FVIII expression via non-viral vector DNA medicine platform results in efficacious levels of FVIII protein and correction of the bleeding phenotype in hemophilia A mice.

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Introduction

Clinical proof of concept for the *in vivo* production of functional proteins was recently demonstrated in a Phase 1 study after local delivery of plasmid DNA (pDNA) to the muscle with electroporation (EP)[1] (**Figure 1**). This data supports the platform's potential to develop next generation protein replacement therapeutics, including FVIII to treat hemophilia A (HA). Here we describe employing this gene delivery technology to a synthetic DNA plasmid (INO-D001) encoding human FVIII protein (huFVIII) with modifications to boost expression and activity (**Figure 2**).

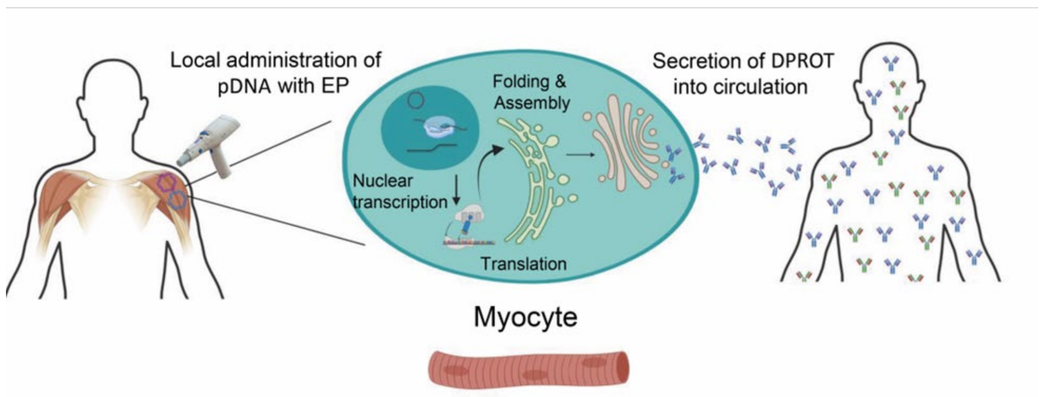


Fig. 1: Schematic of DNA medicine technology platform. Synthetic plasmid DNA constructs are administered IM followed by *in vivo* EP for the local expression of the transgenes in the deltoid muscle. DNA-encoded proteins (DPROTs) or DNA-encoded monoclonal antibodies (DMAbs) are expressed in the myocytes and secreted into circulation.

Fig. 2: Drug design. INO-D001 is comprised of a DNA plasmid encoding human Factor VIII with an IgE leader sequence, a partial B domain, and modifications to boost expression and activity of FVIII protein (red triangles represent the positions of the modifications).

[1]Tebas, P., et al., Safety and pharmacokinetics of SARS-CoV-2 DNA-encoded monoclonal antibodies in healthy adults: a phase 1 trial. Nat Med, 2025. (12):4150-4159.

Phenotypic bleeding correction in FVIII KO model

Preclinical *in vivo* proof of concept studies were performed using the FVIII knockout (FVIII KO (HA)) mice to recapitulate the bleeding phenotype of severe HA, displaying below 1% of FVIII activity and extended clotting times compared to wild-type (WT) mice. The ability of INO-D001 treatment to correct the bleeding phenotype was measured based on the time required to achieve hemostasis and the total amount of blood loss after excising a 6 mm section at the end of the tail. Male FVIII KO mice were treated with empty DNA plasmid (pGX0001) or INO-D001 on day 0 delivered IM + CELLECTRA EP. Untreated C57BL/6 WT (WT B6) mice were added as a control. Fifteen (15) days following treatment, mice were weighed and the tail was transected 6 mm from the tip (**Table 1**).

