

## **Clinical Pharmacology BLA Review**

Division of Clinical Evaluation and Pharmacology/Toxicology (DCEPT)

Office of Tissues and Advanced Therapy (OTAT)

**Submission Number:** 125772.00

**Product Name:** Recombinant adeno-associated viral vector serotype 5 (rAAV5) vector containing a codon-optimized version of the naturally occurring Padua variant of the FIX gene (hFIXco-Padua); [HEMGENIX]

**Proposed Indication:** Treatment of adults with hemophilia B (congenital Factor IX deficiency) (b) (4)

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**Date Submitted:** March 24, 2022

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## 1. Executive Summary

HEMGENIX is a gene therapy product that consists of a codon-optimized coding DNA sequence of the gain-of-function Padua variant of human Factor IX (FIX), under control of a liver-specific LP1 promoter and encapsulated in a non-replicating recombinant adeno-associated viral vector of serotype 5 (AAV5). In this BLA submission, the applicant proposes HEMGENIX (etranacogene dezaparvovec-frdm) for treatment of adult patients with hemophilia B with a (b) (4)

[REDACTED]

[REDACTED]

[REDACTED]

The clinical pharmacology of HEMGENIX is supported by two clinical studies employing the Padua variant of a codon optimized human Factor IX gene (Study #CT-AMT-061-01 and Study #CT-AMT-061-02). The supporting clinical data from the predecessor product that used similar vector (AAV5) with HEMGENIX was used to inform dosing for later clinical studies, and viral kinetics assessments of the vector.

From clinical pharmacology perspective the viral kinetics, FIX protein expression and FIX activity support the proposed single dose of  $2 \times 10^{13}$  gc/kg of HEMGENIX. Limited data exist for subjects with NAb titers >1:350 and a trend in decreased FIX activity with higher pre-existing anti-AAV5 NAb titers should be incorporated in deciding threshold for NAb titers. The final clinical pharmacology decision on target threshold of NAb titers depend on (b) (4)

[REDACTED]

## 2. Recommendations

The clinical pharmacology information in this BLA is acceptable. The applicant adequately addressed clinical pharmacology labelling comments.

### 3. Background

Hemophilia B is an X-chromosome-linked inherited bleeding disorder of a coagulation factor deficiency resulting from reduced levels or absence of factor IX (FIX). The disorder affects approximately one in 30 000 males worldwide. Traditionally, a residual baseline FIX level is used to classify patients as having severe (< 1 IU/dL), moderate (1–5 IU/dL) or mild (5–40 IU/dL) hemophilia B. Currently, there is no available cure for hemophilia B, and treatment focuses on prophylactic intravenous (IV) infusion of either plasma-derived or recombinant FIX products. A major limitation of these conventional FIX therapies is the short half-life (18 to 22 hours), which requires every 3-4 day infusions. This short half-life of conventional FIX products is in part resolved by development of newer FIX therapies with extended half-life (86-104 hours) that allow a longer dosing interval of every 7-10 days.

In this BLA submission, the applicant proposes HEMGENIX (etranacogene dezaparvovec) for the treatment of adult patients with hemophilia B to reduce the frequency of bleeding episodes (b) (4)

HEMGENIX is a gene therapy product that consists of a codon-optimized coding DNA sequence of the gain-of-function Padua variant of human FIX, under control of a liver-specific LP1 promoter and encapsulated in a non-replicating recombinant adeno-associated viral vector of serotype 5 (AAV5). Following single intravenous administration, HEMGENIX preferentially targets liver cells for transduction and results in expression of Factor IX-Padua protein.

This BLA submission is supported by the results of three clinical studies:

- **Study 1** (#CT-AMT-060-01): A phase I/II, open-label, uncontrolled, single-dose, dose-ascending, multi-center trial investigating an adeno-associated viral vector containing a codon-optimized human Factor IX gene (AAV5-hFIX) administered to adult patients with severe or moderately severe haemophilia B.
- **Study 2** (#CT-AMT-061-01): Phase IIb, open-label, single-dose, single-arm, multi-center trial to confirm the factor IX activity level of the serotype 5 adeno-associated viral vector containing the Padua variant of a codon-optimized human factor IX

gene (AAV5-hFIXco-Padua, AMT-061) administered to adult subjects with severe or moderately severe hemophilia B.

- **Study 3** (#CT-AMT-061-02): Phase III, open-label, single-dose, multi-center multinational trial investigating a serotype 5 adeno-associated viral vector containing the Padua variant of a codon optimized human Factor IX gene (AAV5-hFIXco-Padua, AMT-061) administered to adult subjects with severe or moderately severe hemophilia B.

#### 4. Summary of Clinical Pharmacology Findings

The major clinical pharmacology findings are summarized based on the two relevant clinical studies employing the Padua variant of a codon optimized human Factor IX gene (AAV5-hFIXco-Padua, AMT-061). The first-in-human (FIH) study (#CT-AMT-060-01) was based on wild-type hFIXco without the gain-of-function Padua variant which was considered the predecessor product (AMT-060). From a clinical pharmacology perspective, the data from AMT-060 were reviewed to inform dose selection and viral vector DNA kinetics.

#### Vector DNA Biodistribution and Shedding

**Study #CT-AMT-060-01 (N=10 subjects):** Two dose levels (low:  $5 \times 10^{12}$  gc/kg and high:  $2 \times 10^{13}$  gc/kg) were explored in 10 subjects with hemophilia B.

- During the 5 year follow-up period, negative shedding (i.e., three subsequent measurements below limit of quantification of vector DNA) was achieved from blood, saliva, nasal secretions, urine, and feces in 10 subjects.
- Shedding from semen was achieved in 9/10 subjects. One subject was unable to produce semen due to a historical medical condition and, therefore, shedding from semen could not be assessed.
- The mean time to reach a negative shedding was longest in blood which was 509 days (low dose group) and 705 days (high dose group).

- The vector clearance from semen was the second longest and it lasts up to 228 days (low dose group) and 157 days (high dose group).
- The highest observed blood concentration (C<sub>max</sub>) was 6.7x10<sup>8</sup> copies/mL (low dose cohort) and 4x10<sup>9</sup> copies/mL (high dose cohort) that occurred within median T<sub>max</sub> of 24 hours following administration of AMT-060.
- The lowest mean quantifiable viral blood concentration (C<sub>last</sub>) was 1411 copies/mL (low dose) and 1108 copies/mL (high dose).

**Study #CT-AMT-061-01 (N=3 subjects): Dose= 2 × 10<sup>13</sup> gc/kg**

- Two subjects (67%) no longer shed vector DNA at a mean of 183 days for semen samples and at 218 days for blood samples.
- One subject had positive test results at all post-dose visits during the 2.5 year follow-up period.
- The C<sub>max</sub> in blood was 2.7x10<sup>10</sup> copies/mL that occurred within a median T<sub>max</sub> of 4 hours following administration of AMT-06.

**Study #CT-AMT-061-02 (N=54 subjects): Dose=2 × 10<sup>13</sup> gc/kg**

- The T<sub>max</sub> for vector DNA in blood was observed at 4 hours with mean C<sub>max</sub> of 2.2x10<sup>10</sup> copies/mL and C<sub>last</sub> of 1293 copies/mL.
- Clearance of vector DNA from blood (i.e., absence of shedding) was confirmed in 30 out of 54 subjects (56%) at 2-years post-treatment.
- T<sub>max</sub> for vector DNA in semen was observed between Weeks 5 to 27 with C<sub>max</sub> of 3.9 x 10<sup>5</sup> copies/mL.
- Clearance of vector DNA from semen was achieved in 37 subjects (69%) at 2.5 years.

**FIX Activity and Protein Expression:**

The one-stage activated partial thromboplastin time (aPTT-based) assay is used as a primary assay for PK assessment of FIX activity. The FIX activity measured by



chromogenic assay was consistently lower by ~2-fold than that measured by one-stage assay across all the clinical studies. The FIX antigen (b) (4) kit is used for quantitative determination of FIX protein concentration and results are reported as % FIX protein of normal human levels.

**Study #CT-AMT-061-01:**

- At 12 months following AMT-061 administration the mean uncontaminated FIX activity level was  $40.8 \pm 9.45\%$  of normal measured by the one-stage assay and all three subjects achieved a FIX activity  $> 30\%$ .
- The mean FIX protein expression ranged from 3.6 to 4.2% and the ratio of FIX activity to protein ranged from 5.1 (Week 3) to 11.9 (Month 30).

**Study #CT-AMT-061-02:**

- At 6 and 12 months post-AMT-061 treatment, the mean FIX activity was  $38.9 \pm 18.7\%$  (range: 8.2 to 97.1%) and  $41.5 \pm 21.7\%$  (range: 5.9 to 113.0%), respectively. At Month 18, the mean FIX activity was  $36.9 \pm 21.4\%$  (range: 4.5 to 122.9%).
- A higher variability was observed in FIX protein concentration as compared to FIX activity. The mean FIX protein levels ranged from 19% to 24% across visits with a high standard deviation (38%).

**Effect of Intrinsic and Extrinsic Factors on FIX Activity (Study #CT-AMT-061-02):**

Although limited sample size and confounding factors preclude definitive conclusions, the following are major summary of the effects of intrinsic and extrinsic factors on FIX activity:

- A trend of higher mean FIX activity was observed with increased age. The mean FIX activity levels were 1.5 to 2-fold lower in the  $< 40$  years subgroup compared to  $\geq 60$  years of age at the evaluated time points. It should be noted that all treated subjects were adults ( $41 \pm 16$  years of age).

- The FIX activity increased by 32 % in overweight (BMI 25-29, n=29 subjects) and by 49 % in obese (BMI  $\geq$  30, n=10 subjects) as compared to normal BMI (<25, n=11 subjects).
- Subjects with mild renal impairment (N = 7/53) had higher mean FIX activity (up to 37% higher) compared to those with normal renal function during Month 6 to 18 post-dose period. However, confounding factors such as age and BMI should be considered in interpreting the effects of mild renal impairment. For example, 5 out of 7 subjects with mild renal impairment are  $\geq$  60 years of age and 6 out of 7 subjects have BMI  $\geq$  25 kg/m<sup>2</sup>.
- A trend for lower FIX activity with liver impairment (about 31% lower FIX activity) was observed.
- Based on the applicant's assessment of ALT elevation, subjects with ALT elevation (13 out of 53; 24%) had approximately 44% lower mean FIX activity at Month 18 compared to those that did not have ALT elevation.
- The 9/53 subjects (17%) that were treated with corticosteroid for ALT elevations exhibited approximately 63% lower mean FIX activity at Month 18 compared to those who did not receive corticosteroid coadministration.

## Immunogenicity Risk Assessments

### 1. Anti-AAV5 Neutralizing Antibodies:

- In Study #CT-MT-061- 01, all 3 subjects had anti-AAV5 NAbs before dosing. However, the NAbs titer level was below 50 and all the three subjects achieved FIX activity >30%.
- In Study #CT-MT-061- 02, 38.9 % (21/54 subjects) had anti-AAV5 NAbs before dosing with a median titer of 1:57 (range: 1:9 to 1:3,212).
- After HEMGENIX administration, all subjects in both studies developed detectable anti-AAV5 NAbs within 2-3 weeks.
- The mean FIX activity at Month 12 was  $42 \pm 22$  % in subjects with NAbs titer  $\leq$  1:100 (n=45) and FIX activity was  $36 \pm 17$ % in subjects with NAbs titer >1:100 to < 1:700 (n=5). The mean FIX activity at Month 12 was  $42 \pm 22$ % in subjects with

NAbs titer  $\leq$  1:350 (n=47) and  $27 \pm 17\%$  in subjects with NAbs titer  $>1:350$  to  $<1:700$  (n=3). It should be noted that only one subject had a titer  $> 700$  and the uncontaminated FIX activity for this subject was 1.5%.

- Overall, only limited data (n=3) is available with a target threshold  $> 1:350$  NAbs and the Month 12 FIX activity is lower by 36% in subjects with NAbs titer  $>1:350$ . It should also be noted that final clinical pharmacology decision on target NAbs level depends on the assay performance (b) (4). Currently, there is no validated neutralizing anti-AAV5 antibody assay and the titer level determined by the clinical trial assay may not be reliable.

## **2. AAV5 Capsid-specific T-cell Response:**

- In Study # CT-AMT-061-02, the majority (88.9%) of subjects with interpretable results did not have specific AAV5 capsid T-cell response prior to dosing (at the baseline assessment).
- The number of subjects who developed a specific AAV5 capsid T-cell response varied during the study, reaching the highest number at Week 6 following AMT-061 treatment (15/38 [39.5%] subjects).
- Specific T-cell response was observed in 39/54 subjects (72%) in at least one follow-up visit. Uncontaminated FIX activity ranged between 6.0 and 97% of normal for visits where subjects also had a specific T-cell response.
- Overall, it is difficult to interpret the results of the current cellular immune response against AAV5 capsid due to high variability and missing data, and no conclusions can be reached.

## **Dose-Response, Exposure-Response and Durability Assessments**

- In the Study #CT-AMT-060-01, two dose levels (low:  $5 \times 10^{12}$  gc/kg and high:  $2 \times 10^{13}$  gc/kg) were explored in 10 patients with hemophilia B. Both dose levels were found to be safe, and the higher dose of  $2 \times 10^{13}$  gc/kg was selected for subsequent clinical development.

- Exploratory viral vector dose versus exposure (C<sub>max</sub>) analysis showed a trend of increasing C<sub>max</sub> with total dose; however, the narrow range in the total dose and inter-individual variability in C<sub>max</sub> did not allow for establishing a clear relationship of dose versus vector DNA kinetic parameter, C<sub>max</sub>.
- Exploratory evaluation between vector DNA kinetic parameters in blood and visit-matched FIX activity did not reveal a significant correlation.
- The relationship between estimated ABR and mean FIX activity across the Month 7 to 18 post-treatment period was explored with a generalized additive model assuming negative binomial distribution of the bleeding episode count. Based on the limited data, no clear relationship was established between FIX activity and ABR presumably due to the observed higher FIX activity (> 12%) in over 90% of the subjects and low frequency of bleeding events.
- Durability analysis was conducted using a linear mixed-effects repeated model for change from baseline and percent change from baseline of FIX activity or protein concentration over time using Month 6 post-dose data as the reference baseline level. The Month 6 post-dose level is selected as a reference since stable level of FIX expression and activity is achieved within 3-6 months post AMT-061 administration. It should be noted that the main efficacy period for ABR was Months 7 to 18 after HEMGENIX treatment.
- Durability analysis of FIX activity and FIX protein concentration showed that FIX levels were stable up to 18 months with no statistically significant difference in the least square mean FIX levels at Months 7-18 compared to values obtained at Month 6. It should be noted that these durability analyses cannot be extrapolated outside of the current evaluation period i.e., 18 months following treatment with AMT-061.

## 5. Clinical Pharmacology Labeling Comments

The following are summary of clinical pharmacology labeling comments that were communicated to the Applicant. The updated labeling is acceptable from the clinical pharmacology perspective.

## **Section 5: WARNINGS AND PRECAUTIONS**

### **5.3 Immune-mediated neutralization of the AAV5 vector capsid**

- Requested to include statements about lack of validated assay for quantitative determination of NAbs titer.
- Requested to Include statement about development of NAbs after treatment with HEMGENIX.

## **Section 8: USE IN SPECIFIC POPULATIONS**

**Requested to update information about hepatic and renal impairment based on the limited clinical data.**

## **Section 12: CLINICAL PHARMACOLOGY**

### **12.1 Mechanism of Action**

- Recommended revision to provide more specific information based on mechanistic data derived from relevant nonclinical and clinical studies.

### **12.2. Pharmacodynamics**

- Recommended to include FIX activity as part of section 12.2.
- Recommended update on FIX activity reflecting results obtained from Phase 3 study with inclusion of descriptive statistics.
- Recommended revision of statements about pharmacodynamics in specific population.

### **12.3. Pharmacokinetics**

- Recommended to include biodistribution and shedding data for the predecessor product.
- Recommend revision of vector biodistribution and viral shedding with inclusion of results obtained during the 120 safety update.

### **12.6. Immunogenicity**

- Recommended to include information about lack of data to support re-administration of HEMGENIX in the presence of high NAb
- Included statement about lack of validated assay for NAb.

## 6. Comprehensive Clinical Pharmacology Review

### 6.1. General Pharmacology and Pharmacokinetics

HEMGENIX is a gene therapy consists of a codon-optimized coding DNA sequence of the gain-of-function Padua variant of human Factor IX (FIX), under control of a liver-specific promoter (LP1), encapsulated in a non-replicating recombinant adeno-associated viral vector of serotype 5 (rAAV5).

