PROTOCOL

INVESTIGATOR-DRIVEN RANDOMIZED CLINICAL TRIAL

PROTOCOL TITLE: INdividualized ITI based on fviii(ATE) protection by VWF (INITIATE)

Product: Wilate®

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SIGNATURE PAGE

Title of protocol: **<u>IN</u>dividualized <u>ITI</u> based on fviii(<u>ATE</u>) protection by VWF (INITIATE)**

Sponsor Approval

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Jonathan Ducore, MD, MPH Principal Investigator	Date
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INVESTIGATOR AGREEMENT

By signing this page, I attest that I have read and understand the contents of Clinical Protocol INdividualized ITI based on fviii(ATE) protection by VWF (INITIATE) and any current amendments. I agree to adhere to the design, conduct, and reporting requirements of the study as stated in the clinical protocol and to my obligations to the Sponsor as described in the protocol and executed contracts between myself, my Institution, and the Sponsor. I also agree to adhere to any subsequent amendments to the clinical protocol.

I understand that no deviations from this protocol may be made without the written permission of the Study Coordinating Center PI, except where necessary to eliminate immediate hazard(s) to trial subjects, or when the change(s) involve only logistical or administrative aspects of the trial.

Investigator's Signature: _____

Investigator's Name: _____

Institution: _____

Date: _____

SYNOPSIS

PROTOCOL TITLE: Individualized ITI Based on FVIII Protection by VWF (INITIATE)

OVERVIEW: The primary goal of the INITIATE trial is to compare the clinical outcome of individualized lot selection to random lot selection utilizing one plasma-derived von Willebrand factor (VWF)/coagulation factor (FVIII) complex concentrate for immune tolerance induction (ITI) in subjects with congenital Hemophilia A, FVIII activity ≤2%, and a historical high-titer inhibitor [≥5 Bethesda Unit (BU)].

TREATMENT ARMS:
Participants will be randomized on a two-to-one basis between one of two study arms, individualized lot selection (alternative treatment arm) and random lot selection (standard treatment arm, current US clinical practice in ITI). Study sites, participants and investigators will be blinded to the assigned treatment arm.

Alternative Treatment Arm:
Two-thirds of the participants will be randomized to blinded individualized lot selection for ITI. The target initial dose of FVIII for ITI is 200 IU/kg/day intravenously. Wilate® will be the VWF/FVIII complex concentrate (Octapharma USA, Inc.; U.S. License No. 1646) prescribed during the trial. A FVIII dose reduction will be used for subjects receiving Hemlibra® (See Appendix IV for specifics).

Individualized lot selection will be performed according to a modified Oxford method in a central laboratory, by testing subject's plasma against 4-6 lots of Wilate® and selecting the one with the highest residual FVIII activity remaining after incubation (lowest Oxford titer). The same lot will be used throughout the entire ITI course for each subject. If the selected lot is depleted prior to the completion of ITI, a second individualized lot selection will be performed using the original plasma sample provided at baseline.

At six months, participants in the standard treatment arm who still have an inhibitor titer >0.6 may be switched to the alternative treatment arm.They will be treated as new participants who have failed prior ITI and a second individualized lot selection will be performed using the original plasma sample provided at baseline.

Standard Treatment Arm:
One-third of the participants will receive random lot selection for ITI. The dose and concentrate (Wilate®) used will be the same as that for the alternative treatment arm. Concentrate will be randomly selected from available lots. The same lot will be used throughout the entire ITI course for each subject. If the random lot is depleted prior to the completion of ITI, a second lot will be randomly selected. In both cases the random lot will be tested against the subject's plasma to measure the residual FVIII activity after incubation. The results of this test will not be used for lot selection.

OBJECTIVES:
Primary Objective: The primary objective of the INITIATE trial is the **time** to achieve negative inhibitor (<0.6 BU) comparing individualized lot selection to random lot selection.

Secondary Objectives: Secondary objectives of this study include the **time** to achieve partial and complete success, maintenance of inhibitor eradication, monthly break-through bleeding

rates, cost of ITI including cost of bypassing agents for bleeding control, and adherence to the ITI treatment regimen comparing the standard to the alternative therapy. The impact of inhibitor titer at start of ITI and during the course of ITI, including the peak titer of the inhibitor, will be investigated. Additionally, biologic assays will be performed to understand factors related to ITI success.

HYPOTHESIS:
The primary hypothesis is that the **time** to negative inhibitor (<0.6 BU) will be shorter with individualized lot selection compared to random lot selection.

INCLUSION/EXCLUSION CRITERIA:

Inclusion Criteria: Subjects must meet all of the following criteria to be eligible for enrollment into the study:

1) Diagnosis of congenital Hemophilia A and baseline FVIII ≤2%.
2) Weight ≥ 5 kg
3) History of FVIII inhibitor titer ≥5 BU
4) Current FVIII inhibitor titer ≥5 BU or ≥0.6 BU and failed ITI defined by FVIII recovery <66% normal and half-life <6 hours
5) Adequate venous access for daily concentrate infusions
6) For participants <18 years, a parent or guardian willing and able to provide informed consent with verbal or written assent from the child if require by the local institution. For participants ≥18 years, a willingness and ability to provide informed consent from the subject.
7) Ability to comply with study related treatments, evaluations, and follow-up.

Exclusion Criteria: Subjects meeting any of the following criteria are ineligible for enrollment in the study:

1) Acquired hemophilia
2) Congenital or acquired bleeding disorder in addition to Hemophilia A
3) ITI factor replacement regimen within the past one month unless there is clear evidence of ITI failure with no reduction in inhibitor titer over the past two months
4) HIV positive with viral load ≥200 particles/μL or ≥400,000 copies/mL
5) Rituximab within the past 3 months
6) IVIG within the past 1 month
7) Treatment with other immunosuppressive drugs within the past 1 month (excluding intermittent steroid use for asthma)
8) Concomitant experimental treatment
9) History of hypersensitivity to plasma-derived VWF- or FVIII-containing concentrates
10) Elective surgery planned in the next 6 months (excluding vascular access procedure)
11) Any condition or chronic illness, which in the opinion of the investigator makes participation ill-advised
12) Inability or unwillingness to complete required screening, follow-up, and exit studies

CRITERIA FOR EVALUATION:

Efficacy:

Primary Endpoint:
The primary endpoint is **time** to inhibitor <0.6 BU.

This endpoint was chosen because a shorter time to negative inhibitor should decrease monthly break-through bleeding frequency in the early phase of ITI, reduce morbidity and cost, and improve quality of life.

Secondary Endpoints:
Secondary endpoints include **time** to achieve partial and complete success as defined according to the following criteria:

- Inhibitor titer <0.6 BU.
- Incremental *in vivo* FVIII recovery in the normal range [≥66% of normal (1.5% per IU/kg), equal to 0.99%per IU/kg] with samples taken prior to and 15 or 30 minutes after concentrate treatment. The recovery assessment should be done without any wash-out period.
- Half-life of FVIII ≥6 hours. The half-life assessment should be done in a non-bleeding status without any wash-out period.

Complete Success (CS) of ITI:

All three criteria above met.

Partial Success (PS) of ITI:

The first two of the three criteria above met.

Partial Response (PR) of ITI:

One of the three criteria above met.

Partial Failure (PF) of ITI:

Inhibitor still present, but titer is decreased to <5 BU in contrast to ≥5 BU before start.

Complete Failure (CF) of ITI:

None of the above mentioned criteria met, and the inhibitor titer is still ≥5 BU.

The following additional secondary endpoints will be evaluated:

- Absence of relapse, up to 12 months after achievement of complete or partial ITI success
- The number of break-through bleeding events during the course of ITI-treatment
- Cost of ITI – including bleeding control using bypassing agents prior to start and during ITI
- Health-Related Quality of life (HRQoL)
- Adherence with the ITI treatment regimen
- The impact of inhibitor titer at start of ITI and during the course of ITI, including the peak titer of the inhibitor
- Additional biologic assays will be performed to understand factors related to ITI success.

STUDY DESIGN: The INITIATE trial is a post-marketing investigator-initiated double-blinded randomized clinical study, comparing alternative therapy to standard therapy to reduce the time to inhibitor titer <0.6 BU. Participants from any Hemophilia Treatment Center in the US will be eligible. Each clinical site will be led by a hematologist experienced in the management of congenital hemophilia.

STUDY POPULATION: Participants of any age with congenital hemophilia, FVIII activity ≤2% and historical high-inhibitor (≥5 BU).

SAMPLE SIZE: 120 participants will be enrolled.

RANDOMIZATION: Participants are randomized to treatment arm in a 2:1 ratio. A central laboratory will test each alternative treatment arm subject's plasma sample against 4-6 factor lots and the lot with the lowest Oxford titer will be assigned. Subjects in the standard treatment arm will have lot assignment performed by random lot selection. The treating physician, treatment site staff, and participant will be blinded to the randomization allocation.

DATA MANAGEMENT: Clinical sites enter data via a secure, internet-based electronic data capture (EDC) system.

HUMAN SUBJECTS: The most important risk for the participants is bleeding; high rates of bleeding are expected in individuals with Hemophilia A and inhibitors.

