Development of a universal circulating biomarker of a nuclease-free genomic integration technology, GeneRide™

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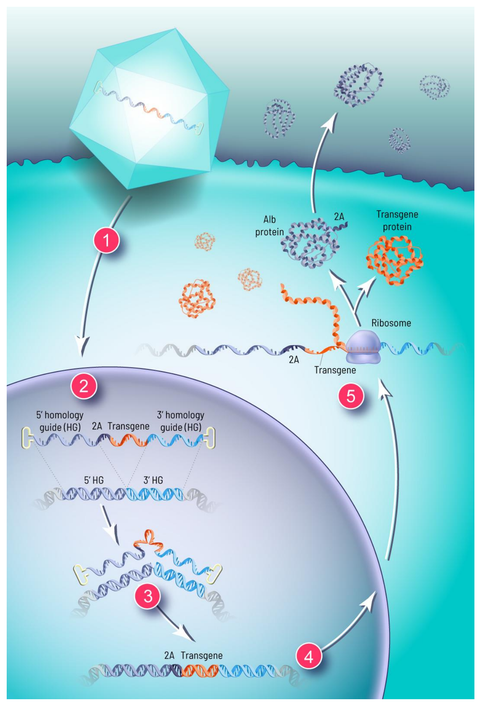
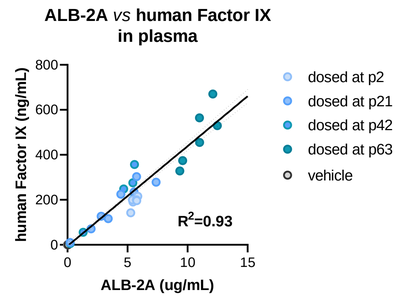
GeneRide™, a nuclease-free promoterless AAV genome-editing technology

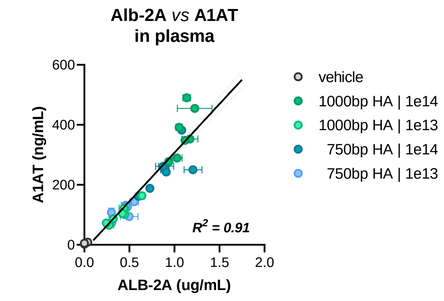


GeneRide™ is a novel AAV-based genome-editing technology that leverages the natural process of homologous recombination to insert a therapeutic transgene into the genome. For liver-directed targets, the transgene is integrated into the *Albumin* locus. The integrated transgene can thus "hitch a ride" on the highly active *Albumin* endogenous promoter, which results in high transgene expression selectively in hepatocytes. The transgene is precisely inserted in-frame between the penultimate and the stop codons of *Albumin*, and utilizes a P2A peptide sequence that induces ribosomal skipping. This results in the production of two separate proteins: a C-terminal tagged albumin (ALB-2A) and the therapeutic transgene. The percentage of modified *Albumin* allele in liver can be directly determined by a qPCR-based assay, and ALB-2A levels in circulation can be quantified by ELISA. Herein we show the linear correlation between genomic integration in liver and transgene expression with ALB-2A in circulation. ALB-2A thus becomes a universal circulating biomarker for monitoring non-invasively GeneRide-mediated genome-editing in the liver.

Excellent linear correlation between levels of secreted transgenes and ALB-2A in plasma

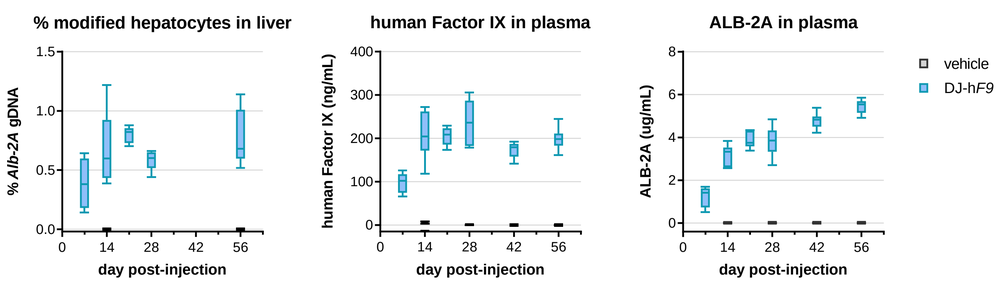


Neonate (p2), juvenile (p21) and adult (p42 and p63) FvB/NJ mice were dosed i.v. with 1e14vg/kg of GeneRide™-human Factor IX (DJ-h*F9*), and harvested 4 weeks post-injection. Linear regression of plasma ALB-2A and the transgene, human Factor IX, yields a R^2=0.93, thus supporting the use of ALB-2A in plasma as a surrogate for transgene levels.