HEMGENIX is intended as a one-time intravenous (IV) infusion in patient with hemophilia B expected to result in secretion of functional human FIX and reduction of bleeding episodes.

The FIH dose exploration study (Phase 1/2) was conducted using the predecessor product AMT-060 that employed the wild-type hFIXco without the gain-of-function Padua variant. In this Phase 1/2 study, two dose levels (low:  $5 \times 10^{12}$  gc/kg and high:  $2 \times 10^{13}$  gc/kg) were explored in 10 patients with hemophilia B patients. The mean FIX activity ranged from 2.8% to 8.2% (low dose) and 4.0% to 10.7% (high dose). Both dose levels were found to be safe and the higher dose of  $2 \times 10^{13}$  gc/kg was selected for subsequent clinical development (Phase 2b & 3) based on the trend in increased FIX activity. However, the small increase in FIX activity is confounded by baseline endogenous secretion of FIX and potential contamination by exogenous treatment with FIX replacement. In subsequent clinical studies (Phase 2b and 3), the wild-type hFIXco in AMT-060 is replaced with the gain-of-function Padua variant resulting in a different PK profile of transgene expression and activity. Thus, the results of the FIX expression, FIX activity and immunogenicity data from the Phase 1 study were not included as part of the clinical pharmacology assessment. The Phase 1 results are useful to provide supportive

viral vector kinetic information up to 5 years since the viral vector (i.e., rAAV5) remains the same throughout the development of HEMGENIX.

### **Viral Vector DNA Biodistribution and Shedding:**

Vector DNA in biological matrices was measured using a (b) (4) -based assay with limit of detection (LOD) of 760 copies/mL. The vector DNA kinetic profile in blood exhibited a rapid distribution phase followed by slow elimination and a higher inter-individual variability.

In Study #CT-AMT-060-01, viral vector DNA kinetics and shedding were evaluated in blood, saliva, nasal secretion, urine, and feces. All 10 subjects stopped shedding vector DNA (i.e., negative shedding defined as three subsequent measurements below limit of quantification), from blood, saliva, nasal secretions, urine, and feces during the 5 year follow-up period. Negative shedding from semen was achieved in 9/10 subjects. One subject was unable to produce semen due to a historical medical condition and, therefore, shedding from semen could not be assessed. The time to reach a negative shedding result was longest in the blood, with mean time to reach a negative result of 509 days in the low dose group and 705 days in the high dose group. The vector clearance from semen was the second longest and it lasted up to 228 days in the low dose group and 157 days in the high dose group. The highest observed blood concentration (C<sub>max</sub>) was  $6.7 \times 10^8$  copies/mL in the low dose cohort and  $4 \times 10^9$  copies/mL in the high dose cohort that occurred within a median T<sub>max</sub> of 24 hours following administration of AMT-060. The lowest mean quantifiable viral blood concentration (C<sub>last</sub>) was 1411 copies/mL (low dose) and 1108 copies/mL (high dose).

In Study #CT-AMT-061-01, vector DNA levels were determined in blood and semen samples. Two subjects no longer shed vector DNA at a mean of 183 days for semen samples and at 218 days for blood samples. One subject had positive test results at all post-dose visits during the 2.5 years follow-up period. The C<sub>max</sub> in blood was  $2.7 \times 10^{10}$  copies/mL that occurred within median T<sub>max</sub> of 4 hours following administration of AMT-061 and the C<sub>last</sub> was 2232 copies/mL.

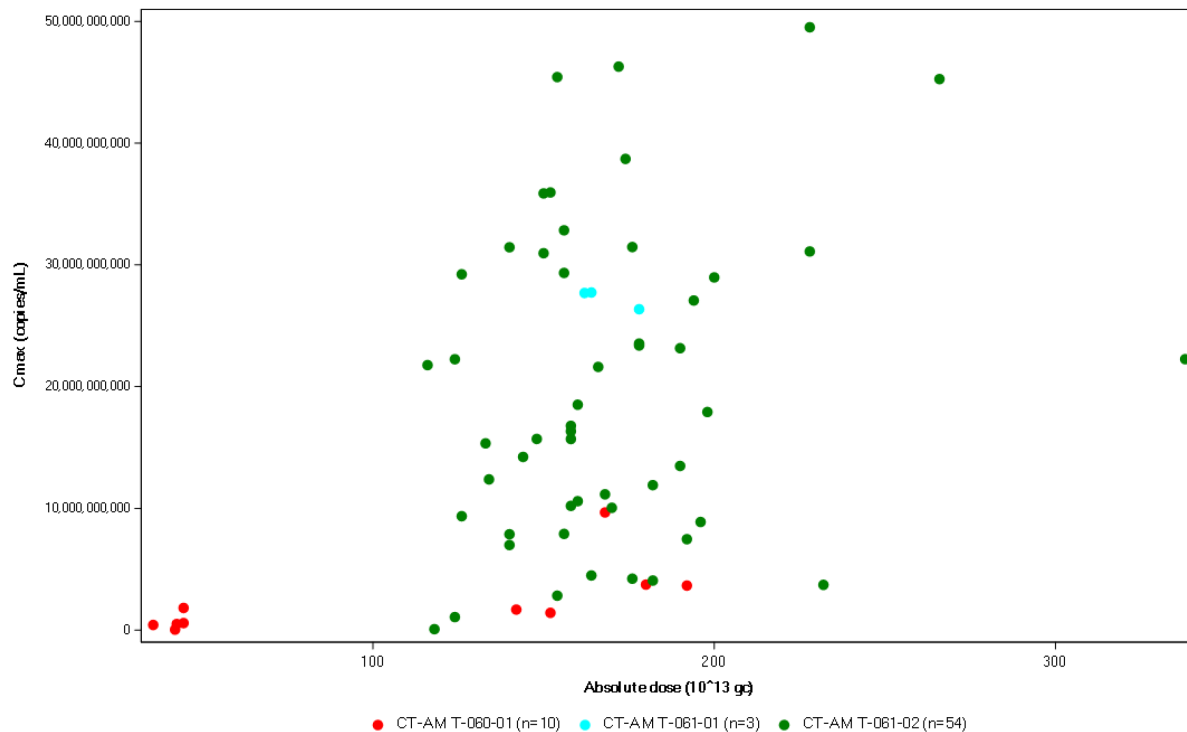
In Study CT-AMT-061-02, the T<sub>max</sub> for vector DNA in blood was observed at 4 hours with mean C<sub>max</sub> of  $2.2 \times 10^{10}$  copies/mL and C<sub>last</sub> of 1293 copies/mL. Clearance of vector DNA from blood (i.e., absence of shedding) was confirmed in 30/54 subjects (56%) at 2-years post-treatment with AMT-061 and median time to absence of shedding from blood was 52.3 weeks. The T<sub>max</sub> for vector DNA in semen was observed between Weeks 5 to 27 with C<sub>max</sub> of  $3.9 \times 10^5$ . Clearance of vector DNA from semen was confirmed in 37/54 subjects (69%) at 2.5-years, and the median time to vector clearance from semen was 47.3 weeks.

Overall, vector kinetic parameters were comparable following HEMGENIX administration for the two studies (CT-AMT-061-01 and CT-AMT-061-02). However, the mean C<sub>max</sub> in semen was about 7-fold higher in Study CT-AMT-061-01 compared to that in Study CT-AMT-061-02 which was attributed to one subject with very high values in CT-AMT-061-01 study and the overall higher inter-individual variability observed with blood and semen viral DNA kinetic data. The mean vector DNA C<sub>max</sub> in blood following administration of  $2 \times 10^{13}$  gc/kg in Study #CT-AMT-060-01 was approximately 5-fold lower compared to that observed with Study #CT-AMT-061-02 while mean vector DNA C<sub>last</sub> in blood was comparable. Typically, the first sampling time point in blood corresponds to T<sub>max</sub> for vector DNA in blood following IV administration and this difference in C<sub>max</sub> can be due to the difference in first sampling time (i.e., 24 hours for #CT-AMT-060-01 and 1 hour for #CT-AMT-061-02).

Exploratory viral vector dose versus exposure (C<sub>max</sub>) analysis was conducted using combined data from the three clinical studies (Figure 1). For HEMGENIX, generally a trend of increasing C<sub>max</sub> with total dose was observed; however, the narrow range in the total absolute dose and inter-individual variability did not allow to establish a clear relationship between dose- vector DNA shedding parameters. For Study #CT-AMT-061-02, the administered doses ranged from 116 -  $338 \times 10^{13}$  gc (~3-fold range) and a slight trend of increasing C<sub>max</sub> in blood with increasing total dose was observed. However, the inter-individual variability in C<sub>max</sub> was over 10-fold, suggesting that other in vivo disposition characteristics of the viral vector may contribute to variability in vector DNA kinetics.



**Figure 1: Scatter Plot of Vector DNA Cmax (copies/mL) in Blood vs. Total Administered Dose (gc)**



Source: clinical-info-amend; Figure 2

## 6.2. Pharmacodynamic Assessments

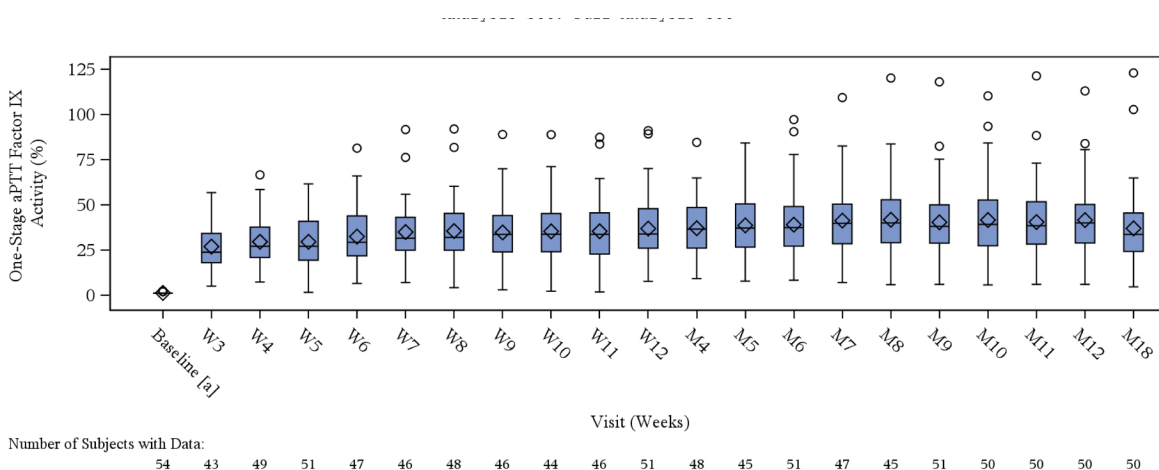
### FIX Activity and Protein Expression:

The one-stage activated partial thromboplastin time (aPTT-based) assay was used as a primary assay for pharmacodynamic (PD) assessment of FIX protein expression and FIX activity. FIX activity values that were measured more than 5 half-lives after most recent FIX-replacement administration is used to support efficacy evaluation and referred as “uncontaminated FIX levels”. The FIX activity measured by chromogenic assay was consistently lower by ~2-fold than those measured by one-stage (aPTT-based) assay across all the clinical studies.

In Study #CT-AMT-061-01, at Week 52 following AMT-061 administration the mean uncontaminated FIX activity level was  $40.8 \pm 9.45\%$  of normal measured by the one-stage assay and all the three subjects achieved a FIX activity  $> 30\%$ . The FIX protein expression was detected within one week following administration of AMT-061. The mean FIX protein expression ranged from 3.6 to 4.2% and the ratio of FIX activity to protein ranged from 5.1 (Week 3) to 11.9 (Month 30).

In Study #CT-AMT-061-02, at 6 months post-AMT-061 treatment, the mean FIX activity was  $38.95 \pm 18.72\%$  (range: 8.2 to 97.1%). The FIX activity was maintained through Month 12, with a mean FIX activity of  $41.48 \pm 21.71\%$  (range: 5.9 to 113.0%). At Month 18, the mean FIX activity was  $36.90 \pm 21.40\%$  (range: 4.5 to 122.9%; Figure 2). The time to onset of FIX protein expression occurred at Week 3 as evaluated by first uncontaminated measurement. FIX protein levels during the post-treatment period followed a similar trend as FIX activity by one-stage (aPTT-based) activity; however, more variability was observed in the protein concentrations. The mean FIX protein levels ranged from 19.3% to 23.6% across visits with high standard deviation ( $\pm 38\%$ ). The mean ratio of uncontaminated FIX activity to protein concentration was 5.9 % at Week 3 and remain stable at approximately 7 to 8.5% between Month 6 and Month 18.

**Figure 2: Box Plot of One-Stage aPTT Uncontaminated Factor IX Activity (%) Over Time During the Post-Treatment Period**



[a] Baseline factor IX is imputed based on subject's historical hemophilia B severity documented. Source: efficacy-data-tables-and-figures; Figure 2.2.1.9

### **Effect of Intrinsic and Extrinsic Factors on FIX Activity:**

The effect of intrinsic and extrinsic factors on uncontaminated FIX activity was evaluated in the PK population (defined as subjects receiving a full dose of AMT-061 and have at least 1 post-dose FIX activity measurement in the Study CT-AMT-061-02).

A trend of significantly higher mean FIX activity was observed with an increase in age. It should be noted that all subjects were adult ( $41 \pm 16$  years of age), and 58% of subjects (31/53) were below 40 years of age. The mean FIX activity levels were 1.5 to 2-fold lower in the < 40 years subgroup compared to  $\geq 60$  years of age at the evaluated time points. However, confounding factors (e.g., corticosteroids treatment, renal impairment) and limited sample size preclude a meaningful interpretation of the age effect on FIX activity following treatment with AMT-061.

The FIX activity increased by 32 % in overweight subjects (body mass index, BMI= 25-29) and by 49 % in obese (BMI  $\geq 30$ ) as compared to normal BMI (<25). This increased FIX activity with increased BMI can be in part explained by the body weight-based dosing and the corresponding increase in administered total dose. There is a trend for increase in FIX activity with increasing bodyweight or total dose administered. However, these relationships are not statistically significant presumably due to limited sample size, narrow bodyweight, and dose range ( $1.16\text{-}3.38 \times 10^{15}$  gc).

Subjects with mild renal impairment (N = 7/53) had higher mean FIX activity (up to 37% higher) compared to those with normal renal function during Months 6 to 18 post-dose. One subject with moderate renal impairment in the study had similar FIX activity as subjects with normal renal function. There is no mechanistic explanation for this increase in FIX activity with mild renal impairment since FIX is a macromolecule that is not expected to be excreted by renal route. However, confounding factors such as age and BMI should be considered in interpreting the effects of mild renal impairment. For example, 5 out of 7 subjects with mild renal impairment are  $\geq 60$  years of age and 6 out of 7 subjects have BMI  $\geq 25$  kg/m<sup>2</sup>. As described above there is a trend for increase in FIX activity with increasing age and BMI that makes it difficult to determine the impact of

renal impairment independent of age and BMI. Considering the limited information on mild/moderate renal impairment group, confounding factors, and no subjects with severe or end stage renal disease are included in the study, the effect of renal impairment could not be fully evaluated.

Based on the limited clinical data, there is a trend for lower FIX activity with moderate to severe liver impairment as evaluated based on baseline (b) (4) or equivalent (b) (4). Subjects were considered to have moderate to severe liver impairment with CAP score  $\geq S2$  [ $\geq 260$  dB/m] and mild to no impairment with CAP score  $< S2$  [ $< 260$  dB/m]. The 18 months mean FIX activity is  $29 \pm 14\%$  (n=11 subjects with  $\geq S2$ ) and  $42 \pm 26\%$  (n=28 subjects with  $< S2$ ), respectively. This observed ~31% reduction in mean FIX activity in moderate to severe hepatic impairment is mechanistically plausible since AMT-061 is a liver-targeted gene therapy and transgene expression is expected to depend on status of the liver. Since the data from the current study are limited, future assessment of liver impairment on the impact of FIX expression and activity is recommended.

Thirteen out of 53 subjects (24%) who received a full dose of HEMGENIX experienced alanine aminotransferase (ALT) elevation (ALT > upper limit of normal [ULN] when the baseline ALT is below ULN, or ALT > 2  $\times$  baseline value, occurring over the initial 90 days postdose). Nine subjects were treated with corticosteroids for ALT elevation of either > ULN (n = 8) or > 2  $\times$  baseline value (n = 1). In general, ALT elevations in the first few months after gene delivery have been suggested as a class effect of AAV gene therapies including clinical studies of other similar products in hemophilia B. The cause of transaminase elevations remain unclear but published studies suggest correlation of transaminase elevations with cytotoxic T cell response toward capsid proteins.