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ABBREVIATIONS

AE	Adverse Event
AHCDC	Association of Hemophilia Clinic Directors of Canada
APC	Antigen Presenting Cell
aPCC	activated prothrombin complex concentrate
ASH	American Society of Hematology
ASPHO	American Society of Hematology/Oncology
BU	Bethesda Unit
CF	Complete Failure
CR	Complete Response
CV	Curriculum Vitae
DSMC	Data Safety Monitoring Committee
EDC	Electronic Data Capture
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
FVIIa	Activated Factor VII
FVIII	Factor VIII
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
Haem-A-QoL	Haemophilia-specific Quality of Life Questionnaire for Adults
Haemo-QoL	The Quality of life assessment instrument for children and adolescents with haemophilia
HRQoL	Health-Related Quality of Life
ICH	International Committee on Harmonization
ISTH	International Society on Thrombosis and Haemostasis
ITI	Immune Tolerance Induction
IU	International Unit
IVR	*In vivo* recovery

kg	Kilogram
MASAC	Medical & Scientific Advisory Council
mL	Milliliter
NHF	National Hemophilia Foundation
OU	Oxford Unit
pd	Plasma-derived
PF	Partial Failure
PI	Principal Investigator
PR	Partial Response
PUPs	Previously Untreated Patients
rVIIa	Recombinant activated factor VIIa
rFVIII	Recombinant factor VIII
SAE	Serious Adverse Event
SC	Study Coordinator
SCC	Study Coordinating Center
TDH	Terminal Dry-Heat
UKHCDO	UK Haemophilia Central Doctors Organization
VWF	Von Willebrand factor
VWD	Von Willebrand disease

DEFINITIONS

Low responding inhibitor	Inhibitor level is remains <5 BU/mL despite ongoing factor exposure.
High responding inhibitor	Inhibitor level is ≥5 BU/mL at any time
Joint bleed	Any complaint requiring treatment located in a joint
Soft tissue bleed	Any complaint requiring treatment located outside the joints
Minor bleed	Bleed characterized by mild pain, minimal swelling, minimal restriction of motion, resolving with 24 hours of treatment and not resulting in a life-threatening condition
Major bleed	Any bleed not considered minor
Negative inhibitor	A negative inhibitor is defined as a titer <0.6 BU
FVIII half-life	Time (in hours) to 50% decrease in FVIII activity
Inhibitor titer	The inhibitor titer is the reciprocal of the dilution of subject plasma which results in 50% of residual FVIII
Normal *in vivo* FVIII recovery	FVIII recovery ≥66% normal
Bypassing agents	Clotting factor concentrates used for the treatment or prevention of bleeding in hemophilia subjects with inhibitors. The action of the bypassing agent is independent of FVIII.
Exposure day (ED)	Any day during which factor therapy is given, regardless of the number of treatments in that day.

BACKGROUND AND SIGNIFICANCE

Inhibitor formation in patients with hemophilia

Anti-FVIII inhibitor (antibody) formation is the most serious complication of Hemophilia A treatment today, being estimated to occur in up to 10-30%, or more, of those with severe Hemophilia A. Use of FVIII is generally futile in this group, because the inhibitor will rapidly inactivate the infused concentrate.

More than 90% of inhibitors occur within the first 50 exposure days and 50% before the first 15 exposure days. Several risk factors for the development of inhibitors to FVIII have been identified in patients with Hemophilia A. The incidence of inhibitors depends on both,

genetic factors:

- severity of hemophilia
- type of mutation[1]
- ethnicity[2]
- family history of inhibitors[3]
- the HLA genotype[4,5]

and non-genetic factors:

- age at first FVIII treatment[6,7]
- intensity of FVIII treatment[8]
- continuous infusion of FVIII[9]
- type of FVIII concentrate used[10]

Walsh and colleagues from the Centers for Disease Control and Prevention recently demonstrated a higher rate of mortality in FVIII inhibitor patients.[11] In multivariate analysis the odds of death were 70% higher among patients with a current inhibitor compared to those without an inhibitor ($p<0.01$), and the deaths among inhibitor patients were primarily attributed to bleeding complications.

Management of high-titer inhibitors

Management of Bleeding

Treatment options for bleeding in patients with high-titer inhibitors include bypassing agents, activated recombinant coagulation factor VII (rFVIIa; NovoSeven®) and/or plasma-derived activated prothrombin complex concentrate (aPCC; FEIBA®), or porcine recombinant coagulation factor VIII (rFVIII; OBIZUR®). The efficacy of these agents is unpredictable and the results are suboptimal compared to direct FVIII replacement.

Prevention of Bleeding

Ideally, patients with hemophilia should receive continuous, long-term prophylaxis to prevent spontaneous bleeding and hemophilic arthropathy. Inhibitors complicate the current standard of care, being three times weekly (or individualized) FVIII prophylaxis (preventive FVIII replacement therapy) to prevent joint bleeding and arthropathy long-term. Bypassing agents can be used for prophylaxis, but must be given more frequently, are associated with high costs and are less efficacious compared to FVIII prophylaxis in patients without inhibitors.

Immune Tolerance Induction

ITI therapy is a program of regular infusions over a prolonged period of time to eradicate inhibitors. ITI is the treatment of choice to eliminate inhibitors and restore normal FVIII pharmacokinetics (recovery and half-life of FVIII). This enables regular FVIII treatment in the case of bleeding and surgery, as well as prophylactic treatment to prevent hemophilic arthropathy and life-threatening bleeding. Successful ITI leads to improved quality of life and reduced costs.[12]

Current recommendations for ITI are based on data from ITI registries, systematic reviews [12-15] and the International Immune Tolerance Study (NCT00212472).[16] Several publications include guidance in terms of choice of product (VWF-containing plasma-derived (pd) FVIII).[17-21] Expert groups in North America and Europe have published treatment experience and recommendations.[22,23,24] The Association of Hemophilia Clinic Directors of Canada[25] and UK Haemophilia Centre Doctors Organization)[26] have published national clinical practice guidelines. The National Hemophilia Foundation (NHF) Medical & Scientific Advisory Council (MASAC) has not published clinical practice guidelines for the US.

ITI Treatment Strategy

The dose of FVIII used varies from 50 IU/kg on alternate days to 100 IU/kg twice daily. The International Immune Tolerance Study (NCT00212472) demonstrated a lower bleeding rate and shorter time to ITI success in participants treated with 200 IU/kg/day compared to 50 IU/kg three times per week.[16]

Today rFVIII concentrates are the products of choice for standard Hemophilia A treatment in the US, also for 1st-line ITI treatment of inhibitors. Currently, no pdVWF/FVIII products are licensed specifically for ITI treatment by the Food and Drug Administration (FDA). Clinicians may choose to start with a pdVWF/FVIII product or switch to this product on an individual patient basis.[22]

The REScue Immuntolerance Study (RESIST) (NCT01051544 and NCT01051076) aimed to compare ITI outcomes between rFVIII and pdVWF/FVIII ITI regimens.[27] These studies were recently closed due to poor accrual. Data have not been published. The European PEDNET Hemophilia includes evaluation of etiology of inhibitors and ITI outcomes.[28] The PRospectIve Observational Study of PlasMa-derived FVIII/VWF in Immune Tolerance Induction (PRISM) Registry is an observational study in the US evaluating inhibitor outcomes in patients treated with the pdVWF/FVIII concentrate, Alphanate®.[29]

Duration of ITI

ITI success is defined by a negative inhibitor titer (<0.6 BU), improved FVIII *in vivo* recovery (≥66 or 80% of calculated FVIII recovery) and increased FVIII half-life (≥6 or 7 hours).[16,26,29]

ITI may be achieved within 6-12 months, but can take as long as 1-3 (even more) years to achieve tolerance.[30] Reported efficacy is 50-80% depending on the product used for ITI.

