PO025

Analysis of Long-Term Expression Up To 72 Weeks of a Liver-Specific rAAV Gene Therapy Expressing the Human FVIII Transgene from Dirloctocogene Samoparvovec in Adult Mice

Emmeline L. Blanchard*[1], Thomas Antrilli[1], Gabrielle Jones[1], Drew Peterson[1], Graciela Rivera-Pena[1], Joseph Silverberg[1], Erich Heinzel[1], Sylvie Smith[1], George Atkins[1], Jennifer Frick[1], Charlie Li[1], Heena Beck[1], Hayley Hanby[1], Jeffrey M. Alexander[1], and Christopher R. Riling[1]

*Presenting Author: emmeline.blanchard@sparktx.com

Affiliation: [1] Spark Therapeutics, Inc., Philadelphia, PA, USA

Summary

1. Liver-directed, recombinant adeno-associated virus (rAAV) gene therapy is a promising method of treating genetic diseases such as haemophilia A.

2. Establishment of durable transgene expression in a healthy, adult liver from an rAAV vector is a complex and multifactorial process involving immunological and non-immunological factors.

3. To explore long-term liver-directed AAV expression in mice of the B-domain deleted human Factor VIII (BDD-hFVIII) transgene contained in dirloctocogene samoparvovec, we analyzed BDD-hFVIII protein, DNA, and RNA up to 72 weeks.

4. No evidence of a decline of BDD-hFVIII protein was observed over 72 weeks. BDD-hFVIII DNA and RNA were preserved at 72 weeks.

Background

- Haemophilia A is an X-linked bleeding disorder with impaired coagulation due to a partial or complete loss of human factor VIII (hFVIII) activity.[1]
- Liver-directed, recombinant adeno-associated virus (rAAV) gene therapy is a promising method for treating haemophilia A.[1]
- Establishment of durable transgene expression in a healthy, adult liver from an rAAV vector is a complex and multifactorial process involving immunological and non-immunological factors.[1]
- While pre-clinical and clinical data show that expression can be maintained for more than 10 years in a canine model of haemophilia A and more than 8 years in haemophilia B patients, respectively, loss of previously stable transgene levels has also been reported in both animals and humans in other studies with rAAV vectors.[1,2,3,4]
- Here, we explore the long-term expression of our liver-directed AAV gene therapy platform, particularly the B-domain deleted (BDD)-human Factor VIII (hFVIII) transgene contained in dirloctocogene samoparvovec in mice over a period of up to 72 weeks.

Methods

AAV Vector Generation

- An AAV-Spark100 encapsidated rAAV vector using a vector genome identical to that found in dirloctocogene samoparvovec (SPK-8011) was generated via triple transient transfection in adherent HEK293 cells.

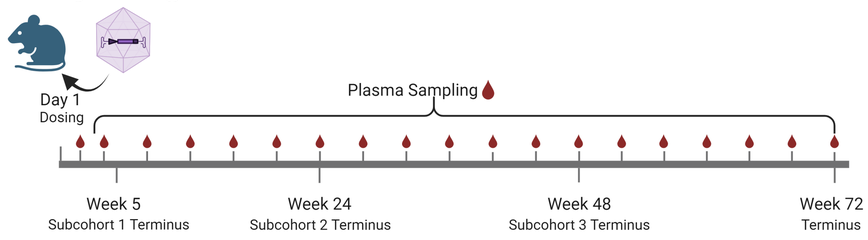
Study Design

- Adult male C57BL/6 mice were dosed with $3.5x10^{10}$ vector genomes (vg)/mouse (equivalent to $1.4x10^{12}$ vg/kg) and followed up to 72 weeks. Plasma sampling and takedowns are described in Figure 1.