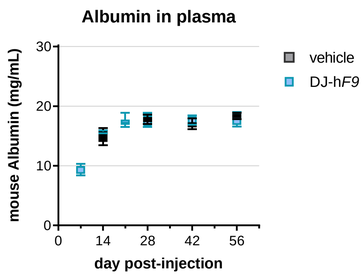


Neonate FvB/NJ mice (p2) were injected i.v. with GeneRide™-A1AT (DJ-A1AT) for a final dose of 1e13 or 1e14vg/kg. The transgenes were designed with 750bp or 1000bp homology arms. Animals were harvested 6 weeks post-injection. Linear regression of plasma ALB-2A and transgene, A1AT, yields a R^2=0.91, thus supporting the use of ALB-2A in plasma as a surrogate for transgene levels.

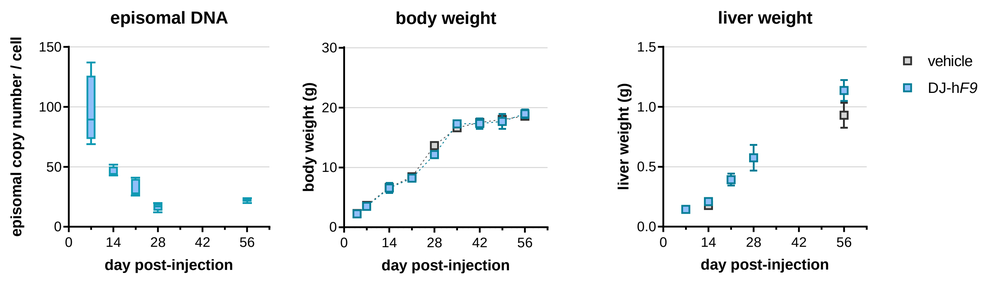
GeneRide™ kinetics: Early detection of integration, transgene expression and ALB-2A in plasma



Neonate C57 mice (p2) were injected i.v. with GeneRide™-human Factor IX (1e14vg/kg DJ-h*F9*) and harvested at 1, 2, 3, 4 and 8 weeks post-injection.
Genomic integration can be detected as early as after 1 week post-injection, and it reaches a stable plateau at 2 weeks. ALB-2A protein in circulation increases over the first 3-4 weeks; this increase is associated with upregulation of endogenous albumin after birth. The stably integrated transgene, Factor IX, follows a similar kinetics to genomic integration and ALB-2A in plasma.

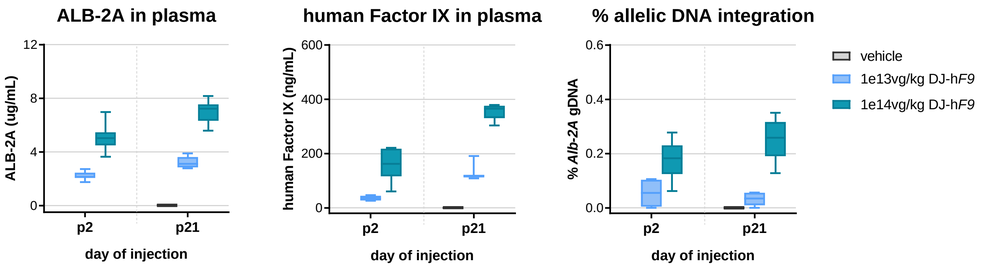


Stable transgene expression over time despite dilution of episomal DNA with liver growth