Once tolerance is achieved, dose and frequency of factor infusions are gradually tapered to a prophylactic regimen. Inhibitor titers and FVIII levels should be tested monthly during the first half year, subsequently every second month. After tolerance is achieved, it is recommended to follow the patients closely for relapses in order to restart ITI immediately.[26]

Alternative strategies may be considered if the inhibitor titer fails to drop at least 20% over each 6-months period after the peak titer has been reached.[26]

Costs of Inhibitors and ITI

Treating patients with high-titer inhibitors is costly.[31] In addition to cost of FVIII during ITI, there are costs of bypassing agents to prevent and treat bleeding. ITI treatment is expensive in the short run. However, successful eradication of an inhibitor pays off in the long run. Once a negative inhibitor titer (<0.6 BU) is achieved the monthly bleeding rate drops by up to 90% (86.1-86.4% in the Octanate® study; data not published; Octanate® in ITI-BLA package submitted to FDA), and the use of bypassing agents and associated costs decrease. After successful ITI, prophylaxis is resumed. Overall, the cost of prophylaxis is three times less expensive than prophylaxis with bypassing agents[32]. Compared with patients on on-demand or prophylactic treatment using bypassing agents, subjects who have gone through a successful ITI have lower drug and hospitalization costs ($ 22.2 million vs. $ 38.7 million or $ 42.2 million), longer life expectancy (74.3 years vs. 69.6 years for both regimens), and fewer/almost equal bleeding events (801 vs. 1819 or 694), respectively.[33]

The average direct medical costs for treatment of children and adults with inhibitors can be quantified in a cost of care analysis. Such an estimate has been done for Octanate® in ITI versus the on-demand use of NovoSeven®.[34] The costs for on-demand pre-ITI phase management with NovoSeven® for one year was compared with the time period after start of high-dose ITI with Octanate®, and the break-even point analysis to estimate the number of years after which treatment with Octanate® pays off was done. The median age of initiation of high-dose ITI with Octanate® was 8 years for children and 23 years for adults. During the period with on-demand treatment of bleeding episodes with NovoSeven® before ITI with Octanate®, the children (n=8) had a mean body weight of 36 kg, bled 17 times per year, were treated for 6 days per bleed – twice per day – using 120 µg/kg, and were hospitalized 19 times per year. The ITI treatment was done with a mean of 230 IU/kg daily, bled 5 times per year, and were hospitalized zero times per year. The time until complete success was 11 months (range: 6-17) and the prophylactic dose after tolerance was 47 IU/kg three times per week. The treatment with high-dose ITI, including prophylaxis after tolerance, with Octanate® over 10 years had average costs of € 2.5 million, which is € 7.5 million less than the €10 million costs calculated for the on-demand NovoSeven® option over 10 years. The mean break-even point was 1.2 years, after which ITI followed by prophylaxis with Octanate® was cheaper. For adults (n=8) the average cost saving was € 2.5 million and the break-even point was 3 years.

Another break-even point calculation between ITI costs and the use of NovoSeven® on-demand in 10 high-responder children with inhibitors (mean age: 6 years) confirmed the above findings.[35] The use of a bypassing agent might increase the costs of inhibitor treatment up to 12-times over the patient's lifetime, and the break-even point of ITI versus NovoSeven® on-demand was 1.4 years after treatment start.[35]

Effect of VWF on ITI

The development of inhibitory antibodies to FVIII requires the interaction of antigen presenting cells (APCs), T-cells, and B-cells with the ability to recognize immunogenic peptides of FVIII.[36] VWF forms a non-covalently linked complex with FVIII to stabilize and protect it from protease degradation in the circulation.[37] In addition, VWF may partially block the binding of immune cells to FVIII – especially the C2, A3, and maybe also A2 domains – and, thus, reduce the potential for inhibitor formation.[37]

In vitro studies

VWF reduces the immunogenicity of FVIII in Hemophilia A mice.[38] Delignat et al hypothesize that VWF exerts at least two non-mutually exclusive immunoprotective roles towards FVIII – 1) VWF prevents (in a dose-dependent manner) the endocytosis of FVIII by professional APCs by blocking the interaction of FVIII with as yet unidentified endocytic receptor(s), a phenomenon also shown for human APCs;[39] and 2) hypothetically, VWF, by virtue of increasing the half-life of FVIII in the circulation, may allow for an increased contact time with tolerogenic marginal B-cells in the spleen.[38]

VWF-containing pdFVIII products protect FVIII better than nude FVIII against neutralizing antibodies *in vitro*,[40] and that this causes a better recovery and half-life of FVIII *in vivo*.[41] Furthermore, pdVWF/FVIII concentrates added to FVIII-deficient plasma with the presence of an inhibitor generate more thrombin *in vitro* than nude FVIIIs – both plasma-derived and recombinant.[42] Finally, it has been demonstrated – both *in vitro* and by a tail clip survival test – that VWF has a dose-dependent protective effect on FVIII, limiting the inhibitor inactivation of FVIII, and that a preformed complex of VWF with FVIII provides more effective protection from inhibitors than a competitive binding of antibodies and VWF to FVIII.[43]

Most recently, Bravo et al. studied the differential sensitivity to inhibitors of the native pdFVIII/VWF complex vs. the combination of purified, isolated FVIII and VWF proteins. They conducted *in vitro* inhibitor assays (BU) using various combinations of VWF and FVIII concentrates. Inhibitor titers were higher (i.e. residual FVIII lower) for all nude FVIII concentrates compared to plasma and pdFVIII/VWF concentrates despite pre-incubation with VWF. Thrombin generation was lower with nude FVIII concentrates compared to plasma and PDFVIII/VWF concentrates.[44] Based on these findings, the authors recommend that Bethesda assay titration using different FVIII concentrates be used to select the optimal concentrate for ITI.[44] This recommendation supports the INITIATE study design.

Clinical Data of VWF/FVIII Concentrates for ITI

Octapharma conducted a comparison of 26 studies comprising 511 patients undergoing ITI, of which 315 subjects participating in 13 studies received pdVWF/FVIII[21,45-54] and 196 patients in another 13 studies received nude (recombinant) FVIII.[55-67] The patient cohorts in all these publications were incompletely described, but there is a trend towards improved ITI results in patients who previously failed ITI in the pdVWF/FVIII trials than the rFVIII studies. It is clear from this comparison that both pdVWF/FVIII and rFVIII products are different in terms of the ITI success rates. The overall success rate (i.e. both complete and partial success) ranged from 57.5% to 94.1% for the pdVWF/FVIII concentrates, with a complete success rate ranging from 32.0% to 87.9%. When the still ongoing studies[53,54] were excluded, the weighted average complete, partial, and overall success rates were 61.0%, 17.4%, and 78.4%, respectively. The two products Alphanate® and Fanhdi® (being essentially the same product, using the same manufacturing method) impacted the success rates significantly with a low complete success

rate of 43.8% and a high partial success rate of 30.8% (to a total of 74.7%), a difference which was statistically significant versus the other pdVWF/FVIII products (p<0.001). When these products were excluded from the analysis, the complete, partial, and overall success rates were improved to 76.7%, 5.0%, and 81.8%, respectively. The Octanate® figures were 70.8%, 8.3%, and 79.1%, respectively (total n=48), whereas the Wilate® studies are ongoing (see below 'ITI treatment experience with Octanate® and Wilate®'). Haemoctin® from Biotest (identical to Octanate®) and Factane® from LFB (Octanate® without heat-treatment, but with nanofiltration) showed similar complete response rates to Octanate® with 64.3% and 87.5%, respectively. None of the success rates were dependent on the actual amount of VWF in the products. Thus, the differences seen are most likely related to the quality of VWF and how the FVIII is protected by VWF in the various pdVWF/FVIII concentrates.

The rFVIII products had an overall success rate ranging from 25.0% to 81.8%, with a complete success rate in the range from 0.0% to 81.8%. The weighted average complete, partial and overall success rates were 57.1%, 9.2%, and 66.3%, respectively, which is not different from the pdVWF/FVIII except for more partial success in the pdVWF/FVIII group. Among the rFVIIIs, Kogenate®/Kogenate FS® had a complete response rate higher than Recombinate®/Advate® (68.1% vs. 43.2%; p<0.05). Alphanate® and Fanhdi® had fewer complete responses and more partial responses than the rFVIII concentrates – 43.8% vs. 57.1% and 30.8% vs. 9.2% (both p values <0.05), respectively. When these products were excluded from the pdVWF/FVIII products, the complete, partial, and overall success rates were improved to 76.7%, 5.0%, and 81.8%, respectively, which revealed a significantly better performance for the pdVWF/FVIII concentrates over the rFVIIIs with 76.7% (+34.3%) vs. 57.1% for the complete responses and 81.8% (+23.4%) vs. 66.3% for the overall responses (both p values <0.05; the partial response rate was not different). Based on all these studies it is reasonable to assume that the complete success rate in ITI is at least 45-75% for pdVWF/FVIII (excluding Alphanate® and Fanhdi®) versus 30-60% for rFVIII preparations, i.e. around 33% higher for pdVWF/FVIII.

Individualized Selection of VWF/FVIII Lots for ITI

Individualized selection involves measuring residual FVIII activity when patient plasma is mixed with VWF/FVIII concentrate *in vitro*. The lot of VWF/FVIII resulting in the highest residual FVIII activity is selected for use in ITI.

Today, several methods, including the modified Oxford method, are used for measuring residual FVIII activity after incubation of a FVIII source (concentrate or plasma) with the inhibitor patient plasma.[68] The inhibitor titer is the reciprocal of the dilution of patient plasma which results in 50% of residual FVIII, and the difference between 1 Bethesda unit (BU) and 1 Oxford unit (OU) is 1 BU ≈ 1.21 OU – which equals 1 OU ≈ 0.83 BU. The relationship between the residual factor VIII values and BU or OU is described by the equations $FVIII_{res}=e^{(-0.69)BU}$ and $FVIII_{res}=e^{(-0.83)OU}$. The principle of lot selection is to first test the inhibitor-containing plasma against a FVIII standard to establish the patient's inhibitor titer (normal BU determination) and then to test several lots of a product against the same inhibitor plasma to find a lot which gives the highest residual FVIII content (lowest OU titer). Ideally, the lot providing the highest residual FVIII titer after infusion to the patient, i.e. after being subjected to the anti-FVIII, will challenge the immune system more and provide better prevention and control of bleeding.