Study Endpoints and Analyses

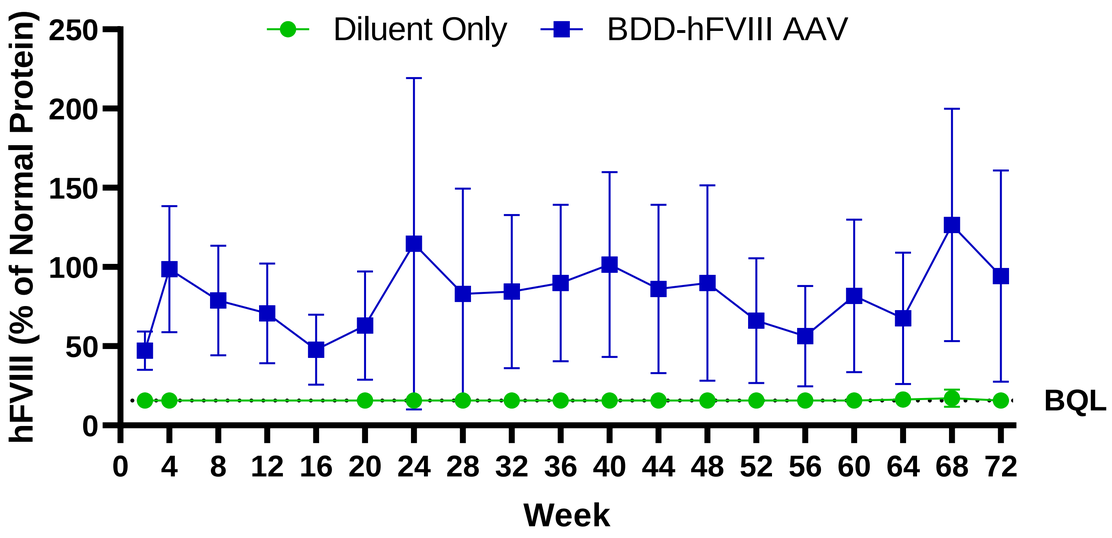
- Circulating BDD hFVIII levels were assessed via enzyme-linked immunosorbent assay (ELISA).
- Vector genome copy number (VGCN) and transgene RNA levels in the liver were assessed via real-time quantitative polymerase chain reaction assays.
- A linear mixed effect model was fit after week 4 to circulating BDD-hFVIII protein levels to evaluate protein over time after the initial incline. Linear regressions were fit to BDD-hFVIII VGCN and RNA data to evaluate change over time.
- Descriptive statistics included mean and standard deviation (SD).

Figure 1: Study Design



Results

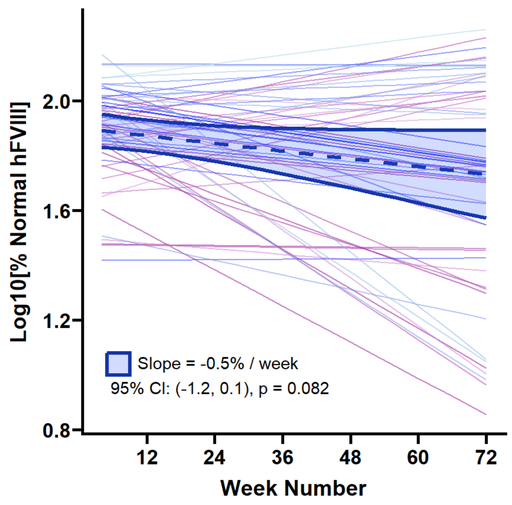
Figure 2. Circulating BDD-hFVIII protein was preserved through 72 weeks



Circulating BDD-hFVIII protein was analyzed at week 2, 4 and then every 4 weeks for 72 weeks. Subcohorts of animals were taken down at weeks 5, 24, 48, and 72; therefore, n was 60 for the first 5 weeks, 50 for 5-24 weeks, 40 for 24-48 weeks, 30 for 48-72 weeks. A reference value of 150 ng/mL was set as 100% normal hFVIII protein. Values below the quantitative limit (BQL) were set to the lower limit of quantification, 15.63%. Data points indicate means ± SD. BQL values were included for mean calculation.

- BDD-hFVIII plasma protein expression varied over time but demonstrated mean ± SD circulating levels of 81.52 ± 21.08% of normal protein (150 ng/mL = 100%) at $1.4x10^{12}$ vg/kg ($3.5x10^{10}$ vg/mouse) AAV vector. Importantly, **circulating BDD-hFVIII protein was preserved at 72 weeks at a clinically relevant dose in this preclinical model.**

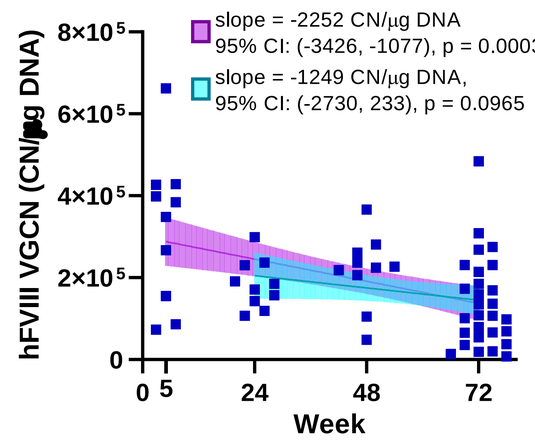
Figure 3. No evidence of a decline of BDD-hFVIII protein was demonstrated over 72 weeks in mice



A linear mixed effects model was set for circulating BDD-hFVIII protein (150 ng/mL as 100% normal hFVIII) over time. Thin lines represent slopes for individual animals, while dotted line represents the overall slope of all animals. Shaded region represents 95% confidence interval (CI).

