Protocol Title: Weight-based Dosing in Hemophilia A: A randomized, controlled, open-label, crossover trial to measure factor VIII recovery following factor VIII concentrate dosing based on total body weight, ideal body weight, and lean body mass

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Abstract

The increasing prevalence of obesity has not spared the hemophilia population with rates reflecting those of the general population. Obesity alters the pharmacokinetic properties of many drugs making it difficult to determine the dose to use when administering medications based on body weight. Under-or overdosing of clotting factor concentrates in hemophilia may result in catastrophic bleeding or heart attack and stroke, respectively. Alternative descriptors of body weight, such as lean body mass (LBM) and ideal body weight (IBW) are sometimes considered in these situations. It has been demonstrated that when recombinant factor VIII (rFVIII) is dosed according to total body weight (TBW) in overweight and obese hemophiliacs, FVIII recovery values are much greater than the expected 2 IU/dl per IU/kg. This value is only valid if the plasma volume is assumed to be 0.5 dL/kg; however, this assumption does not hold true in individuals whose weight and height differ markedly from the norm. These findings suggest the need for alternative methods of rFVIII dosing in individuals whose morphometric characteristics differ from the ideal and, further, suggest we are overdosing hemophilia A patients, which, considering the high price of rFVIII concentrates, is an economic issue contributing to rising healthcare costs.

We propose a single center, randomized, controlled, open-label, crossover trial to determine if rFVIII dosed according to LBM and IBW achieves a targeted FVIII recovery with better precision than based on TBW. We hypothesize the use of LBM and IBW to determine the dose of rFVIII necessary to attain a desired FVIII recovery of 2 +/- 0.2 IU/dl per IU/kg (100 +/- 10%) in overweight and obese (body mass index greater than or equal to 25 mg/m^2), adult males (age 18 or older) with hemophilia A (FVIII activity 40% or less) will result in a 50% greater proportion of subjects within this range when compared to TBW. Eligible patients receiving care at the Hemophilia Center of Western Pennsylvania (HCWP) will be enrolled during clinic visits. Following enrollment and completion of screening assessment, subjects will present to HCWP for three study visits with each study visit occurring on successive weeks. Subjects will not have received any rFVIII for a period of at least 72 hours prior to each study visit. Recombinant FVIII infusion based on TBW, LBM, or IBW will take place during each study visit, and the order will be determined by randomization. During each study visit, FVIII levels will be assessed by obtaining blood samples before and at 10 and 30 minutes and 1 hour after infusion. Outcomes include the proportion of subjects achieving a desired peak FVIII recovery value of 2 +/- 0.2 IU/dl per IU/kg (100 +/- 10%) at 10 minutes following infusion of rFVIII dosed according to LBM and TBW, IBW and TBW, and LBM and IBW. We will use mixed effects logistic regression to investigate the effect of using different weight-based dosing methods on attaining target FVIII levels. In conclusion, if alternative weight-based FVIII dosing achieves acceptable FVIII recovery, the findings would be noteworthy and practice-changing, resulting in significant healthcare cost savings.

1.0 Background

1.1 Obesity and Hemophilia

Obesity in the United States has reached epidemic proportions resulting in a major public health crisis. Current estimates reveal more than 35% of adults to be obese with the prevalence increasing steadily over the past few decades.[1] Obesity is responsible for a number of health problems, including cardiovascular disease, hypertension, hyperlipidemia, type II diabetes mellitus, and osteoarthritis among many others.[1] Medical costs related to obesity account for 10% of all medical spending and total approximately $147 billion per year.[2] Obesity is defined as a body mass index (BMI) greater than or equal to 30 mg/m^2.[1] BMI is calculated as follows: [(weight in kg)/(height in m^2)].[1] The increased frequency of obesity in hemophilia has been documented. The CDC found 23.5% of hemophilia patients 20 years of age or older in the United States were obese.[3] Individuals with hemophilia are at risk for the same obesity related comorbidities as those in the general population, such as cardiovascular disease, hypertension, hyperlipidemia, etc.[3] Additionally, obesity in hemophilia increases the risk of hemophilic joint disease, which leads to chronic arthritis, joint replacement surgeries, and lifelong disability.[3]

