In vivo mRNA therapy for Argininosuccinic Aciduria


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 (Fig 1). Inherited ASL deficiency causes argininosuccinic aciduria, the second most common urea cycle defect causing hyperammonaemia, chronic liver and cerebral diseases

Fig 1: Urea cycle

Lipid nanoparticles (LNPs) encapsulating mRNA (Fig 2) are in phase III clinical trials for liver inherited metabolic diseases e.g. ornithine transcarbamylase deficiency, proline and methylmalonic acidemia.

Fig 2: Lipid Nanoparticles

Methods

In vitro: Fibroblasts

In vivo: ASLD mouse model

In vivo efficacy study in healthy and ASL deficient (ASLD) patient fibroblasts.

Fig 3: Knock in hypomorphic Acsas mouse.

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.

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=3,7), 7d (N=3-4).

Weekly treatment from birth show normalisation of phenotype

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=5-7), 72h (N=3,7), 7d (N=4).

Fig 8: Normalisation of disease biomarkers with ammonia (A), ASA (B), citrulline (C), C13 ureagenesis (D) and ornithine levels (E) not significantly different from WT littermates.

Fig 9: Normalisation of disease biomarkers with ammonia (A), ASA (B), citrulline (C), C13 ureagenesis (D) and ornithine 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.


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