In Study CT-AMT-061-02, subjects with ALT elevation had approximately 44% lower mean FIX activity at Month 18 compared to those that did not have ALT elevation. The 9/53 subjects (17%) that were treated with corticosteroid for ALT elevations exhibited approximately 63% lower mean FIX activity at Month 18 compared to those who did not receive corticosteroid coadministration. All subjects discontinued steroid use prior to

Week 26, and the mean duration of corticosteroid use for elevated transaminases was  $79.8 \pm 26.6$  days (range: 51 to 130 days).

### **Exposure-Response Analysis**

Exploratory evaluation between vector DNA shedding in blood and visit-matched FIX activity did not reveal a clear correlation between blood vector DNA shedding parameter (C<sub>max</sub>) and FIX activity. The relationship between estimated ABR and mean FIX activity across the Month 7 to 18 post-treatment period was explored with a generalized additive model assuming negative binomial distribution of bleeding episode count. Overall, based on the limited data, no clear relationship was established between FIX activity and ABR presumably due to the observed higher FIX activity (> 12%) in over 90% of the subjects and low frequency of bleeding events.

### **Durability of FIX Protein Expression and FIX Activity**

The integrated comparative FIX activity and FIX protein expression over time for Study #CT-MT-061- 01 and CT-MT-061- 02 are provided in Table 1. The mean uncontaminated FIX activity was within the range of 0.9-1.3-fold in Study CT-AMT-061-01 compared to Study CT-AMT-061-02 (Table 1). However, the mean uncontaminated FIX protein level was approximately 4-fold lower in Study CT-AMT-061-01 compared to Study CT-AMT-061-02 at all the time points post-dose. This can be due to the limited number of subjects (N = 3) in Study CT-AMT-061-01 and the overall higher variability of FIX protein expression in Study CT-AMT-061-02. The source of higher FIX protein variability is unknown, but the applicant indicated that the FIX protein level is not an accurate representation of FIX activity due to the presence of non-functional protein conferred by mutations in the FIX gene in hemophilia B patients and the bioanalytical assay cannot differentiate between the Padua variant expressed by HEMGENIX and wild-type protein.

An integrated exploratory analysis on durability of FIX expression and FIX activity following HEMGENIX treatment in patients with hemophilia B was conducted including pooled data from Studies CT-AMT-061-01 and CT-AMT-061-02. Durability analysis was conducted using a linear mixed-effects repeated model for change from baseline and

percent change from baseline of FIX activity or protein over time using Month 6 post-dose data as the reference baseline level for HEMGENIX. The Month 6 post-dose level was selected as a reference since stable level of FIX expression and activity is achieved within 3-6 months post AMT-061 administration. Durability analysis of FIX activity and FIX protein concentration derived from HEMGENIX showed that FIX levels were stable up to 18 months with no statistically significant difference in the least square mean FIX levels at Months 7, 8, 9, 10, 11, 12, and 18 compared to value obtained at Month 6.

In subjects with and without baseline anti-AAV5 neutralizing antibodies (NAbs), the durability of FIX activity response measured as change from baseline and percent change from baseline of FIX activity levels at Months 7, 8, 9, 10, 11, 12, and 18 was not statistically significantly different from the 6 months post-dose durability baseline with the exception of 4% reduction from durability baseline (Month 6) at Month 18 in Anti-AAV5 NAb positive subgroup (22 subjects) and a 4% increase at Month 12 in the Anti-AAV5 NAb negative subgroup (33 subjects). These data suggest that Anti-AAV5 NAb appears to have minimal effect on the durability of FIX activity up to Month 18 as compared to the value obtained at Month 6. However, the overall mean FIX activity and FIX protein levels at 6 to 18 months post-dose were consistently lower in subjects with preexisting anti-AAV5 NAbs compared to those in subjects without preexisting anti-AAV5 NAbs (see immunogenicity section for details).

The durability of FIX activity response measured as change from durability baseline (Month 6) of FIX activity was generally not significantly different up to Month 18 in corticosteroid treated (9 subjects) and untreated subgroups (46 subjects) except for about 3 % reduction at 10, 12 and 18 months in subjects treated with corticosteroids for ALT elevation. These results suggest a trend for decrease in FIX activity in corticosteroid treated subjects beginning from Month 10, but the sample size is limited (9 subjects) to meaningfully interpret the results of the durability assessment for this subgroup analysis. As discussed previously the overall FIX activity is lower by 63% at Month 18 in corticosteroid treated subjects for ALT elevation as compared to those who did not receive corticosteroids. Overall, the durability analysis provides supportive evidence on

FIX activity level up to 18 months, but these results don't allow extrapolation beyond 18 months following treatment with AMT-061.

**Table 1: Summary of Mean (SD) % FIX Activity and Protein Level Over Time following Administration of  $2 \times 10^{13}$  gc/kg of HEMGENIX**

| Study                                   | FIX Activity (%) |               |                |                | FIX Protein Level (%) |                |                |                |
|-----------------------------------------|------------------|---------------|----------------|----------------|-----------------------|----------------|----------------|----------------|
|                                         | Month 6          | Month 9       | Month 12       | Month 18       | Month 6               | Month 9        | Month 12       | Month 18       |
| <b>CT-AMT-061-01 (N=3)<sup>a</sup></b>  | 47.1<br>(12.4)   | 47.7<br>(5.7) | 40.8<br>(9.4)  | 46.9<br>(12.7) | 4.2<br>(1.1)          | 4.3<br>(0.8)   | 4.9<br>(0.7)   | 3.7<br>(1.3)   |
| <b>CT-AMT-061-02 (N=51)<sup>b</sup></b> | 38.9<br>(18.7)   | 40.3<br>(21)  | 41.5<br>(21.7) | 36.9<br>(21.4) | 21<br>(30.8)          | 21.6<br>(32.7) | 19.6<br>(30.7) | 23.1<br>(36.3) |

<sup>a</sup>includes all 3 subjects except at Month 18 where measurement was available from 2 subjects. <sup>b</sup> includes 51 subjects except at Month 12 & 18 where assessment was available from 50 subjects

**Source:** Table 10 & 23; Module 2.7.2

### 6.3. Immunogenicity Assessments

Prior to AAV-vector based gene therapy administration, some subjects can be exposed to wild-type AAV virus and may have circulating baseline neutralizing antibodies (NAbs) that may jeopardize gene transduction and transgene synthesis. After AAV-vector based gene therapy administration, NAbs are produced and expected to persist for several years. Also, the viral capsid and transgene product can activate T-cell response. Thus, immunogenicity assessment of AAV-based gene therapy generally includes humoral and T cell-mediated immunity to the vector and transgene.

### **Anti-AAV5 Neutralizing Antibodies:**

A (b) (4) assay with limit of detection (LOD) of 1:7 titer was used for detection of anti-AAV5 NAb for both Study CT-MT-061- 01 and Study CT-MT-061-02. Currently, this assay is not validated (b) (4).

In Study CT-MT-061- 01, all 3 subjects had anti-AAV5 NAb before dosing. However, the NAb titer level was below 50 and all the three subjects achieved FIX activity >30%. In Study CT-MT-061- 02, 38.9 % (21/54 subjects) had anti-AAV5 NAb before dosing with a median titer of 1:57 (range: 1:9 to 1:3,212). After HEMGENIX administration, all subjects in both studies developed detectable anti-AAV5 NAb by Week 2 or 3 and remained elevated through to Month 12 (Study CT-MT-061-02) or 24 (Study CT-MT-061-01) post-treatment.

The impact of baseline anti-AAV5 NAb on FIX activity was evaluated based on four selected thresholds of anti-AAV NAb titer: 1:10, 1:50, 1:100 and 1:350. When higher target threshold of 1:350 NAb titer level is selected, the number of subjects with positive anti-AAV5 NAb were limited ( $n=3$  subjects). The mean uncontaminated FIX activity at Month 12 was  $42 \pm 22\%$  in subjects with NAb titers  $\leq 1:350$  ( $n=47$ ) and  $27 \pm 17\%$  in subjects with NAb titers  $>1:350$  ( $n=3$ ).

### **Anti-FIX Antibodies and FIX Inhibitors:**

In Study CT-MT-061- 02, the majority (53/54 [98.1%]) of subjects tested negative for anti-FIX antibodies prior to dosing (at the baseline assessment) and at Month 12 post-AMT-061 treatment. One subject tested positive for anti-FIX antibodies prior to dosing and periodically during the study post-AMT-061 treatment to Month 6; the subject had a FIX activity level of 8.4% at Month 6 and 11.4% at both Month 12 and Month 18 post AMT-061 treatment. The baseline anti-AAV5 NAb level was below LOD for this subject indicating that the NAb level is not contributing to the lower FIX activity. The FIX inhibitor level was below LOD (Nijmegen-Bethesda units [NBU]/mL = 0.415) for all 54 subjects



prior to dosing (at the baseline assessment) and remained below LOD through to Month 12 post-AMT-061 treatment.

### **AAV5 Capsid-specific T-cell Response:**

For characterization of cellular immune response, AAV5 reactive T-cells were measured using an (b) (4) assay. This method relies on detection of (b) (4) upon stimulation with AAV5.

In Study CT-AMT-061-02, the majority (88.9%) of subjects with interpretable results did not have specific AAV5 capsid T-cell response prior to dosing (at the baseline assessment). The number of subjects who developed a specific AAV5 capsid T-cell response varied during the study, reaching the highest number at Week 6 following AMT-061 treatment (15/38 [39.5%] subjects). There were missing data due to issues related to insufficient number of cells and nonconformance in the analysis. Specific T-cell response was observed in 39/54 subjects (72%) in at least one follow-up visit. Uncontaminated FIX activity ranged between 6.0 and 97 % of normal for visits where subjects also had a specific T-cell response. Specific T-cell responses concurrent with TEAEs of ALT and/or AST increased were noted for 6 subjects and concurrent with corticosteroid treatment in 2 subjects. Overall, it is difficult to interpret the result of the current cellular immune response against AAV5 capsid due to high variability, and missing data.

## 7. Appendix

7.1. **Study#1-** A phase I/II, open-label, uncontrolled, single-dose, dose-ascending, multi-center trial investigating an adeno-associated viral vector containing a codon-optimized human Factor IX gene (AAV5-hFIX) administered to adult patients with severe or moderately severe haemophilia B (Study #CT-AMT-060-01)

The primary objective of the study was to evaluate the safety of systemic administration of AMT-060 (AAV5-hFIX), an adeno-associated viral vector (AAV) containing a codon-optimized human Factor IX (hFIX) gene, to adult subjects with severe or moderately severe hemophilia B.

The secondary objectives of the study were to evaluate efficacy and safety of systemic administration of AMT-060 to adult subjects with severe or moderately severe hemophilia B. The following are clinical pharmacology relevant endpoints included as part of secondary objectives of the study:

- Factor IX (FIX) activity level
- Use of FIX replacement therapy
- Shedding of the vector in various body matrices such as blood, urine, faces, nasal secretion, saliva and semen
- Immune responses against AAV5 capsid proteins in response to AMT-060
- Immune responses against FIX protein after administration of AMT-060
- Effect of AMT-060 on inflammatory markers

**Overall Study Design:** Subjects were adult with severe (i.e., FIX activity <1 %) or moderately severe (i.e., FIX activity  $\geq 1$  to  $\leq 2\%$ ) hemophilia B. A maximum of 2 subjects with moderately severe hemophilia B phenotype were enrolled per cohort. Eligible subjects were allocated to 2 consecutive dose cohorts and received a single IV dose of AMT-060:

- Cohort 1 received the low dose of  $5.0 \times 10^{12}$  genome copies (gc)/kg
- Cohort 2 received the high dose of  $2.0 \times 10^{13}$  gc/kg.

**FIX Protein and Activity Assessment:** Blood samples for FIX protein concentration and activity measurement were collected as shown in Table 2. Also, additional blood samples for FIX protein and FIX activity were collected bi-weekly between Visits 22-31 (i.e., Weeks 39- 156) and monthly between Visits 32-35 (i.e., Weeks 182-260). When subject was on prophylactic FIX replacement therapy, blood sampling took place on days just prior to the administration of the replacement therapy to the extent the visit windows allowed. FIX protein concentration and activity were measured at the central laboratory with the following assays:

- one-stage activated partial thromboplastin time (aPTT)-based assay
- chromogenic assay/(b) (4) assay

**FIX Replacement Therapy:** From Visit 1 and throughout the entire trial, subjects were asked to document use of FIX replacement therapy in an e-diary. Information recorded included date and time of FIX administration, the drug name, and dose. At each visit, the Investigator reviewed e-diary entries, FIX replacement therapy, and the subject's medical/hospital records on FIX replacement therapy (if any). After Visit 21, e-diary data review was performed at least monthly, by means of a phone call, in addition to the review at each visit.

**Vector DNA Viral Shedding and Biodistribution Assessment:** Samples from blood, saliva, nasal secretions, urine, feces and semen were collected for vector DNA measurement (Table 2). Sampling continued for the individual subject and for a specific matrix until 3 consecutive negatives samples had been detected for the subject for that matrix.

**AAV5 Antibodies:** Blood sampling for measurement of total (immunoglobulin G [IgG] and immunoglobulin M [IgM]) antibodies to AAV5 and neutralizing antibodies (NAbs) to AAV5 took place at the timepoints indicated in Table 2.

**AAV5 Capsid-Specific T cells:** Sampling for AAV5 capsid-specific T cells took place at the timepoints indicated in the schedules of assessments in Table 2. Possible responses

included the following, where cutoff represents the background threshold for positivity of 17 spot forming cells (SFC)/million peripheral bloodmononuclear cells (PBMCs):

- Positive, specific AAV5 response if  $\geq 5$  SFC/300,000 PBMCs above cutoff
- Equivocal AAV5 response if 2 to  $< 5$  SFC/300,000 PBMCs above cutoff
- No specific (negative) AAV5 response if  $< 2$  SFC/300,000 PBMCs above cutoff
- Results uninterpretable due to control result

**Anti-FIX Antibodies and FIX Inhibitors:** Blood samples for measurement of anti-FIX antibodies and FIX inhibitors were collected at the timepoints indicated in Table 1. The Investigator has the option of collecting additional blood samples for FIX inhibitors in case (of suspicion) of detection of inhibitors. A subject had FIX inhibitors if a subject tested positive for inhibitors at 2 consecutive tests, performed preferably within 2 weeks. If a subject tested positive for FIX inhibitors, continued with no change to treatment type for 6 weeks, and the FIX inhibitor test was negative after that time, the inhibitor was classified as transient.

**Inflammatory Markers:** Blood sampling schedule for interleukin-1 beta (IL-1 $\beta$ ), interleukin-2 (IL-2), interleukin-6 (IL-6), interferon gamma (IFN $\gamma$ ), and monocyte chemotactic protein-1 (MCP-1) were as follows:

- Visit 2 (prior to IMP administration)
- Visits 3 to 14 (weekly in Weeks 1-12)
- Visits 15 to 17 (bi-weekly in Weeks 14, 16, 18).