1.2 Obesity and the Pharmacokinetics of Drug Therapy

Obesity may significantly alter the pharmacokinetic properties of drugs, particularly volume of drug distribution and clearance, and is concerning, especially when administering weight-based medications with a narrow therapeutic index or high cost.[4] Alternative descriptors of body weight have been used with a number of medications, including aminoglycosides, antineoplastic agents, and general anesthetics, in obesity.[4] Two such methods are ideal body weight (IBW) and lean body mass (LBM). IBW is calculated in males as follows: [(50 kg) + (2.3 kg x height in inches > 60)].[5] LBM is calculated in males as follows: [(9.27 x 10^3 x TBW in kg)]/[(6.68 x 10^3) + (216 x BMI)].[6] It is not known if LBM or IBW, or some other descriptor of body weight, such as adjusted body weight, is more precise in determining drug dose when administering weight-based therapies.[7] What is known is that while there is no single best descriptor for describing all of a drug's pharmacokinetic properties in obesity, certain descriptors fare better than others in certain situations.[7] Total body weight (TBW) appears to be more appropriate when describing volume of distribution, particularly important with moderate to highly lipophilic drugs, and LBM appears to be more accurate when describing clearance, significant in chronically-dosed drugs.[7]

1.3 Pharmacokinetics of FVIII concentrates

Hemophilia A is an inherited X-linked recessive disease resulting from deficiency of clotting factor VIII (FVIII) which manifests as spontaneous and/or traumatic bleeding involving joints, muscles, mucocutaneous surfaces, and the central nervous system.[8] The prevention and treatment of bleeding in hemophilia A is accomplished by the intravenous infusion of FVIII concentrates.[8] Dosing is weight-based, and the calculation used to determine FVIII dosing is as follows: [(weight in kg x desired FVIII increase in IU/dL)/(2)] where 2 is the expected FVIII recovery value in IU/dL per IU/kg.[9] Ingram first validated this formula in 1981 after Rizza observed in 1976 that the following calculation resulted in an expected FVIII recovery value of 2: [weight in kg x observed FVIII recovery in IU/dL)/(dose in IU)].[10, 11] It is not known if FVIII dosing should continue to be based on TBW in overweight and obese individuals. Recent studies have shown that the observed FVIII recovery value is dependent on TBW with one study reporting median FVIII recovery values of 2.30 and 2.70 in patients with a BMI of 25-29 and 30 mg/m^2 or higher, respectively, using current TBW-based dosing.[9] Similarly, another study reported median FVIII recovery values of 2.18 and 2.68 in patients with a BMI of 25-29 and 30 mg/m^2 or higher, respectively.[12] These results differ from the expected FVIII recovery value of 2. Ingram cautioned this value was only valid if the patient's plasma volume is assumed to be 0.5 dL/kg, with approximately 85% of FVIII confined to the intravascular space in the steady state.[10, 13] This assumption, however, does not hold true in individuals whose weight and height differ markedly from the norm.[9, 13] Plasma volume correlates well with lean body mass but does not vary proportionally with extremes in weight.[14, 15] Adipose tissue, which accounts for the majority of increased TBW in obese individuals, is less vascular, therefore, contains less plasma

volume, than lean body mass.[14, 15] Moreover, 20-40% of the excess TBW in obesity is due to supporting tissue, e.g. muscle and connective tissue, which contains more plasma volume than adipose tissue alone.[14, 15] Thus, obese patients are expected to have less plasma volume than their average weight counterparts.[14, 15]

The above findings support alternative methods of FVIII dosing in individuals whose morphometric characteristics differ from the ideal, i.e. overweight and obese individuals, and suggest among this group, the dose given may exceed what is required, resulting in an unjustified higher cost, as compared with non-obese individuals.

1.4 Preliminary Information

We performed a retrospective pharmacokinetic analysis of hemophilia A patients administered recombinant FVIII (rFVIII) based on IBW, which showed peak levels and half-life comparable to standard dosing based on TBW (Table 1).[16] One subject, with a BMI of 45.8, required an additional 25% of rFVIII to achieve an adequate peak FVIII level. It is hypothesized that LBM-based dosing would have been more accurate in this subject, and all morbidly obese patients because it accounts for weight due to supporting tissue rather than assuming excess weight is solely due to adipose tissue.

Table 1. Pharmacokinetic analysis of rFVIII utilizing IBW based dosing in hemophilia.

	BMI	TBW based rFVIII dose	IBW based rFVIII dose	Peak FVIII level
Patient 1	45.8	7550	3800	0.78
Patient 2	30.0	4550	3535	0.97
Patient 3	33.8	5000	3350	1.04
Patient 4	31.5	5750	4225	1.23
Patient 5	35.1	5900	3900	1.06
Patient 6	39.7	6100	3535	0.97

Given these results, along with the changes in plasma volume that occur in obesity, coupled with Ingram's findings, and the fact that rFVIII is predominantly confined to the intravascular space, we hypothesize that LBM and IBW are better descriptors of body weight than TBW when determining rFVIII dosing in overweight and obese patients with hemophilia A.