This strategy is being evaluated in the ObsITI study. ObsITI is an observational study of ITI in Europe (www.obsiti.com). The objective is to investigate the role of *in vitro* tests including lot

selection according to the modified Oxford method (described above) on individual ITI success rates in patients undergoing ITI.

Data on the Role of Lot Selection in ObsITI

Octanate® Data

An analysis of 42 Octanate® ITI patients from ObsITI with complete details on the lots used was performed. Thirteen (31.0%) subjects used only selected lots, whereas 29 (69.0%) received random lots (i.e. could be either ideal or suboptimal lots used, details not known). Among the patients with selected lots 11 (84.6%) had a complete response, the remaining 2 (15.4%) had a partial response. There were no failures in this group. In the random lot cohort, 21 (72.4%) had a complete response and 3 (10.3%) had a partial response, whereas there were 5 (17.2%) failures in this group. None of these differences was significant statistically. Two of the patients had inhibitor titers above 200 BU, both of them were in the random group – 1 complete success and 1 failure. These were defined as outliers. Based on the time to reach a negative inhibitor titer (<0.6 BU; criteria 1), FVIII incremental recovery ≥80% (criteria 2), and FVIII half-life ≥7 hours (criteria 3), Kaplan-Meier curves for the two cohorts were made, displaying the days versus the probability of reaching each of the three criteria. The time to reach a negative inhibitor titer (<0.6 BU; criteria 1) was shorter for the patients with lot selection, although the difference did not reach completely the significance level (p=0.061). The time to reach a FVIII incremental recover ≥80% (criteria 2) was again shorter for the subjects in the lot selection group and this difference was significant (p=0.013), whereas the time to reach a FVIII half-life ≥7 hours (criteria 3) was the same for the two groups (p=0.78). The median time to reach a negative inhibitor titer (<0.6 BU; criteria 1) with a probability of 50% (0.5) was calculated to be 49 days versus 132 days for the batch selection versus the random lot group, respectively. The median time to reach a FVIII incremental recovery ≥80% (criteria 2) with a probability of 50% (0.5) was of course slightly longer, but showed the same roughly three months difference with 78 days versus 151 days for the lot selection versus the random lot group, respectively. The hazard risk ratios calculated were 5.3 and 2.85 for the time to criteria 1 and 2, respectively. With a hazard ratio of 2 (and outliers excluded) combined with e.g. a power of 85% and an alpha of 2.5% or a power of 90% and an alpha of 5%, roughly 50 patients in each arm (100 in total) would be needed to show a difference between lot selection and random lots in ITI, using time until reaching a 'negative inhibitor titer (<0.6 BU; criteria 1) with e.g. a ≥80% reduction in the monthly bleeding rate' and/or reaching a 'FVIII incremental recover ≥80% (criteria 2)' as the end-point(s). To use 'complete success' (i.e. fulfilled criteria 3) as end-point is unrealistic, as there were no difference between lot selection and random lots in this analysis, although the reason for that can be that some of the random lot patients probably got 'good lots' too.

Wilate® for Use in ITI

Wilate® was launched for the first time in Germany in 2005, and is approved for the treatment of both von Willebrand disease (VWD) and Hemophilia A in Europe.[69] In the US, Wilate® is approved only in VWD (since 2009).[70] Wilate® is a high-purity (i.e. 100 IU FVIII/mg total protein) pdVWF/FVIII complex concentrate, purified by the use of ion-exchange chromatography columns, viral safeguarded by a combination of the chemical solvent/detergent (S/D) treatment (gold-standard for enveloped viruses such as HIV, HBV, and HCV) and terminal dry-heat (TDH) treatment (state-of-the-art method for both enveloped and non-enveloped viruses – the latter like HAV and parvovirus B19).[71] Wilate® is further purified by size exclusion chromatography (SEC) and a special ultra-/dia-filtration (UF/DF) technique. Parvovirus B19 responds completely different to heat inactivation than other viruses. The externalization of the viral DNA, leaving an empty – but intact capsid, renders the virus non-infective – but still immunogenic.[72] Thus,

seroconversion with formation of anti-B19 IgG is possible in recipients of heat treated pdVWF/FVIII concentrates, but disease is not transmissible at the conditions used, including a specified residual moisture.[73] The TDH treatment of Wilate® is 2 hours during manufacturing.[74] Wilate® possesses all the important features asked for in ITI, namely high purity, a very high pathogen safety profile, and an excellent protection of its FVIII by VWF – all achieved through unique, novel, and innovative techniques such as: SEC and special UF/DF with a high cut-off, as well as an optimized lyophilization and full residual moisture testing combined with a prolonged TDH treatment.

Octapharma has reserved lots of Wilate® for this trial.

Wilate® Data

From the ObsITI database there are two German patients who received Wilate® for ITI, both completed successfully. The two single cases represent one case each of 1st- and 3rd-line ITI. The number of poor prognosis factors was 3 and 4, respectively, including a historical peak titer of ≥200 BU. The 3rd-line ITI patient was switched to Wilate® only after 16.5 years, with the two previous attempts being done with a pdFVIII (almost devoid of VWF) and pdVWF/FVIII product other than Octanate® or Wilate® – with 2.4-fold more VWF than FVIII. The 1st-line ITI patient achieved a negative inhibitor after 2.8 months, normal FVIII recovery after 9.2 months, and a normal FVIII half-life after 10.2 months. The 3rd-line ITI achieved a negative inhibitor titer after 3.3 years, normal FVIII recovery after 3.6 years and a normal FVIII half-life after 6.2 years.

Two abstracts at the 2014 American Society of Hematology Annual meeting described ten Canadian patients who were prescribed Wilate® for ITI in Canada.[53,54] Eight patients had 1 one or more poor prognostic factors. One patient (with 3 poor prognostic factors) has been defined as a failure, whereas the nine other subjects have achieved a reduction in the inhibitor titer and monthly bleeding rate, including one complete success after four months.

STUDY OBJECTIVES AND PURPOSE

Rationale

The rationale for this study is that an optimal regimen for ITI has not been defined, and patients with Hemophilia A and inhibitors have high morbidity, mortality, and associated costs. There are several ongoing registries to evaluate the etiology of inhibitors and to explore ITI outcomes, but there are no open ITI clinical trials at the current time.

Primary and Secondary Objectives

Primary Objective: The primary objective of the INITIATE trial is to compare individualized lot selection to random lot selection in **time** to negative inhibitor (<0.6 BU).

Secondary Objectives: Secondary objectives of this study include the comparison of standard to alternative therapy for **time** to achieve partial and complete success, maintenance of inhibitor eradication, monthly break-through bleeding rates, cost of ITI – including bleeding control using bypassing agents prior to start and during ITI, quality of life, and adherence with ITI treatment regimen. Additionally, the impact of inhibitor titer at start of ITI and during the course of ITI,

including the peak titer of the inhibitor, will be investigated; and biologic assays will be done to understand factors related to ITI success.

Hypothesis

The primary hypothesis is that the **time** to negative inhibitor (<0.6 BU) will be shorter with individualized lot selection compared to random lot selection and that this will impact monthly break-through bleeding and reduce costs.

STUDY DESIGN

Description of Study Design

The primary goal of the INITIATE trial is to compare the clinical outcome of individualized lot selection to random lot selection utilizing one plasma-derived VWF/FVIII complex concentrate for immune tolerance induction (ITI) in patients with congenital Hemophilia A, FVIII activity ≤2%, and a historical high-titer inhibitor (≥5 BU).

The INITIATE trial is a post-marketing investigator-initiated double-blinded randomized clinical study comparing alternative therapy to standard therapy to reduce the time to partial or complete ITI success. Participants from any Hemophilia Treatment Center in the US will be eligible. Each clinical site will be led by a hematologist experienced in the management of congenital hemophilia.

TREATMENT ARMS:

Participants will be randomized on a two-to-one basis between one of two study arms, individualized lot selection (alternative treatment arm) and random lot selection (standard treatment arm, current US clinical practice in ITI). Study sites, participants, and investigators will be blinded to the treatment status assigned.

Alternative treatment arm:
Two-thirds of the participants will be randomized to blinded individualized lot selection for ITI. The target initial dose of FVIII for ITI is ~200 IU/kg/day intravenously, See Appendix II. The suggested maximum dose is 20,000 IU/day. Investigators may adjust the dose to a minimum dose of 150 units/kg if infusion volume is not feasible in patients without central venous access or in patients with von Willebrand factor levels >250%. Splitting dose into two infusions per day must be approved by the Steering Committee, and if approved, will be considered a protocol deviation. For subjects using Hemlibra®, the dose will be reduced to 50 IU/kg 3 times/week. Wilate® will be the VWF/FVIII complex concentrate (Octapharma USA, Inc., U.S. License No. 1646) prescribed for ITI.

Individualized lot selection will be performed according to a modified Oxford method in a central laboratory, by testing subject's plasma against 4-6 lots of Wilate® and selecting the one with the highest residual FVIII (lowest Oxford titer) activity remaining after incubation. The same lot will be used throughout the entire ITI course for each subject. If the selected lot is depleted prior to the completion of ITI, a second individualized lot selection will be performed using the original plasma sample provided at baseline.

