In vivo exosome based therapy for liver inherited metabolic disease: ASL deficiency

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Introduction

- **Argininosuccinic lyase (ASL)** is a cytosolic enzyme involved in the **urea cycle** where it detoxifies ammonia by converting **argininosuccinic acid (ASA)** to **L-arginine** and fumarate[1] **(Fig 1)**.
- ASL is also involved in the synthesis of nitric oxide (NO) from L-arginine.

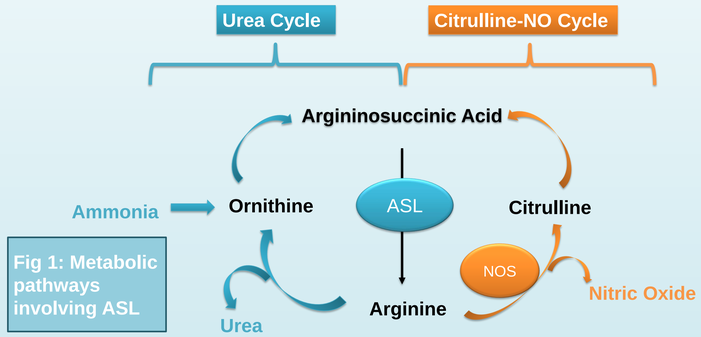


Fig 1: Metabolic pathways involving ASL

- Deficiency in ASL results in **argininosuccinic aciduria**, an autosomal recessive disorder characterised by hyperammonaemic decompensation, neurocognitive impairment and elevated ASA levels.
- Standard of care aims to normalise ammonia levels with protein restricted diet, ammonia scavenger drugs and in severe cases liver transplantation.
- **Early death rate and neurocognitive deficiency in >15% and >90% patients respectively asserts the need for novel therapeutic strategies[2].**

- **Exosomes** are nanospheres (30-200nm diameter) and transport functional proteins and genetic contents to recipient cells[3].
- They are of great interest to be exploited as therapeutic cargo carriers.
- Exosomes are biocompatible, protect cargoes, can be modified to enhance selectivity and cross the blood brain barrier[4].

- **We aim to use exosomes as a novel therapeutic strategy to deliver functional human ASL protein (hASL) in a hypomorphic mouse model of ASL deficiency.**

Methods

- The knock-in *Asl*$^{Neo/Neo}$ hypomorphic mouse model recapitulates the phenotype of the human disease showing increased plasma ammonia and ASA levels and early death at 2-3 weeks of age[1,5].
- Efficacy assessment of **SINGLE** administration of therapeutic **hASL protein containing exosome construct (EXO-hASL)** either via **intraperitoneal (IP)** injection (2x10^{11} particles per mouse aged 12±2 days) or **intravenous (IV)** injection (1.5x10^{11} particles per mouse aged 3 days) for 24 hours.
- Efficacy assessment of **REPEATED** daily **IP** administration on mice from 8 days of age (5x10^{10} particles/ gram of mouse) for up to 10 days.
- Two controls: Untreated Wild-Type **(WT)** mice and ASL hypomorphic mice treated with exosomes not overexpressing hASL **(EXO).**
- Efficacy endpoints: measurements of plasma ammonia levels, plasma ASA levels and survival.

Results

Efficacy studies following SINGLE IP administration of therapeutic protein ASL loaded exosomes (EXO-hASL) after 24 hours

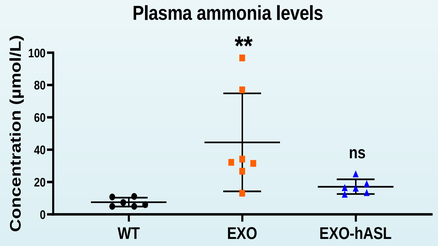
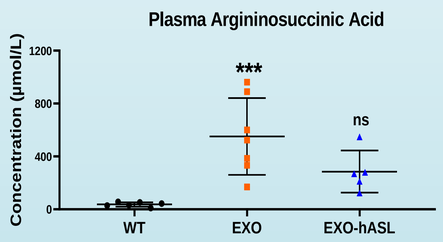


Fig 2: Reduction in plasma ammonia levels. 24h single IP administration of EXO-hASL resulted in 65% reduction in the plasma ammonia levels compared to ASL hypomorphic mice treat given control exosomes (EXO). One way ANOVA with Dunnett's multiple comparisons test against the WT control; ***p=0.0052*, ns *p=0.5893*.