We, therefore, propose a single-center, randomized, controlled, open-label, crossover trial to determine if rFVIII dosing based on LBM and IBW achieves a targeted FVIII recovery, 2 +/- 0.2 IU/dl per IU/kg (100 +/- 10%), with better precision than based on TBW in overweight and obese, adult males with hemophilia A (FVIII activity 40% or less). We hypothesize using LBM and IBW to determine the dose of rFVIII necessary to attain a desired FVIII recovery of 2 +/- 0.2 IU/dl per IU/kg (100 +/- 10%) in overweight and obese, adult males with hemophilia A will result in a 50% greater proportion of subjects within this range when compared to TBW.

2.0 Significance

The prevalence of obesity in the general population has increased significantly over the course of the past few decades with greater than 1/3 of the United States adult population considered obese.[1] The obesity epidemic has not spared the hemophilia population with obesity affecting close to 25% of adults in the United States.[3] Obesity may significantly alter the pharmacokinetic properties of drugs.[4] FVIII concentrates are dosed according to TBW and calculated as follows: [(weight in kg x desired FVIII increase in IU/dL/(2)] where 2 is the expected FVIII recovery value in IU/dL per IU/kg and considered valid only when the plasma volume is assumed to be 0.5 dL/kg.[9] However, plasma volume does not increase proportionally with TBW as a result of the decreased vascularity of adipose tissue, so plasma volume per kg of body weight in obesity is less than that observed in normal weight individuals.[14, 15] Recent studies have shown that current FVIII concentrate dosing in overweight and obese individuals results in FVIII recovery values greater than desired suggesting that current dosing may exceed required levels for treatment of bleeds, surgery, or trauma in individuals with hemophilia A.[9, 12]

If dosing exceeds requirement in hemophilia A, there are significant economic implications. The current standard of care for the treatment of hemophilia A in the United States is rFVIII, for which the average

wholesale cost is $1 per unit infused, which for a 70 kg male on prophylaxis, is several hundred thousand units and several hundred thousand dollars annually.[17] One United States center estimated a monthly cost savings of $120,000 if 20 overweight and/or obese pediatric patients receiving prophylactic therapy were dosed using IBW rather than TBW, which equates to an approximate $1.5 million cost savings annually.[17] If alternative weight-based FVIII dosing achieves acceptable FVIII recovery, the findings would be noteworthy and practice-changing, resulting in significant healthcare cost savings.

3.0 Objectives

3.1 Primary Objective

To demonstrate that rFVIII dosed according to LBM achieves a targeted FVIII recovery, 2 +/- 0.2 IU/dl per IU/kg (100 +/- 10%), with better precision than based on TBW in overweight and obese (body mass index greater than or equal to 25 mg/m^2), adult males (age 18 or older) with hemophilia A (FVIII activity 40% or less).

Primary hypothesis: The use of LBM to determine the dose of rFVIII necessary to attain a desired FVIII recovery of 2 +/- 0.2 IU/dl per IU/kg (100 +/- 10%) in overweight and obese, adult males with hemophilia A will result in a 50% greater proportion of subjects within this range when compared to TBW.

3.2 Secondary Objective

To demonstrate dosing rFVIII based on IBW achieves a targeted FVIII recovery, 2 +/- 0.2 IU/dl per IU/kg (100 +/- 10%), with better precision than based on TBW in overweight and obese (body mass index greater than or equal to 25 mg/m^2), adult males (age 18 or older) with hemophilia A (FVIII activity 40% or less).

Secondary hypothesis: Determining the rFVIII dose necessary to achieve a desired FVIII recovery of 2 +/- 0.2 IU/dl per IU/kg (100 +/- 10%) in overweight and obese, adult males with hemophilia A utilizing IBW will result in a 50% greater proportion of subjects within this range when compared to TBW.

4.0 Selection and Enrollment of Subjects

4.1 Inclusion criteria

The following criteria are required for study inclusion:

1. Adult males age 18 or older.

2. Hemophilia A (FVIII activity 40% or less).

3. Overweight or obesity defined as a BMI of 25.0-29.9 and ≥ 30 mg/m^2, respectively.

4.2 Exclusion criteria

Presence of any of the following criteria will result in study exclusion:

1. Prior history of, or currently detectable, FVIII inhibitor defined as greater than or equal to 0.6 Bethesda Units (BU); however, a subject with a past low-level non-responding inhibitor defined as less than 5 BU, with no increase in titer following FVIII exposure, and not detectable within 12 months of the study, despite FVIII exposure during that period, will be allowed to enroll on study.

