Hemophilia B Gene Therapy in Mice Using a Novel Chimeric AAV Capsid
Combined With the Potency Enhanced CB 2679d-GT FIX Variant

Grant E. Blouse1, Katja Pekrun2, Tom Knudsen1, Jeff Landau1 and Mark A. Kay2
1Catalyst Biosciences, South San Francisco, United States. 2Stanford University School of Medicine, Departments of Pediatrics and Genetics, Stanford, United States

Results

The in vivo performance of the novel capsid / transgene at 8x10^{11} vg/kg

![Graphs showing FIX antigen and activity levels over time for different treatments.]

- FIX antigen and activity levels were stable by week 5 and remained durable for the remaining 12 weeks of the study. (A) FIX antigen as determined by ELISA, (B) FIX activity levels as determined by aPTT (C) FIX specific activity calculated as the ratio of FIX activity/antigen. Data are presented as mean ± S.D.

The in vivo performance of the novel capsid / transgene at 8x10^{10} vg/kg

![Graphs showing FIX antigen and activity levels over time for different treatments.]

- FIX antigen and activity levels increased over the first three weeks and remained stable through the 6.5 weeks of the ongoing study (data cut-off). (A) FIX antigen as determined by ELISA, (B) FIX activity levels as determined by aPTT (C) FIX specific activity calculated as the ratio of FIX activity/antigen. Data are presented as mean ± S.D.

Combination of the CB 2679d-GT transgene with a novel capsid provides superior FIX levels at 1/10 the AAV dose compared to previous studies and currently reported constructs using the Padua transgene.

Methods

- Codon optimized Wild-Type, CB 2679d-GT and R338L Padua FIX AAV constructs were prepared on the T448 background downstream of a robust hepatocyte-specific promoter in the ApeE4HT-AAT-FIX minigene-IgG4 (aA vector and packaged into novel chimeric AAV capsid identified through DNA shuffling.

- FIX minigene constructs were packaged into a novel AAV capsid designed through DNA shuffling.

- C57BL/6 FIX-deficient mice (3-5 mice/group) were injected via the tail vein with either 2.0x10^6, 2.0x10^7 or 2.0x10^8 vector genomes per mouse (vg/mouse) corresponding to 8x10^1, 8x10^7 or 8x10^8 vg/kg assuming a nominal mouse weight of 25 grams with of AAV FIX vectors expressing either CB 2679d-GT, Padua, or wild-type FIX. Blood was collected for examination of the antigen levels and FIX activity levels.

- FIX activity was assessed by an activated partial thromboplastin time (aPTT) Factor IX single-stage clotting assay on an ACL-Top instrument (Instrumentation Laboratories) using the recommended HemosIL® or SynthasIL® reagents and calibrators.

Bibliography


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