Table 1: Evaluation of the efficacy of INO-D001 treatment in FVIII KO 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	INO-D001	0.076	3.4
C57BL/6 WT (WT B6)	None	0	3.1

FVIII KO mice treated with pGX0001 empty DNA plasmid negative control had a median blood loss of 0.49 g and bleeding time of 26.4 min. In contrast, WT mice had a median blood loss of 0.0 g and bleeding time of 3.1 min, respectively. FVIII KO mice treated with INO-D001 displayed significantly reduced blood loss (0.076 g, $p < 0.05$ vs FVIII KO mice + pGX0001) and bleeding time compared to pGX0001 (3.4 min, $p < 0.05$ vs FVIII KO mice + pGX0001), displaying a comparable bleeding phenotype as WT mice (**Table 1, Figure 3 A,B**). Mean FVIII activity level of 20% was measured by the two-stage chromogenic assay in INO-D001-treated mice (**Figure 3C**). This data supports the potential of INO-D001 treatment to correct the bleeding phenotype in a FVIII deficient background.

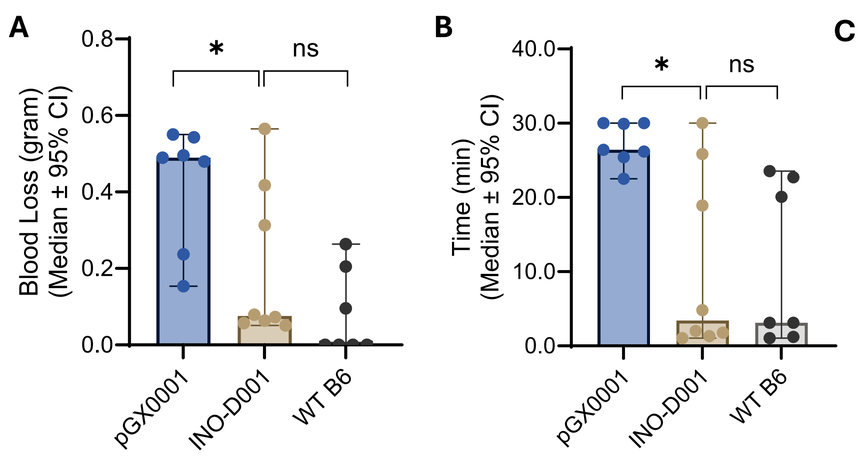


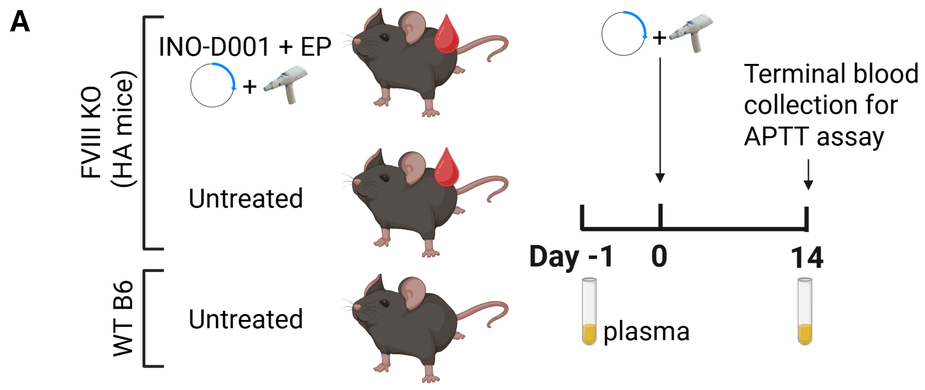
Fig. 3: Evaluation of the efficacy of INO-D001 treatment in FVIII KO mice by tail bleeding model. Blood loss (change in mouse weight) (A) and bleeding time (B) were measured. Data is shown as median with 95% confidence interval. *$p < 0.05$; ns, not significant. (C) Mean FVIII activity for INO-D001 and pGX0001 treatment groups on day 15 as measured by the two-stage chromogenic assay.