2. Allergy to FVIII products

3. Current rFVIII requirements do not include at least a 72-hour period without rFVIII administration

4.3 Enrollment

All subjects will be enrolled at the Hemophilia Center of Western Pennsylvania (HCWP). We aim to enroll 24 subjects over two years (12 per year). Potentially eligible patients will be approached to determine interest in study participation during clinic visits at HCWP. If a patient is interested in the study, he will discuss the study in further detail during the screening visit, which does not need to be a separate visit if the patient is already at HCWP for clinic visit or another reason. Discussion will include the purpose, protocol specifics as they relate to the patient, potential adverse effects, and risks and benefits of the study. Each potential subject

will be encouraged to ask all questions that he feels are necessary to enable him to make an informed decision regarding participation in the study. Each potential subject will be encouraged to take the time needed for thoughtful consideration whether or not to participate in the study. Subjects who read the consent form are free to refuse enrollment. The principle investigator (PI) or co-investigator will obtain informed consent. No experimental interventions will occur until after informed consent is obtained. If any new information occurs during the conduct of the study, subjects who have been consented will be informed and will be re-consented with this information at the next study visit.

The PI or a member of the research staff will conduct subject enrollment and screening. Subjects will be considered enrolled in the study after informed consent is obtained. Once enrolled, each subject will receive a unique subject identification number (i.e. PK001, PK002, etc.). No subject may begin the study prior to enrollment and assignment of a unique subject identification number. Screening assessment will be completed following enrollment. All individuals receiving a unique subject identification number will have any reason for study non-participation documented. Any subject identification numbers that are assigned will not be reused even if the subject does not receive treatment.

Study participants will be free to withdraw at any time. All data collected prior to the time of withdrawal will be analyzed, but no additional information will be collected. Processed blood sample results will continue to be used; however, remaining samples will be destroyed or used as indicated by the subject. The reason and date of study withdrawal for all subjects will be recorded.

5.0 Randomization

Subjects will undergo randomization within 24 hours of enrollment and completion of screening assessment. Randomization is for the order of TBW, LBM, and IBW dosing for each subject. Permuted block randomization will be performed by the Center for Research on Healthcare Data Center (CRHC DC) web-based data entry system. Subjects will be randomized to 1 of 6 possible dosing scenarios based on 3 different weight-dosing regimens being evaluated (TBW, LBM, and IBW) (Table 2).

Table 2. Randomization Scenarios.

	1st Week (study visit 1)	2nd Week (study visit 2)	3rd Week (study visit 3)
Scenario 1	TBW	LBM	IBW
Scenario 2	LBM	IBW	TBW
Scenario 3	IBW	TBW	LBM
Scenario 4	TBW	IBW	LBM
Scenario 5	LBM	TBW	IBW
Scenario 6	IBW	LBM	TBW

6.0 Study Intervention

Recombinant FVIII concentrate is an FDA approved, and both efficacious and safe, therapy for the treatment and prevention of bleeding in hemophilia A. Given the preliminary data described above, standard of care at HCWP now includes pharmacokinetic testing to determine which weight-based dosing method achieves targeted FVIII recovery with the greatest precision in overweight and obese patients with hemophilia A; therefore, each subject will use his own rFVIII for the purposes of the study. Recombinant FVIII includes all recombinant FVIII brand products. Subjects will receive rFVIII during each of three study visits occurring on successive weeks and will not have received rFVIII for a period of at least 72 hours prior to each study visit (Table 3). Potential subjects whose current rFVIII requirements do not include at least a 72-hour period without rFVIII administration will not be eligible for study participation until rFVIII is being given in a routine, which includes a 72-hour period without rFVIII treatment. If at anytime during the study, rFVIII is necessary for non-research purposes, the next study visit will be postponed until at least 72 hours following the last non-research related rFVIII treatment. Study participation will not limit the amount of rFVIII necessary for clinical purposes.

Recombinant FVIII will be administered according to current FDA approved indications and dosing. The rFVIII infusion dose will be calculated as follows: [(weight in kg x desired FVIII increase of 100 IU/dL)/(2)]. TBW will be used to determine weight in kg, or it will be determined using LBM or IBW (see Appendix 1 for details

regarding BMI, LBM, and IBW calculations). Subjects whose FVIII recovery is less than the desired 2 +/- 0.2 IU/dl per IU/kg will not receive additional rFVIII as this is not done in routine clinical practice outside of active bleeding or bleeding prophylaxis prior to surgery. The HCWP nurse, per standard of care, will record details of the storage, lot number, stability, production, and expiration dates. A member of the research staff will ensure documentation of date, time, and amount of rFVIII infusion administered are recorded for data submission.

