Durable CYP21A2 Gene Therapy in Non-Human Primates for Treatment of Congenital Adrenal Hyperplasia

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Abstract

Severe Congenital Adrenal Hyperplasia (CAH) is most commonly caused by genetic defects in CYP21A2 gene, which leads to a deficiency of the 21-hydroxylase enzyme and disruption in the biosynthesis of Adrenal corticosteroids. Despite treatment with corticosteroids, patients remain at significant risk for adrenal crisis, experiencing a 3-fold higher mortality rate than age matched controls. They also suffer from significant infertility, bone, metabolic, and cardiovascular disease, and hyperandrogenism in women leading to genital abnormalities, hirsutism, and other complications. We are developing an AAV5- based gene therapy (BBP-631) that will provide a functional copy of the CYP21A2 gene to the adrenal glands of CAH patients. To determine the durability of this therapy we treated cynomolgus monkeys with increasing doses of BBP-631 via intravenous injection. At 4-, 12- and 24-weeks post treatment, expression of hCYP21A2 mRNA and vector genome copies (VGC) in the adrenals and other peripheral tissues was measured. VGC was present in the liver and adrenals at 4 weeks, with durable detection through 24 weeks and total vg levels were dose dependent. hCYP21A2 RNA expression in adrenal and liver tissues was also dose dependent and continued to increase from 4 weeks through 12 weeks. There were no adverse safety signals in any of the treated animals. This data combined with efficacy data of BBP-631 in a Cyp21-/- mouse model supports our continued clinical development of BBP-631 as a treatment for congenital adrenal hyperplasia.

Study Design

Group	Test Article	Dose Level (vg/kg)	Necropsy 4 Weeks (Day 29) Male	4 Weeks (Day 29) Female	12 Weeks (Day 85) Male	12 Weeks (Day 85) Female	24 Weeks (Day 168) Male	24 Weeks (Day 168) Female
1	BBP-631	5E12	2	2	2	2	0	0
2	BBP-631	1.5E13	1	1	2	2	0	0
3	BBP-631	4.5E13	0	0	2	2	1	1

Selection Criteria

- Cynomolgus macaques >2.5 years old and 2-3 kg in weight
- AAV5 neutralizing antibody negative (1:5 dilution)

Measurements:

- Animals were routinely monitored for changes in hematology, clinical and urine chemistry
- Biodistribution of vector genomes and transgene measured by ddPCR and qRT-PCR
- Antibodies against AAV5 and hCYP21 in the serum by ELISA
- Immune response against AAV5 and hCYP21 in PBMCs
- Histopathology for immune infiltrates

Background

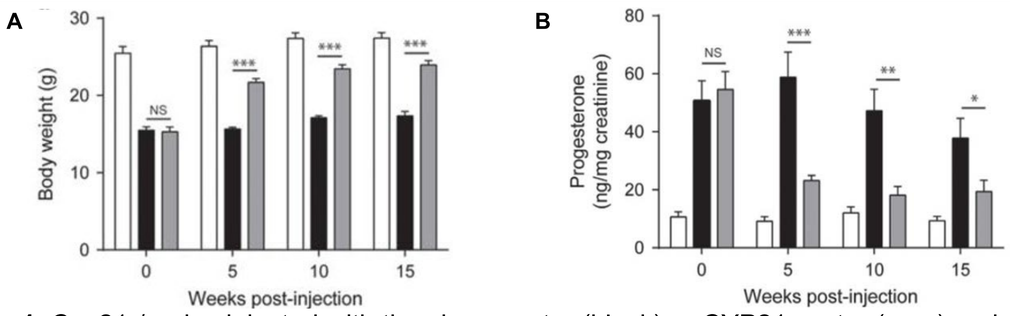

Figure 1. Cyp21-/- mice injected with the sham vector (black) or CYP21 vector (grey) and a control mouse (white). A) Mice injected with the CYP21 vector (grey) had a substantial recovery of body weight 5, 10 and 15 weeks post-injection, and B) a major decrease in urinary progesterone.

- **Congenital Adrenal Hyperplasia (CAH) encompasses a group of rare autosomal recessive disorders, it is most commonly caused by genetic defects in the CYP21A2 gene, leading to deficiency in 21-hydroxylase, a key enzyme for both cortisol and aldosterone synthesis.**
- **Cyp21 -/- mice are phenotypically frail with decreased body weight and elevated urinary progesterone compared to wild type mice (Figure 1).[1]**
- **AAV-based gene therapy to introduce a functional copy of human CYP21 in the Cyp21-/- mouse model led to phenotypic correction, specifically normalization of body weight (Figure 1A) and urinary progesterone levels (Figure 1B).[1]**

Results

BBP-631 has dose-dependent and persistent vector genomes in adrenal gland and liver tissue