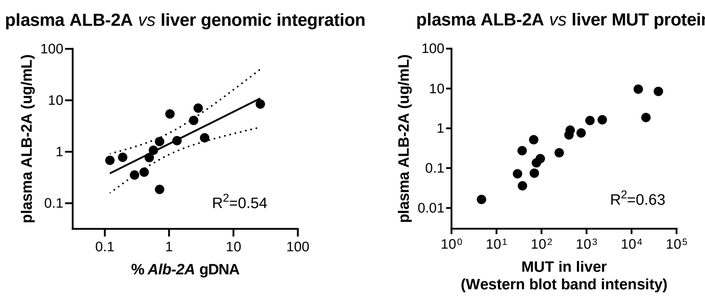
Episomal copy numbers decrease exponentially over time after injection in neonatal C57 mice (p2) with GeneRide™-human Factor IX (1e14vg/kg DJ-hF9). The decrease in episomal copies correlates with the progressive growth of the liver after birth. Despite the loss of episomes over time, transgene expression remains stable through integration of GeneRide™ in the genome within the first week after injection.

Integration efficiency, transgene and ALB-2A not affected by the age of the animals at dosing



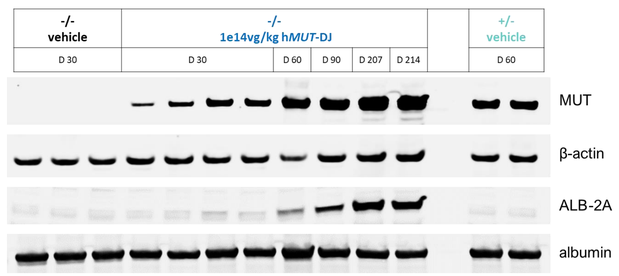
Neonate FvB/NJ mice (p2) or juvenile mice (p21) were injected i.v. with GeneRide™-human Factor IX (h*F9*-DJ) at 1e13 or 1e14vg/kg and harvested 6 weeks post-injection. Integration efficiency and transgene expression are not affected by the age at which the animals are dosed with GeneRide™.

Plasma ALB-2A as a surrogate for integration and expression of intracellular transgenes



Neonatal animals of the mouse model for methylmalonic acidemia (MMA), *Mut*$^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$, were injected i.v. at p1 with GeneRide™-human methylmalonyl-CoA mutase (DJ-h*MUT*) at different doses (1e13, 3e13 or 1e14vg/kg) and harvested over a period of 3 months.
Circulating levels of ALB-2A show a linear correlation with the levels of genomic integration as well as the levels of MUT protein in liver, a mitochondrial protein. These data demonstrate that plasma ALB-2A can be used as a circulating biomarker to monitor expression of liver intracellular transgenes, in real-time, without requiring invasive biopsies.

ALB-2A as a surrogate for changing levels of intracellular transgene expression over time



Neonatal *Mut*$^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$ mice (p2) were injected i.v. with 1e14vg/kg of GeneRide™-MUT (DJ-h*MUT)*, and livers were harvested over a period of 7 months.
Human MUT and ALB-2A proteins in liver were analyzed by Western blot. Vehicle-treated *MUT*$^{+/-}$ is shown as reference.
Hepatocytes edited by GeneRide™ express functional MUT, which gives them selective growth advantage over *Mut*$^{-/-}$ endogenous hepatocytes (Chandler *et al.* ASGCT 2018; Venturoni *et al.* ASGCT 2019). This selective expansion can be detected by the increased levels of the transgene expression and ALB-2A.

CONCLUSIONS

- GeneRide™ enables **targeted** genomic integration of therapeutic transgenes leading to **durable** expression despite rapid rAAV episome dilution.
- The efficiency of integration and level of transgene expression are independent of the age of the animal at the time of treatment.
- An ELISA-based assay was developed to monitor the expression of a C-terminus 2A-tagged albumin, **ALB-2A**, in circulation.
- Circulating ALB-2A correlates linearly with expression of therapeutic transgenes, for both secreted and intracellular proteins, thus validating the use of **ALB-2A as a universal circulating biomarker** for GeneRide™ liver targets.
- ALB-2A becomes a key biomarker to monitor genome editing and transgene expression non-invasively in **pre-clinical and clinical development**.