CB 2679d-GT - A Novel Human Factor IX Variant Shows Enhanced Activity After Delivery Into Hemophilic Mice Using an AAV Capsid With High Liver Transduction

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Catalyst Biosciences has developed a next generation subcutaneously delivered engineered coagulation Factor IX, dalcinonacog alfa, with increased catalytic activity, resistance to antithrombin inhibition and improved affinity for activated FVIII. The triplet substitutions (R318Y, T343R and R338E) provide this FIX variant with a 22-fold enhanced potency over wild-type (wt) FIX. We previously presented the superiority of this molecule by AAV delivery (CB 2679d-GT) over the R338L Padua FIX variant in a preclinical proof of concept study in hemophilia B mice using a self-complementary rAAV-DJ8 FIX expression construct (Blouse et al., Hemophilia, 2019, vol. 25: S1, P124). The FIX Padua variant was originally discovered in a patient with juvenile thrombophilia having an 8- to 9-fold enhanced FIX specific activity compared to wildtype and has since garnered usage as the leading FIX construct in haemophilia B gene therapy trials. In the present study we tested delivery of different FIX rAAV genes using the novel chimeric KP1 AAV capsid, which had previously been shown to exhibit high transduction rates in murine and human hepatocytes in vivo (Pekrun et al., JCI Insight, 2019, vol. 4, 22).

Codon optimized wildtype (wt), CB 2679d-GT, and Padua FIX sequences containing a truncated version of the first FIX intron were cloned downstream of a robust hepatocyte-specific ApoE/SerpinA promoter and packaged into AAV-KP1. The in vivo performance of the constructs was assessed in FIX-deficient hemophilia B mice injected with 2 x10¹⁰ vg/mouse, 2 x10⁹ vg/mouse, and 2 x10⁸ vg/mouse and followed over several months. FIX antigen and activity levels were assessed by ELISA and aPTT assays, respectively. As expected, FIX activity levels were significantly increased in plasma from mice receiving the CB 2679d-GT and Padua FIX rAAV constructs as compared to those that had received the wt FIX rAAV. The FIX antigen and thus activity levels using the KP1 capsid and the new single-stranded vector construct were between 5- and 10-fold higher compared to the previous study that employed the DJ8 capsid and a self-complementary vector construct. Specific FIX activity in plasma from mice injected with CB
2679d-GT was 2- to 3- fold higher than in plasma from Padua rAAV injected mice, similar to the expected improvement observed in the previous study. This study demonstrates that combining a next generation AAV vector with the potency enhanced FIX variant CB 2679d-GT has the potential to improve transgene expression and effectively lower the viral dose to one tenth or even one hundredth of the dose currently needed to achieve therapeutically relevant FIX activity levels. This would not only substantially reduce the cost of rAAV based FIX gene therapy but would importantly reduce the risk of adverse immune responses that even now limit this therapeutic approach.