LogicBio THERAPEUTICS

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Novel Genome Editing Therapy Improves Disease Phenotype and Survival in a Mouse Model of Methylmalonic Acidemia

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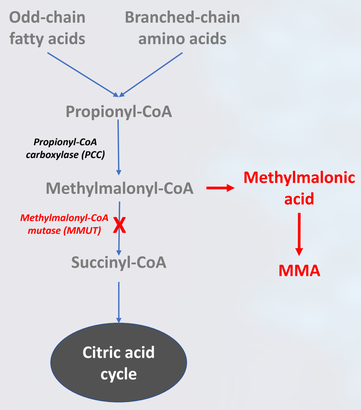
1 Methylmalonic Acidemia and Gene Therapy

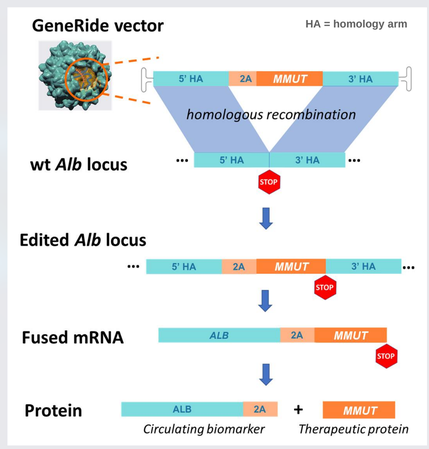
Methylmalonic acidemia (MMA) is a rare genetic disorder most commonly caused by mutations in the gene encoding mitochondrial methylmalonyl-CoA mutase, *MMUT*. MMA patients suffer from frequent episodes of metabolic instability, leading to developmental delay and other neurological abnormalities and are potentially lethal. There is no cure for MMA; the current standard of care mainly consists of a highly restricted diet. However, even under strict dietary management, many patients remain metabolically unstable, and liver transplantation becomes the only therapeutic alternative for these patients.

The recent advance of AAV-based gene therapy has brought hope to patients of many severe monogenic diseases. However, significant challenges remain for treating children and young adults with inborn diseases such as MMA, which feature neonatal onset and require early intervention. This is because conventional AAV therapies rely on episomal-driven transgene expression, which is gradually lost during liver growth. LogicBio has been pioneering a novel AAV-based gene editing technology, GeneRide, which has shown superior durability in the growing liver. In this study, we demonstrated the therapeutic potential of GeneRide technology to treat MMA.



2 GeneRide™, a Nuclease-free Promoterless AAV Genome Editing Technology

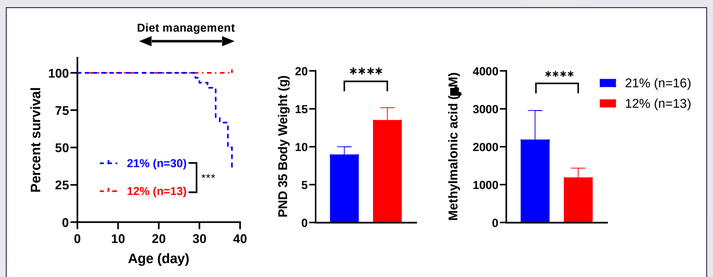
GeneRide is a novel AAV-based gene editing technology that leverages the natural process of homologous recombination to insert a therapeutic transgene into the genome (1). A GeneRide cassette consists of a transgene flanked by homology arms (HA) that match the sequences of the targeted species. For liver-directed indications, the transgene (e.g., *MMUT*) is integrated into the albumin locus (*ALB*) and can thus "hitch a ride" on the highly active endogenous *ALB* promoter, resulting in high transgene expression in hepatocytes. The transgene is precisely inserted in-frame between the penultimate and the stop codons of *ALB*. A P2A peptide is utilized for polycistronic protein expression, resulting in the production of two separate proteins: a C-terminal P2A-tagged ALB (ALB-2A) and the therapeutic protein (e.g., MMUT). The percentage of modified *ALB* allele and the level of ALB-2A are quantified by qPCR and ELISA, respectively. Levels of genomic integration, transgene expression and circulating ALB-2A linearly correlate with one another, making ALB-2A an easily accessible biomarker to monitor GeneRide-mediated gene editing in the liver.



3 Low Protein Diet Significantly Improves Survival of Methylmalonic Acidemia Mice

In this study, we employed a murine model of MMA, *Mmut*$^{-/-}$;*Tg*$^{INS\text{-}MCK\text{-}Mmut}$ (MMA mice), which displays pathophysiological conditions observed in MMA patients (2). The MMA mice are deficient in the endogenous *Mmut* gene and express *Mmut* cDNA driven by the muscle-specific creatine kinase (MCK) promoter. Fed on standard rodent chow with 21% protein content, the MMA mice displayed lower body weight than healthy heterozygous littermates starting at 2 weeks of life. After weaning (PND28), animals started to experience sudden metabolic decompensation with increased mortality. We evaluated the effect of dietary protein content and found that lower protein content extended the life of the MMA mice, which is consistent with the clinical management of MMA patients. The low protein diet allowed us to evaluate the long-term therapeutic efficacy of the GeneRide vector mLB-001 in the MMA mice.

