Bleeding phenotype correction in hemophilia A mice following in vivo Factor VIII gene transfer by electroporation in skeletal muscle cells

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Introduction

Long term expression of the clotting Factor VIII (FVIII) is the therapeutic goal for people with hemophilia A (HA) (PwHA). Although viral-vectored gene therapies for the treatment of HA have been approved, uptake has been limited due to variable efficacy, declining FVIII activity, hepatotoxicity, high cost, and inability to redose.

Non-viral gene delivery to skeletal muscle followed by electroporation (EP) using CELLECTRA™ is an attractive therapeutic modality which offers long-term *in vivo* protein expression. Clinical proof-of-concept of this approach was demonstrated in a Phase 1 study (NCT05293249), where durable *in vivo* production of complex proteins, functional monoclonal antibodies, persisted at sustained levels for at least 72 weeks in all treated participants. The platform allowed for redosing and none of the participants presented with a host immune response against the encoded proteins. These data support the platform's potential to develop durable DNA-encoded therapeutic protein (DPROT) replacement therapies, including FVIII to treat HA. Here, the platform is being employed to deliver a plasmid DNA-encoding human FVIII (pGX9436) to muscle cells towards the long-term, sustained production of FVIII in PwHA.

Methods

The pGX9436 construct was designed for enhanced FVIII stability, improved muscle cell expression and secretion. *In vivo* functionality of the expressed FVIII was assessed in the B6;129S-F8^{tm1Kaz}/J HA FVIII -/- knockout (KO) mice (n=8/group) after administration of a single dose of pGX9436 or empty vector (pGX0001) by intramuscular (IM) injection followed by EP. WT C57BL/6 mice served as phenotype controls. In all mice, bleeding phenotype correction was evaluated 15 days post-treatment using a tail-clip bleeding assay and plasma FVIII activity was measured using the two-stage chromogenic assay and one-stage activated partial thromboplastin time (APTT)- based assay.

Results

pGX9436 IM-EP delivery in mice resulted in durable therapeutic FVIII activity. In the tail-clip assay, compared to pGX0001 -treated FVIII -/- KO mice, those treated with pGX9436 displayed significantly reduced median blood loss (0.076 g vs pGX0001: 0.49 g, $p < 0.05$) and bleeding time (3.4 min vs pGX0001: 26.4 min, $p < 0.05$) compared to pGX0001. No significant differences were observed between pGX9436-treated KO and WT mice (blood loss: 0.076 g vs WT: 0.0 g; bleeding time 3.4 min vs WT: 3.1 min) (Figure 1). Therapeutic levels with mean FVIII activity of 20% were measured by the two-stage chromogenic assay in all pGX9436-treated mice that effectively controlled bleeding. As a secondary readout, activity measured by APTT-based clotting assay in plasma of pGX9436- treated FVIII -/- KO mice showed clotting activity (clotting time) of 97% compared to WT mice (100%). A statistically significant difference in mean clotting time between plasma from pGX9436- treated FVIII-/- KO mice and naïve FVIII-/- KO mice was recorded.

Conclusion

We provide preclinical proof-of-concept for a novel human FVIII replacement therapeutic modality, demonstrating *in vivo* production of functional FVIII displaying high levels of activity and correction of

bleeding phenotype. Data support continued development of pGX9436 as a next generation hemophilia A therapeutic.

Figure 1

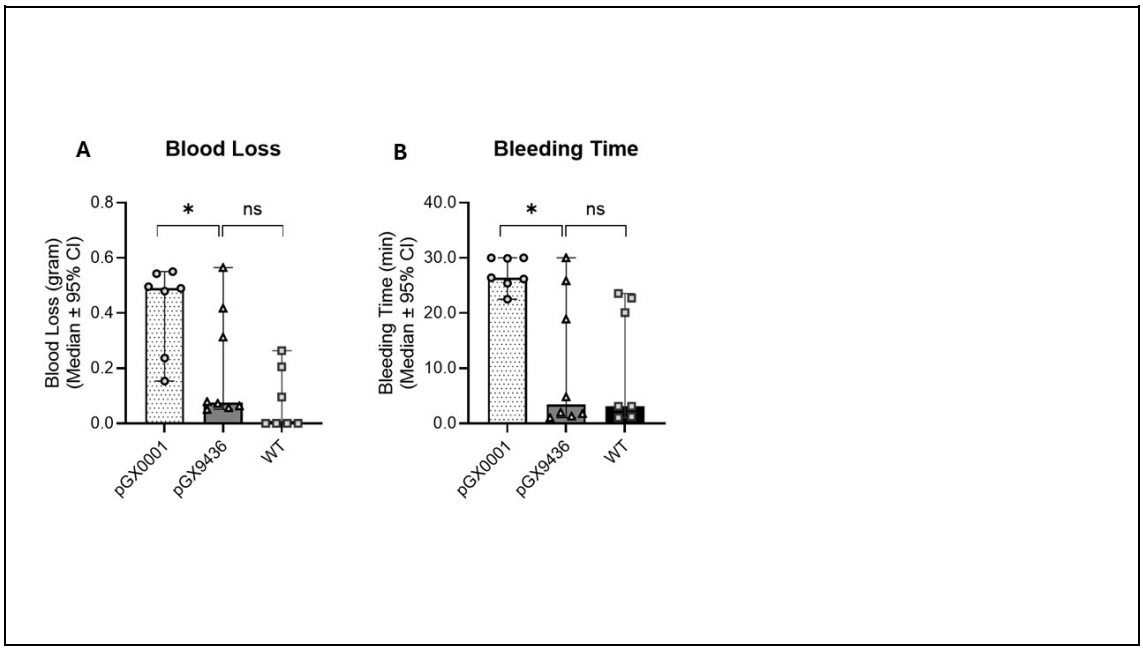

Figure Legend

Figure 1: Evaluation of the efficacy of pGX9436 treatment in hemophilia A mice by tail clip bleeding model. (A) Male FVIII -/- KO mice were treated with pGX0001 or pGX9436 on day 0 delivered IM + EP. Untreated C57BL/6 WT mice were added as a control. Fifteen (15) days following treatment, the tail was transected 6 mm from the tip. Blood loss (change in mouse weight) (A) and bleeding time (B) were measured. Data is shown as median with 95% confidence interval. Statistically significant differences between compared groups are indicated: *$p < 0.05$; NS, not significant; as measured by Ordinary one-way ANOVA.