PO057

One Stage Assay Analysis Demonstrates that Gene Therapy-Derived Factor VIII (FVIII) from Dirloctocogene Samoparvovec Reduced Clot Times Compared to Endogenous FVIII at Comparable FVIII Levels

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Summary

1. Liver-directed gene therapies expressing factor VIII (FVIII) have demonstrated the potential for sustained, therapeutic expression of FVIII, improving patient outcomes in multiple clinical trials.

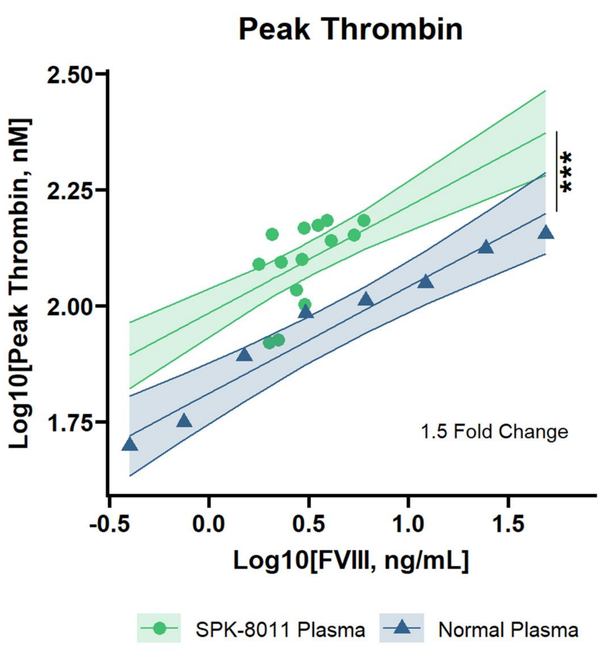
2. Expanding on observations of increased thrombin generation, we investigated differential activity of SPK-8011 FVIII in study participant plasma samples compared to endogenous FVIII and Xyntha® using the One Stage Assay (OSA).

3. Plasma from a subset of haemophilia A Phase I/II study participants who received SPK-8011 had reduced clotting times by the OSA when compared with plasma from a pool of healthy individuals at comparable levels of FVIII antigen.

4. Findings from this clotting time study combined with previous thrombin generation results suggest an improved function from gene therapy-derived FVIII compared to FACT and Xyntha® which warrants further investigation and characterization.

Background

- Liver-directed, adeno-associated virus (AAV)-mediated transfer of a B-domain deleted (BDD) Factor VIII (BDD-FVIII) transgene via **dirloctocogene samoparvovec (SPK-8011)** has demonstrated the potential to achieve sustained, circulating levels of FVIII with a single infusion in patients with haemophilia A.[1]
- Previously, we reported increased measures of thrombin generation—a global coagulation assay—in **a subset of Phase I/II study participants** who received SPK-8011 compared with pooled plasma from healthy individuals containing similar levels of FVIII antigen (right)[2].
- **Hypothesis:** We hypothesized that the effect seen in the Thrombin Generation Assay (TGA) should be measurable in orthogonal FVIII activity assays as well. To investigate this further, we evaluated clotting times of plasma samples containing gene therapy-derived, endogenous, or recombinant FVIII using **the One Stage Assay (OSA).**