7.0 Clinical and Laboratory Evaluations

Eligible patients receiving care at HCWP will be enrolled during clinic visits. Following enrollment, completion of screening assessment and randomization, subjects will present to HCWP for three study visits with each study visit occurring on successive weeks (Table 3). Subjects will not have received any rFVIII for a period of at least 72 hours prior to each study visit. Recombinant FVIII infusion based on TBW, LBM, or IBW will take place during each study visit, and the order will be determined by randomization (Table 2). The HCWP nurse will perform rFVIII infusion over 5 to 10 minutes using a winged (butterfly) infusion needle. One-stage trough, peak, and recovery FVIII levels will be obtained on 0.5 ml citrate blood samples drawn before infusion and 10 minutes (+/- 1 minute), 30 minutes (+/- 5 minutes), and 1 hour (+/- 5 minutes) after infusion. If at anytime during the study, rFVIII is necessary for non-research purposes, the next study visit will be postponed until at least 72 hours following the last non-research related rFVIII treatment.

A member of the research staff will collect all data (Table 3). During the screening visit, FVIII inhibitor status will be assessed and based on routine comprehensive clinic anti-FVIII titer within the last 12 months. If no anti-FVIII titer has been performed during that time period, FVIII inhibitor status will be assessed during the screening visit using the Bethesda assay for which one 5 ml blood sample will be obtained when applicable. Also, documentation of an allergy to FVIII products will be obtained from the subject's medical record. Subjects meeting exclusion criteria during screening visit will return for study visits 1, 2, and 3. During study visit 1, weight and height will be recorded followed by calculation of BMI, TBW, LBM, and IBW (see Appendix 1). During each study visit, one-stage trough, peak, and recovery FVIII levels will be obtained on 0.5 ml citrate blood samples drawn before and 10 minutes (+/- 1 minute), 30 minutes (+/- 5 minutes), and 1 hour (+/- 5 minutes) after infusion. All blood samples will be obtained by a research nurse with a maximum anticipated volume of 2 ml needed for each visit, which will be sent to the Institute for Transfusion Medicine Coagulation Laboratory in Pittsburgh, PA. During each study visit, adherence will be measured by the proportion of FVIII levels obtained (12 FVIII levels are needed – 4 per study week x 3 study weeks).

Table 3. Study Calendar.

	Screening Visit	**TBW Week** **Study Visit 1**	**LBM Week** **Study Visit 2**	**IBW Week** **Study Visit 3**
FVIII inhibitor status assessed	X			
Allergy to FVIII products recorded	X			
Height and weight recorded		X		
BMI, TBW, LBM, and IBW calculated		X		
rFVIII infused according to TBW		X		
rFVIII infused according to LBM			X	
rFVIII infused according to IBW				X
FVIII level checked prior to rFVIII infusion		X	X	X
FVIII level checked 10 minutes (+/- 1-minute following rFVIII infusion		X	X	X
FVIII level checked 30 minutes (+/- 5 minutes) following rFVIII infusion		X	X	X
FVIII level checked 1 hour (+/- 5 minutes) following rFVIII infusion				
Adherence recorded		X	X	X
AEs recorded		X	X	X

8.0 Adverse Events

All subjects enrolled in the study and having received any FVIII concentrate infusion will be included in the safety evaluation and all adverse events (AEs) will be recorded. Potential AEs include risk of blood drawing, including bleeding; allergic reaction; FVIII inhibitor development; and thrombosis.

1. Blood drawing

There may be discomfort, which is common, with drawing blood, which may include pain, lightheadedness, dizziness, syncope, ecchymosis, bleeding, or infection in the tissue surrounding the vein. Appropriate medical intervention may consist of applying pressure to resolve bleeding or antibiotics in the setting of infection.

2. Allergic reaction

Allergic reactions are rarely, if ever, reported with the use of rFVIII. Adverse events will be classified using the Common Terminology Criteria for Adverse Events (CTCAE) system, volume 4.03, 2010.[18] Allergic type symptoms may include fever, chills, pruritis, rash, urticaria, paresthesias, chest pain, dyspnea, wheezing, nausea, vomiting, edema, tachycardia, hypotension, anaphylaxis, or death. Subjects will be monitored for the development of these symptoms. If these symptoms occur, and are felt to be the result of rFVIII, the infusion will be discontinued, and appropriate medical interventions will be administered. Anaphylaxis will result in study exclusion. The decision regarding study exclusion in less severe, or minor, allergic reactions will be at the discretion of the PI.

3. FVIII inhibitor

FVIII inhibitors develop in up to 25% of individuals with severe hemophilia A, usually occurring during the first 20 exposures, in childhood, with the incidence decreasing substantially after 150 treatment days and rarely, if ever, occurs among highly exposed adults.[19, 20] Thus, there is a rare possibility of FVIII inhibitor development. Subjects will be monitored closely and if FVIII inhibitor development is suspected, assessment will be performed with the Bethesda assay. If FVIII inhibitor development does occur, the subject will be excluded from further study participation and appropriate medical treatment will be provided.