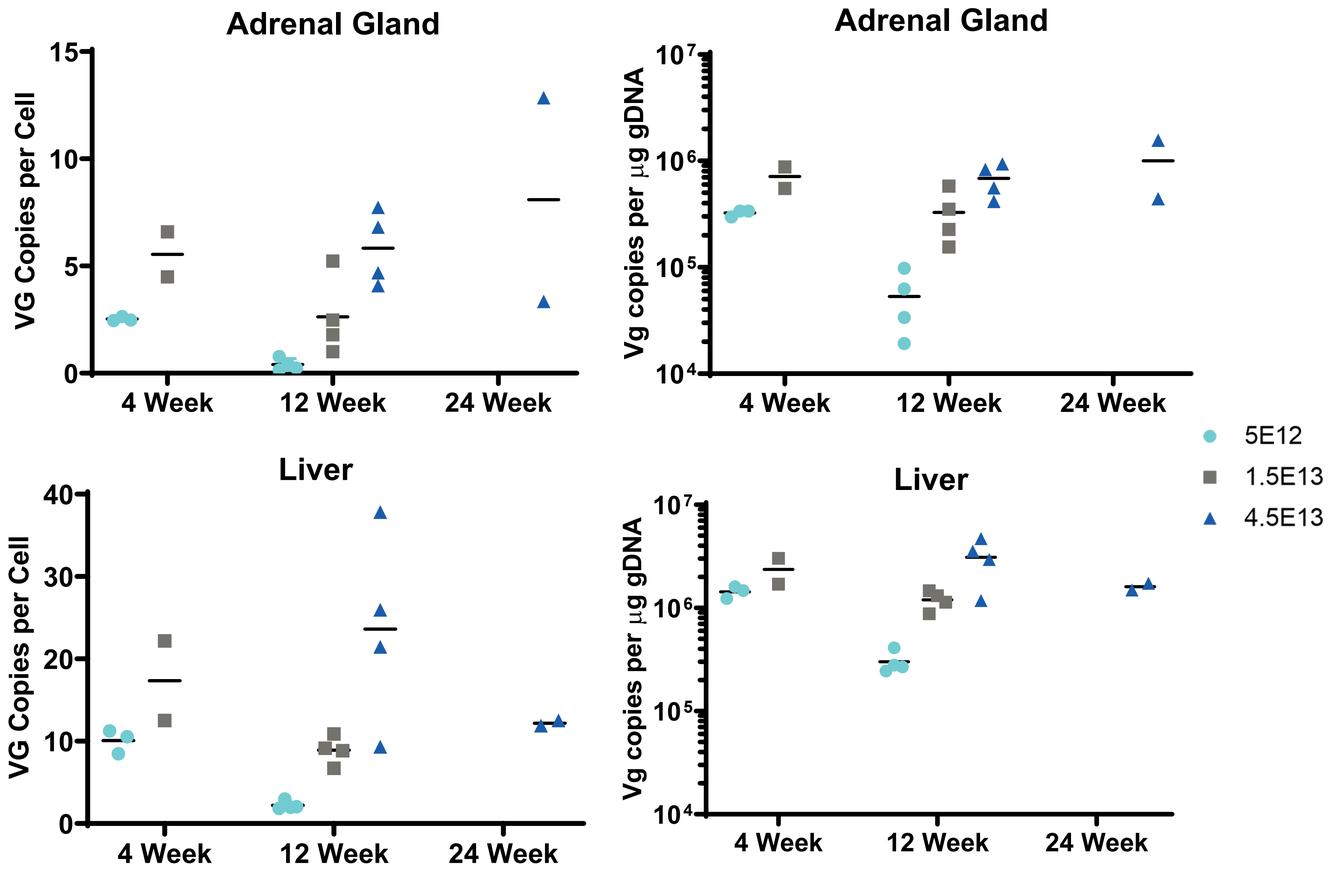

Figure 2. Vector genome copies (VGC) per µg DNA in adrenal gland and liver tissue at 4, 12 and 24 weeks post dosing, measured via ddPCR.

