In vivo mRNA therapy for Argininosuccinic Aciduria

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Aim: Assess therapeutic potential of systemically delivered LNPs encapsulating hASL-mRNA.

Background

Argininosuccinic lyase (ASL) is a urea cycle enzyme, which detoxifies ammonia by converting argininosuccinic acid (ASA) to L-arginine and fumarate[1] (**Fig 1**). Inherited ASL deficiency causes **argininosuccinic aciduria**, the second most common urea cycle defect causing hyperammonaemia, chronic liver and cerebral diseases[2]. Standard of care aims to normalise ammonaemia with protein-restricted diet, ammonia-scavenger drugs and in severe cases liver transplantation.

Fig 1: Urea cycle

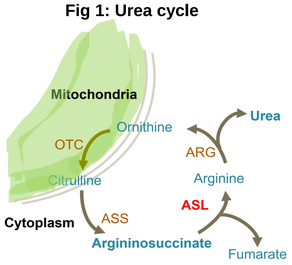
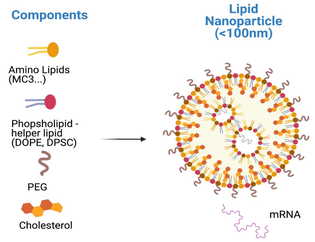


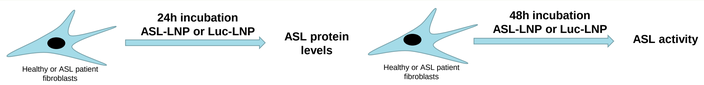
Fig 2: Lipid Nanoparticles



Lipid nanoparticles (LNP) encapsulating mRNA (**Fig 2**) are in phase I/II clinical trials for liver inherited metabolic diseases *e.g.* ornithine transcarbamylase deficiency, propionic and methylmalonic acidaemias[3].

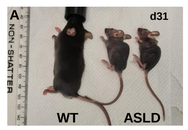
Methods

In vitro: Fibroblasts



EXPERIMENT 1: *In vitro* efficacy study in healthy and ASL deficient (ASLD) patient fibroblasts

In vivo: ASLD mouse model



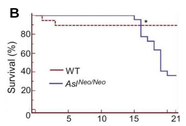
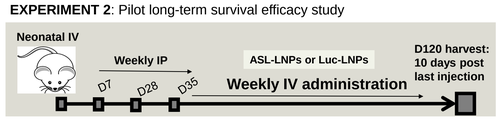
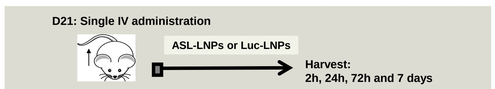


Fig 3: Knock in hypomorphic *Asl*$^{Neo/Neo}$ mouse[4]. Recapitulates human disease phenotype including impaired growth (**A**) and early death (**B**).

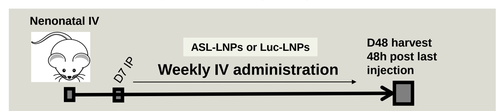
EXPERIMENT 2: Pilot long-term survival efficacy study



EXPERIMENT 3: Pharmacokinetics and Pharmacodynamics study (PK/PD)



EXPERIMENT 4: Efficacy *in vivo* following weekly treatment from birth



IP= Intraperitoneal administration; IV= Intravenous administration; **All doses 1mg/kg**

Results

In vitro ASL levels and activity increase post ASL-LNP treatment

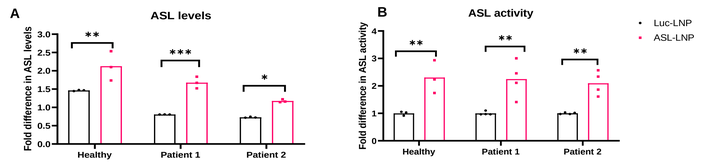


Fig 4: **Significant increase in ASL levels** (**A**) and **activity** (**B**) in healthy and ASLD fibroblasts post 24h and 48h incubation respectively with ASL-LNP vs Luc-LNP.

Pilot survival study showed improved growth and survival

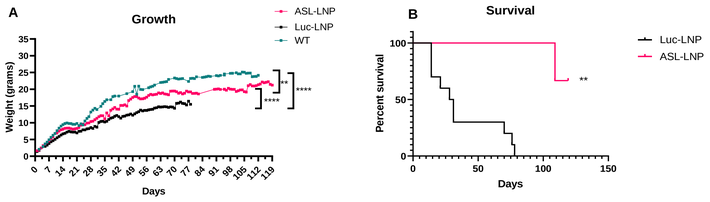


Fig 5: Pilot long-term survival study showed sustained efficacy with significant increase in growth (**A**) and survival (**B**) ASL-LNP (N=3) vs Luc-LNP (N=9). WT N=7.