**Table 2: Schedule of Assessment for clinical pharmacology end points**

| Analyte                                                | Matrix                                 | Sampling Time                                                                                                                                                                                                                                                               |
|--------------------------------------------------------|----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| FIX activity and FIX protein                           | Plasma                                 | Predose and postdose weekly starting from Week 1 to Week 12, every 2nd week from Week 14 to 26, every 3 months until 3 years (Weeks 39, 52, 65, 78, 91, 104, 117, 130, 143, 156), and then every 6.5 months until 5 years (Weeks 182, 208, 234, 260).                       |
| Vector DNA                                             | Blood, urine, saliva, nasal secretions | Predose and postdose at 24 hours, weekly starting from Week 1 to Week 12, every 2nd week from Week 14 to 26, every 3 months until 3 years (Weeks 39, 52, 65, 78, 91, 104, 117, 130, 143, 156, 182, 208), and then every 6.5 months until 5 years (Weeks 182, 208, 234, 260) |
|                                                        | Semen, feces                           | Predose and postdose at Weeks 1, 3, 6, 9, 12, 14, 16, 18, 20, 22, 24, 26, every 3 months until 3 years (Weeks 39, 52, 65, 78, 91, 104, 117, 130, 143, 156, 182, 208), and then every 6.5 months until 5 years (Weeks 182, 208, 234, 260).                                   |
| NAbs to AAV5 capsid and total antibodies (IgG and IgM) | Serum                                  | Predose and postdose weekly starting from Week 1 to Week 4, at 6.5 months (Week 26), and then every year until 5 years (Weeks 52, 104, 156, 208, 260).                                                                                                                      |
| Anti-FIX antibodies                                    | Serum                                  | Predose and postdose at 6.5 months (Week 26), and then every year until 5 years (Weeks 52, 104, 156, 208, 260).                                                                                                                                                             |
| FIX inhibitors                                         | Serum                                  | Predose and postdose at 3 months (Week 12), 6.5 months (Week 26), and then every year until 5 years (Weeks 52, 104, 156, 208, 260).                                                                                                                                         |
| AAV5 Capsid-specific T-cell response                   | Peripheral blood mononuclear cells     | Predose and postdose weekly starting from Week 1 to Week 12, and then every 2nd week from Week 14 to Week 26.                                                                                                                                                               |

Source: Module 2.7.2; Table 2

## Demographic and Dosing of AMT-060

The study enrolled adult males with median age of 44 years (range 33 to 72) and median body mass index (BMI) of 26 kg/m<sup>2</sup>(range 20 to 31). The mean age of subjects in Cohort 1 was higher than for those in Cohort 2 (60.2 years vs. 38.2 years, respectively). This was because 3 of the 5 males (60%) in Cohort 1 were approximately 70 years old, while all subjects in Cohort 2 were less than 50 years old. During the last 12 months prior to screening, treated bleeding episodes were reported by 5 (100%) subjects in Cohort 1 and 2 (40%) subjects in Cohort 2; all 7 subjects were on a prophylactic FIX regimen. The number of bleeding episodes in the year prior to screening were higher for Cohort 1 and ranged from 7 to 22 in Cohort 1 (N = 5) and from 0 to 7 in Cohort 2 (N = 3). During the 7 days prior to screening, all subjects except 1 in Cohort 1 and 2 in Cohort 2 received 1 or more infusions of any FIX replacement therapy. At screening, 9 subjects were taking FIX prophylactic regimen with doses ranging from 2000 IU one time per week to 4000 IU every 3 days.

AMT-060 (also known as AAV5-hFIXco) is a recombinant AAV5 vector containing the codon-optimized human FIX complementary deoxyribonucleic acid (cDNA) under the control of LP1, a liver-specific promoter. All subjects received a single IV infusion of AMT-060 at Visit 2 (Day 1). The required volume of AMT-060 depended on the cohort and the subjects body weight. The following dose levels were administered of AMT-060:

- Cohort 1 (5 subjects):  $5 \times 10^{12}$  gc/kg
- Cohort 2 (5 subjects):  $2 \times 10^{13}$  gc/kg

**Reviewer comments: Considering the body weight of the subjects, the mean administered dose of AMT-060 was:**

- Cohort 1:  $41.8 \times 10^{13}$  gc (range:  $35.6-44.5 \times 10^{13}$ )
- Cohort 2:  $166.8 \times 10^{13}$  gc (range:  $142-192 \times 10^{13}$ )

**Thus, due to the comparability in body weight range among the two cohorts the four-fold difference in dose range was maintained.**

## **Factor IX Activity and Protein Expression**

The secondary efficacy endpoint was FIX-replacement-therapy-free FIX activity or endogenous FIX activity. FIX-replacement-therapy-free FIX activity was defined as measurement taken at least 10 days after a preceding FIX replacement therapy administration. Measurements taken within 10 days of preceding FIX replacement therapy were considered as “contaminated” and thus not included in this analysis.

Local monitoring data (one-stage [aPTT-based] FIX assay) were used by the Investigator to determine whether FIX prophylaxis could be discontinued within 12 weeks following AMT-060 treatment. All subjects who were on FIX prophylaxis at study entry could discontinue use as they were able to maintain their endogenous FIX activity level  $\geq 2\%$ . The mean endogenous FIX activity levels of the subjects in Cohort 1 ranged from 2.8% to 8.2% of normal and were stable during the post-tapering period (i.e., from discontinuation of FIX prophylaxis until data cutoff). Three of the 5 subjects in Cohort 1 achieved a mean FIX activity level of  $>5\%$ . The mean endogenous FIX activity level in Cohort 2 ranged from 4.0% to 10.7% of normal and were stable during the post-tapering period. Four of the 5 subjects in Cohort 2 achieved a mean FIX activity of  $> 5\%$ . The FIX protein concentration varied during the study with the mean FIX protein levels ranging between Month 6 to Year 5 from 6 % to 31 % of normal in Cohort 1 and 22 % to 32 % of normal in Cohort 2.

During the study between dosing and study completion, all subjects received a prophylactic FIX regimen and the majority (9 subjects; 4 subjects in Cohort 1 and 5 subjects in Cohort 2) used an on-demand FIX regimen at least once. However, the amount of prophylactic FIX therapy used during the study was considerably less than that used in the year prior to screening. In Cohort 1, the mean (SD) dose of all FIX infusions was 326,532 (234,900) IU in the year prior to screening and decreased to 252,950 (222,790) IU during the observed post-tapering period of 1760 days. In Cohort 2, the mean (SD) dose of all FIX infusions was 233,778 (156,873) IU in the year prior to screening and decreased to 85,800 (84,482) IU during the observed post-tapering period of 1753 days.

**Reviewer comments: Treatment with AMT-060 resulted in 2.8-8.2% and 4-10.7% FIX activity in Cohort 1 and Cohort 2, respectively. On average the one-stage assay provides about 1.5-fold higher FIX activity versus the chromogenic assay. The observed small increase in FIX activity following treatment with AMT-060 is potentially cofounded by:**

- 1. Baseline endogenous secretion of FIX i.e., patients are able secreting baseline FIX in the range of 1-2%**
- 2. Exogenous treatment with FIX replacement therapy and the proposed 10-day washout period may not be adequate for some FIX products with extended half-life. For example, one subject was treated with IDELVION that has half-life of 4.3 days and a washout period of up to 21 days is expected (~5xhalf-life).**

### **Vector DNA Viral Clearance and Biodistribution**

A validated (b) (4) method was used for the detection of vector DNA in urine, blood, nasal swabs, faeces, saliva, and semen. Clearance of vector DNA was confirmed by 3 subsequent measurements below limit of detection (LOD), achieved in all subjects at both dose levels from all the matrices except for semen, where clearance was achieved in 9/10 subjects. One subject was unable to produce semen due to a historical medical condition and, therefore, shedding from semen could not be assessed.

The first absence of shedding (negative shedding) was defined for a specific matrix when three consecutive negative samples had been confirmed for the subject for that specific matrix. A negative shedding result from blood was reached within 191 to 911 days (Cohort 1) and 484 to 1111 days (Cohort 2). A negative shedding result of vector DNA from urine was achieved within 23 to 79 days (Cohort 1) and 57 to 155 days (Cohort 2). Negative shedding result from feces was reached within 43 to 111 days (Cohort 1) and 112 to 282 days (Cohort 2). For semen, time to negative shedding ranged from 65 to 365 days in Cohort 1 and 84 to 282 days in Cohort 2. The vector DNA clearance from blood for individual subjects is displayed in Figure 3. The summary noncompartmental analysis (NCA) derived PK parameters are displayed in Table 3.



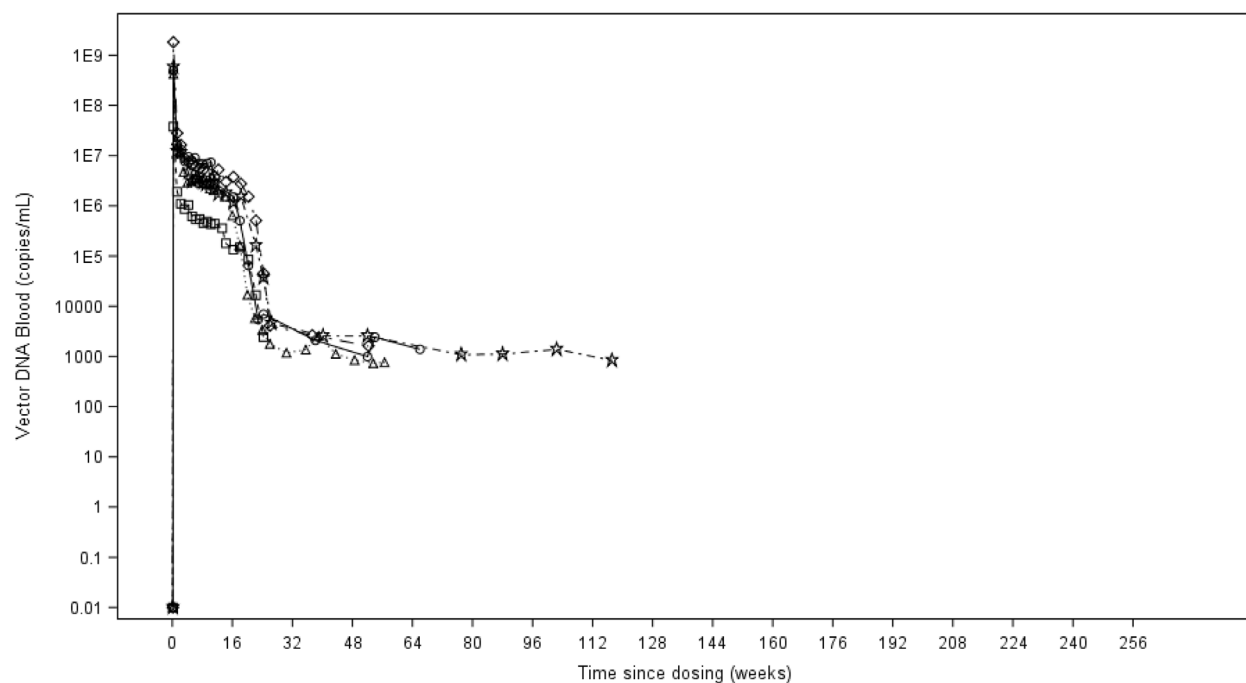
**Table 3: Summary Statistics of PK parameters for Blood and Semen Vector DNA Kinetics (#CT-AMT-060-01)**

| <b>Matrix &amp; Parameter</b> | <b>Cohort 1:<br/>5.0 x 10<sup>12</sup> gc/kg (N=5)</b> | <b>Cohort 2:<br/>2.0 x 10<sup>13</sup> gc/kg (N=5)</b> |
|-------------------------------|--------------------------------------------------------|--------------------------------------------------------|
| <b>Blood</b>                  |                                                        |                                                        |
| Clast (copies/mL)             |                                                        |                                                        |
| Mean (SD)                     | 1410.8 (683.3738)                                      | 1108.4 (312.5089)                                      |
| CV (%)                        | 48.439                                                 | 28.195                                                 |
| Cmax (copies/mL)              |                                                        |                                                        |
| Mean (SD)                     | 6.717E+08 (6.741E+08)                                  | 4.032E+09 (3.328E+09)                                  |
| CV (%)                        | 100.362                                                | 82.541                                                 |
| Tmax (days)                   |                                                        |                                                        |
| Median                        | 1.02                                                   | 0.98                                                   |
| Min                           | 0.98                                                   | 0.74                                                   |
| Max                           | 1.03                                                   | 1.05                                                   |
| <b>Semen</b>                  |                                                        |                                                        |
| Clast (copies/mL)             |                                                        |                                                        |
| Mean (SD)                     | 5239.5 (5684.2612)                                     | 10512.4 (6734.9182)                                    |
| CV (%)                        | 108.489                                                | 64.066                                                 |
| Cmax (copies/mL)              |                                                        |                                                        |
| Mean (SD)                     | 2.602E+06 (4.753E+06)                                  | 7.297E+06 (6.530E+06)                                  |
| CV (%)                        | 182.684                                                | 89.486                                                 |
| Tmax (days)                   |                                                        |                                                        |
| Median                        | 13.795                                                 | 7.4                                                    |
| Min                           | 7.5                                                    | 5.55                                                   |

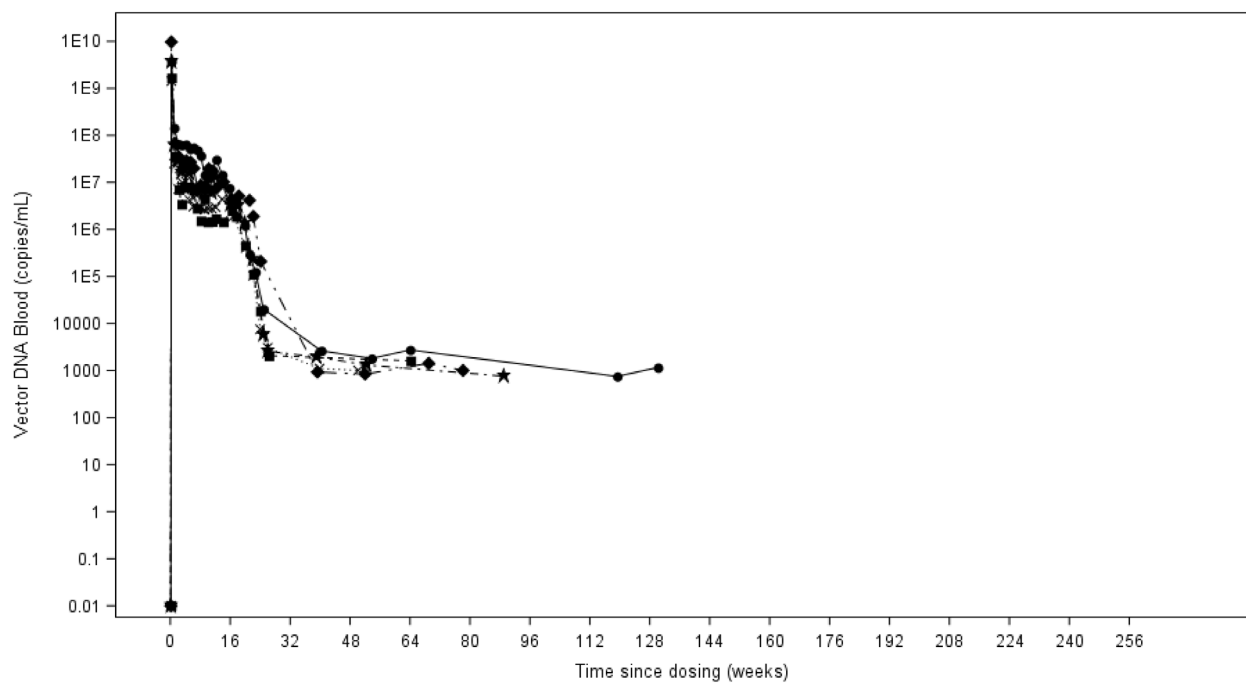
Source: clinical-info-amend; Table 26.1.1

**Figure 3: Individual subject profile of vector DNA clearance from blood**

(A): Cohort 1



(B): Cohort 2



Source: CT-AMT-060-01 5-year CSR Figure 14.3.5.3, Figure 14.3.5.4

## **Immunogenicity Assessment**

### **Neutralizing and Total (IgG and IgM) antibodies to AAV5:**

None of the subjects had pre-existing NABs to AAV5 using (b) (4) NAB assay at screening or prior to AMT-060 administration. After reanalysis of the serum samples with the more sensitive, research-grade, (b) (4) anti-AAV5 NAB assay, 1 out of 10 subjects was found to be positive for anti-AAV5 NABs before treatment. As expected, all subjects developed a humoral immune response to AAV5 within 1 week of exposure to AMT-060.

Two subjects in Cohort 1 had detectable IgG anti-AAV5 antibodies before dosing. After AMT-060 administration, 5/10 subjects developed detectable IgG anti-AAV5 antibodies by Week 1 (Visit 3) postdose and all subjects developed detectable IgG anti-AAV5 antibodies by Week 2 (Visit 4) postdose. The titer increased to > 1:109,350 as early as Week 1 (Visit 3) to Week 26 (Visit 21) in all subjects from both the cohorts and remained elevated through to Year 5 postdose.

One subject in Cohort 1 and 2 subjects in Cohort 2 had detectable IgM anti-AAV5 antibodies before dosing (Visit 2). After AMT-060 administration, IgM anti-AAV5 antibodies were detectable as early as Week 1. The titers increased after Week 1 postdose and then gradually declined over time.

### **Anti-FIX Antibodies and FIX Inhibitors:**

One subject in Cohort 1 tested positive for antibodies to FIX at Week 52 (Visit 23) following dosing which persisted until study completion. This subject's FIX activity was 7.1% before testing positive for anti-FIX antibodies and ranged between 5.2 to 21.8% after testing positive. None of the subjects developed inhibitory antibodies against FIX during the study.

### **AAV5 Capsid-specific T-cell Response:**

One subject in Cohort 1 had a AAV5 capsid-specific T-cell measurement of 33.3 SFC/million peripheral blood mononuclear cells at only 1 time point of 9 weeks after

treatment, which was considered a positive, specific AAV5 response (results >17 SFC/millionPBMCs were regarded as positive).