Survival | Body weight | Circulating methylmalonic acid

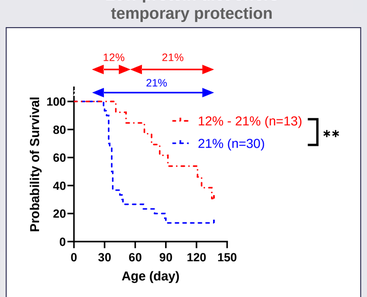


- Low protein diet provided to MMA mice at 2 weeks of age improved survival and body weight, and reduced circulating levels of methylmalonic acid at 1 month old

*** p<0.001, log-rank test. Mice fed standard chow (21% protein) had a median survival of 38 days.

**** p<0.0001, Student t-test

Low protein diet offers temporary protection



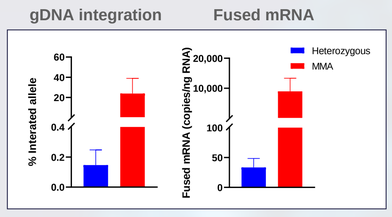
- Low protein diet provided between 2- and 7-weeks increased animal survival

** p<0.01, log-rank test. Mice fed 21% protein diet had a median survival of 38 days, mice fed 12% protein diet for 5 weeks had a median survival of 121 days

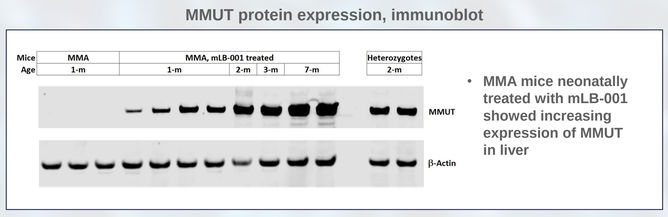
4 GeneRide™-edited Hepatocytes Expand in MMA Mice Through Selective Advantage

The GeneRide vectors utilize natural homologous recombination to insert the gene encoding the therapeutic protein into the genome for expression. Despite low level of on-target integration initially, restoration of MMUT expression and activity in edited, MMUT-expressing hepatocytes conferred a selective advantage over Mmut-deficient hepatocytes. In MMA mice dosed with mLB-001 either neonatally or as adults, there was a time-dependent increase in the percentage of integrated allele, level of fused *Alb-P2A-MMUT* mRNA, concentration of circulating Alb-2A, expressed MMUT protein and MMUT mutase activity. Such an increase was not observed in the healthy heterozygous littermates.

gDNA integration | Fused mRNA

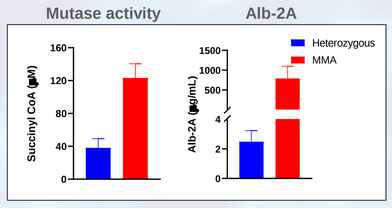


MMUT protein expression, immunoblot

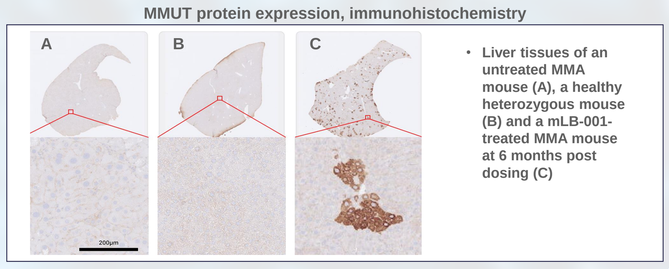


- MMA mice neonatally treated with mLB-001 showed increasing expression of MMUT in liver

Mutase activity | Alb-2A



MMUT protein expression, immunohistochemistry



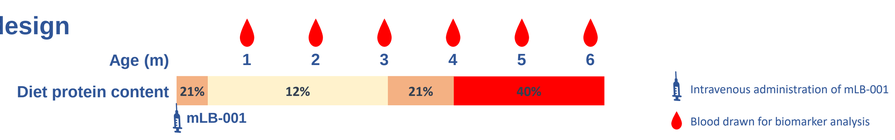
- Liver tissues of an untreated MMA mouse (A), a healthy heterozygous mouse (B) and a mLB-001-treated MMA mouse at 6 months post dosing (C)

- Liver genomic DNA integration, fused *Alb-P2A-MMUT* mRNA, MMUT mutase activity and circulating Alb-2A at 6 months post dosing