FVIII activity by one-stage APTT-based clotting assay in FVIII KO model

Mechanistic studies were performed to elucidate the role design modifications may have on the activity of INO-D001-encoded FVIII (**Figure 2**). For example, we have included the R571S modification associated with increased thrombin generation[2]. The impact of this modification cannot be measured in the two-stage chromogenic assay, therefore, FVIII activity was measured by one-stage activated partial thromboplastin time (APTT)-based clotting assay (**Figure 4A**). The plasma of INO-D001-treated FVIII KO mice showed activity (clotting time) comparable to WT mice indicating complete correction of the bleeding phenotype. In contrast, a statistically significant difference in mean clotting time between plasma from INO-D001- treated and untreated FVIII KO mice was recorded (**Figure 4B**).

Three mechanisms have been reported for the enhanced activity associated with the R571S mutation: resistance to FVIII degradation by activated protein C (APC), enhanced A2 domain stability leading to prolonged activation of Factor X (FX), or rapid dissociation of activated FX (FXa) resulting in increased thrombin generation[2]. Thus, we evaluated the role of APC in the APTT assay. Results showed that APC had no significant effect on clotting activity in the plasma of INO-D001-treated FVIII KO mice compared to WT mice (**Figure 4C**), suggesting a modified FX and FXa interaction rather than APC degradation resistance may be the important mechanisms associated with a gain of function mutation present in INO-D001. There results support the findings of complete correction of bleeding in the tail clip assay at the lower levels of FVIII activity measured by the two-stage chromogenic assay (**Figure 3C**).

[2]Wischmeyer, J.T., et al., A naturally occurring gain-of-function mutation in Factor VIII. N Engl J Med, 2025. 393(7):722-724.



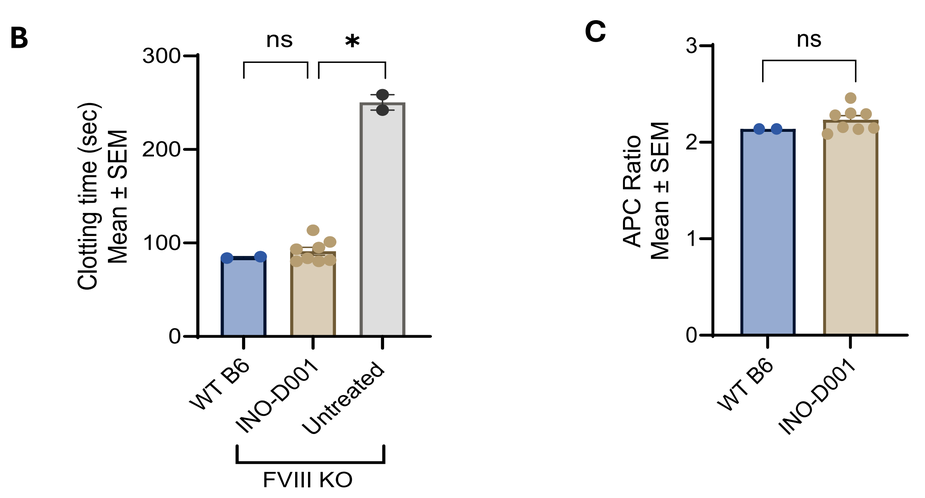


Fig. 4: Functional FVIII activity measurements in INO-D001-treated FVIII KO mice by APTT-based assay. (A) FVIII KO mice (HA mice) were treated with INO-D001 delivered IM + CELLECTRA EP. (B) FVIII clotting activity was measured with the one-stage APTT-based assay in the presence of APC in INO-D001-treated plasma samples collected on day 14 and in plasma samples collected from untreated WT C57BL/6 (WT B6) and untreated FVIII KO mice. (C) APC ratios were calculated based on the clotting times in the presence of APC/ clotting time in the absence of APC. Data represent Mean ± SEM. *$p < 0.05$; ns, not significant.

Conclusions

- We provide preclinical proof-of-concept for a novel human FVIII replacement therapeutic modality, demonstrating *in vivo* production of functional FVIII and correction of the bleeding phenotype in FVIII KO HA mice.
- Plasma from INO-D001-treated HA mice showed clotting activity comparable to WT mice measured by the one-stage APTT assay, consistent with gain of function mutation.
- Data support continued development of INO-D001 as a next generation hemophilia A therapeutic.

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