[00179] Figure 55 shows the Factor VIII variant amino acid sequence (SEQ ID NO: 106) encoded by the CS01m12 codon-altered nucleotide sequence in accordance with some embodiments ("CS01m12-FL-AA").

[00180] Figure 56 shows the Factor VIII variant amino acid sequence (SEQ ID NO: 107) encoded by the CS04m12 codon-altered nucleotide sequence in accordance with some embodiments ("CS04m12-FL-AA").

[00181] Figures 57A and 57B show the CS01m12 codon-altered nucleotide sequence (SEQ ID NO: 108) encoding a Factor VIII variant with m1 (F328S) and m2 amino acid substitutions in accordance with some embodiments ("CS01-FL-NAm12").

[00182] Figures 58A and 58B show the CS04m12 codon-altered nucleotide sequence (SEQ ID NO: 109) encoding a Factor VIII variant with m1 (F328S) and m2 amino acid substitutions in accordance with some embodiments ("CS04-FL-NAm12").

DETAILED DESCRIPTION OF DISCLOSURE

I. Introduction

[00183] AAV-based gene therapy holds great promise for the treatment of hemophiliacs. For hemophilia B, first clinical data are encouraging in that FIX levels of about 10% can be maintained in at least some patients for more than 1 year. For hemophilia A however, achieving therapeutic expression levels of 5-10% with AAV vectors remains challenging for various reasons. First, the Factor VIII coding sequence is too large for conventional AAV-based vectors. Second, engineered B-domain deleted or truncated Factor VIII constructs suffer from poor expression in vivo, even when codon-optimized. Third, these B-domain deleted or truncated Factor VIII variant constructs have short half-lives in vivo, exacerbating the effects of poor expression. Fourth, even when expressed, FVIII is not efficiently secreted from cells, as are other coagulation factors, such as Factor IX.

[00184] Moreover, these challenges cannot be addressed by simply administering higher doses of the gene therapy construct. According to current knowledge, the vector dose of an AAV-based gene therapy vector should be increased above $2x10^{12}$ vg/kg bodyweight. This is