Durability of factor IX expression in neonate mice treated with GeneRide™

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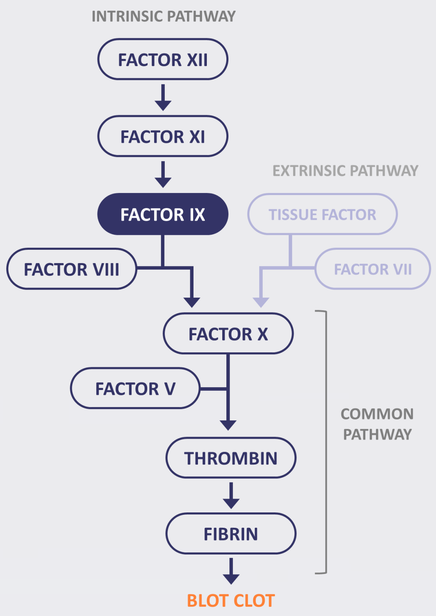
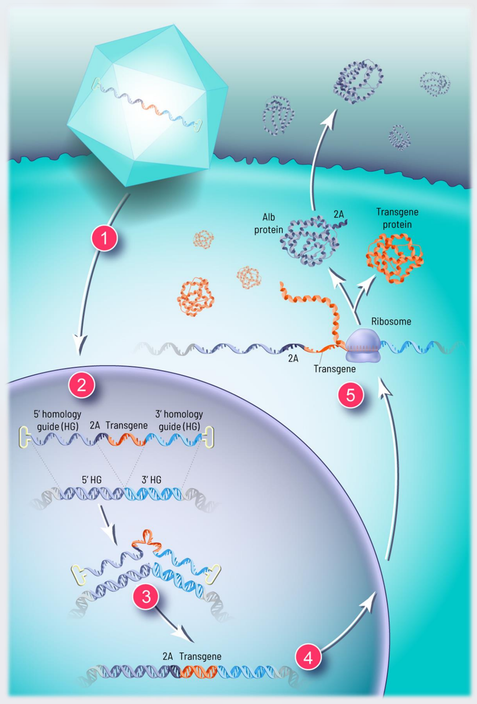
Hemophilia B and gene therapy



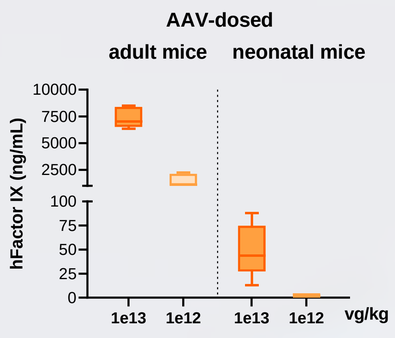
Hemophilia B is an X-linked bleeding disorder caused by loss-of-function mutations in blood clotting factor IX. Due to their inability to activate the coagulation cascade, hemophilia B patients suffer from easy bruising and long bleeding episodes that can affect internal organs and deep tissue such as joints or muscle, causing uncontrolled swelling and eventually leading to permanent tissue damage. Current standard care consists of chronic intravenous infusion of plasma-derived or recombinant factor IX. However, factor replacement is not always suitable or accessible to patients and has been often associated with inhibitor formation. Several clinical trials using canonical AAV gene therapy in adult hemophilia B patients are on-going and have shown promising results, with the potential to provide long-lasting production of factor IX from hepatocytes following a single injection (1-4). While this is an exciting prospect for adult patients, pediatric patients will not benefit from this approach due to progressive loss of therapeutic levels of factor IX as hepatocytes divide and the liver grows.

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

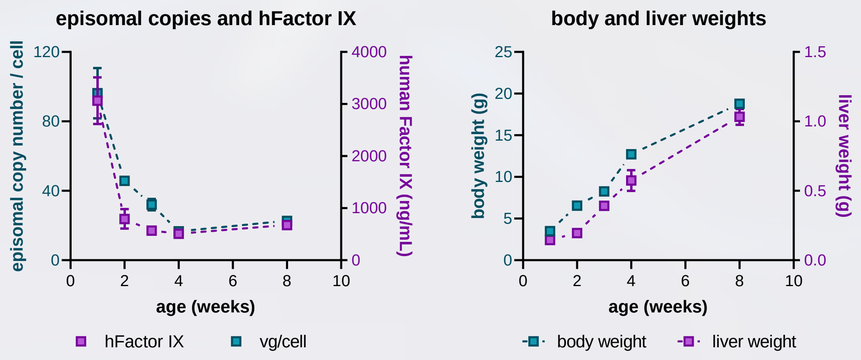


GeneRide is a novel AAV-based genome-editing technology that leverages the natural process of homologous recombination to insert a therapeutic transgene into the genome (5). For liver-directed targets, the transgene is integrated into the *albumin* locus. The integrated transgene (*e.g. F9*) can thus "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 allows for polycistronic protein expression. This results in the production of two separate proteins: a C-terminal 2A-tagged albumin (ALB-2A) and the therapeutic transgene (*e.g.* factor IX). 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. Levels of genomic integration in liver and transgene expression as well as ALB-2A in circulation linearly correlated with each other, making ALB-2A a universal circulating biomarker for monitoring non-invasively GeneRide-mediated genome-editing in the liver.