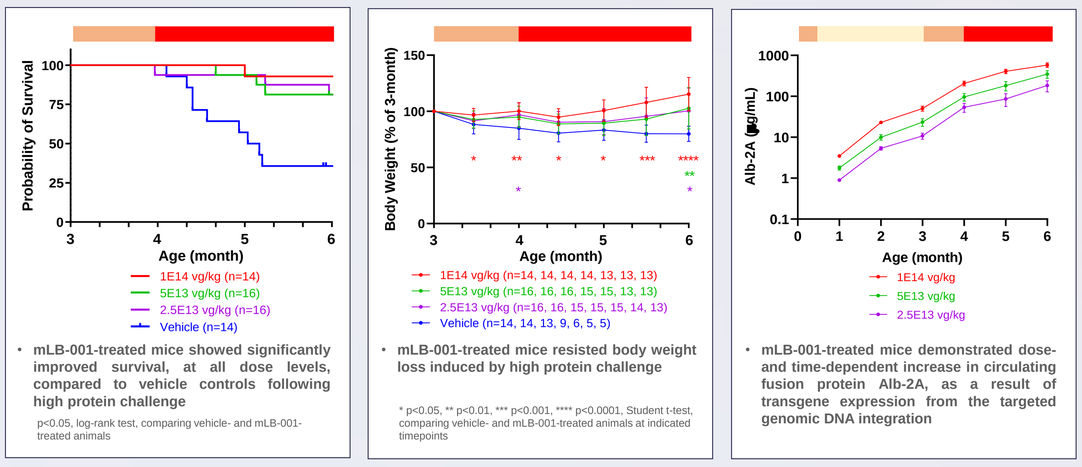
5 GeneRide™ Treatment Provides MMA Mice Resistance to a High Protein Diet Challenge

Neonatal mice were dosed with a GeneRide vector encoding *Mmut* cDNA and mouse homology arms (mLB-001). They were maintained on a 12% protein diet for three months, followed by stepwise increase in dietary protein content to mimic metabolic decompensations driven by increased protein flux. Under 12% protein diets, no significant difference in animal survival or body weight was observed between vehicle- and mLB-001-treated groups. However, in response to a high protein diet challenge, the mLB-001-treated MMA mice in all three dosage groups were protected from diet-induced weight loss and demonstrated significantly better survival than the vehicle-treated animals ($p<0.05$, Log-rank test). In addition, the mLB-001-treated animals demonstrated dose- and time- dependent increases in circulating Alb-2A, as a result of the expansion of MMUT-expressing hepatocytes over time. This allowed lower doses of mLB-001 to achieve therapeutic effects.

Study design



Survival | Body weight | Circulating Alb-2A



- mLB-001-treated mice showed significantly improved survival, at all dose levels, compared to vehicle controls following high protein challenge

p<0.05, log-rank test, comparing vehicle- and mLB-001-treated animals

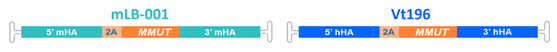
- mLB-001-treated mice resisted body weight loss induced by high protein challenge

* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001, Student t-test, comparing vehicle- and mLB-001-treated animals at indicated timepoints

- mLB-001-treated mice demonstrated dose- and time-dependent increase in circulating fusion protein Alb-2A, as a result of transgene expression from the targeted genomic DNA integration

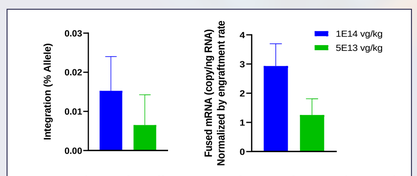
6 GeneRide™ Vector Successfully Edits Human Hepatocytes in Mice with Humanized Liver

To translate efficacy shown in the MMA mouse model to the human setting, we treated chimeric mice with a humanized liver (PXB, Phoenix Bio) with a GeneRide vector encoding MMUT cDNA flanked by human homology arms, Vt196. Adult PXB mice injected with the human specific GeneRide vector demonstrated on-target genomic DNA integration, fused mRNA expression and MMUT protein expression in transplanted human hepatocytes. These results demonstrate that the GeneRide vector is capable of editing human hepatocytes in vivo, supporting its application in treating pediatric MMA patients.



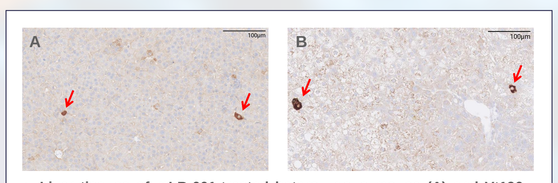
- GeneRide vector contains either mouse or human homology arms flanking the albumin stop codon

gDNA integration | Fused mRNA



- Dose-dependent liver genomic DNA integration and fused *Alb-P2A-MMUT* mRNA expression in adult humanized PXB mice at 12 weeks post dosing

MMUT protein expression, immunohistochemistry



- Liver tissues of mLB-001-treated heterozygous mouse (A) and Vt196-treated PXB mice (B), showing strong transgene expression of MMUT (arrows) above the endogenous protein level

7 Conclusions

- GeneRide is a novel AAV-based gene editing technology featuring durable therapeutic transgene expression through homologous recombination. The technology is especially attractive for its potential to treat monogenic diseases with neonatal onset that require early intervention.
- In a mouse model of MMA, a GeneRide vector encoding MMUT (mLB-001) led to on-target integration into the albumin locus. GeneRide-edited hepatocytes expressing functional MMUT had a selective advantage in the regenerating liver and showed expansion over time. The treatment protected the animals from high protein diet-induced metabolic crisis.
- The combined efficacy data in MMA mice and translational data in mice with a humanized liver support the clinical development of hLB-001 as a therapeutic agent for this devastating disease.

References

1. Barzel A. et al. Nature (2015)
2. Manoli et al. JCI Insight (2018)

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