**7.2. Study#2-** Phase IIb, open-label, single-dose, single-arm, multi-center trial to confirm the factor IX activity level of the serotype 5 adeno-associated viral vector containing the Padua variant of a codon-optimized human factor IX gene (AAV5-hFIXco-Padua, AMT-061) administered to adult subjects with severe or moderately severe hemophilia B (Study #CT-AMT-061-01)

The primary objective was to confirm that a single dose of  $2 \times 10^{13}$  genome copies (gc)/kg AMT-061 resulted in factor IX (FIX) activity levels of  $\geq 5\%$  at 6 weeks after dosing.

The clinical pharmacology relevant secondary objectives focused on evaluating:

- endogenous FIX activity at 52 weeks following administration of AMT-061.
- discontinuation of previous prophylaxis treatment.
- total usage of FIX replacement therapy.

The clinical pharmacology relevant exploratory objectives focused on evaluating:

- FIX activity level based on chromogenic assay.
- ratio of one-stage (aPTT-based) assay FIX activity level and chromogenic assay FIX activity level.
- FIX protein-to-activity ratio.
- correlation of FIX activity levels and observed anti-AAV5 antibody titers.
- immunogenicity: formation of anti-AAV5 antibodies (total IgM and IgG, NABs). AAV5 capsid-specific T-cell response, formation of anti-FIX antibodies, formation of FIX inhibitors.
- shedding of vector DNA in blood and semen.
- inflammatory markers.

**Overall Study Design:** The Study CT-AMT-061-01 is a Phase IIb, open-label, single-dose, single-arm, multi-center trial consisting of a screening phase, a treatment plus post-treatment follow-up phase, and a long-term follow-up phase. After a maximum 6-week screening period, 3 eligible subjects received a single intravenous (IV) dose of  $2 \times 10^{13}$  gc/kg AMT-061. Subjects were monitored for tolerance to AMT-061 and detection of

immediate AEs for 24 hours (overnight stay) after dosing. The dosing of the subjects was separated by a minimum of 14 days to allow for subject safety monitoring and to ensure appropriate action could be taken in case any acute reactions were observed. After dosing, subjects are being followed for a total of 5 years (60 months) for evaluation of efficacy parameters and safety. Post-treatment follow-up visits occurred weekly up to Week 12, every second week from Week 12 to Week 26, and every month from Week 26 to Week 52. All subjects continue to be followed every 6 months from Week 52 to 60 months (4 years long-term follow-up). This report is based on 2.5 years follow-up after AMT-061 administration.

### **Demographics and Dosing of AMT-061**

The study subjects were male and, aged 43, 47, and 50 years old at the screening. The mean bodyweight of the subject was 84 kg (range: 81-89 kg). Two subjects were African American and 1 subject was Caucasian. The study subjects first presented with symptoms of hemophilia B when they were <1 year-old. All were diagnosed with severe hemophilia B the same year their first symptoms appeared with corresponding circulating FIX levels  $\leq 1\%$  of normal. In the 12 months prior to the Screening Visit, all 3 subjects used prophylactic and on-demand FIX replacement therapy, and all had >150 days of exposure to FIX. In the 1 year prior to the Screening Visit (Visit 1), subjects received Alprolix® as prophylactic or on-demand FIX therapy; 1 subject also received Idelvion® as prophylactic or on-demand FIX therapy. All subjects were administered the same IV dose of AMT-061 ( $2 \times 10^{13}$  gc/kg).

**Reviewer comments: Considering the body weight of the subjects, the actual administered dose ranged from 1.62 to 1.78 X 10<sup>15</sup> gc.**

### **Efficacy Assessment**

The primary efficacy endpoint was the uncontaminated FIX activity (i.e., more than 5 half-lives of exogenous FIX use) at Week 6 post AMT-061 administration. FIX activity or FIX protein concentration values that were measured more than 5 half-lives after most recent FIX-replacement administration is included here and are referred to as uncontaminated

values. At Week 6, the mean  $\pm$  SD uncontaminated FIX activity level was  $30.6 \pm 6.97\%$  of normal measured by the one-stage (aPTT-based) assay. Individual FIX activity levels achieved by each subject at Week 6 were 23.9%, 30.0%, and 37.8%.

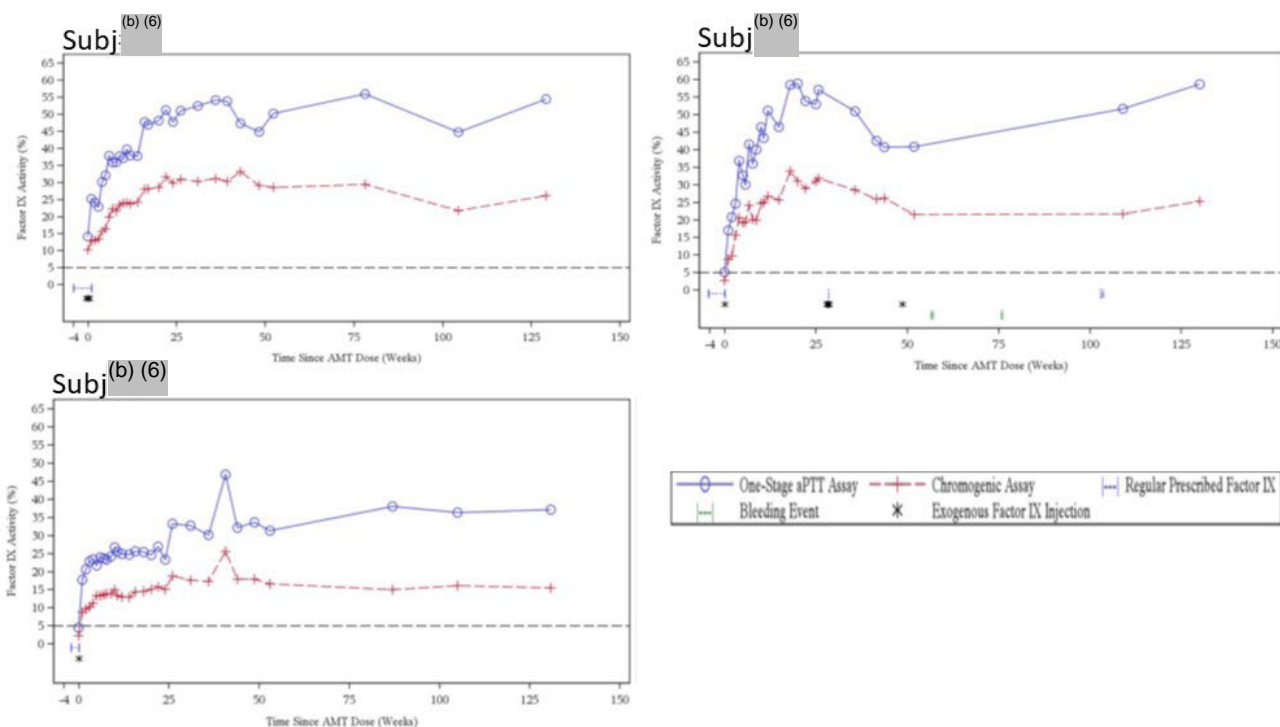
At Week 52, the mean  $\pm$  SD uncontaminated FIX activity level was  $40.8 \pm 9.45\%$  of normal measured by the one-stage (aPTT-based) assay. Individual FIX activity levels achieved by each subject at Week 52 were 31.3%, 40.8%, and 50.2%. The time course of FIX activity, exogenous Factor IX use and bleeding episodes for individual subjects are shown in Figure 3.

Two of the 3 subjects did not experience bleeding episodes post-AMT-061 administration. The third subject had 2 lower leg muscle bleeding episodes that were treated with exogenous FIX; one was spontaneous and the other was traumatic. The ABR over 2.5 years (30 months) of follow-up was 0.27 and the ABRs for spontaneous and traumatic bleeding episodes over 2.5 years (30 months) of follow-up were both 0.14. All 3 subjects discontinued use of continuous prophylaxis FIX during the study within 1 to 4 days of AMT-061 administration. One subject required on-demand FIX replacement therapy post-AMT-061 administration. The annualized mean FIX use for this subject was 689.8 IU/year over 2.5 years (30 months) of follow-up for the post-continuous prophylaxis period. This subject required on-demand FIX replacement therapy per protocol due to elective surgeries (2 major hip surgeries associated with an ongoing SAE of worsening avascular necrosis), 2 reported bleeding episodes, and a single self-administered infusion due to an unreported reason.

### **FIX Activity and Protein Concentration**

The FIX activity, measured by chromogenic assay, was consistently lower than that measured by one-stage (aPTT-based) assay with the mean ratio of FIX activity by Chromogenic assay to one-stage (aPTT-based) assay ranging from 0.4431 to 0.6337 across all post-dose time points (Figure 4 & Table 4). The mean uncontaminated FIX protein concentration ranged from 3.6 to 4.2 % between Week 3 and Month 30 (2.5 years). The mean ratio of FIX activity to protein was 5.1 at Week 3, gradually increasing to 7.2 at Week 6, 8.3 at Week 52 and 11.9 at Month 30 (Table 4).

**Figure 4: Factor IX Activity (%), Exogenous Factor IX Use, and Bleeding Episodes Over Time by Subject (All Subjects Treated)**



Source: CT-AMT-061-01 2.5-year CSR Figure 2.4.1

## Vector DNA Viral Clearance and Biodistribution

Vector DNA levels were determined in blood and semen samples by means of a quantitative (real-time) polymerase chain reaction. Sampling continued for an individual subject and for a specific matrix until 3 consecutive negative samples had been detected for the subject for that matrix. Two subjects no longer shed vector DNA at a mean of 26.21 weeks (range: 26 to 26 weeks) for semen samples and at 31 weeks (range: 31 to 78 weeks) for blood samples. One subject had positive test results at all post-dose visits. Individual subject plots of AAV vector DNA (copies/mL) over time (from AMT-061 administration to data cutoff) are provided in Figure 5.

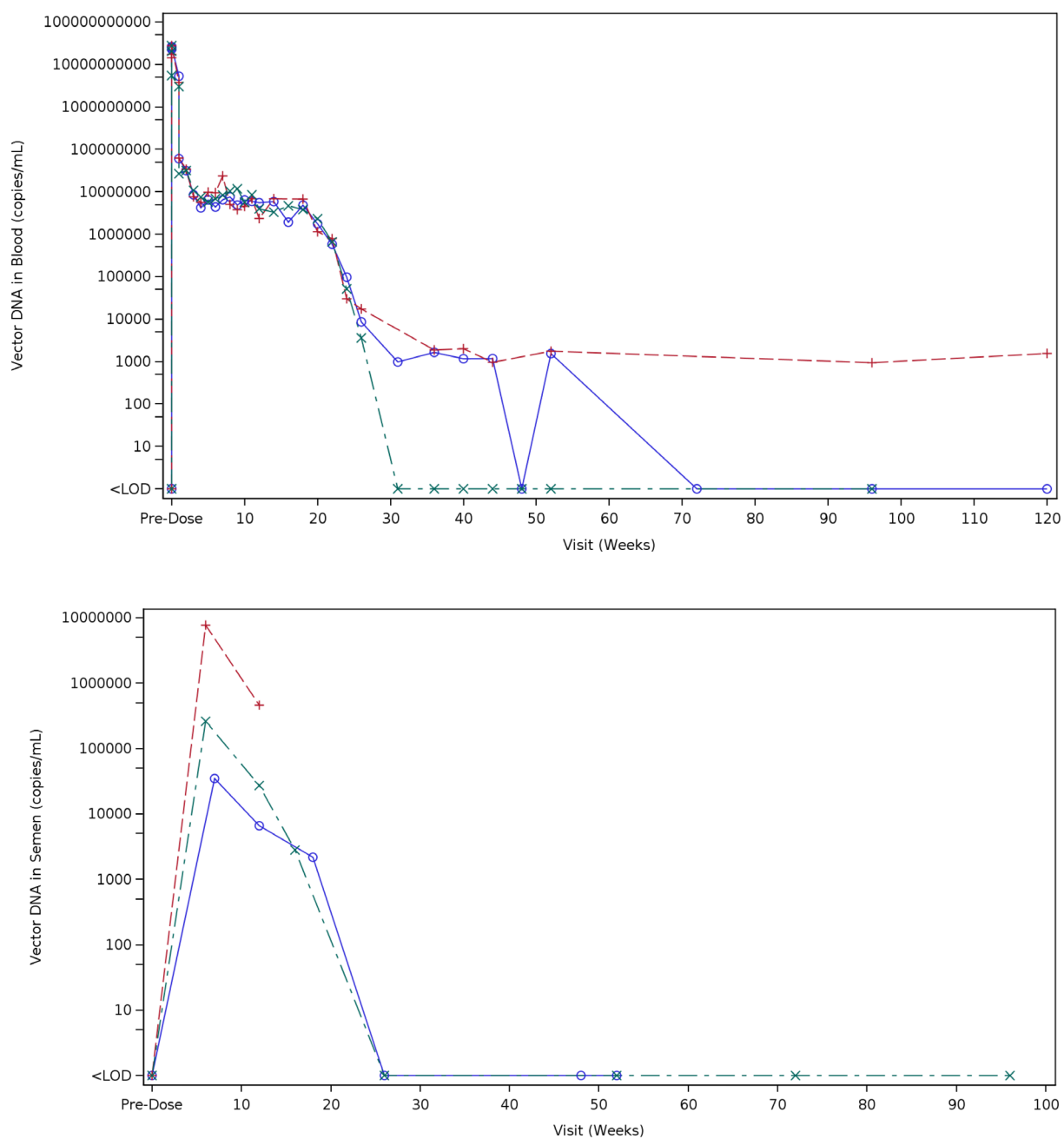


**Table 4: Uncontaminated FIX Activity, FIX Protein, and the Ratio of FIX Activity to FIX Protein in the Post-treatment Period at Selected Visits**

|                    | FIX<br>activity One-<br>stage (%) | FIX<br>Chromoge<br>nic (%) | activity Ratio<br>Chromogen<br>ic to One-<br>Stage | FIX Protein (%)   | Ratio<br>Activity to<br>FIX Protein<br>(One-Stage) |
|--------------------|-----------------------------------|----------------------------|----------------------------------------------------|-------------------|----------------------------------------------------|
|                    | (N = 3)                           | (N = 3)                    | (N = 3)                                            | (N = 3)           | (N = 3)                                            |
| <b>Baseline, n</b> | 1                                 | 1                          | 1                                                  | N/A               | N/A                                                |
| Mean               | 5.10                              | 2.70                       | 0.53                                               | -                 | -                                                  |
| <b>Week 3, n</b>   | 3                                 | 3                          | 3                                                  | 3                 | 3                                                  |
| Mean (SD)          | 23.40 (1.04)                      | 13.07 (2.7)                | 0.56 (0.1)                                         | 4.70 (0.85)       | 5.08 (0.9)                                         |
| <b>Week 6, n</b>   | 3                                 | 3                          | 3                                                  | 3                 | 3                                                  |
| Mean (SD)          | 30.57 (6.97)                      | 17.50 (3.6)                | 0.58 (0.1)                                         | 4.310<br>(1.2372) | 7.17 (0.5)                                         |
| <b>Month 6, n</b>  | 3                                 | 3                          | 3                                                  | 3                 | 3                                                  |
| Mean (SD)          | 47.07 (12.38)                     | 27.17 (7.3)                | 0.58 (0.03)                                        | 4.190<br>(1.0776) | 11.23 (0.4)                                        |
| <b>Month 12, n</b> | 3                                 | 3                          | 3                                                  | 3                 | 3                                                  |
| Mean (SD)          | 40.77 (9.45)                      | 22.20 (6)                  | 0.54 (0.02)                                        | 4.90 (0.67)       | 8.27(1.0)                                          |
| <b>Month 18, n</b> | 2                                 | 2                          | 2                                                  | 2                 | 2                                                  |
| Mean (SD)          | 46.95 (12.66)                     | 22.20 (10.18)              | 0.4603 (0.09)                                      | 3.72 (1.3)        | 12.79(1.0)                                         |
| <b>Month 24, n</b> | 3                                 | 3                          | 3                                                  | 3                 | 3                                                  |
| Mean (SD)          | 44.20 (7.7)                       | 19.83 (3.2)                | 0.450(0.03)                                        | 4.35 (0.5)        | 10.15                                              |
| <b>Month 30, n</b> | 3                                 | 3                          | 3                                                  | 3                 | 3                                                  |
| Mean (SD)          | 50.03 (11.4)                      | 22.30 (5.9)                | 0.44 (0.03)                                        | 4.24 (1.0)        | 11.87 (0.4)                                        |

Source: CT-AMT-061-01 2.5-year CSR, Table 2.1.1 and Table 2.1.