At six months, participants in the standard treatment arm who still have an inhibitor titer >0.6 may be switched to the alternative treatment arm.They will be treated as new participants who have failed prior ITI and a second individualized lot selection will be performed using the original plasma sample provided at baseline.

Each Wilate® batch includes 1.6-1.8 million IU and is expected to last for about 3-57 months depending on the weight of the subject and prescribed dose. See dosing chart. (Appendix II)

Standard treatment arm:
One-third of the participants will receive random lot selection for ITI. The dose and concentrate used will be the same. Concentrate will be randomly selected from available Wilate® lots. The same lot will be used throughout the entire ITI course for each subject. If the random lot is depleted prior to the completion of ITI, a second lot will be randomly selected. In both cases the random lot will be tested against subject's plasma to measure the residual FVIII activity left after incubation but this result will not affect lot selection.

Participants in the standard treatment arm will be offered the option of switching to the alternative treatment after 6 months if they have an inhibitor titer >0.6 BU.

Description of Primary and Secondary Endpoints

Efficacy

Primary Endpoint:
The primary endpoint is **time** to inhibitor <0.6 BU.

Secondary Endpoints:
Secondary endpoints include the **time** to achieve partial and complete success.

The success of ITI is defined according to the following criteria:

- Inhibitor titer <0.6 BU.
- Incremental *in vivo* FVIII recovery in the normal range [≥66% of normal (1.5 %/IU/kg), equal to 0.99%/IU/kg] with samples taken prior to and 15 or 30 minutes after FVIII treatment. The recovery assessment should be done after the regular ITI treatment, without any wash-out period.
- Half-life of FVIII ≥6 hours (blood samples for FVIII determination should be taken 6 ± 2 hours and 24 ± 2 hours after FVIII treatment). The half-life assessment should be done in a non-bleeding status, e.g. after concentrate treatment, without any wash-out period interrupting the ITI.

Complete Success of ITI:

All three criteria above met.

Partial Success of ITI:

The first two of the three criteria above met.

Partial Response of ITI:

One of the three criteria above met.

Partial Failure of ITI:

Inhibitor still present, but titer has decreased to <5 BU in contrast to ≥5 BU before start.

Complete Failure of ITI:

None of the above mentioned criteria met, and the inhibitor titer is still ≥5 BU.

The following additional secondary endpoints will be evaluated:

- Absence of relapse, up to 12 months after achievement of complete or partial ITI success
- The number of break-through bleeding events during the course of ITI-treatment
- Cost of ITI – including bleeding control using bypassing agents prior to start and during ITI
- Quality of life
- Adherence with the ITI treatment regimen
- The impact of inhibitor titer at start of ITI and during the course of ITI, including the peak titer of the inhibitor
- Additional biologic assays will be performed to understand factors related to ITI success.

SELECTION OF PARTICIPANTS

Inclusion Criteria: Subjects must meet all of the following criteria to be eligible for enrollment into the study:

1) Diagnosis of congenital Hemophilia A and baseline FVIII ≤2%.
2) Weight ≥ 5 kg
3) History of FVIII inhibitor titer ≥5 BU
4) Current FVIII inhibitor titer ≥5 BU or ≥0.6 BU and failed ITI defined by FVIII recovery <66% normal and half-life <6 hours
5) Adequate venous access for daily concentrate infusions
6) For participants <18 years, a parent or guardian willing and able to provide informed consent with verbal or written assent from the child if require by the local institution. For participants ≥18 years, a willingness and ability to provide informed consent from the subject.
7) Ability to comply with study related treatments, evaluations, and follow-up.

Exclusion Criteria: Subjects meeting any of the following criteria are ineligible for enrollment in the study:

1) Acquired hemophilia
2) Congenital or acquired bleeding disorder in addition to Hemophilia A

3) ITI factor replacement regimen within the past one month unless there is clear evidence of ITI failure with no reduction in inhibitor titer over the past two months
4) HIV positive with viral load ≥200 particles/μL or ≥400,000 copies/mL
5) Rituximab within the past 3 months
6) IVIG within the past 1 month
7) Treatment with other immunosuppressive drugs within the past 1 month (excluding intermittent steroid use for asthma)
8) Concomitant experimental treatment
9) History of hypersensitivity to plasma-derived VWF- or FVIII-containing concentrates
10) Elective surgery planned in the next 6 months (excluding vascular access procedure)
11) Any condition or chronic illness, which in the opinion of the investigator makes participation ill-advised
12) Inability or unwillingness to complete required screening, follow-up, and exit studies

Enrollment Procedures

Clinical sites will enroll research participants according to their institutional practices. Verification of eligibility will be conducted. The completed eligibility checklist and signed informed consent documents should be available as source documents for study monitor review.

The eligibility screening period can only begin after the informed consent is obtained.

TREATMENT OF PARTICIPANTS

High-Dose ITI Strategy

High-dose ITI strategy will be used for this study. Participants will be prescribed 200 IU/kg infused intravenously once daily. Doses will be rounded up to nearest 1000 units. See Appendix II for dosing chart. For subjects using Hemlibra®, the dose will be reduced to 50 IU/kg 3 times/week. Treatment will be prescribed for a minimum of six months.

Randomization

Participants are randomized to either the Standard or Alternative Treatment Arm at a 2:1 ratio.

Upon confirmation of eligibility, participants will be randomized by the Clinical Coordinating Center to maintain blinding of the site to randomization assignment. Randomization envelopes will be used. Based on randomization, lot assignments will be provided by the Clinical Coordinating Center to the sites.
Randomization will occur approximately 1 month prior to treatment initiation to allow for lot selection and distribution of the assigned lot.

Treatment Concentrate

Standard Treatment Arm
Wilate® will be the concentrate used for ITI in the Standard Treatment Arm. Concentrate from the randomly selected lot will be provided.

Alternative Treatment Arm
Wilate® will be the concentrate used for ITI in the Alternative Treatment Arm. Concentrate from the individually selected lot will be provided.

Packaging and Labeling of Factor Concentrate

Wilate® is available as 500 and 1000 IU VWF/FVIII (ratio 1:1) vials. The vials are distributed with a Mix2Vial™ needle-free transfer device.

Drug Ordering, Storage, and Accountability

Drug Distribution

Once subjects have enrolled, been randomized to receive a matched or random lot of product, the information as to which product lot has been assigned to that subject, using the subject's identifying code, will be transmitted to ASD Healthcare. The subject's treating institution will order product from ASD Healthcare through its usual distribution pharmacy using the subject's identifying code in the same manner as any other patient product would be ordered. ASD Healthcare has committed to provide factor at or below what the institution would be charged if product were ordered for a non-study patient. ASD Healthcare will ship product to the pharmacy routinely used by the institution for that subject and product will be delivered to the subject in the usual manner for that pharmacy.

Wilate® will be stored at the participant's home either in the myCubixx refrigerator or their own refrigerator. The myCubixx refrigerator is also provided by ASD Healthcare.

Prior and Concomitant Therapy

Prior Therapy
Prior ITI regimens are allowed. Prior ITI regimen must have been discontinued within 1 month of study enrollment unless there is clear evidence of ITI failure with no reduction in inhibitor titer over the past 2 months.

Bypassing Agents
As long as the subject's inhibitor level is ≥0.6 BU, we expect that prevention and treatment of bleeding episodes may require bypassing agents.

Prophylactic Therapy with Bypassing Agents (anti-inhibitor coagulant complex [FEIBA®],rVIIa, [NovoSeven®RT], and Hemlibra®)
Bypassing agents mnay be prescribed as prophylaxis according to the participant's bleeding tendency based on prior reports. The use of bypassing agents should be stopped once the subjects' inhibitor level is <0.6 BU, but continued use is at the discretion of the treating physician.

Bleed Treatment with Bypassing Agents (anti-inhibitor coagulant complex [FEIBA®] and rVIIa, [NovoSeven®RT])

As guidance, the following treatment regimens might be used for treatment of hemorrhages or in subjects with a high bleeding tendency for prevention of bleeding [1,75] FEIBA®: 75-100 IU/kg, twice daily, dosage depending on severity of hemorrhage. Same dosage recommendation is given for prophylactic treatment in subjects with a high bleeding tendency.

1) NovoSeven®RT)=: 90 µg/kg every 2 hours, dosage and frequency may be adjusted based on physician discretion depending on severity of hemorrhage.

2) In case of insufficient effect with FEIBA® and/or NovoSeven®RT, recombinant porcine FVIII (rpFVIII,OBIZUR®) might be an alternative bleeding treatment: 50-100 IU/kg up to twice daily, dosage depending on severity of hemorrhage (only recommended in subjects with porcine FVIII inhibitor levels <20 BU). OBIZUR® is not approved by the FDA for the treatment of congenital Hemophilia A.

Prophylactic Therapy with Non-factor replacement (emicizumab-kxwh, Hemlibra®)

Hemlibra® is now FDA approved to prevent bleeding in patients with hemophilia A and FVIII inhibitors. There are no reports of use in combination with ITI and safety is unproven. If an investigator elects to use Hemlibra® along with ITI in patients with significant and frequent breakthrough bleeds then the dose of ITI should be reduced to 50 units/kg three times weekly. Investigators should follow prescribing information for Hemlibra® in regards to treatment of bleeding episodes with other Bypassing Agents (anti-inhibitor coagulant complex [FEIBA®] and rVIIa, [NovoSeven®RT]).