Fig 3: Reduction in plasma ASA levels. 24h single IP administration of EXO-hASL resulted in 48% reduction in the plasma ASA levels compared to ASL hypomorphic mice treat given control exosomes (EXO). One way ANOVA with Dunnett's multiple comparisons test against the WT control; ****p=0.0007*, ns *p=0.1058*.



SINGLE IV administration with EXO-hASL after 24 hours reduces hyperammonaemia

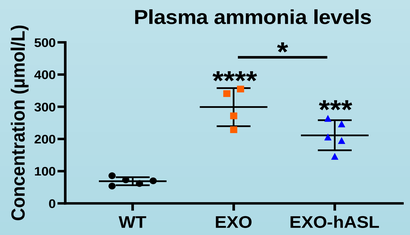


Fig 4: Reduction in plasma ammonia levels. Following 24h single IV administration of EXO-hASL, there was a significant 38% reduction in the plasma ammonia levels compared to ASL hypomorphic mice treat given empty exosomes (EXO). One way ANOVA with Dunnett's multiple comparisons test; WT vs EXO *****p>0.0001*, WT vs EXO-hASL ****p=0.0005*, EXO vs EXO-hASL **p=0.019*.

Survival curve following REPEATED IP administration of EXO-hASL

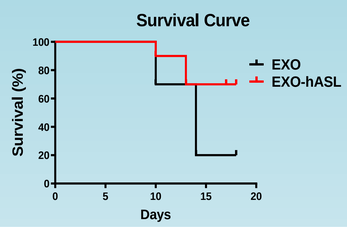


Fig 5: Improvement in the survival of ASL deficient mice. Repeated IP administration of EXO-hASL resulted in an increasing survival trend against the ASL hypomorphic mice treat given empty exosomes (EXO). Increase in survival was prominent past the critical phase of 2 weeks of age (d14). N=10. Log-rank (Mantel-Cox) test; *p=0.0745*.

Results

Efficacy studies following REPEATED IP administration of EXO-hASL

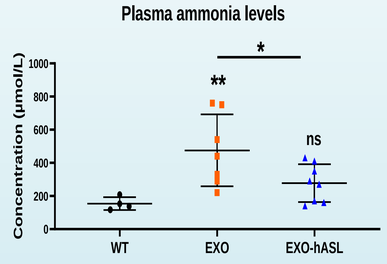


Fig 6: Reduction in plasma ammonia levels. Repeated IP administration of EXO-hASL resulted in significant 62% reduction in the plasma ammonia levels compared to ASL hypomorphic mice treat given control exosomes (EXO) when measured at day 18. One way ANOVA with Dunnett's multiple comparisons test; WT vs EXO ***p=0.0074*, WT vs EXO-hASL ns *p=0.3221*, EXO vs EXO-hASL **p=0.044*.

Discussion

- Hyperammonaemia, hallmark of ASL deficiency, was reduced significantly in all the IV and IP treatments.

- Single IP administration of EXO-hASL reduced plasma ASA levels which requires higher ASL activity compared to ammonia[1].

- Repeated IP injections resulted in marked improvement in the survival of ASL hypomorphic mice and decrease in plasma ammonia levels.

- Future work includes additional efficacy studies, further dose response studies and studies looking at ASL expression levels *in vivo.*

Successful proof of concept of therapeutic potential of biological protein hASL loaded engineered exosomes in ASL deficiency *in vivo*.

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. Pegtel DM, et al. Exosomes. Annu Rev Biochem. 2019;88:487-514. 4. Murphy DE, et al. Extracellular vesicle-based therapeutics: natural versus engineered targeting and trafficking. Exp Mol Med. 2019;51(3):1-12. 5. Erez A, et al. Requirement of argininosuccinate lyase for systemic nitric oxide production. Nat Med. 2011;17(12):1619-26.