**Figure 5: Individual Plot of AAV Vector DNA in Blood and Semen (copies/mL) Over Time (From IMP to Cut-Off Analysis Set)**



Source: CT-AMT-061-01 2.5-year CSR, Figure 3.5.1&2.

## Immunogenicity Assessment

### Antibodies to AAV5:

All subjects were positive for NAb to AAV5 (titer  $\geq 7$ ) at Screening and titers were 19.5, 22.1, and 33.0 for the 3 subjects, respectively, at the Baseline Visit prior to administration of AMT-061. Following administration of AMT-061, titers increased, with levels  $>36450$  (the upper limit of quantification) in all 3 subjects by Week 2; titers remained  $>36450$  through to Month 24. With pre-existing NAb, subjects still achieved a mean FIX activity of approximately 30.6% by Week 6, 40.8% at Week 52, and 50.0% at Month 30.

All subjects developed anti-AAV5 IgG within one week following administration of AMT-061. The titer increased to  $> 1:109,350$  by Week 5, 12 or 26 in the 3 subjects and remained elevated through to Month 24.

**Reviewer comments: The 26-Week FIX activity was 57%, 51% and 33.2 % for the three subjects (displayed as subject 1 to 3 in Figure 3) with NAb titers of 19.5, 22.1 and 33, respectively. These limited data and the overall FIX activity profile (Figure 3) suggest a trend for negative correlation between FIX activity and baseline NAb. However, the FIX activity is over 30% for all subjects and the NAb level is still considered low for all three subjects ( $<1:50$ ).**

### Anti-Factor IX Antibodies and Factor IX Inhibitors:

One subject had a positive result in the anti-FIX antibody screening assessment at pre-dose, Baseline, Week 6, Week 12, Week 26, and Week 52, but had negative results in the subsequent confirmation assays. Negative results were noted at Month 24. Levels of FIX inhibitors were below the detection limit for all 3 subjects.

### AAV5 Capsid-specific T-cell Response:

A single AAV5 capsid-specific T-cell response was observed at Week 48 for one subject, but results were uninterpretable at Week 52. No specific AAV5 responses were detected in the other 2 subjects.

**Inflammatory Markers:**

The inflammatory markers assessed included IFN $\gamma$ , IL-1 $\beta$ , IL-2, IL-6, and MCP-1. Elevated IFN $\gamma$  was noted in two subjects at Week 1 (13.48-28.11 ng/L) and then declined in one subject but remain elevated for the second subject until Week 40. High IL-6 levels were observed with one subject at Week 40 but were within normal range at other visits. High MCP-1 levels were noted with one subject at Day 1 (815.6 ng/L), Week 1 (759.4 ng/L), and Week 8 (747.5 ng/L) but were within normal range at other visits.

7.3. **Study #3-** Phase III, open-label, single-dose, multi-center multinational trial investigating a serotype 5 adeno-associated viral vector containing the Padua variant of a codon optimized human Factor IX gene (AAV5-hFIXco-Padua, AMT-061) administered to adult subjects with severe or moderately severe hemophilia B (Study #CT-AMT-061-02)

The primary objective was to demonstrate the non-inferiority of AMT-061 ( $2 \times 10^{13}$  gc/kg) during the 52 weeks following establishment of stable Factor IX (FIX) expression (months 6 to 18) post-treatment (AMT-061) follow-up compared to standard of care continuous routine FIX prophylaxis during the lead-in phase, as measured by the annualized bleeding rate (ABR).

The secondary objective was to demonstrate additional efficacy and safety of systemic administration of AMT-061. The clinical pharmacology secondary objectives focused on evaluating:

- endogenous FIX activity at 6,12 and 18 months after a single dose of AMT-061.
- annualized consumption and infusion rate of FIX replacement therapy.
- discontinuation of previous continuous routine prophylaxis.
- trough FIX activity.
- correlation of baseline anti-AAV5 antibody titers using the luciferase based NAb assay on factor FIX activity levels after AMT-061 dosing.
- formation of anti-AAV5 antibodies (IgM, IgG, NAb).
- AAV5 capsid-specific T-cell response.
- formation of anti-FIX antibodies.
- formation of FIX inhibitors and recovery.
- aspartate aminotransferase (AST) and alanine aminotransferase (ALT) level and use of corticosteroids.
- shedding of vector deoxyribonucleic acid (DNA) in blood and semen.
- inflammatory markers.

**Overall Study Design:** Study CT-AMT-061-02 is an ongoing Phase 3, open-label, single-dose, multicenter and multinational in male adult patients with moderately severe or severe FIX deficiency and who were on continuous routine FIX prophylaxis. The study consisted of a screening period, lead-in period, treatment and post-treatment follow-up period including a long-term follow-up period. A total of 75 subjects were screened and 54 subjects received a single dose of HEMGENIX and were followed for 1.5 years post-treatment to evaluate safety and efficacy during this reporting period.

FIX activity or FIX protein concentration values that were measured more than 5 half-lives after the most recent FIX-replacement administration are included here and referred to as “uncontaminated values”. Blood samples for determination of FIX activity and FIX protein were collected per the assessment schedule described in Table 5. FIX activity was measured by both one-stage (aPTT-based) FIX assay and chromogenic FIX assay. Samples for determination of vector DNA in blood and semen, and samples for immunogenicity assessments were collected per the schedule described in Table 5.

### **Demographic and Dosing of AMT-061**

Most subjects enrolled in the Post-treatment Safety Population were White (74.1%) and not Hispanic or Latino (83.3%). Subjects in the Post-treatment Safety Population had a mean (SD) age of 41.5 (15.8) years, mean (SD) body weight of 85.1 (19.3) kg, and mean (SD) BMI of 27.2 (5.1) kg/m<sup>2</sup>.

At the time of initial diagnosis, 44/54 (81.5%) subjects had severe hemophilia B and 10/54 (18.5%) subjects had moderately severe hemophilia B. All subjects received prophylactic FIX replacement therapy in the year prior to screening and 5/67 (7.5%) subjects received on demand FIX replacement therapy.

From the 54 subjects that enrolled to receive AMT-061, 53 subjects received a single full dose of  $2 \times 10^{13}$  gc/kg AMT-061. One subject received a reduced dose (approximately 10% of the expected dose) before AMT-061 was withdrawn due to a TEAE of hypersensitivity that occurred during infusion.

**Reviewer comments:** Considering the body weight of the subjects, the administered mean total dose was  $1.7 \times 10^{15}$  gc (range  $1.16$ - $3.38 \times 10^{15}$  gc).

**Table 5: Sampling Schedule for Assessment of FIX Activity, FIX Protein, Vector DNA Levels and Immunogenicity**

| <b>Analyte</b>                                                 | <b>Matrix</b> | <b>Time point</b>                                                                                                                                                                                                                                                                                  |
|----------------------------------------------------------------|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| FIX activity and FIX protein                                   | Plasma        | Predose and postdose weekly starting from Week 1 to Week 12, every month from Month 4 until 1 year (Week 52), and then every 6 months until 5 years (Months 18, 24, 30, 36, 42, 48, 54, 60).                                                                                                       |
| Vector DNA                                                     | Blood         | Predose and postdose at 3 hours after dosing, weekly starting from Week 1 to Week 12, monthly from Week 13 to Week 20, and at the Week 52 visit and any additional visits.<br><br>Every visit during the Long-term Follow-up period from Month 18 to Month 60, including at any additional visits. |
|                                                                | Semen         | Predose and postdose at weeks 6, 12, 13, 15 and at any additional visits.<br><br>Every visit during the Long-term Follow-up period from Month 18 to Month 60, including at any additional visits.                                                                                                  |
| NAbs to AAV5 capsid and total antibodies (IgG and IgM) to AAV5 | Serum         | Predose and postdose every 3 weeks starting from Week 3 to Week 12, at 6 months, and then every year until 5 years (Week 52, Months 24, 36, 48, 60).                                                                                                                                               |
| Anti-FIX antibodies                                            | Serum         | Predose and postdose at Week 6, Week 12, 6 months, and then every year until 5 years (Week 52, Months 24, 36, 48, 60).                                                                                                                                                                             |
| AAV5 Capsid-specific T-cell                                    | Peripheral    | Predose and postdose weekly starting from Week 1 to Week 12,                                                                                                                                                                                                                                       |

response blood and then every month from Month 4 until 1 year  
mononucle(Week 52).  
ar cells

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Source: CT-AMT-061-02 18-month CSR, Section 9.1.

### **FIX Activity and FIX Protein Concentration**

At Baseline, mean  $\pm$  SD FIX activity from the one-stage (aPTT-based) assay was  $1.19 \pm 0.39\%$  based on FIX activity imputed according to subjects' severity of hemophilia B at screening (severe:  $<1\%$  and moderately severe:  $\geq 1\%$  and  $<2\%$ ). At 6 months post-AMT-061 treatment, the mean (SD) FIX activity was  $38.95 \pm 18.72\%$  (range: 8.2 to 97.1%). The FIX activity was maintained through Month 12, with a mean FIX activity of  $41.48 \pm 21.71\%$  (range: 5.9 to 113.0%). At Month 18, the mean (SD) FIX activity was  $36.90 \pm 21.40\%$  (range: 4.5 to 122.9%).

By the end of the  $\geq 6$ -month lead-in period, 43/54 (79.6%) subjects had FIX activity  $<12\%$  of normal. At Month 3, 12 and 18 following treatments with AMT-061, FIX activity was  $<12\%$  of normal in 4/51 (7.8%), 4/50 (8.0%) and 3/50 (6.0%) subjects, respectively. Treatment with AMT-061 was associated with lower odds of having FIX activity  $<12\%$  of normal for the Month 6 to 18 post-treatment period compared to the lead-in period (odds ratio: 0.027; 95% CI: 0.009, 0.080;  $p < 0.0001$ ).

FIX activity, as measured by chromogenic assay, was consistently lower than those measured by one-stage (aPTT-based) assay and the mean ratio of FIX activity by chromogenic assay to one-stage (aPTT-based) assay ranged from 0.41 to 0.52 up to Month 18 (Table 6). FIX protein levels during the post-treatment period followed a similar trend as FIX activity by one-stage (aPTT-based) activity; however, more variability was observed in the protein concentrations (Figure 6 & Table 6). The mean ratio of uncontaminated FIX activity to protein concentration was 5.9 at Week 3, 8.1 at Week 10 and remain stable at approximately 7 to 8.5 between Month 6 and Month 18 (Table 6).



**Reviewer comment:** There is high inter-subject variability in FIX activity. The source of this variability includes ALT elevation, corticosteroid treatment and age (for details see section on intrinsic and extrinsic factors affecting FIX activity). Most subjects (> 90%) achieved a stable FIX activity  $\geq 12\%$  following treatment with AMT-061.

### **Effect of Intrinsic Factors on FIX activity**

**Age:** The effect of age on FIX activity was evaluated by comparison of FIX activity levels at Weeks 6, Months 3, 6, 12, and 18 post-dose in the < 40 years, 40 to < 60 years, and  $\geq 60$  years of age subgroups. Most subjects (31/53, 58%) were below 40 years of age. There was a trend of higher FIX activity with increase in age; a higher FIX activity was observed in the 40 to < 60 year age subgroup (up to 30% relative difference) compared to the < 40 year age subgroup. Mean FIX activity levels were comparable between the 40 to < 60 year age and  $\geq 60$  year age subgroups at the evaluated time points except for Month 18, which had 1.6-fold lower FIX activity in 40 to < 60 year age subgroup compared to  $\geq 60$  year age subgroup). Mean FIX activity levels were 1.5 to 2-fold lower in the < 40 years subgroup compared to  $\geq 60$  years subgroup at the evaluated time points.

**Reviewer comments:** There was no scientific justification of the selected age stratification. This reviewer conducted additional analyses considering age as a continuous variable. There was statistically significant relationship between age and FIX activity level at Month 12 ( $p=0.02$ ). The age effect on the FIX activity level is visually displayed in Figure 7.

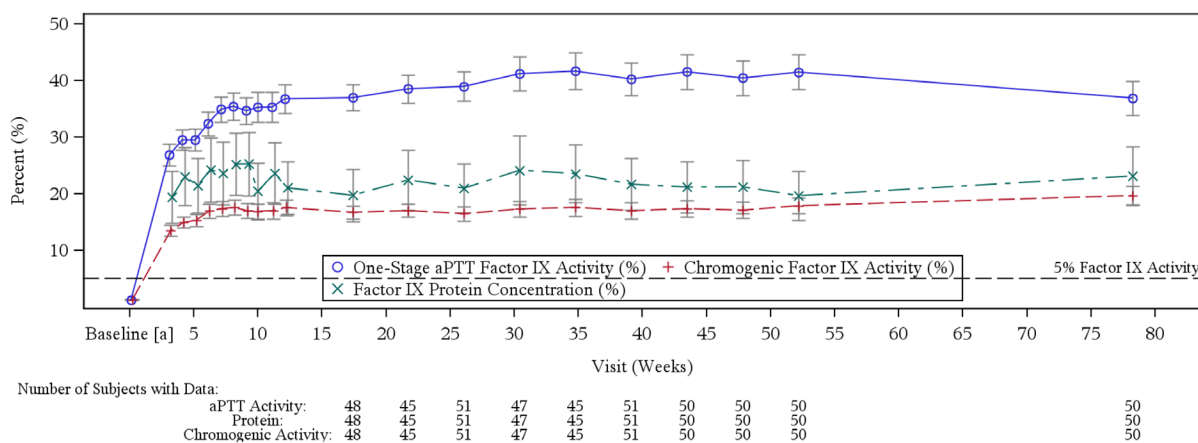
**Table 6: Uncontaminated FIX Activity (%), FIX Protein (%), and the Ratio of FIX Activity to FIX Protein in the Post-treatment Period at Selected Visits**

| Visit              | One-stage activity | FIX Chromogenic FIX activity) | Ratio chromogenic to one-stage | FIX Protein   | Ratio FIX activity to Protein levels |
|--------------------|--------------------|-------------------------------|--------------------------------|---------------|--------------------------------------|
| <b>Baseline, n</b> | 54                 | 54                            | N/A                            | N/A           | N/A                                  |
| Mean (SD)          | 1.19 (0.39)        | 1.19 (0.39)                   |                                |               |                                      |
| <b>Week 3, n</b>   | 43                 | 43                            | 43                             | 43            | 43                                   |
| Mean (SD)          | 26.83 (12.71)      | 13.47 (6.11)                  | 0.512 (0.071)                  | 19.35 (30.01) | 5.87(5.2)                            |
| <b>Week 12, n</b>  | 51                 | 51                            | 51                             | 51            | 51                                   |
| Mean (SD)          | 36.78 (18.17)      | 17.52 (9.90)                  | 0.479 (0.103)                  | 21.02 (33.02) | 7.60 (5.2)                           |
| <b>Month 6, n</b>  | 51                 | 51                            | 51                             | 51            | 51                                   |
| Mean (SD)          | 38.95 (18.72)      | 16.45 (8.82)                  | 0.414 (0.051)                  | 20.96 (30.84) | 7.52(4.8)                            |
| <b>Month 12, n</b> | 50                 | 50                            | 50                             | 50            | 50                                   |
| Mean (SD)          | 41.48 (21.71)      | 17.86 (10.05)                 | 0.422 (0.046)                  | 19.64 (30.73) | 8.48 (5.5)                           |
| <b>Month 18, n</b> | 50                 | 50                            | 50                             | 50            | 50                                   |
| Mean (SD)          | 36.90 (21.40)      | 19.66 (11.72)                 | 0.521 (0.060)                  | 23.11 (36.30) | 7.13 (5.1)                           |

\* Ratio of one-stage FIX activity (%) to FIX protein (%) was calculated for each subject and the mean ratio was then reported.

Source: CT-AMT-061-02 18-month CSR, [Table 2.1.1.1](#) and [Table 2.3.1](#).

**Figure 6: Mean ( $\pm$ SE) of Uncontaminated Central Laboratory FIX Activity (%) by One-Stage (aPTT-based) and Chromogenic Assay and FIX Protein Concentration Over Time During the Post-Treatment Period**



Source: CT-AMT-061-02 18-month CSR, [Figure 2.1.1.2](#).

**Race, Ethnicity and Body mass index (BMI):** Most subjects were White (75%) and Non-Hispanic or Latino (84%). The subgroup analysis did not reveal a major difference for race and ethnicity. The effect of BMI on FIX activity was evaluated by comparing FIX levels at Week 6, Months 3, 6, 12, and 18 post-dose in subgroups with BMI < 25, 25 to < 30, and  $\geq 30$ . Thirty of 53 subjects in Study CT-AMT-061-02 were in the BMI range 25-29. There was a trend of increase in FIX activity with increase in BMI. The mean FIX activity at Month 18 for the BMI subgroups < 25, 25 to 29, and  $\geq 30$  were 28.7%, 38.0%, and 42.7%, respectively.

