Young mice administered adult doses of AAV5-hFVIII-SQ achieve therapeutic factor VIII expression into adulthood

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Background

- Hemophilia A (HA) is an X-linked genetic bleeding disorder caused by a deficiency in the activity of coagulation factor VIII (FVIII). Individuals with severe deficiencies (i.e., <1 IU/dL) experience spontaneous bleeding internally into joints or muscles.
- Hemarthrosis typically develops before 2 years of age in children with severe HA and, if untreated, leads to recurrent bleeds with musculoskeletal deformity and loss of mobility.
- Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) gene transfer provided reduced bleeding for adult clinical trial participants with severe hemophilia A. However, pediatric clinical feasibility and outcomes are unknown.
- Previous preclinical research in neonatal mice and non-human primates with AAV vectors indicates robust liver cell proliferation early in life can prevent stable transgene expression.

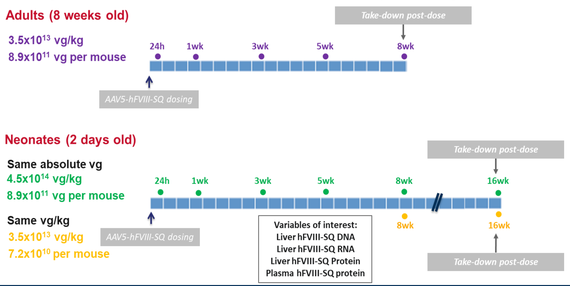
Objectives

- To investigate the effect of vector dose on transgene production and persistence in neonatal vs adult mice using a mouse model of Hemophilia A.

Study design

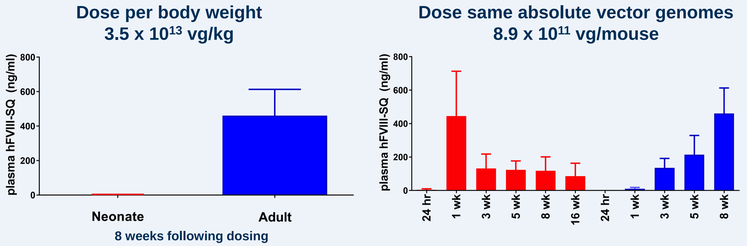
- RAG2$^{-/-}$ x FVIII$^{-/-}$ double-knockout (DKO) mice were used in the study.
- The adult cohort (5 groups of 10 eight-week-old mice) were dosed with AAV5-hFVIII-SQ by intravenous (IV) injection via tail vein at a dose of 3.5x10^{13} vg/kg (i.e., an absolute dose of 8.9x10^{11} vg/mouse).
  - Adult mice were then euthanized at 24 hours or 1, 3 , 5, or 8 weeks after dosing.
- The neonatal cohort (8 groups of 10 two-day-old mice) were dosed with AAV5-hFVIII-SQ by IV injection via temporal vein either: 1) at the same absolute vector genomes as adults (i.e., 8.9x10^{11} vg/mouse; 6 groups) or 2) based on body weight (i.e., 3.5x10^{13} vg/kg; 2 groups).
  - Neonatal mice were then euthanized at 24 hours or 1, 3, 5, 8, or 16 weeks after dosing.

Figure 1. Adult vs. Neonate Study Design



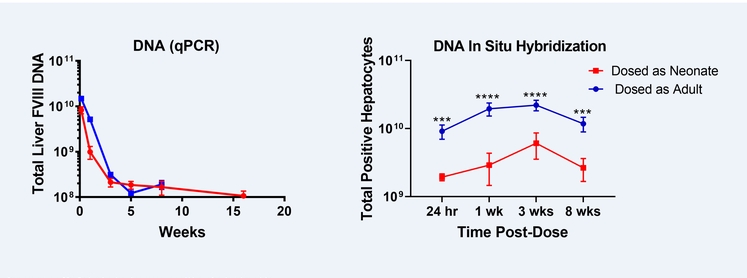
Results

Figure 2. Neonatal mice require same total vector genomes as adult to achieve therapeutic levels of hFVIII-SQ in plasma



- **Dose based on body weight**
  - Neonatal mice did not achieve meaningful plasma hFVIII-SQ protein levels.
- **Dose at same absolute vector genomes (adult dose)**
- Neonates maintained therapeutic levels of hFVIII-SQ expression into adulthood.
- Neonatal mice initially exhibited high hFVIII-SQ plasma levels followed by a partial decline potentially due to age-related body mass and blood volume increases.

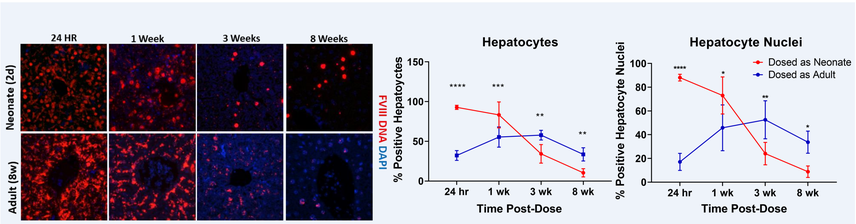
Figure 3. Neonatal mice are capable of taking up the same amount of vector genomes as adult mice



Data are mean ± SD; Red= mice dosed as neonates and blue=mice dosed as adults.

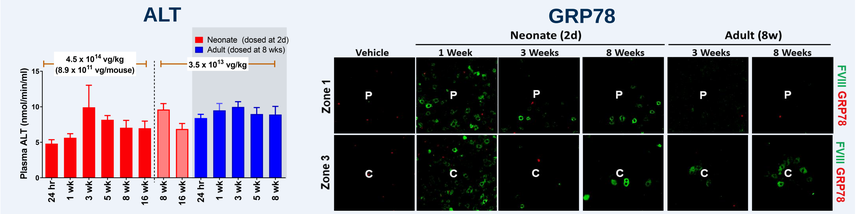
- The majority of total vector genomes in the liver disappeared over the first few weeks following dosing.
  - Despite liver growth in the neonatal cohort**, the slope of decline** in vector genomes over time is **similar between young and adult animals**.
- The **total number of hepatocytes** that stained positive for hFVIII-SQ DNA **remained stable** in neonatal mice, suggesting hFVIII-SQ genomes are **not lost due to mitosis**.

Figure 4: Kinetics of vector genome trafficking differ in neonatal mice



- hFVIII-SQ **vector genome detected** in the majority of hepatocyte nuclei by **24 hours** in **neonates**, compared to **3-8 weeks** in **adult mice**.
- This difference may explain the more rapid onset of expression of hFVIII-SQ protein observed in younger mice.

Figure 5: AAV5-hFVIII-SQ does not induce hepatoxicity



- **No significant increase in ALT** was detected in either adult or neonate cohorts.
- **No increase in GRP78** expression in hepatocytes expressing hFVIII-SQ was detected in either adult or neonatal cohort.

Conclusions

- Results demonstrate the capacity for AAV5-hFVIII-SQ transduction and rate of DNA decline is similar between neonates and adult mice when administered the same total quantity of vector.
- No features of hepatotoxicity or ER stress were observed.
- These data suggest that young mice require the same total vector genomes as adult mice to sustain hFVIII-SQ plasma levels.

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Disclosures:

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