***, $p < 0.001$.

Methods

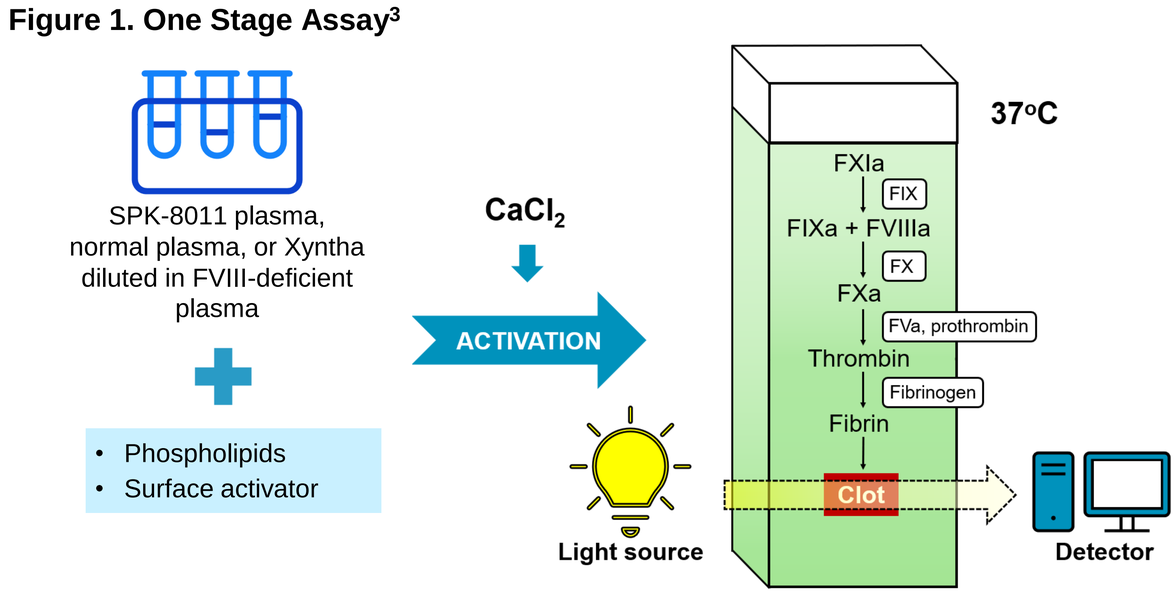
SPK-8011 plasma collection and clinical analysis of FVIII antigen concentration

- Plasma from participants dosed on the **SPK-8011 Phase I/II trial** (NCT03003533/NCT03432520) was collected as previously reported (George et al. NEJM 2021).[1]
- Samples tested for FVIII activity by OSA represented **12-, 26- and 40-week post-injection** timepoints. These samples contained FVIII antigen levels ranging from 7–23.8 ng/mL as determined by a human FVIII immunoassay (MSD technology using Green Mountain Antibodies, GMA8023/GMA8024).

One Stage Assay (OSA)

- **Study participant plasma samples**, **normal human plasma** (**i.e. Factor Assay Control Plasma [FACT])** George King Bio-Medical, Overland Park KS, USA), **and Xyntha®,** were diluted in 1X Tris buffered saline (TBS, 50 mM Tris, 150 mM NaCl) with 1% bovine serum albumin (BSA).
- All samples were further diluted into **FVIII deficient plasma** (George King Bio-Medical, Overland Park KS, USA) prior to testing using the assay protocol.
- Clotting times in plasma samples were measured using the TriniCLOT aPTT reagent (TCoag Ltd., Wicklow, Ireland) on a STart analyzer (Stago, Parsippany, NJ, USA).

Figure 1. One Stage Assay[3]



Statistical analyses

- Analyses performed included a whole model fit to the log transformed experimental results and **ANCOVA, or Analysis of Covariance**, which tested to see if there were any significant effects in this model: Log(aPTT)=Sample Type+Log(FVIII)+Sample Type*Log(FVIII).
- A **linear model fit to FACT and Xyntha® sample clot times** was chosen to compare mean differences in residual clotting times between control samples over a range of FVIII antigen levels and study participant samples with antigen levels that fall within the control sample range.
- The **Welch's non-paired t-test** was performed to assess statistical significance in clot time reduction.

Results

Figure 2. SPK-8011 Plasma Exhibits a Distinct Response Curve Compared to Either Endogenous FVIII or Xyntha® in the One Stage Assay