Reviewer comments: The FIX activity increased by 32 % in overweight (BMI 25-29, n=29 subjects) and by 49 % in obese (BMI  $\geq 30$ , n=10 subjects) subjects as compared to normal BMI (<25, n=11 subjects). This increased FIX activity with increased BMI can be in part explained by the body weight-based dosing and the corresponding increase in administered total dose. There is a trend for increase in FIX activity with increasing body weight. A similar trend is observed with dose vs. FIX activity (Figure 7). However, these

relationships are not statistically significant presumably due to limited sample size, narrow bodyweight, and dose range ( $1.16\text{-}3.38 \times 10^{15}$  gc).

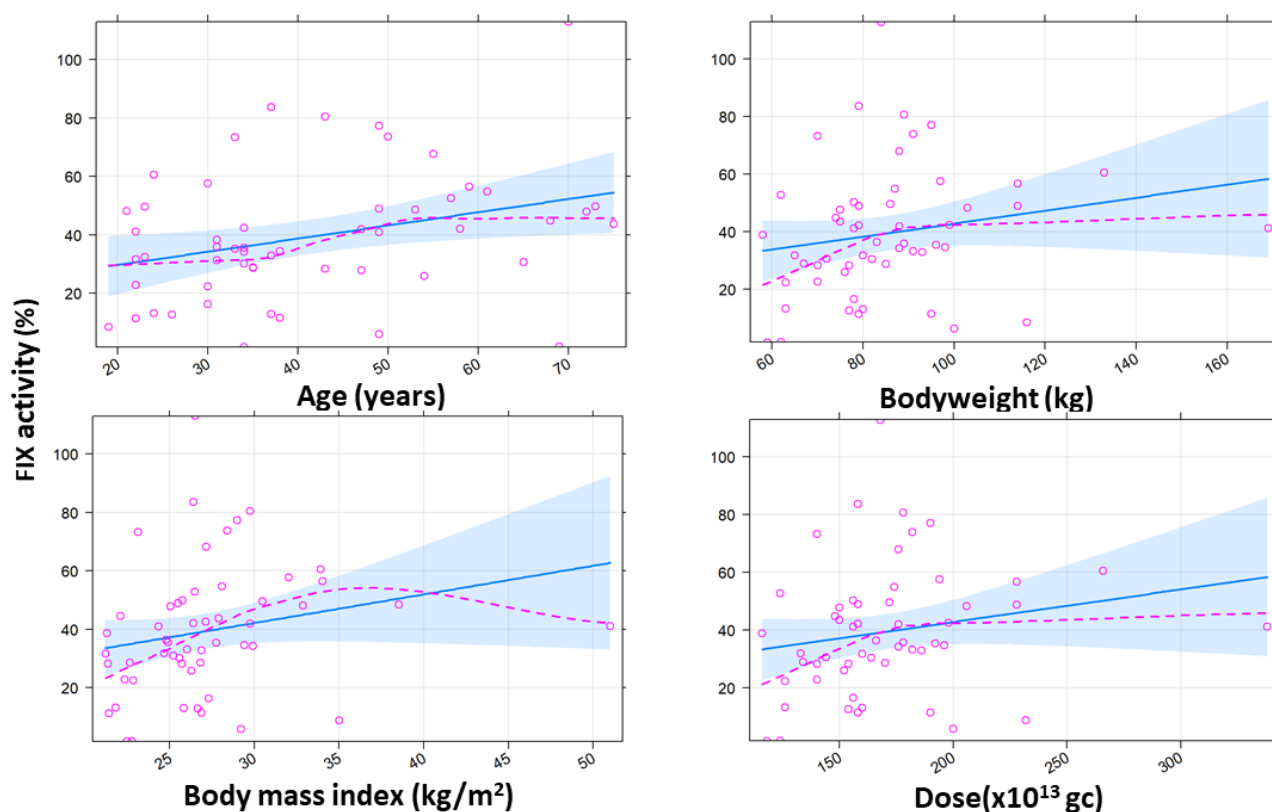
**Effect of hepatic impairment:** The effect of baseline liver pathology on the FIX activity was evaluated by Baseline (b) (4) or equivalent (b) (4) and classified as follows:

- Degree of fibrosis ( $\geq 9$  kilopascals [kPa] versus  $< 9$  kPa);
- Degree of steatosis (moderate to severe impairment with CAP score  $\geq S2$  [ $\geq 260$  dB/m] versus mild to no impairment  $< S2$  [ $< 260$  dB/m]).

All subjects had less than 9kPa degree of fibrosis and 28/53 subjects had steatosis with a CAP score  $< S2$ , 11 subjects had steatosis with a CAP score of  $\geq S2$ , and 14 subjects had missing information on CAP score. The differences in FIX activity between  $< S2$  and  $\geq S2$  groups at the evaluated time points were less than 35%. Also, the adjusted ABR during the study period compared to the Lead-in Period in  $\geq S2$  subgroup (1.04, 95% CI: 0.32, 3.40) versus that in  $< S2$  subgroup (2.01, 95% CI: 0.59, 6.84) at 7 to 18 months. These observations may be partly influenced by lower sample size in  $\geq S2$  subgroup.

Reviewer comments: No formal study was conducted to evaluate the impact of hepatic impairment. Based on the limited clinical data, there is a trend for lower FIX activity with moderate to severe liver impairment ( $\geq S2$ ) versus  $< S2$  (Figure 8). The 18-month mean FIX activity was  $29 \pm 14\%$  ( $n=11$  subjects with  $\geq S2$ ) and  $42 \pm 26\%$  ( $n=28$  subjects with  $< S2$ ), respectively. This observed  $\sim 31\%$  lower mean FIX activity in moderate to severe hepatic impairment is mechanistically plausible since AMT-061 is a liver-targeted gene therapy and the transgene expression is expected to depend on status of the liver. Since the data from current study are limited, future assessment is recommended.

**Figure 7: A plot evaluating effect of age, bodyweight, body mass index and dose on FIX activity measured at Month 12.**



The dashed line represents a loess nonparametric-regression smooth of the observed data. The blue line shows the regression line, and the shaded region represents 95% confidence interval.

Source: Reviewer analysis

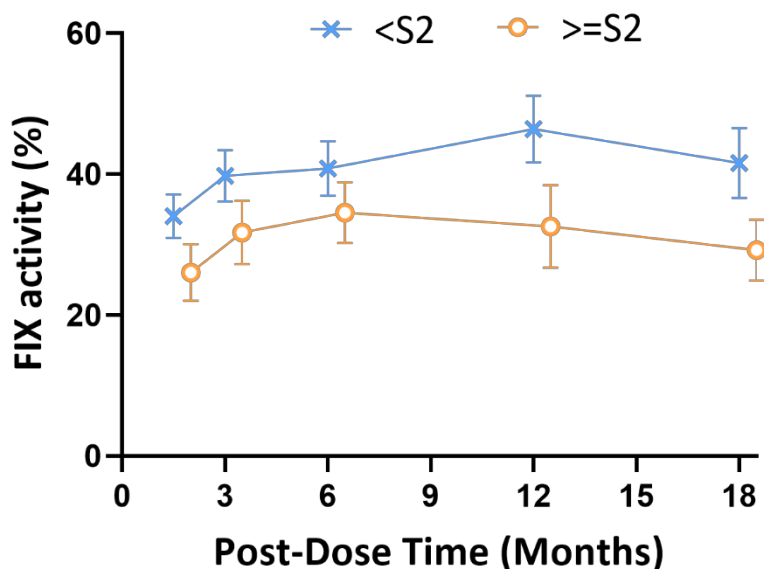
**Effect of Renal impairment:** The effect of renal impairment on FIX activity was evaluated by comparing FIX levels in mild and moderate renally impaired subjects to those with normal renal function at Week 6, Months 3, 6, 12, and 18 post-dose. Renal impairment was classified using the renal function evaluation by creatinine clearance:

- $\geq 90$  mL/min; normal renal function (control)
- 60-89 mL/min; mild impairment
- 30-59 mL/min; moderate impairment
- 15-29 mL/min; severe impairment

- <15 mL/min; kidney failure

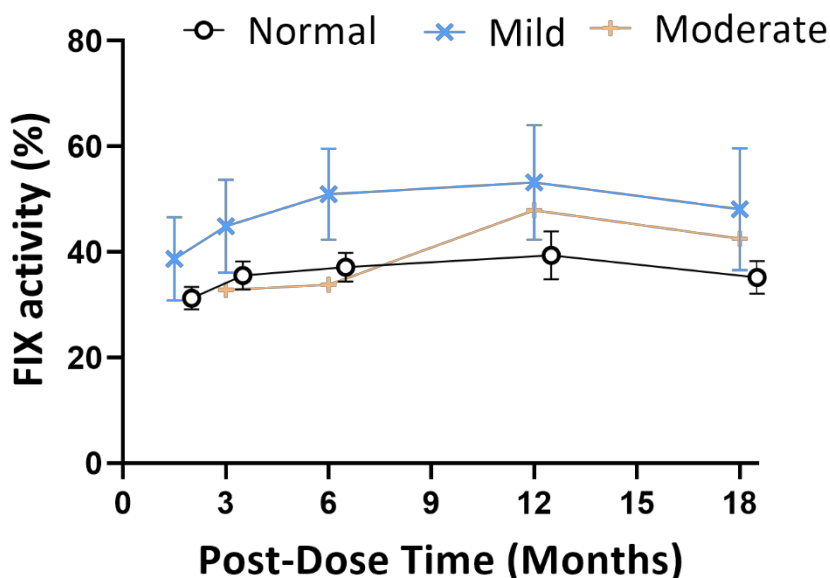
Most of subjects (45/53) had normal renal function, 7 subjects had mild renal impairment, and 1 subject had moderate renal impairment. No subjects with severe renal impairment or kidney failure (end stage renal disease) were enrolled in the study. Subjects with mild renal impairment had slightly higher mean FIX activity (up to 37% relative difference) compared to subjects with normal renal function at the evaluated time points post-dose (Figure 9). One subject with moderate renal impairment had similar FIX activity compared to the normal renal function group.

**Figure 8 : Effect of Baseline Liver Pathology by Steatosis Grade Category on Uncontaminated FIX Activity (%) (PK Population)**



**Source:** Reviewer analysis using data provided in durability and PK analysis (Table 1.5.2)

**Figure 9 : Effect of Baseline Renal Impairment on Uncontaminated FIX Activity (%) (PK Population)**



**Source:** Reviewer analysis using data provided in durability and PK analysis (Table 1.6)

Reviewer comments: No formal study was conducted to evaluate the impact of renal impairment on FIX activity. Based on the limited clinical data there is a trend for higher FIX activity with mild renal impairment as compared to subjects with normal renal function (Figure 9). However, confounding factors such as age and BMI should be considered in interpreting the effects of mild renal impairment. For example, 5 out of 7 subjects with mild renal impairment are  $\geq 60$  years of age and 6 out of 7 subjects have BMI  $\geq 25$  kg/m<sup>2</sup>. As described above there is a trend for increased FIX activity with increasing age and BMI that makes it difficult to determine the impact of renal impairment independent of age and BMI. Also, none of these 7 subjects with mild renal impairment had NABs. Considering the limited information on mild and moderate renal impairment group and confounding factors and no subjects with severe or end stage renal disease are included in the study, the effect of renal impairment could not be fully evaluated.

**Effect of baseline FIX activity level:** The effect of baseline FIX activity at the time of historical diagnosis on post-dose FIX activity was evaluated by comparing FIX activity in moderately severe (1-2% of normal FIX activity) and severe (< 1% of normal FIX activity) hemophilia B subgroups at Week 6, Months 3, 6, 12, and 18. Most subjects had severe hemophilia B with < 1% of normal FIX activity at the time of diagnosis (44/54, 81.5%). No notable differences were observed in the mean FIX activity between moderately severe and severe hemophilia B subgroup of subjects across different time points after dosing.

### **Effect of Extrinsic Factors on FIX activity**

#### **Effect of co- administration of FIX replacement therapy:**

Administration of FIX-replacement therapy after HEMGENIX administration has been shown to augment the increased FIX activity levels following HEMGENIX. For evaluating FIX activity levels following HEMGENIX administration, values within 5 half-lives of the exogenous FIX replacement therapies were excluded.

**Effect of corticosteroid use for ALT elevation:** Per the study protocol, corticosteroids were administered to subjects with ALT elevation following treatment with HEMGENIX. Nine out of 53 subjects (PK population) received corticosteroids for ALT elevation. The subjects treated with corticosteroids in response to ALT elevations are a subset of the subjects with ALT elevation (defined as > ULN or > 2 × baseline per local or central lab occurring within 90 days of dosing) except for one subject that did not fit the definition of ALT elevation.

The mean FIX activity levels at 6 months, 12 months, and 18 months post-dose were 57%, 64%, and 63% lower, respectively, in the 9 subjects with ALT elevation who received corticosteroids compared to the rest of the 44 subjects who did not receive corticosteroid treatment. The Month 18 mean FIX activity was  $15.6 \pm 8$  % for subjects treated with corticosteroids and 41.6 % for subjects without corticosteroid treatment. Five subjects had ALT elevations but did not receive steroids. Of these 5 subjects, 4 had > 18% of normal



FIX activity at all time points post-dose and only one subject had lower FIX activity between 6% and 10% of normal FIX activity during the post treatment period.

Reviewer comments: Based on the applicant's assessment of ALT elevation, ALT elevation was reported in 13/53 subjects (24%). Subjects with ALT elevation had approximately 44% lower mean FIX activity at Month 18 compared to those who did not have ALT elevation. The 9/53 subjects (17%) that were treated with corticosteroid for ALT elevations exhibited approximately 63% lower mean FIX activity at Month 18 compared to those who did not receive corticosteroid coadministration. From this analysis, it appears that subjects that met the criteria of ALT elevation and received corticosteroid treatment had the lowest FIX activity.

**Effect of drug product batches:** The in vitro potency range was 0.7 – 1.4 RU across the 5 batches used in the study. Comparison of FIX activity across these batches showed no notable differences in mean FIX activity between the batches at 6, 12, and 18 months post-dose. A sensitivity analysis performed by excluding subjects who experienced infusion site reactions and may have had infusion interruptions or partial dose infusion (1 subject) confirmed no notable differences across the batches. Batch A18P002 showed a slightly larger variability in FIX measurements across the time points compared to the other batches.

### **Annualized Consumption of FIX Replacement Therapy**

During the  $\geq 6$ -month lead-in period, subjects used a mean (SD) of 257,338.8 (149,013.1) IU/year (range: 83,541 to 755,892 IU/year) of FIX replacement therapy. Use of FIX replacement therapy decreased between Month 7 to 12 and Month 13 to 18 of the posttreatment period, with subjects using a mean (SD) of 8399.1 (29,720.9) IU/year (range: 0 to 156,536 IU/year) and 8486.6 (28,770.2) IU/year (range: 0 to 180,618 IU/year), respectively. Consumption of FIX replacement therapy was significantly lower following treatment with AMT-061 after the first year (i.e., between Month 7 and 18) following establishment of stable FIX expression as compared to standard of care during the lead-in period, with a mean FIX consumption decrease of 248,825.0 IU/year ( $p < 0.0001$ ).

## **Correlation of Annualized Bleeding Rate (ABR) and FIX Activity**

The relationship between estimated ABR and mean FIX activity across the Month 7 to 18 post-treatment period was explored with a generalized additive model assuming negative binomial distribution of bleeding episode count. Limited data suggested a non-linear relationship between ABR and FIX activity with a shallow downward trend of ABR with an increase in FIX activity but higher uncertainty in ABR toward higher FIX activity.

**Reviewer comments:** No clear relationship is established between FIX activity and ABR presumably due to the observed higher FIX activity (> 12%) in over 90% of the subjects and low frequency of bleeding events.

## **Vector DNA Viral Clearance/Shedding**

The time of maximum levels of vector DNA (Tmax) in blood was observed between 4 to 7 hours with mean Cmax of  $2.2 \times 10^{10}$  copies/mL following AMT-061 administration (Table 7). A subject was no longer shedding vector DNA if they had a negative laboratory result for 3 or more consecutive time points. Clearance of vector DNA from blood, indicating the absence of shedding, was confirmed in 25 subjects (46.3%) in the post-treatment period. Median time to vector clearance in blood was not achieved at the time of the 18-month follow-up. Individual subject plots of AAV vector DNA in blood (copies/mL) over time (from AMT-061 administration to data cutoff) are provided in Figure 10.

