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POSTER ABSTRACTS

801.GENE THERAPIES

Childhood Treatment with Adeno-Associated Viral Gene Therapy Results in Stable FVIII Expression and Improved Bleeding Phenotype in Adult Severe Hemophilia A Dogs

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Introduction: In patients with severe hemophilia A, adeno-associated virus (AAV)-mediated factor VIII (FVIII) gene therapy is associated with reduced bleeding compared with FVIII prophylaxis. AAV5-FVIII gene therapy is approved in the U.S. and conditionally approved in Europe for the treatment of adults with severe hemophilia A. Gene therapy during childhood could potentially prevent the development of arthropathy and improve quality of life. However, there are unknowns regarding long-term safety and whether the predominant episomal nature of AAV persistence would lead to loss of transgene expression with hepatocyte divisions and liver expansion during childhood. The inflammatory response to AAV gene therapy in children is also largely uncharacterized.

We have previously reported the treatment of five neonatal or infant dogs with a single vector infusion of a codon-optimized AAV5-B-domain deleted canine FVIII (cFVIII) construct with a hybrid liver promoter (AAV5-cFVIII). Dogs treated at 2 weeks of age demonstrated improved whole blood clot time (WBCT) over a 6-month period despite minimal cFVIII expression (<3%). Dogs treated at 2 months of age demonstrated stable cFVIII expression measured over a 6-month period and decreased WBCT.

Aims: To describe the early inflammatory response to AAV5-cFVIII in infant and neonatal dogs, and to provide an update on the safety and efficacy of AAV5-cFVIII in these animals 12-16 months post-treatment.

Methods: Hemophilia A dogs were treated with a single vector infusion (dose=2.0e14 vg/kg) of AAV5-cFVIII at 2 weeks (n=2) or 2 months (n=3) of age. Epigenomic data suggests that 2 months of age in dogs is equivalent to 9 months in humans. Liver volume was measured by magnetic resonance imaging at baseline, 3, 6, and 12 months post-AAV5-cFVIII treatment. Plasma cytokine levels were measured using a cytokine array pre-treatment (day 0) and post-treatment (days 2-28). Percutaneous liver biopsy samples were obtained at 2 weeks, 6 months, and 12 months post-treatment.

Results: Cytokine array analyses of samples demonstrated that one dog who received AAV5-cFVIII at 2 weeks experienced transient post-treatment plasma elevation of IL-6, KC-like, MCP-1, TGFβ-1, and TGFβ-2 that returned to baseline levels by day 18. Two dogs treated at 2 months experienced a transient increase in GMC-SF, IL-2, IL-6, IL-7, IL-15, and IL-18 that returned to baseline levels by day 21. No changes in levels of IFNγ, IL-8, IL-10, IP-10, TNFα, or TGFβ-3 were observed. Changes in plasma cytokine levels were not associated with an elevation in plasma alanine transaminase levels.

Hemophilia A dogs treated with AAV5-cFVIII at 2 weeks of age maintained an improved WBCT 12 to 16 months post-treatment, although cFVIII expression as measured by one-stage (OSA) and chromogenic substrate assays (CSA) remained minimal (<3%). Body weight and liver volume increased by 10.6-fold (measured at 16 months) and 12-fold (measured at 12 months) respectively.

Dogs treated with AAV5-cFVIII at 2 months of age demonstrated sustained cFVIII expression by OSA and CSA after 12 to 16 months (8.9-13.39% measured at 16 months by OSA), despite a 3.2-fold increase in body weight (measured at 16 months), and a 3.9-fold increase in liver volume (measured at 12 months). This was associated with improved WBCT observed over the 12 to 16-month period for all three dogs.

Overall, a reduction in bleeding events (BEs) was observed in all dogs. No BEs were recorded prior to AAV5-cFVIII treatment for dogs treated at 2 weeks of age. During the 16 months post-treatment, one dog experienced two spontaneous BEs, while no BEs were observed for the second dog. The dogs treated at 2 months experienced 4 (3 spontaneous and 1 traumatic) BEs

prior to AAV5-cFVIII treatment, and 1 traumatic BE 48 hours post-treatment prior to the detection of FVIII expression. For the remaining 16 months post-treatment, no BEs were observed in this group.