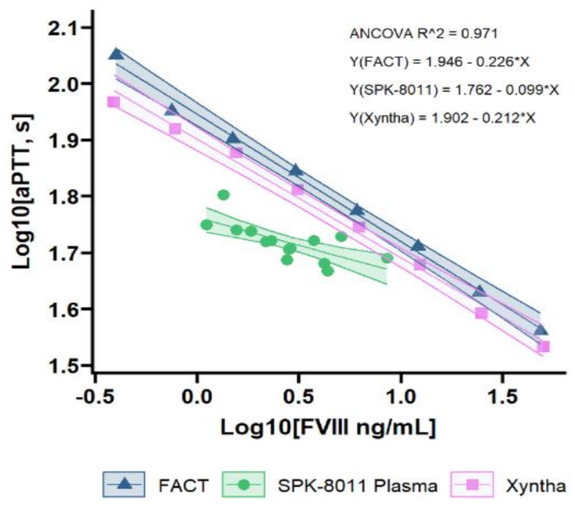


Fig. 2. Plasma samples from 14 HemA study participants treated with SPK-8011 (green circles) were tested in the OSA along with defined antigen levels of normal plasma (blue triangles) and Xyntha (violet squares). All study participant samples had FVIII antigen levels within the antigen range of normal plasma and Xyntha samples. Log transformed values of clotting times versus FVIII antigen values were plotted in the graph above.

- Gene therapy-derived FVIII in **SPK-8011 study participants** resulted in **shorter clotting times in the OSA** when compared to endogenous FVIII (FACT plasma) and Xyntha® at similar antigen levels.
- A linear relationship was demonstrated between mean FVIII antigen and clot times, and **the slope of the linear regression for SPK-8011 plasma is significantly different** from both that of Xyntha® and normal (FACT) plasma by Tukey's HSD.

Figure 3. SPK-8011 Plasma Results in Significantly Reduced Clotting Times Compared to Either Endogenous FVIII or Xyntha®

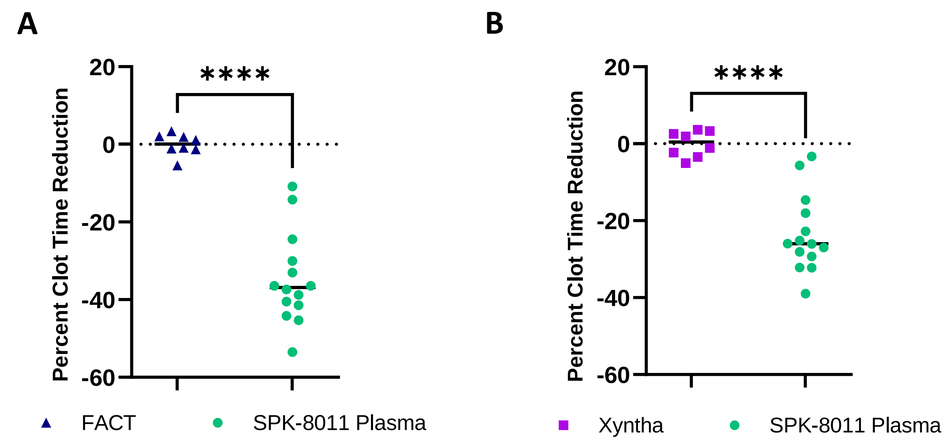


Fig. 3. Mean reduction in clotting times (horizontal lines) calculated for plasma samples from 14 HemA study participants treated with SPK-8011 (green circles) were compared to changes from predicted times of normal plasma (blue triangles) in Panel A, and Xyntha (violet squares) in Panel B. ****, $p < 0.0001$.

- Using a linear model fit to control sample clot times, **SPK-8011 plasma samples demonstrated a 34.7% ± 11.7 (mean ± SD) reduction in clot times compared to normal plasma, and a 23.5% ± 10.0 (mean ± SD) compared to Xyntha.**
- This **reduction in clot time for SPK-8011 plasma was significantly different** from both normal plasma ($p < 0.0001$) and Xyntha ($p < 0.0001$) by Welch's t-test.

Conclusions

- Plasma from individuals with haemophilia A who received SPK-8011 demonstrated **shorter clot times** compared to pooled plasma from healthy donors and recombinant FVIII containing similar antigen levels.
- These findings corroborate our previous observation that SPK-8011 FVIII may have **enhanced activity** compared with endogenous FVIII in a global coagulation assay (TGA).
- Taken together, these observations emphasize **the importance of further characterizing gene therapy derived FVIII.**

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References

1. L. George et al, *NEJM* 2021
2. I. Rojas et al *EAHAD* 2023
3. F. Peyvandi et al, *J Thromb Haemost* 2016

Acknowledgements

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Disclosures

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