Limitations of canonical AAV gene therapy in neonates

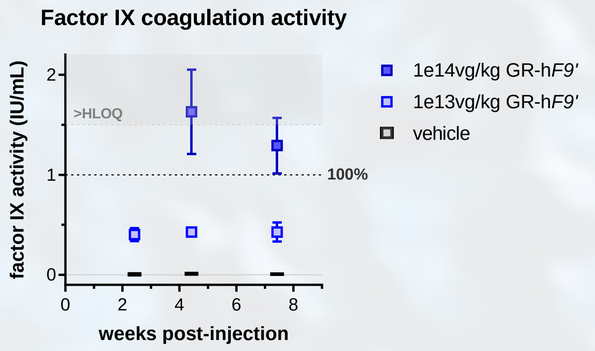


Engineered rAAV vectors encoding liver specific promotor driven human factor IX (DJ-LSP1-h*F9*) were administered (iv) to adult mice (8~10-week-old) and neonatal mice (PND1) at the same vg/kg dose level. Plasma levels human factor IX were measured by ELISA 5-6 weeks post-dose. A 100~1000 folds drop of factor IX expression was observed between adult and neonatal dosing.



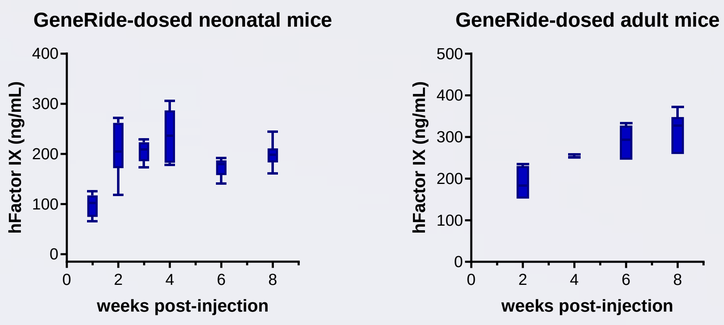
Neonatal mice (PND1) were dosed (iv) with 1e14vg/kg of DJ-h*F9*. Body weight, liver weight, vector episomal copy number per cell in liver, and plasma hFactor IX levels were monitored periodically after dosing. Consistent with previous reports (6,7), a dramatic decrease of vector episomal copy number and thus transgene expression level is observed within the first few weeks, as corresponding to the rapid growth of the animal.

GeneRide achieves therapeutic effect in the hemophilia B mouse model



Efficacy of GeneRide was demonstrated in the mouse model of hemophilia B (*B6.129P2-F9^{tm1Dws}/J*). Male hemizygous mice (n=6 per group) were dosed (iv) with GeneRide encoding a hyperactive variant of human factor IX (h*F9'*) at 1e14 or 1e13vg/kg. Plasma was collected at various time points after dosing and hFactor IX activity was measured in the one-stage coagulation assay (Stago CK-PREST 5). GeneRide-treated animals showed dose-dependent hFactor IX activity. The activity levels achieved with this hyperactive Factor IX already reach ~50% of normal activity at 1e13vg/kg, and supraphysiological level with the higher dose. The activity achieved by GeneRide exceeds the therapeutic level that will prevent bleeding episodes and joints damage in severe hemophilia B (8).

Durability of GeneRide in neonatally-treated animals



GeneRide-hFactor IX (DJ-GR-h*F9*) was administered (iv) to neonatal (PND1) and adult mice (6-week-old). Plasma levels of hFactor IX were measured at various time points following dosing. In contrast to canonical AAV where a significant loss of transgene expression was observed when dosed to neonates, GeneRide provides durable transgene expression regardless of the age at dosing. GeneRide thus overcomes the current challenge of canonical gene therapy and presents a great potential in providing durable treatment to pediatric patients.

CONCLUSIONS

- GeneRide is a novel rAAV-based genome editing technology enabling targeted genomic integration of therapeutic transgenes.
- Using a neonatal mouse model, we have demonstrated durable expression of transgene (human factor IX) via GeneRide, whereas canonical AAV shows rapid reduction of transgene expression due to episomal dilution/degradation in response to liver growth.
- Using a hyperactive human factor IX variant, GeneRide delivers 50% normal coagulation activity at 1e13vg/kg and a supraphysiological activity at 1e14vg/kg in a mouse model of hemophilia B.
- Taken together, GeneRide could provide an early cure for pediatric patients suffering from hemophilia B.

1. *Nathwani et al. NEJM (2011)*
2. *Nathwani et al. NEJM (2014)*
3. *George et al. NJM (2017)*
4. *Miesbach et al. Blood (2018)*
5. *Barzel et al. Nature (2015)*
6. *Cunningham et al. Mol Ther (2008)*
7. *Wang et al. Mol Ther (2011)*
8. *Soucie et al. Blood (2018)*