ABSTRACT: Eurogin International Multidisciplinary HPV Conference

Title: Ectopic FVIII expression via non-viral vector DNA medicine platform results in e icacious levels of FVIII protein and correction of the bleeding phenotype in Hemophilia A mice.

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Introduction and Objective: Replacement therapy with recombinant FVIII or non-factor agents remains the standard-of-care for people with Hemophilia A (HA) (PwHA). AAV-based gene therapies aim to restore FVIII levels, but uptake has been limited due to variable e icacy, declining FVIII activity, hepatotoxicity, high cost, and inability to redose.

Non-viral gene delivery to skeletal muscle with electroporation (EP) using CELLECTRA™ o ers an attractive potential alternative to current treatments. Clinical proof-of-concept of this modality was demonstrated in a Phase 1 study, where durable *in vivo* production of complex proteins, functional monoclonal antibodies, persisted 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 protein replacement therapies, including FVIII to treat HA. Here, the platform has been employed to deliver plasmid DNA-encoding human FVIII (pDFVIII) to muscle cells, for long-term FVIII replacement in PwHA.

Materials and Methods: The pDFVIII construct incorporates combined modifications for enhanced FVIII stability, improving ectopic muscle cell expression and secretion. pDFVIII intramuscular (IM) EP (IM-EP) delivery in mice resulted in durable therapeutic FVIII activity. E icacy was assessed in FVIII knockout (KO) mice (n=8/group) receiving a single dose of pDFVIII or empty vector (pGX0001) by IM-EP. WT C57BL/6 mice served as phenotype controls. Bleeding phenotype correction and plasma FVIII activity were evaluated 15 days post-treatment using tail-clip bleeding and two-stage chromogenic assay, respectively.

Results: pDFVIII-treated FVIII KO mice 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 di erences were observed between pDFVIII and WT (blood loss: 0.076 g vs WT: 0.0 g; bleeding time 3.4 min vs WT: 3.1 min) (Table 1). Therapeutic levels of plasma FVIII activity were measured in all pDFVIII-treated mice that e ectively controlled bleeding.

Conclusions: We provide preclinical proof-of-concept for a novel human FVIII replacement therapy modality, demonstrating *in vivo* production of functional FVIII and correction of bleeding phenotype. Data support continued development of pDVFIII as a next generation HA therapeutic.

Table 1: Evaluation of the efficacy of pDFVIII treatment in hemophilia A mice by tail bleeding model

Mouse Model	Treatment	Median Blood Loss (grams)	Median Bleeding Time (min)
FVIII KO	pGX0001	0.49	26.4
FVIII KO	pDFVIII	0.076	3.4
C57BL/6 WT	None/naive	0	3.1