4. Thrombosis

FVIII concentrates are rarely, if ever, associated with the development of thrombotic complications. Subjects will be monitored closely and if thrombosis is suspected, the appropriate clinical evaluation will be performed. If thrombosis does occur, the subject will be excluded from further study participation, and appropriate medical treatment will be provided.

5. Bleeding

Because study subjects have hemophilia, there is a risk of bleeding. In the event that bleeding occurs at the infusion site, and cannot be stopped with pressure, it may be necessary to use an adhesive material to stop the bleeding. It is also possible that changing rFVIII schedule could cause bleeding. Additional rFVIII will be administered if this occurs. If bleeding is major, defined as a decrease in hemoglobin 2 grams per deciliter or more; transfusion of 2 or more units of red blood cells; or bleeding in a critical organ, such as intracranial, intraspinal, intraocular, pericardial, or retroperitoneal, the subject will be excluded from further study participation. If bleeding is minor, defined as all bleeding episodes that do not meet criteria for major bleeding, further study participation will be at the discretion of the PI.

6. Inadvertent Disclosure

There is a potential for disclosure or breach of confidentiality of collected data. In order to reduce these risks, research-related documents and clinical information stored in research files will be assigned an alphanumeric identifier. A linkage key for linking the assigned alphanumeric identifier with the subject's name will be stored in locked files at HCWP.

7. Potential benefits

Study participation may not result in any direct clinical benefit; however, it may provide data supporting the efficacy of rFVIII dosed according to LBM and/or IBW for the prevention and treatment of bleeding in overweight and obese hemophiliacs, which will lead to significant healthcare-related cost savings.

9.0 Statistical Considerations

9.1 Outcomes

9.1.1 Primary Outcome: The proportion of subjects achieving a desired peak FVIII recovery value of 2 +/- 0.2 IU/dl per IU/kg (100 +/- 10%) at 10 minutes following infusion of rFVIII dosed according to LBM and TBW.

9.1.2 Secondary Outcome: The proportion of subjects achieving a desired peak FVIII recovery value of 2 +/- 0.2 IU/dl per IU/kg (100 +/- 10%) at 10 minutes following infusion of rFVIII dosed according to IBW and TBW.

9.1.3 Secondary Outcome: The proportion of subjects achieving a desired peak FVIII recovery value of 2 +/- 0.2 IU/dl per IU/kg (100 +/- 10%) at 10 minutes following infusion of rFVIII dosed according to LBM and IBW.

The proportions above will be determined by dividing the number of subjects attaining the desired peak FVIII recovery value by the total number of subjects. This will be done for TBW, LBM, and IBW. FVIII recovery values will be calculated as follows: [(weight in kg x observed FVIII recovery in IU/dL)/(dose in IU)] and expressed as IU/dL per IU/kg.

9.2 Statistical Analysis

Preliminary analyses will focus on data checks for completeness and accuracy and address any issues with data quality. Descriptive summaries will be examined including the mean and standard deviations (or medians and quartiles for skewed distributions) for continuous variables and frequencies and proportions for categorical variables. Since the crossover design involves 6 sequence groups with 3 intervention groups and 3 periods, distributions of baseline characteristics and peak FVIII recovery value will be compared between the sequence groups to assess effectiveness of the randomization. Peak FVIII recovery value and the proportion achieving the target levels will be summarized by intervention group and by period. The primary outcome, attaining a peak FVIII recovery value of 2 +/- 0.2 IU/dl per IU/kg (100 +/- 10%) is binary. We will use mixed effects logistic regression to investigate the effect of using different weight-based dosing methods on attaining target FVIII levels. By having a random subject effect, this model would account for non-independence of observations due to the crossover design. In addition, it validly handles ignorable missing data, although dropout is expected to be minimal given the short duration of the study. Independent variables would include fixed intervention group effect and fixed period effect. Though we do not expect them to be significant, treatment by period interaction and carry-over effects would be included and tested for in the initial model. If these terms are significant, then adjusted intervention group effects will be reported. Appropriate linear contrasts will be constructed to compare TBW and LBM (Aim 1) and TBW and IBW (Aim 2) if the overall test for the intervention effect was found to be significant. Residual analyses will be conducted to identify sources of model misspecification, outliers, and possibly influential observations. As an exploratory aim, we will also determine if differences exist between LBM and IBW. Confidence intervals will be constructed and used for inference. All primary analyses for intervention group comparisons will use an intention-to-treat approach whereby all patients randomized are included in the analysis. All tests will be conducted at the 5% level.