Immunosuppression
Immunosuppressant and immune modulating agents are not allowed with the exception of intermittent steroid use for asthma.

Participant Adherence with ITI

Adherence during ITI is very important, as interruptions of regular injections have the potential to reduce the chance for ITI success. Through a system like myCubixx from ASD Healthcare, with RFID-tagged product in a refrigerator with an RFID reader, information on whether subjects are following the treatment regimen can be accessed via a portal. In addition, the product on stock at each participant's residence is monitored constantly, which allows for notification of the product stocks in real time. Smart tags in the myCubixx refrigerators use a secure cellular network, so no extra network or installation costs are needed. All information in the myCubixx portal is de-identified and do not contain Protected Health Information.

In addition, sites will be performing drug accountability. Participants will be asked to bring their empty vials and boxes to their follow-up visits.

Interruption of Study Drug

ITI should not be interrupted for any reason, specifically not for recovery or pharmacokinetic assessments. If a participant is admitted the hospital, the assigned Wilate® lot should be utilized whenever possible. No more than 7 consecutive days of alternative concentrate will be allowed.

Dose Reduction Based on Response to ITI (See Appendix III for the Subject Flow Diagram)

In cases where complete success is achieved, ITI will be terminated. The participant may start decreasing the ITI dose to eventually receive a prophylactic treatment regimen of ≤50 IU FVIII/kg every second day after complete inhibitor elimination as determined by his treating physician.

In cases where partial success is achieved, ITI may be terminated or continued at discretion of the treating physician.

In cases where partial response is achieved, ITI will be continued.

Protocol-Directed Study Treatment Discontinuation

Participants may decide to discontinue participation at any time during the study by withdrawing consent. Investigators may discontinue protocol-directed study treatment for any subject, if in their professional opinion, the subject's health, safety, and/or well-being is threatened by continued protocol-directed study treatment. The following circumstances require discontinuation of protocol-directed study treatment:

- ITI will be suspended in case of insufficient subject's adherence (<80% prescribed doses in a 3-month period or a single interruption in treatment of ≥7 days). The choice of further therapeutic options remains the responsibility of treating physician.

- In cases of partial or complete treatment failure after a maximum study treatment period of 18 months, ITI will be terminated. The choice of further therapeutic options is the responsibility of the treating physician. At six months, participants in the standard treatment arm who still have an inhibitor titer >0.6 may be switched to the alternative treatment arm.

CLINICAL AND LABORATORY EVALUATIONS

The following clinical information will be collected during the screening period:

a) Hemophilia type
b) Hemophilia severity
c) Baseline FVIII activity
d) Medical co-morbidities
e) Date of birth
f) Race/ethnicity
g) FVIII mutation (if known)
h) Date inhibitor ≥0.6 BU first detected
i) Inhibitor titer when first detected
j) Date of peak inhibitor titer prior to any ITI
k) Peak inhibitor titer prior to any ITI

l) Prior ITI courses:

- Titer at start
- Peak titer
- Product used
- Dose
- Outcome

m) Prior immunosuppressant or immune modulatory agents used for ITI
n) Prior bypassing agents (type and frequency)
o) In vitro reactivity of FVIII inhibitors against lots of concentrate

Efficacy Studies and Assessments

The following efficacy studies will be performed during the study:

- FVIII inhibitor assay
- FVIII recovery
- FVIII half-life
- Bleeding history

Safety Assessments

The following safety assessments will be performed during the study:

- Hemophilia-related events
- ITI-related AEs and SAEs
    - Infections
    - Thrombotic events

Special Studies

Several laboratory studies on participant blood samples will be performed in centralized laboratories as part of the secondary endpoint analyses. Samples will be collected for special laboratory studies, which will include the following:

- Epitope mapping
- Immunogenotyping/HLA genotyping
- FVIII genetic testing

Subjects participating in the Special Studies will provide approximately 5mL additional blood.

SCHEDULE OF EVENTS (See Appendix I)

Evaluations at Eligibility Screening

Prior to performance of any study-related procedures, subjects and/or their legally authorized representative must sign the informed consent document and minor subjects may be required to give assent as determined by the IRB of Record for the site. The subject will then be screened for eligibility prior to randomization. The eligibility screen period includes the analysis of the subject's plasma in the central lab and must be completed in no more than 5 weeks.

Screening Evaluations

- Medical history/co-morbidities
- Prior/concomitant medications
- Weight and height
- HRQoL assessment
- Diary distribution
- FVIII activity
- FVIII inhibitor
- VWF antigen/activity
- Epitope mapping
- Immunogenotyping/HLA genotyping
- FVIII genetic testing
- FVIII reactivity to Wilate® lots*

*Three (3) tubes of blood will be collected and sent to the central lab. One (1) tube will be used for initial FVIII reactivity to Wilate® lot/s. The second and third tubes will be processed and stored. The additional tubes are to be used in the event that a lot is depleted by a subject and/or participants in the standard treatment arm who still have an inhibitor titer >0.6 are switched to the alternative treatment arm. A tube will be tested against Wilate® lot/s so a new lot can be assigned to the subject in both circumstances. If any of the additional tubes is not used during the subject's participation in the study, the sample will be destroyed.

Laboratory Evaluations during ITI Treatment

The following tests will be done at 2 and 4 weeks after ITI initiation and then on a monthly basis:

- FVIII inhibitor titer; if a negative result is obtained, a repeat test should be drawn within 1 week

The following tests will be done at 2 and 4 weeks after ITI initiation and then on a monthly basis through week 24:

- VWF activity and antigen

The following tests will be done based on response to ITI:

- FVIII assay
- FVIII recovery (once FVIII inhibitor negative)
- FVIII half-life (once FVIII recovery at ≥66%)

Laboratory tests will be collected at the research site.

Clinical Evaluations during ITI Treatment

Participants will have evaluations on a monthly basis until ITI is completed. Evaluations must be performed at the clinical site at least every 8 weeks. Clinical evaluations will be performed by telephone call 4 weeks after every in-person visit performed at the clinical site.

The following information will be collected:

- Concomitant medications
- Changes to medical history/co-morbidities
- Bleeding history
- HRQoL (at first clinical evaluation after negative titer documented)
- ITI-related AEs and SAEs
- Height/weight (as indicated in the schedule of events)
- Diary information

Laboratory Evaluations during Follow-Up

Laboratory tests will be done on an every-other month basis after complete or partial success.

The following tests will be done:

- FVIII inhibitor titer

Clinical Evaluations during Follow-Up

Participants will have evaluations at the clinical site every 8 weeks until week 52 after complete or partial success.

The following information will be collected:

- Concomitant medications
- Changes to medical history/co-morbidities
- Bleeding history

- HRQoL (study exit)
- ITI-related AEs and SAEs

EFFICACY, SAFETY AND SPECIAL STUDIES

Protocol studies will be performed at central laboratories. Investigators desiring rapid laboratory results may perform studies in their local lab but official results to be used in data analysis will be from the central laboratory.

Efficacy

- FVIII inhibitor assay (performed at central lab)
- FVIII activity assay (performed at central lab)

- Incremental *in vivo* FVIII recovery in the normal range [≥66% of normal (1.5 %/IU/kg), equal to 0.99%/IU/kg] with samples taken prior to and 15 or 30 minutes after FVIII treatment. The recovery assessment should be done after the regular ITI treatment, without any wash-out period.
- Half-life of FVIII ≥6 hours (blood samples for FVIII determination should be taken 6 ± 2 hours and 24 ± 2 hours after FVIII treatment). The half-life assessment should be done in a non-bleeding status, e.g. after concentrate treatment, without any wash-out period interrupting the ITI.

In vitro reactivity of FVIII inhibitors against lots of concentrate

Alternative Treatment Arm

For participants in the alternative treatment arm, the *in vitro* reactivity of FVIII inhibitors will be tested against the lots of the study concentrate. The test will be performed according to a modified Oxford method. The batch yielding the highest remaining FVIII activity and the lowest remaining inhibitor titer will be documented. A plasma sample will be retained in case that the first lot is depleted and another batch needs to be identified.

At least three weeks prior to when a participant depletes his lot, a subsequent lot will be selected (randomly for the Standard Arm and based on FVIII reactivity to Wilate® lots for the Alternative Arm).

Standard Treatment Arm

For participants in the standard treatment arm, the *in vitro* reactivity of FVIII inhibitors against the randomly selected concentrate batch will be performed according to a modified Oxford method. This information will be used for secondary analyses. A plasma sample will be retained in case that the first lot is depleted and another batch needs to be identified.

For both arms of the study, the lot/s of study concentrate against which subject plasma samples are tested are randomly chosen by the Coordinating Center from a database containing all lots currently available. Once a lot is assigned to the subject, verification of receipt of correct lot by the subject will be performed by the Coordinating Center through the myCubixx system. Any errors in lot distribution will be escalated and handled appropriately following standard procedures.

