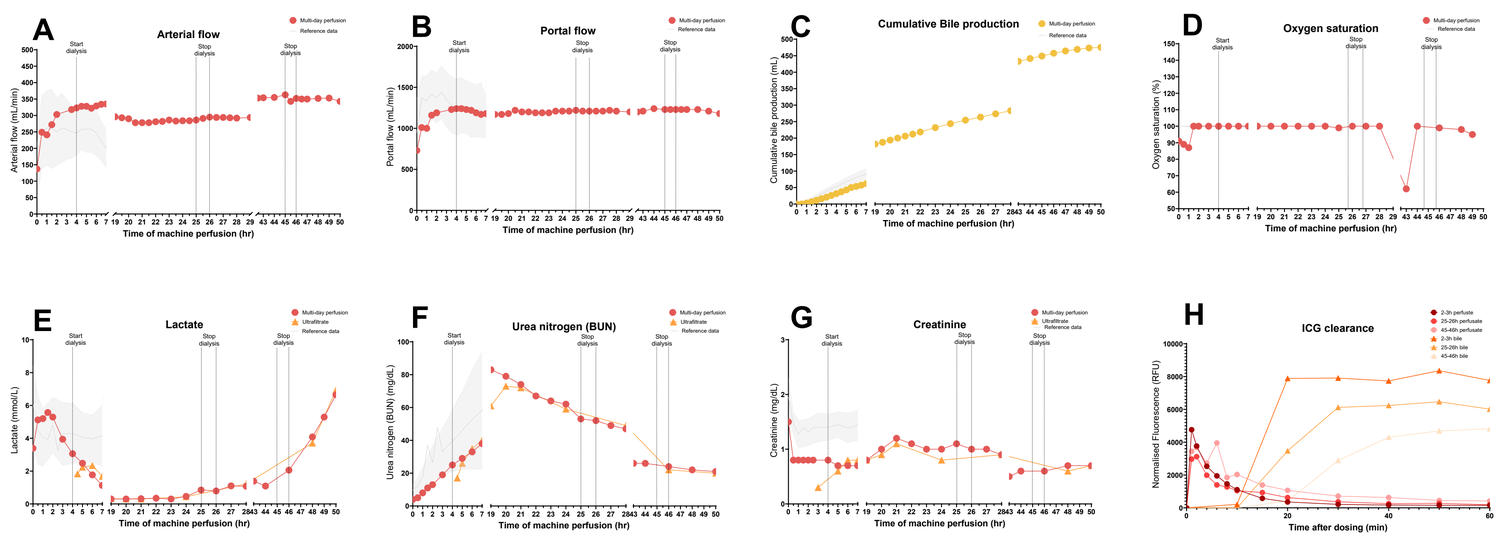

Figure 5. *Ex vivo porcine liver blood gas analysis (1 donor). A-B) Arterial and portal vein perfusate flow. C) Cumulative bile production. D) Perfusate oxygen saturation. E-G) Lactate, Urea and Creatinine concentration in the perfusate and ultrafiltrate. H) ICG clearance from the perfusate into the bile. Reference data represent historic data obtained in liver perfusion experiments ± SD (n = 7)*

CONCLUSIONS AND FUTURE PERSPECTIVES

- We have demonstrated the application of *ex vivo* liver NMP for preclinical screening of the spatiotemporal mRNA editing efficiency, thus efficacy of a novel therapeutic LNP-Oligonucleotide.
- In addition to providing mRNA editing efficiency over time, whole organ perfusion provides extra spatial editing data in a physiological setting.
- We successfully perfused ex vivo liver for 48 hours using haemoconcentration, enabling drug testing, including oligonucleotides, adenoviral therapies and slow-release compounds over an extended therapeutic window.
- Extended perfusion enables physiologically relevant assessment of drug toxicity, such as radiotoxicity.