We base our sample size justifications on computational techniques that match our study design and proposed analytical approach as much as possible within the constraints of already published methodologies (PASS 13. NCSS, LLC. Kaysville, Utah). Accounting for an estimated lost to follow-up of 5%, the effective sample size is 22.8 total or 3.8 patients per sequence group. Using an alpha level of 5% and a two-sided McNemar test to compare LBM and TBW (or IBW and TBW), a sample size of 3 patients per sequence group would provide at least 80% power to detect difference in proportion of 0.50 assuming that that the proportion of discordant pairs is 0.6. A risk difference of 0.50 is a conservative estimate based on prior studies.[3, 4] Since our actual analytical approach (mixed effects logistic model) is more precise, we will have higher power to detect this effect or the same power to detect a smaller effect. We propose to recruit a total of n=30 subjects (15 per year for two years) allowing for 3 screening failures per year.

10.0 Data Collection, Site Monitoring, and Adverse Event Reporting

10.1 Data Collection

The PI or co-investigator will obtain informed consent. The PI will maintain all study data. All data required by the study protocol will be captured and managed by electronic Case Report Forms (CRFs) via

web-based electronic data capture by the CHCR DC. All information will be stored on their server**.** Information will be stored in a secure database. No personal identifiers will be associated with the data. The University of Pittsburgh Research Conduct and Compliance Office may inspect records at any time.

Data collection will cease at the time of withdrawal from the study; however, data collected up to the point of withdrawal will continue to be used. Following the required data retention period, data will be stored for an indefinite period of time on the CRHC DC secure server in its de-identified state. Following completion of the study, linkage codes will be destroyed. Any paper documents will be secured in long-term retention for an indefinite period of time.

10.2 Data Safety Monitoring Plan (DSMP)

The Data Safety Monitoring Committee (DSMC) will meet regularly to review all aspects of the study. The DSMC is comprised of the research team at HCWP consisting of study investigators, research nurses, and regulatory coordinator. Subjects will be closely monitored to ensure subject safety and to that procedures are in place to maintain privacy and confidentiality, progress of the study, integrity of the data, procedure reviews, and for discussion of pertinent scientific literature or events which could affect the benefit to risk ratio. All serious and unexpected adverse events and/or major breaches of confidentiality will be reported to the IRB according to regulations outlined in the IRB *Reference Manual for the Use of Human Subjects*. The DSMC will determine if the risk benefit ratio is sufficiently favorable that it is appropriate to continue the trial. The events are:

- A subject experiences FVIII inhibitor development (defined as greater than or equal to 0.6 BU at ITxM) in association with rFVIII.
- A subject experiences a thrombotic event in association with rFVIII.
- A subject develops severe or catastrophic bleeding.
- A subject develops a Grade 2 or greater allergic reaction in association with rFVIII, defined as follows using the CTCAE grading.
    - Grade 2 - Intervention or interruption of infusion necessary. Responds promptly to symptomatic treatment. Prophylactic medications indicated for less than or equal to 24 hours.
    - Grade 3 - Prolonged, or not rapidly responsive to symptomatic treatment and/or interruption of infusion. Recurrence of symptoms following initial improvement.
    - Grade 4 - Life-threatening consequences. Urgent intervention indicated.
- A subject develops anaphylaxis in association with rFVIII, defined as follows using the CTCAE grading (all anaphylactic events are Grade 3 or higher).
    - Grade 3 - Symptomatic bronchospasm with or without urticaria. Allergy-related edema and/or angioedema. Hypotension. Parenteral intervention needed.
    - Grade 4 - Life-threatening consequences. Urgent intervention indicated.

Such events will be handled as serious adverse events (SAEs) and reported in an expedited time frame to the IRB. The data concerning the event will be reviewed by the DSMC along with all other available data to determine appropriate follow-up, with a decision to continue enrollment and treatment of subjects at that time. Additionally, the following may also stop further subject enrollment and treatment.

- The DSMC warrants temporary suspension of enrollment for further review of data generated to date.
- The PI determines that a medically important event warrants further evaluation by the DSMC.

A report summarizing the above DSMP activities will be submitted to the IRB at the time of annual renewal.