Safety

VWF activity and antigen will be performed at baseline and at interval study visits to determine if there is VWF accumulation during ITI. The rationale is that, although rare, significant thrombotic events have been reported during ITI with VWF/FVIII concentrate.[76]

Special Studies Performed at Central Labs (Optional)

Epitope Mapping

The antibody epitopes on FVIII will be identified by screening peptide libraries and FVIII fragments with inhibitor patient plasma. This will help to identify relevant epitopes recognized by inhibitors in hemophilia A. This research program will give an opportunity to comprehensively study various aspects of inhibitors and the induction of immune tolerance in a relevant number of patients. Knowledge about relevant epitopes from patient plasma rather than from model systems (i.e. monoclonal antibodies or mouse models) will help to understand the immune response to FVIII and to develop novel strategies to deplete inhibitors or inhibitor secreting B cells (University Hospital Frankfurt, Laboratory for Molecular Haemostasis and Immunodeficiencies, Frankfurt, Germany). Results will not be shared with the site investigators.

Immunogenotyping/HLA genotyping

This study will investigate genetic risk factors (gene defect responsible for hemophilia, HLA class II alleles, and immune response genes) as a potential variable with impact on course and outcome of ITI. Recently a significantly higher inhibitor incidence has been found in patients (brothers) with IL-10.G allele 134, TNFA - 308G>A polymorphism within Hap and polymorphisms in the CTLA-4-gene compared to those brothers without the above mentioned polymorphisms[77-79] (University Hospital Bonn, Haemophilia Centre, Bonn, Germany). These tests are desired but not mandatory. Results will not be shared with the site investigators.

Factor VIII genetic testing

Genetic testing to identify the causative FVIII mutation will be done in conjunction with the HLA genotyping. This test is desired but not mandatory. Results will be shared with the site investigators.

Centralized Laboratory Measurements

Specimens sent to the Central Lab are identified by a specimen ID number, and the Central Lab will remain masked to subject ID and treatment assignment.

ITI Success Adjudication Process

INITIATE will employ a masked process for determining ITI success. The INITIATE Steering Committee will review all of the primary efficacy data to determine ITI success.

Economic Impact Analysis

It is expected that participants who achieve criterion 1 (loss of a measurable inhibitor titer) will have a dramatic decrease in the rate of bleeding and the need for and use of bypassing agents. This should have an impact on the cost of therapy. The use of all clotting products will be assessed in all study participants. The use of the myCubixx system should improve the collection of the data as to the number of times factor is used, the date of use, and the type of factor and for what purpose. This will allow calculation of the average number of doses of product used for prophylaxis, for bleeding episodes and the number of units. Average costs/bleeding episode can be calculated as well as total costs over time. The costs will be estimated from the average sales price of each factor product as published by the Centers for Medicare and Medicaid Services (CMS) on the CMS.gov website. This information can be divided by cost early during ITI, cost after reaching criteria 1 and 2. All costs will be calculated in US dollars and average sales prices can be determined by year of use since the CMS data is updated annually.

Satisfaction/Health-Related Quality of Life

The loss of an inhibitor with the concomitant reduction in bleeding episodes, decrease in use of bypassing agents and eventual reduced frequency of dosing of factor VIII should be accompanied by an improved health-related quality of life (HRQoL). HRQoL will be assessed with hemophilia-specific HRQoL questionnaires according to different age groups, which have been linguistically validated and culturally adapted for the respective languages[83].

- The 'Quality of life assessment instrument for children and adolescents with haemophilia' (Haemo-QoL) will be used to assess HRQoL for children and adolescents[84] . It consist of 21-77 items depending on the age group pertaining to 8-13 domains, respectively and a total score with high values implying high impairments in HRQoL.
- The 'Haemophilia-specific Quality of Life Questionnaire for Adults' (Haem-A-QoL)[85] will be used to assess HRQoL in adults. It consists of 46 items pertaining to 10 domains and a total score. High values imply high impairments in HRQoL.

Assessments will be performed at the start of the study, at the first clinical evaluation after negative titer documented, and at study exit. Raw data will be normalized for each study and summary statistics generated for study participants at each time point as well as longitudinally per participant.

INITIATE SAFETY MONITORING PROGRAM

Safety Assessment

Hemophilia-Related Events

A variety of adverse events (AEs) and some serious adverse events (SAEs) are expected to occur during the INITIATE trial due to the nature of the underlying disease and the clinical status

of the enrolled subjects. These AE/SAEs will not necessitate discontinuation of treatment and withdrawal from the study.

ITI-Related Complications

- Infections
- Thrombotic Events

Safety Monitoring Program

Safety monitoring will include data capture for adverse events and adverse reactions, safety reviews, and reporting.

Data Capture

Clinical data will be captured for subject-reported adverse event or suspected adverse reaction information at the time of a study visit. SAEs must be reported in an expedited fashion.

A Data Safety Monitoring Committee (DSMC) will review the reported adverse events.

After reviewing the safety event, the DSMC may recommend a modification of the protocol, termination of the project for an individual center or participant or propose interim analyses.

Any of the following drug safety information shall be collected:

- Adverse events (AEs) and serious adverse events (SAEs) temporally associated with the administration of Wilate® (definitions and reporting requirements see below)
- Other relevant safety information, including post-study safety reports, instances of drug overdose, drug-drug interactions, abuse, misuse, medication error, or lack of efficacy (see below)

Adverse Events

Definitions

An AE is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product. (Definition per International Council on Harmonisation [ICH])

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

Note: The sponsor collects AEs starting with the signing of the ICF.

Adverse Event Reporting

The condition of the subject will be monitored throughout the study. At each scheduled or unscheduled study visit, AEs will be elicited using a standard non-leading question such as "How have you been since the last visit?" In addition, the subject diaries will be checked by study personnel for any documented event.

Any AE or ADR which occurs during the study will be documented in detail on the appropriate CRF. If the subject reports several signs or symptoms representing a single syndrome or diagnosis, the syndrome or diagnosis should be recorded in the CRF. The investigator responsible will grade the severity of all AEs or ADRs (mild, moderate, or severe), the seriousness (non-serious or serious), and the causality as defined below. The Sponsor is responsible for assessing the expectedness of each ADR (expected or unexpected) as defined below.

In the event of clinically significant abnormal laboratory findings, the tests will be repeated and the subject followed up until the laboratory values have returned to normal and/or an adequate explanation for the abnormality has become available.

Diseases, signs, and symptoms and/or laboratory abnormalities already present before the first administration of medicinal product will not be considered AEs unless an exacerbation in intensity or frequency (worsening) occurs.

The responsible investigator will provide detailed information about any abnormalities and about the nature of and reasons for any action taken as well as any observations or comments that may be useful for the interpretation and understanding of an AEs or ADR.

Severity of AEs

The severity of AEs will be graded as follows:

- **Mild:** an AE, usually transient, which causes discomfort but does not interfere with the subject's routine activities
- **Moderate:** an AE which is sufficiently discomforting to interfere with the subject's routine activities
- **Severe:** an AE which is incapacitating and prevents the pursuit of the subject's routine activities.
-

The grading of AEs is up to the medical judgment of the investigator and will be decided on a case-by-case basis.

Causality of AEs

The relationship of AEs to the administered medicinal product will be assessed by the responsible investigator. The causality of AEs will be classified as follows:

- **Probable:** reports including good reasons and sufficient documentation to assume a causal relationship, in the sense of plausible, conceivable, likely, but not necessarily highly probable. A reaction that follows a reasonable temporal sequence from

administration of the medicinal product; or that follows a known or expected response pattern to the suspected medicine; or that is confirmed by stopping or reducing the dosage of the medicine and that could not reasonably be explained by known characteristics of the subject's clinical state.

- **Possible:** reports containing sufficient information to accept the possibility of a causal relationship, in the sense of not impossible and not unlikely, although the connection is uncertain or doubtful, for example because of missing data or insufficient evidence. A reaction that follows a reasonable temporal sequence from administration of the medicinal product; that follows a known or expected response pattern to the suspected medicine; but that could readily have been produced by a number of other factors.
- **Unlikely:** reports not following a reasonable temporal sequence from medicinal product administration. An event which may have been produced by the subject's clinical state or by environmental factors or other therapies administered.
- **Not related (unrelated):** events for which sufficient information exists to conclude that the aetiology is unrelated to the medicinal product.
- **Unclassified:** reports which for one reason or another are not yet assessable, e.g. because of outstanding information (can only be a temporary assessment).

Classification of ADRs

ADRs will be classified as expected or unexpected:

- **Expected:** an AE that is listed in the Wilate® package insert.
- **Unexpected:** an AE that is not listed in the Wilate® package insert, or that differs because of greater severity or greater specificity.

Outcome of AEs

The outcome of all reported AEs has to be documented as follows:

(a) Recovered, resolved
(b) Recovering, resolving
(c) Not recovered, not resolved
(d) Recovered, resolved with sequelae
(e) Fatal
(f) Unknown

A subject's **death** per se is not an event, but an outcome. The event having resulted in the subject's death must be fully documented and reported. Deaths occurring within 4 weeks after medicinal product (Wilate®) treatment end also have to be reported, regardless of whether or not they are considered treatment-related.