PK/PD studies show sustained efficacy up to 7 days

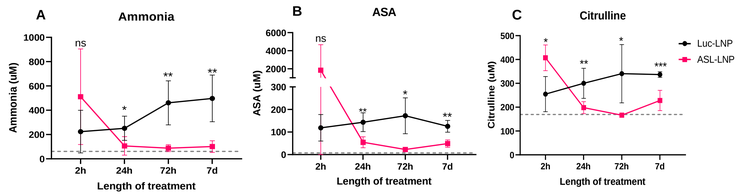


Fig 6: **Significant improvement in disease biomarkers** observed at 24h to 7 days with decrease in plasma ammonia (**A**), ASA (**B**) and citrulline (**C**) levels following single IV administration of 1mg/kg ASL-LNP vs Luc-LNP. Grey dotted lines indicate WT levels. 2h (N=3), 24h (N=5-7), 72h (N=3), 7d (N=3-4).

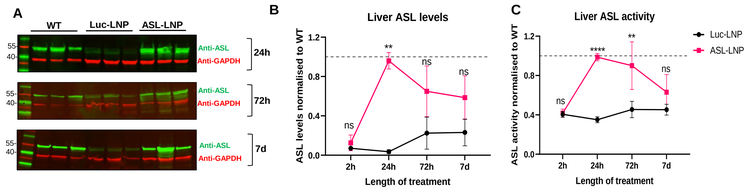


Fig 7: Increase in ASL levels (**A, B**) **and activity** (**C**) observed significantly at 24h post single IV administration of 1mg/kg ASL-LNP vs Luc-LNP. The increase sustained up to 7 days. Values are normalised to WT (grey dotted line). For levels; N=3 per group. For activity; 2h (N=3), 24h (N=5), 72h (N=3), 7d (N=4).

Weekly treatment from birth show normalisation of phenotype

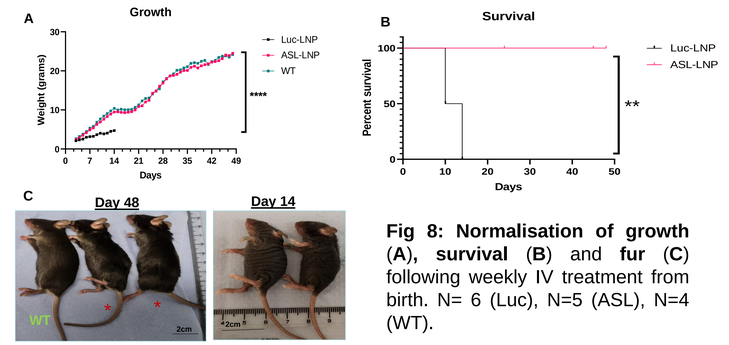


Fig 8: Normalisation of growth (**A**), **survival** (**B**) and **fur** (**C**) following weekly IV treatment from birth. N= 6 (Luc), N=5 (ASL), N=4 (WT).

***ASL-LNP treated mutants** **Luc-LNP treated mutants**

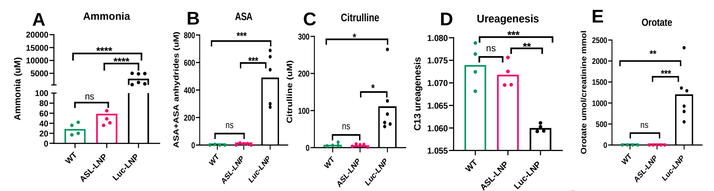
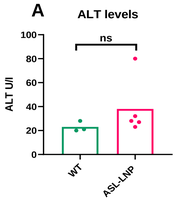


Fig 9: **Normalisation of disease biomarkers** with ammonia (**A**), ASA (**B**), citrulline (**C**), C13 ureagenesis (**D**) and orotate levels (**E**) not significantly different from WT littermates.

Fig 10: Weekly repeat dosing did not significantly increase ALT, a marker of liver toxicity (**A**).



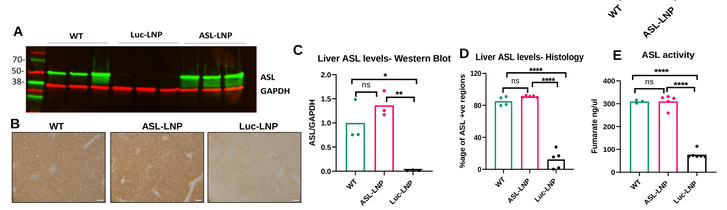


Fig 11: **Liver ASL protein levels** by western blot (**A, C**) and immunostaining (**B, D**) and **activity** (**E**) restored to WT levels.

Conclusion

Successful proof of concept of LNP.mRNA therapy in ASL deficiency *in vitro* and *in vivo* after systemic delivery. This approach could be of benefit for patients affected by argininosuccinic aciduria.

References: 1.Baruteau J, et al. Argininosuccinic aciduria fosters neuronal nitrosative stress reversed by Asl gene transfer. Nat Commun. 2018;9(1):3505. **2**. Baruteau J, et al.. Gene therapy for monogenic liver diseases: clinical successes, current challenges and future prospects. J Inherit Metab Dis. 2017;40(4):497-517. **3**. Hou, X., Zaks, T., Langer, R. *et al.* Lipid nanoparticles for mRNA delivery. *Nat Rev Mater* **6,** 1078–1094 (2021). https://doi.org/10.1038/s41578-021-00358-0. **4**. Erez A, et al. Requirement of argininosuccinate lyase for systemic nitric oxide production. Nat Med. 2011 Nov 13;17(12):1619-26. doi: 10.1038/nm.2544.

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