10.3 Adverse Event Reporting

All AEs experienced by study subjects are to be recorded on the CRF, regardless of the severity of the event or its relationship to study treatment. The AE reporting procedures are based on the CTCAE system, volume 4.03, 2010. Subjects will report any symptoms to the PI or research staff. The PI will determine if symptoms qualify as an AE or SAE with confirmation by one of the co-investigators. AEs will be classified as mild (does not interfere with routine activities), moderate (interferes somewhat with routine activities), or severe

(impossible to perform routine activities). The following algorithm will be used to assess the causality of all AEs:

- **Not related:** The event can readily be explained by factors not involving rFVIII, and a temporal relationship with rFVIII does not exist.
- **Possibly related:** A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of rFVIII but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
- **Probably related:** The temporal relationship between the administration of rFVIII is compelling, and the event cannot be explained by the subject's medical condition or other therapies.
- **Related:** The event follows a reasonable temporal sequence from the administration of rFVIII, follows a known or suspected response pattern to rFVIII, is confirmed by improvement upon stopping rFVIII, and reappears upon repeated exposure to rFVIII.

The PI will follow up all AEs, regardless of severity, until satisfactory resolution. All subjects experiencing AEs will be monitored until symptoms subside and any abnormal laboratory values have returned to baseline, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full pathologist's report will be supplied, if possible. Withdrawal from the clinical study and therapeutic measures shall be at the discretion of the PI. All SAEs will be reported to the IRB according to its guidelines. All events must be assessed to determine the following:

- If the event meets the criteria for an SAE.
- The relationship of the event to study treatment.
- The severity of the event.

An AE is any untoward medical occurrence in a subject in whom a pharmaceutical product is administered but does not necessarily have a causal relationship with the pharmaceutical product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a pharmaceutical product, whether or not related to the pharmaceutical product.[21] A SAE is any untoward medical occurrence that at any dose:

- Results in death.
- In the view of the investigator, places the subject at immediate risk of death.
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Results in a congenital anomaly/birth defect.

A SAE may also be any other medically important event that, in the opinion of the PI, may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above.

11.0 Human Subjects

11.1 Minority Inclusion and Non-Discriminatory Statements

Overweight or obese (BMI greater than 25.0-29.9 and 30 mg/m^2, respectively), adult males (age 18 or older) with hemophilia A (FVIII activity 40% or less) will be eligible for study participation. Children will not be enrolled in the study. As hemophilia A is an X linked recessive genetic disorder, greater than 98% of affected individuals are male, and approximately 90% are Caucasian; therefore, it is anticipated that the majority of subjects will be Caucasian males. There will be no discrimination based on gender, race, or ethnicity. The gender, racial, and ethnic characteristics of the proposed study population will reflect the demographics of the disease, the city of Pittsburgh and surrounding areas, and the University of Pittsburgh Medical Center (UPMC).

11.2 Confidentiality

Study participation and related data will be protected to maintain confidentiality. There is a possibility that the subject's personal information could become generally known. In order to reduce risks of disclosure or breach of confidentiality, research related documents, blood samples, and clinical information stored in the subject's research file will be assigned an alphanumeric identifier, i.e. BW001, which does not contain any

personal identifying information. A linkage key for linking the assigned alphanumeric identifier with the subject's name will be stored in locked files at HCWP. Any publication arising from this study will not contain names or other identifying information.

11.3 Costs and Payments

Subjects will not incur any costs related to study visits, rFVIII infusion, or laboratory testing. The research study is not paying for rFVIII. Each subject will use his own rFVIII for the purposes of the study. Research related costs are outlined in the budget proposal. Subjects will receive compensation up to $300.00 ($100.00 following each study visit completed).

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13.0 Appendices

13.1 Weight Calculations

1. BMI in kg/m^2 calculated as [(weight in kg)/(height in m^2)]
2. LBM in kg calculated as [(9.27 x 10^3 x TBW in kg)]/[(6.68 x 10^3) + (216 x BMI)]
3. IBW in kg calculated as [(50 kg) + (2.3 kg x height in inches > 60)]
4. TBW dose calculated as [(TBW in kg x desired FVIII level of 100 IU/dL)/(2)]
5. LBM dose calculated as [(LBM in kg x desired FVIII level of 100 IU/dL)/(2)]
6. IBW dose calculated as [(IBW in kg x desired FVIII level of 100 IU/dL)/(2)]

13.2 Qualifications of Investigators

Dr. Craig Seaman is an Assistant Professor of Medicine in the Department of Medicine, Division of Hematology and Oncology, at UPMC. His research focuses on hemostasis and thrombosis. He is currently completing his Master of Science degree in Clinical Research at the University of Pittsburgh Institute for Clinical Research Education. He is developing clinical research protocols with the support of his senior research mentor, Dr. Margaret Ragni.

Dr. Margaret Ragni is a Professor of Medicine and the Director of HCWP, and has conducted numerous clinical research studies at the University of Pittsburgh, investigator-initiated and in collaboration with the CDC, FDA, NIH, and pharmaceutical agencies. She is an expert in the area of hemophilia treatment and complications, including AIDS and hepatitis, and management of joint hemorrhages.