BBP-631 has durable and dose-dependent transgene RNA expression through 12 weeks

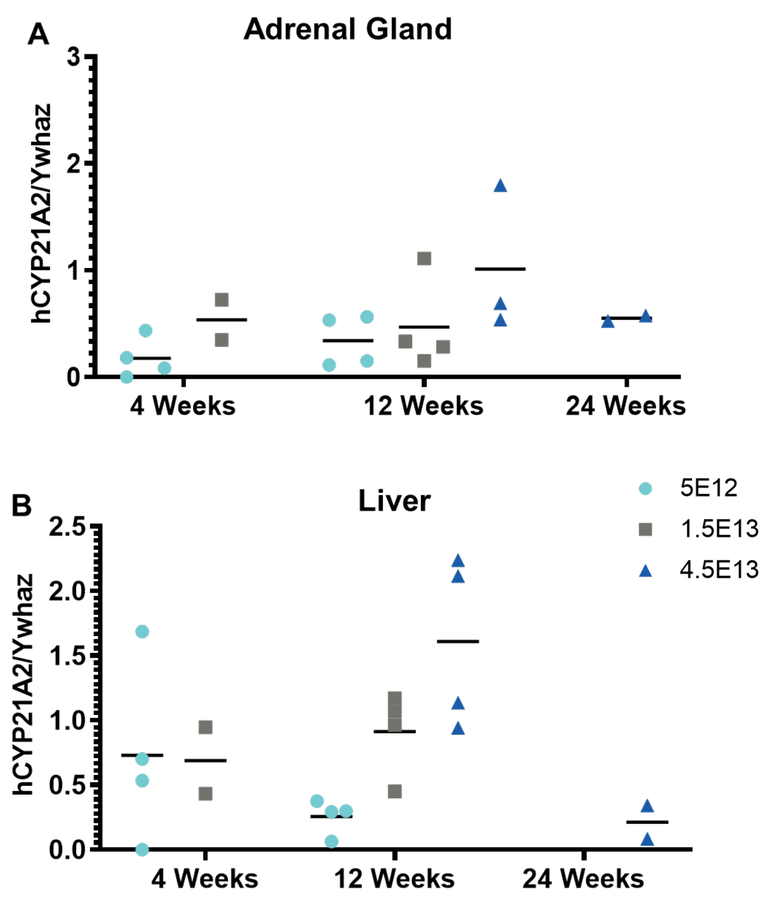

Figure 3. hCYP21 transgene RNA expression was measured in A) adrenal gland and B) liver via ddPCR and displayed as a ratio to the housekeeping gene Ywhaz.

BBP-631 does not impact clinical markers of hepatic, renal or hematologic health

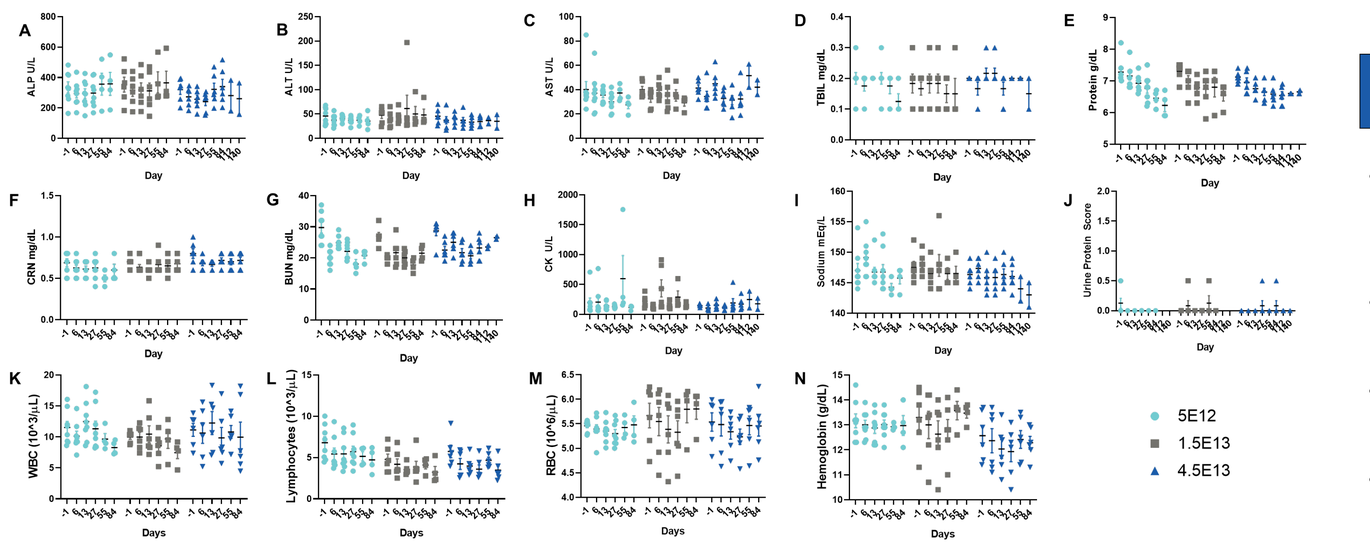

Figure 4. Non-human Primates receiving BBP-631 were monitored for A) alkaline phosphatase (ALP), B) alanine aminotransferase (ALT), C) aspartate aminotransferase (AST), D) total bilirubin (TBIL), E) Protein, F) creatine (CRN), G) urea (BUN), H) creatine kinase (CK), I) sodium, J) urine protein, K) white blood cells (WBC), L) lymphocytes, M) red blood cells (RBC) and N) hemoglobin.

Conclusion

- **No adverse safety signals in any of the treated animals via clinical chemistry, hematology, urinalysis or histopathology (data not shown).**
- **VGC had durable and dose-dependent detection through 24 weeks in the liver and adrenals.**
- **hCYP21A2 RNA expression increased through 12 weeks in a dose-dependent manner in adrenal and liver tissues.**
- **This work supports our continued clinical development of BBP-631 as a treatment for congenital adrenal hyperplasia.**

1 Perdomini M, Dos Santos C, Goumeaux C, et al. An AAVrh10-CAG-CYP21-HA vector allows persistent correction of 21-hydroxylase deficiency in a Cyp21-/- mouse model. Gene Ther. 2017 May;24(5):275-281