Predictable Protein Expression with Enhanced Factor IX Activity Following Administration of a Modified AAV5-hFIX Vector to Nonhuman Primates

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Introduction: Gene therapy for hemophilia B is advancing rapidly and offers the possibility to achieve sustained amelioration of the bleeding phenotype with a single treatment in patients with severe disease. The efficacy and safety of AMT-060, an investigational gene therapy construct comprising an adeno-associated virus (AAV) serotype 5 capsid containing a codon-optimized wildtype FIX gene under control of a liver-specific promoter, were extensively studied during a phase I/II clinical trial. Patients who received AMT-060 showed sustained and durable factor IX (FIX) activity of 3-13% and substantial reduction in spontaneous bleeding without evidence of T-cell mediated hepatotoxicity. The Padua variant has been previously identified as a hyperfunctional variant with an ~8-fold increased specific activity compared to wildtype FIX (Simioni *et al.* 2009). To evaluate levels of FIX activity, this gain-of-function substitution was introduced in the FIX gene portion of the AMT-060 cassette. The efficacy and safety of this variant was assessed in nonhuman primates (NHPs).

Methods: The novel vector is a modified version of AMT-060, an AAV5 vector with a gene cassette containing a liver specific (LP1) and a codon-optimized wildtype FIX gene that has previously been demonstrated to result in durable FIX expression of at least 4 years (Nathwani *et al.* 2014). This variant harbors the Padua substitution R338L in the FIX coding sequence of the gene cassette. Cynomolgus macaques (n=3 per group) received intravenous administrations of AMT-060 (5e12 gc/kg) or the Padua variant in a range of doses (5e11 to 9e13 gc/kg) or the vehicle control. Efficacy assessments included FIX activity and human FIX (hFIX) levels. Safety assessments included the measurement of various markers of coagulation and fibrinolytic activation.

Results: hFIX protein and increased FIX activity levels were achieved in all AAV-injected monkeys. Concentrations of hFIX protein were similar in the plasma of monkeys that received either of the agents, however, as expected, the FIX activity was generally 6-fold higher in the animals that received the Padua variant. There was no significant difference in the levels of hemostatic markers between the groups that received either of the agents, suggesting no increased risk of thrombosis in animals expressing the FIX Padua variant.

Conclusion: Implementation of the Padua R338L substitution in the gene therapy candidate AMT-060 resulted in comparable protein expression with increased specific activity and a favorable safety profile in nonhuman primates. These results suggest that transgene sequences may be optimized while retaining the expression level and safety profile associated with a given vector.

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