Conclusions: Treatment using AAV5-cFVIII at 2 months of age in a hemophilia A dog model resulted in stable FVIII expression into adult life despite significant liver expansion. For some dogs, AAV-cFVIII treatment resulted in an early transient increase in plasma proinflammatory cytokine levels with no evidence of transaminitis that resolved within three weeks.Further studies on liver biopsy samples are ongoing to evaluate cellular implications, vector genome distribution, and mechanisms of AAV persistence.

Disclosures Batty: *CSL Behring:* Consultancy, Honoraria; *Novo Nordisk:* Consultancy, Honoraria; *Pfizer:* Honoraria; *Institute for Nursing and Medication Education (IMNE):* Honoraria; *BioMarin Pharmaceutical:* Consultancy, Honoraria, Research Funding. **Menard:** *Inari Medical:* Consultancy. **Ismail:** *BioMarin Pharmaceutical:* Current Employment, Current equity holder in publicly-traded company. **Yates:** *BioMarin Pharmaceutical:* Current Employment. **Swystun:** *BioMarin Pharmaceutical:* Consultancy. **Fong:** *BioMarin Pharmaceutical:* Current Employment, Current equity holder in publicly-traded company. **Lillicrap:** *Novo Nordisk:* Consultancy, Honoraria; *Pfizer:* Consultancy, Honoraria; *Sanofi:* Consultancy, Honoraria, Research Funding; *CSL-Behring:* Consultancy, Honoraria, Research Funding; *BioMarin Pharmaceutical:* Consultancy, Honoraria, Research Funding.

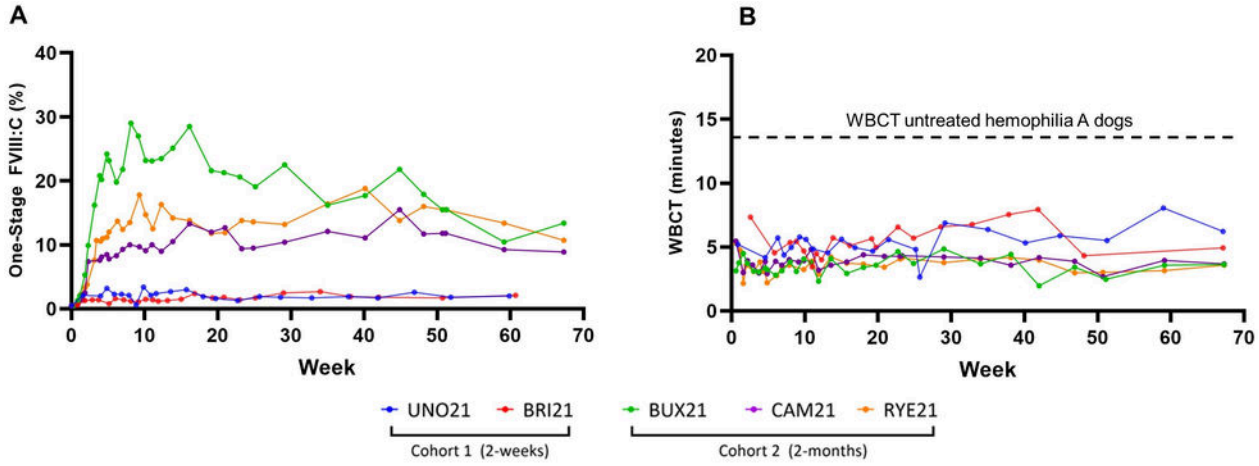

Figure 1: AAV5-cFVIII gene therapy after 16 months follow up in neonatal (cohort 1) or infant (cohort 2) treated severe hemophilia A dogs. (A) FVIII expression measured by one-stage assay (OSA). Lower limit of detection = 2%. (B) Whole blood clotting time (WBCT). WBCT for untreated hemophilia A adult dogs is approximately 13.9 minutes.

Figure 1

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