The time of maximum levels of vector DNA in semen was observed between Weeks 5 to 27 with Cmax of  $3.9 \times 10^5$  following AMT-061 administration. Clearance of vector DNA from semen was confirmed in 33 subjects (61.1%) in the post-treatment period and the median time to vector clearance was 48 weeks (Table 7).

**Reviewer comments:** Over 50 % of subjects did not achieve vector clearance from blood, and a high inter-individual difference is noted in vector kinetic data at 18 months (Figure10). In response to a clinical pharmacology information request, the

applicant evaluated the dose-exposure response. The administered dose ranged from 116 - 338 x 10<sup>13</sup> gc (~3-fold) and a slight trend of increasing C<sub>max</sub> in blood with total dose was observed. However, the blood and semen C<sub>max</sub> varied by over 10-fold suggesting that other biological factors may contribute to variability of vector DNA shedding. Also, as part of the 120-day safety update, the applicant provided additional blood and semen viral vector data. The following are a summary of the blood and semen vector kinetic data based on response to clinical pharmacology IR#3:

- 30 out of 54 subjects (56%) tested below LOD for vector DNA in blood at 3-consecutive time at 2-years.
- On the latest assessment (~2.5 years) 53 out of 54 subjects have at least one measurement below LOD. This needs to be confirmed by two additional assessments.
- 37 out of 54 subjects (69%) tested below LOD for vector DNA in semen at 3-consecutive time at 2.5-years.
- The applicant agreed to collect further samples from the remaining subjects who have not yet achieved 3 consecutive negative vector DNA as part of the 5-year post-dose study period.

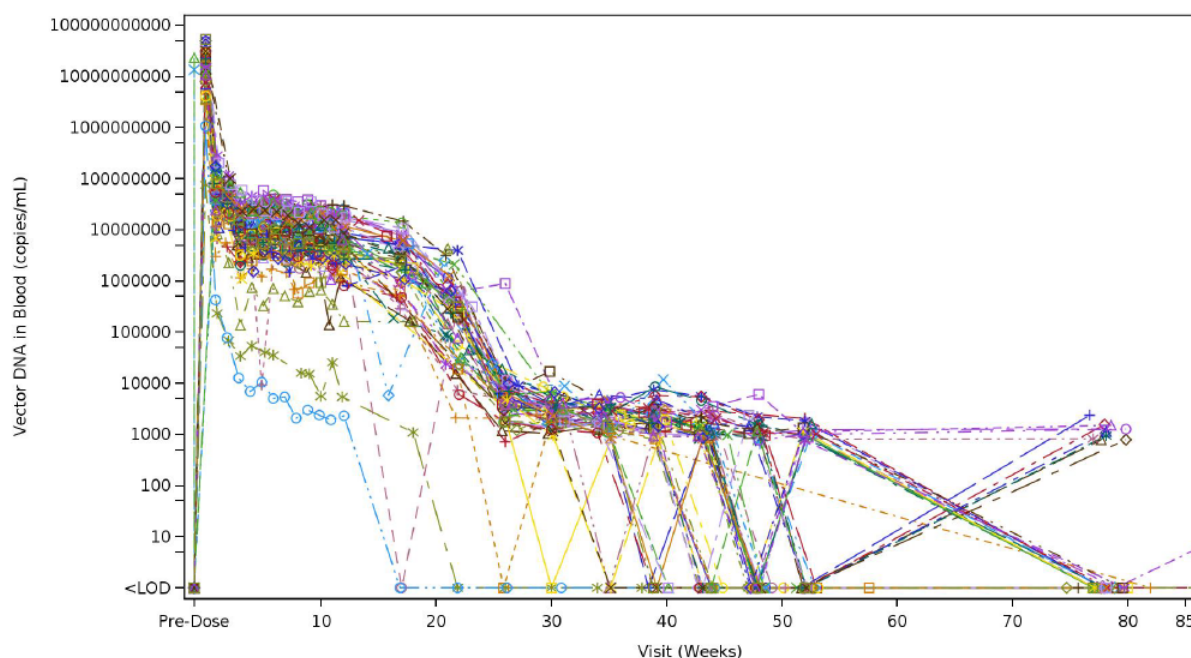
**Table 7: Summary Statistic of Vector DNA Shedding Parameters in Blood and Semen Following Administration of  $2 \times 10^{13}$  gc/kg of HEMGENIX**

| Parameter                                                                                | Blood<br>(n=53)                               | Semen<br>(n=41)                         |
|------------------------------------------------------------------------------------------|-----------------------------------------------|-----------------------------------------|
| Cmax (copies/mL)                                                                         | $2.20 \times 10^{10} \pm 1.45 \times 10^{10}$ | $3.86 \times 10^5 \pm 5.95 \times 10^5$ |
| Tmax <sup>a</sup> (days)                                                                 | 0.17 (0.13, 0.28)                             | 42.0 (38.9, 209)                        |
| Clast (copies/mL)                                                                        | 1293 $\pm$ 488                                | 107,232 $\pm$ 336462                    |
| Proportion of patients with 3 consecutive negative shedding (n/total N (%)) <sup>b</sup> | 25/54 (46.3)                                  | 33/54 (61.1)                            |
| Time to first negative shedding <sup>a,b</sup> (Weeks)                                   | 45 (17, 49)                                   | 30.6 (6, 52)                            |

Cmax corresponds to highest shedding observed; Clast corresponds to lowest shedding observed; Tmax corresponds to the time of highest shedding observed  
Cmax, Tmax and Clast parameters were calculated based on vector DNA values >LOD (760 copies/mL for blood and 800 copies/mL for semen)

<sup>a</sup> presented as median (min, max). <sup>b</sup> Calculated relative to the total number of subjects in the study (N=54).

**Figure 10: Individual Plot of AAV Vector DNA in Blood (copies/mL) Over Time - From IMP to Cut-Off for Safety Population)**



Source: CSR; Figure 3.5.2

## Immunogenicity Assessment

### Anti-FIX Antibodies and FIX Inhibitors:

The majority (53/54 [98.1%]) of subjects tested negative for anti-FIX antibodies prior to dosing (at the baseline assessment) and at Month 12 post-AMT-061 treatment. One subject tested positive prior to dosing and periodically during the study post-AMT-061 treatment to Month 6; the subject had a FIX activity level of 8.4% at Month 6 and 11.4% at both Month 12 and Month 18 post AMT-061 treatment. Levels of FIX inhibitors were <LOD (Nijmegen-Bethesda units [NBU]/mL = 0.415) for all (54/54 [100.0%]) subjects prior to dosing (at the baseline assessment) and remained so through to Month 12 post-AMT-061 treatment (**Table 3.2.1**).

Reviewer comments: It is not clear if pre-existing anti-FIX antibodies results in the observed lower FIX activity of 8.4%.

### **AAV5 Antibodies:**

Levels of anti-AAV5 NAb were <LOD (titer = 7) for 33/54 (61.1%) subjects prior to dosing (at the baseline assessment) and ≥LOD for 21/54 (38.9%) subjects (median titer: 56.9; range: 9 to 3212). When the subject with the titer >3000 was removed from the analysis, pre-dose titers ranged between 9 to 678. By Week 3 post-AMT-061 treatment, anti-AAV5 NAb levels were ≥LOD for all (53/53 [100%]) subjects with the median titer of 8748.0 which is the upper limit of quantification. The Nab levels remained elevated through Month 12 post-treatment.

At Month 6 post-treatment with AMT-061, FIX activity was 35.9% and 40.6% for subjects with and without pre-existing anti-AAV5 NAb, respectively. At Month 12 post-treatment, FIX activity was 35.5% and 44.8% for subjects with and without pre-existing anti-AAV5 NAb, respectively. A linear regression correlative analysis was conducted between baseline anti-AAV5 NAb versus Month 18 FIX activity. The linear regression indicated a trend for lower mean FIX activity in subjects with anti-AAV5 NAb at baseline (Figure 11).

Levels of IgG anti-AAV5 antibodies were <LOD (titer = 50) for the majority (41/54 [75.9%]) of subjects prior to dosing (at the baseline assessment) and ≥ LOD for 13/54 (24.1%) subjects (median titer: 366.0; range: 52 to 8081). By Week 3 post-AMT-061 treatment, levels of IgG anti-AAV5 antibodies were ≥LOD for all (53/53 [100.0%]) subjects (median titer: 43,384.0; range: 5121 to 109,350) and remained elevated through to Month 12 post-treatment.

Levels of IgM anti-AAV5 antibodies were <LOD (titer = 50) for the majority (53/54 [98.1%]) of subjects prior to dosing (at the baseline assessment) and ≥LOD for 1 subject. By Week 3 post-AMT-061 treatment, levels of IgM anti-AAV5 antibodies were ≥LOD for the majority (51/53 [96.2%]) of subjects (median titer: 21,145.0; range: 205 to 109,350). The proportion of subjects with detectable levels of IgM anti-AAV5 antibodies continuously declined from Week 3 to Month 12. At Month 12, 10/53 (18.9%) subjects had levels ≥LOD (median titer: 588.0; range: 305,5215).

**Reviewer comments: The applicant analysis showed a trend for lower FIX activity with subjects that have pre-existing anti-AAV antibodies. However, the applicant's**

initial analysis was only based on a threshold for anti-AAV5 NAb using 1:7 titer (i.e., LOD). We requested an analysis on additional selected thresholds (e.g., 1:10, 1:50, 1:100 and 1:350; Table 8). When the target threshold of 1:350 titer level is selected, the number of subjects with positive anti-AAV5 NAb were limited (n=3 subjects) and the FIX activity is lower by 36%. It should be noted that only one subject had a titer > 700 and the FIX activity for this subject was 1.5%. This reviewer conducted an additional analysis by including one subject with high NAb and all subjects with available FIX activity data. The reviewer analysis is summarized as follows:

- The mean FIX activity at Month 12 was  $42 \pm 22$  % in subjects with NAb titer  $\leq 1:100$  (n=46) and was  $26 \pm 20$ % in subjects with NAb titer  $>1:100$  (n=8).
- The mean FIX activity at Month 12 was  $41 \pm 22$ % in subjects with NAb titer  $\leq 1:350$  (n=49) and it was  $22 \pm 17$ % in subjects with NAb titer  $>1:350$  (n=5).

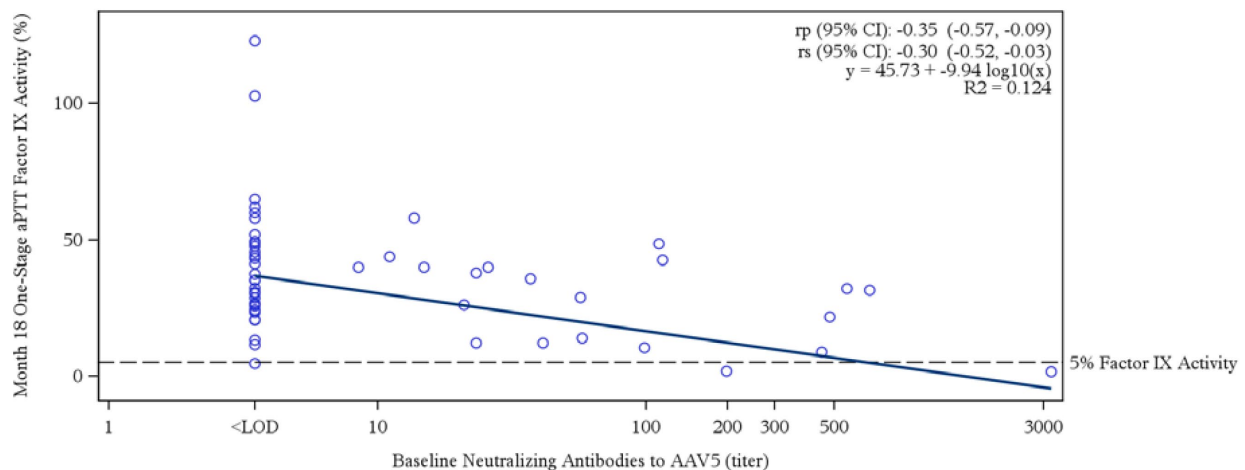
Overall, there are limited number of subjects who have baseline anti-AAV5 NAb above 350 and there is a trend of lower FIX activity with pre-existing anti-AAV5 NAb with higher cutoff values. Note that the clinical trial assay that was utilized is not validated and CDRH review identified issues with the assay. As such, these data should be interpreted cautiously.

**Table 8: Mean Uncontaminated FIX Activity (%) at Month 12 Post-treatment by Baseline Anti-AAV5 NAb Titer**

| Anti-AAV5 NAb Status      | 1:10                 | 1:50                 | 1:100                | 1:350                |
|---------------------------|----------------------|----------------------|----------------------|----------------------|
| Negative ( $\leq$ cutoff) | 45 $\pm$ 23,<br>n=33 | 44 $\pm$ 22,<br>n=42 | 42 $\pm$ 22,<br>n=45 | 42 $\pm$ 22,<br>n=49 |
| Positive ( $>$ cutoff)    | 34 $\pm$ 17,<br>n=16 | 30 $\pm$ 16,<br>n=8  | 35 $\pm$ 17,<br>n= 5 | 27 $\pm$ 17,<br>n= 3 |
| % Mean change             | 24                   | 32                   | 17                   | 36                   |

Source: Applicant response to clinical pharmacology IR#2

**Figure 11: Display of Central Laboratory FIX Activity (%) from the One-stage (aPTT-based) Assay at Month 18 Post-treatment by Baseline anti-AAV5 NAb (titer) with Linear Regression (Full Analysis Set)**



Source: **Figure 2.3.1.1.1**



### **AAV5 Capsid-specific T-cell Response:**

The majority (32/36 [88.9%]) of subjects with interpretable results did not have specific AAV5 capsid T-cell response prior to dosing (at the baseline assessment). The number of subjects who developed a specific AAV5 capsid T-cell response varied during the study, reaching the highest number at Week 6 post-AMT-061 treatment (15/38 [39.5%] subjects). There were missing data due to issues related to insufficient number of cells and nonconformance in the analysis. Overall, 39/54 subjects (72%) had at least one visit with a specific T-cell response. Uncontaminated FIX activity ranged between 6.0 and 97 % of normal for visits where subjects also had a specific T-cell response. Specific T-cell responses concurrent with TEAEs of ALT and/or AST increased were noted for 6 subjects and concurrent with corticosteroid treatment in 2 subjects. Review of specific AAV5 capsid T-cell responses did not identify a correlation with other clinically relevant findings.

**Reviewer comments: It is difficult to interpret the results of the current cellular T-cell response against AAV5 capsid due to high variability and missing data. The applicant may consider further standardization of the assay and conduct of mechanistic correlative assessments between levels of transaminases and T-cell response.**

### **Inflammatory Markers**

The levels of IL-1 $\beta$ , IL-6, and MCP-1 were generally unchanged by AMT-061 treatment. Initial elevations were noted with IL-2 and IFN $\gamma$  levels following AMT-061 treatment but was returned to pre-treatment levels between Week 2 and 12. Levels of IL-2 were <LLOQ (0.72 ng/L) for the majority (53/54 [98.1%]) of subjects prior to dosing (at the baseline assessment) and one subject had levels  $\geq$ LLOQ with a value of 1.320 ng/L. At Week 1 post-AMT-061 treatment, 11/52 (21.1%) subjects had IL-2 levels  $\geq$ LLOQ, with a mean (SD) value of 2.821 (2.803) ng/L (range: 0.78 to 9.04 ng/L). The proportion of subjects with IL-2 levels  $\geq$ LLOQ decreased to 1 to 2 subjects between Week 2 and Week 12. From Month 4 on, IL-2 levels were <LLOQ for all subjects.

Levels of IFN $\gamma$  were  $\geq$ LLOQ (2.86 ng/L) for the majority (44/54 [81.5%]) of subjects prior to dosing (at the baseline assessment) with a mean (SD) value of 7.516 (6.696) ng/L (range: 2.96 to 45.33 ng/L). At Week 1 post-AMT-061 treatment, all subjects (52/52 [100.0%]) had IFN $\gamma$  levels  $\geq$ LLOQ. The proportion of subjects with IFN $\gamma$  levels  $\geq$ LLOQ decreased from Week 2 to Week 8, and the number of subjects with levels  $\geq$ LLOQ ranged between 43 and 47 subjects from Month 4 to Month 12. At Month 12, 45/53 (84.9%) subjects had IFN $\gamma$  levels  $\geq$ LLOQ, with a mean (SD) value of 5.801 (3.316) ng/L (range: 2.95 to 17.94 ng/L).