- A linear mixed effects model was fit to circulating BDD-hFVIII protein levels to evaluate BDD-hFVIII protein in all animals over time after the initial incline in protein through 72 weeks. The linear mixed effects model analysis revealed a slope of -0.5% of normal hFVIII protein per week, which was not significantly less than zero (p = 0.082).
- **Therefore, no evidence of a decline was observed over the 72-week timecourse for circulating BDD-hFVIII protein.**

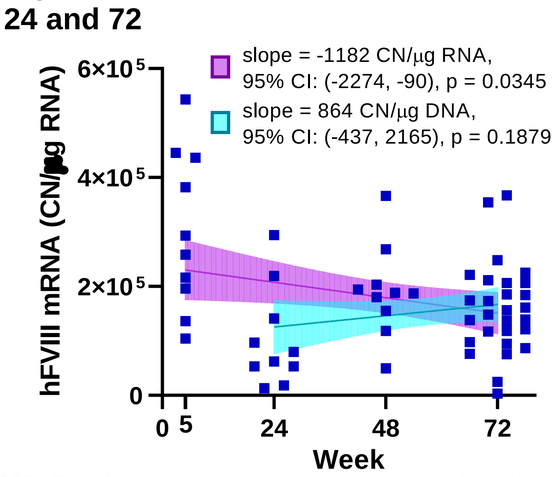
Figure 4. Liver BDD-hFVIII VGCN initially declined but stabilized between weeks 24 and 72



BDD-hFVIII VGCN levels in the liver were analyzed at weeks 5, 24, 48, and 72. n was 10 for week 5, 10 for week 24, 10 for week 48, and 30 for week 72. Data points indicate individual animals. Linear regression for weeks 5-72 (purple) or weeks 24-72 (teal) best fit line and 95% CI bands are displayed.

- A linear regression analysis was performed to analyze BDD-hFVIII VGCN in the liver over time. This analysis revealed a slope from weeks 5 to 72 that was significantly less than zero (p = 0.0003).
- However, when the analysis was performed between weeks 24 and 72, the slope was not significantly less than zero (p = 0.0965). Together, this suggests there was an initial decline of BDD-hFVIII VGCN levels in the liver between weeks 5 and 24, followed by stabilization of the BDD-hFVIII VGCN levels through week 72.
- **Importantly, liver BDD-hFVIII VGCN was preserved at 72 weeks.**

Figure 5. Liver BDD-hFVIII RNA initially declined but stabilized between weeks 24 and 72



BDD-hFVIII RNA levels in the liver were analyzed at weeks 5, 24, 48, and 72. n was 10 for week 5, 10 for week 24, 10 for week 48, and 30 for week 72. Data points indicate individual animals. Linear regression for weeks 5-72 (purple) or weeks 24-72 (teal) best fit line and 95% CI bands are displayed.

- Linear regression analysis was performed to analyze liver BDD-hFVIII VGCN over time. This analysis revealed a slope from weeks 5 to 72 that was significantly less than zero (p = 0.0345).
- However, when the analysis was performed between weeks 24 and 72, the slope was not significantly different than zero (p = 0.1879). Together, this suggests there was an initial decline of BDD-hFVIII RNA levels in the liver between weeks 5 and 24, followed by stabilization of the BDD-hFVIII RNA levels through week 72.
- **Importantly, liver BDD-hFVIII RNA was preserved at 72 weeks.**

Conclusions

- In this study, the long-term expression from an rAAV delivered BDD-hFVIII transgene was observed over the course of 72 weeks in adult male C57BL/6 mice.
- Circulating BDD-hFVIII protein expression demonstrated no sign of a decline over the 72-week timecourse.
- Liver BDD-hFVIII VGCN and RNA were preserved at 72 weeks. For both BDD-hFVIII VGCN and RNA, there was an initial decline in levels between weeks 5 and 24, followed by stabilization of the levels.
- Importantly, these data generated in adult male C57BL/6 mice using the identical vector genome found in dirloctocogene samoparvovec support the potential for FVIII-encoding gene therapies to achieve durable FVIII levels.

Presented at The European Association for Haemophilia and Allied Disorders (EAHAD) 2024 Annual Meeting | 6–9 February 2024 | Frankfurt, Germany

References

1. Samelson-Jones, B.J. et al. *Annu Rev Med* 2023;74:231-247
2. Nguyen, G.N., et al. *Blood* 2019;134:611
3. Reiss, U.M. et al. *Blood* 2023;142:1056
4. Greig, J.A. et al. *Nature Biotechnology* 2023; https://doi.org/10.1038/s41587-023-01974-7

Acknowledgements

This study was sponsored by Spark Therapeutics, Inc.

Disclosures

ELB, JMA, HH, CRR, TA, DRP, GRP, GA, CL: Shareholder: F. Hoffman La-Roche; Employment: Spark Therapeutics; **GJ, HB, JS:** Employment: Spark Therapeutics; **EH:** Employment: J&J Consumer Health (Current); Spark Therapeutics (Previous); **SS:** Employment: Spark Therapeutics (Previous); **JF:** Employment: Century Therapeutics (Current); Spark Therapeutics (Previous)