Action(s) taken

AEs requiring action or therapy must be treated with recognized standards of medical care to safeguard the health and well-being of the subject. Appropriate resuscitation equipment and medicines must be available to ensure the best possible treatment of an emergency situation.

The action taken by the investigator must be documented:

- General actions taken in the event of an AE
  - None
  - Medication (other than Wilate®) or other (e.g., physical) therapy started
  - Test performed
  - Other (to be specified)

The responsible investigator will follow up on each AE until it has resolved or until the medical condition of the subject has stabilized. Any relevant follow-up information will be reported to the Sponsor.

Serious Adverse Events (SAEs)

Serious Adverse Event
A SAE based on ICH and EU Guidelines on Pharmacovigilance for Medicinal Products for Human Use is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (The subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a suspected transmission of any infectious agent via a medicinal product
- Is Medically Important*

* Medical and scientific judgment should be exercised in deciding whether expedited reporting is also appropriate in other situations, such as, important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should usually be considered serious.

One such 'important medical event' is the **suspected transmission of an infectious agent.** These events, therefore, **have to be reported as SAEs**.

A suspected virus transmission means that virus antigen has been detected in the subject. The passive transmission of antibodies alone does not constitute a suspected virus transmission.

SAE Reporting Timelines

Within 24 hours after recognition of the event, a 'Serious Adverse Event Report' must be completed and submitted whether or not they are suspected to be related to the study treatment. All SAEs should be reported to the Coordinating Center.

Exemption from the SAE reporting requirement

Exemptions from the SAE reporting requirement include surgeries that are elective or had been planned before study entry or prolongations of existing hospitalizations for economic or social

rather than medical reasons. Such surgeries or prolongations of hospitalizations should not be considered SAEs.

Other Relevant Safety Information

Instances of drug overdose, drug-drug interactions, abuse, misuse, medication error, or lack of efficacy (items (b) to (g)) should be reported as AEs or SAEs, as applicable.

(a) Post-study safety reports

The investigator should also report any ADR (i.e., any AE with a suspected causal relationship to the medicinal product) occurring after completion of the study. The usual procedure for reporting post-marketing safety information should be followed, but the suspected causal relationship to the clinical study should be stated on the report.

(b) Drug overdose

An overdose is the deliberate or inadvertent administration of a treatment at a dose higher than that specified in the protocol and higher than the known therapeutic dose, and it must be of clinical relevance. The event must be clearly identified as an overdose.

(c) Drug-drug interactions

A drug interaction is a situation where a substance/medicinal product affects the activity of a medicinal product, i.e. increases or decreases the effect(s) of an medicinal product, or produces an effect that none of the products would exhibit on their own. The event must be clearly identified as resulting from a drug-drug interaction.

(d) Abuse

Abuse is the deliberate use of a medicinal product that may lead to addiction accompanied by harmful physical or psychological effects.

(e) Misuse

Misuse is the deliberate administration or use of the medicinal product outside its described indication or outside the current state of the art medical practice (off-label-use). The reaction must be clearly identified as misuse.

(f) Medication error

Medication error involves the inadvertent administration or unintended use of a medicinal product which may be caused by the naming, presentation of pharmaceutical form/packaging, instructions for use/labelling. The reaction must be clearly identified as a medication error.

(g) Lack of efficacy

Lack of efficacy is suspected when the therapeutic result is not as expected, e.g., when patients with haemophilia experience continued bleeding despite the correct administration of coagulation factors.

REGULATORY AND CLINICAL MONITORING

The Site Principal Investigators (PIs) will perform source document verification of eligibility for all study enrollments. Verification also includes appropriate documentation of consent. Monitoring the timeliness of AE/SAE reporting will be done as events are reported. The study will also be monitored for compliance with the International Conference on Harmonization (ICH) Good Clinical Practice Guidelines (GCP).

DATA COLLECTION

Data Entry and Case Report Forms

Clinical sites will enter data onto electronic Case Report Forms (eCRFs) via a secure, internet-based electronic data capture (EDC) system. eCRFs must be completed for each subject screened or enrolled according to the subject's source data on a per-visit basis. Authorized study personnel will each be granted access to the electronic data capture system via provision of a unique password-protected user ID that will limit access to enter and view data specifically for subjects enrolled at their site. Data should be entered into the EDC system within 3 business days of a subject's visit. Sites will be supplied with a set of source document worksheets that correspond to the electronic case report form (eCRF). The worksheets will serve as source documents and are required to be used to enter data into the eCRFs.

In addition, data will be collected from the subject's medical record, subject reports, and subject diaries. Data on adherence to ITI dosing regimen will be collected from myCubixx via the portal. HRQoL questionnaires should be sent to the Study Coordinating Center for data entry in order to protect patients' privacy.

STATISTICAL ANALYSIS

Randomization: Participants will be randomized 2:1. Treatment arm assignments (A-random lot, B-matched lot) will be determined by computer. The assignment will be communicated to the laboratory performing the matching denoting the subject's code number and group assignment.

Data Analysis: Analysis of categorical variables, matched/unmatched lots by time to effect will be performed using Kaplan-Meier plots and log-rank tests. Analysis of continuous and ranked categorical variables (e.g. degree of match) will use Cox regressions or accelerated time failure models. If multiple lots are required for a subject, multilevel models will be used.

Power and Sample Size: A study with 110 participants will have the power to detect a hazard ratio as low as 2.5 at 90% with an α of 0.05. Given the initial hazard ratio estimates of 5 for time to BU < 0.6 and 2.5 for time to recovery > 80%, this should be sufficient. Assuming a drop-out/loss rate of about 10%, the study would try to enroll 120 subjects. An estimate of the number needed to demonstrate that ITI using Wilate® is superior overall (percentage of subjects achieving successful ITI, power of 80% and α of 0.05) is greater than 700 making this as an endpoint unachievable.

Interim Analysis: An interim analysis with the aim of possible early termination will use the alpha spending approach as described by DeMets and Lan.[80] The Lan-DeMets boundary using the O'Brien-Fleming spending function, assuming that most subjects will have achieved criteria 1 or

2 by 6 months of treatment, evaluation 6 months after the 60th subject has been enrolled should include about 55% of the information for the trial and the level at which one could reject the null hypothesis (no difference between the two arms for a one-sided test at a final $p < 0.05$) is $\alpha < 0.008$. In the event that trial accrual is slow, an analysis could be performed after 40 subjects have completed at least 6 months of treatment but this would include only about 36% of the total information and the required $\alpha < 0.001$.[81] Any interim analysis will be evaluated by an independent data monitoring board.

HUMAN SUBJECTS PROTECTION

All aspects of this clinical trial will be conducted according to the protocol, relevant FDA regulations, ICH-GCP Guidelines, and HIPAA for protection of human subjects. IRB approval must be obtained by each participating site. A copy of the IRB approval letter and the approved consent document must be received and approved prior to enrollment of subjects by the site. Copies of approval letters for continuing review must be received by the Coordinating Center prior to expiration of IRB approval so that sites' ability to enroll and treat participants is not interrupted. A copy of the IRB approval letter for each amendment must be received by the Coordinating Center in order for subjects to be enrolled on the protocol amendment. Continuing IRB review will occur annually or more frequently as determined by the IRB of Record for the site.

Risk to the Subjects

Subjects will be randomly assigned to the standard care or alternative arm of the trial. The risk to the subjects involves primarily the risk of bleeding with sustained inhibitor titer if they are randomized to the alternative arm of the trial, but this risk remains with the standard arm as well. Additional risks include: 1) minor risks related to venipuncture; 2) adverse reaction to Wilate®; 3) loss of anonymity.

Adequacy of Protection against Risks

The INITIATE trial will be designed and implemented with great concern for the welfare of participants. Subjects will be recruited by local clinical staff and should be well known to each center since they will already be receiving hemophilia care. If eligible, the participant or participant's parent will be informed about the study and asked to participate. After detailed discussion of the protocol, they will be given a copy of the informed consent document for review. Participation is completely voluntary and parents may withdraw their child from the study at any time. In addition to parental consent, written or verbal assent will be obtained from subjects whenever required by the IRB of Record for the site. Subjects and families will be informed of any information that becomes available during the trial that might impact their continued participation.

Protected Health Information (PHI): Subjects will be given code numbers and these numbers will be used to identify blood samples and matched/random lot allocation. This includes the drug distribution from ASD Healthcare to the distributing pharmacies and the information derived from the myCubixx refrigerator.

Oversight of the Study

Steering Committee

The INITIATE trial will receive overall guidance and oversight from a formal Steering Committee, consisting of the following members: Dr. Ducore, Dr. Thornburg, study site Principal Investigators (PIs) and a representative from the Central Lab. The entire Steering Committee will meet remotely twice during each year to discuss and provide direction for the INITIATE trial. Quarterly, the Steering Committee will have a telephone conference call to discuss problems and issues related to subject recruitment, enrollment, treatment, and data collection; minutes will be distributed.

Study Coordinating Center

The Study Coordinating Center will oversee the day-to-day issues and problems that arise during the INITIATE trial and will be critical to the smooth functioning of this clinical trial.

The Study Coordinating Center will meet with site staff by teleconference on a weekly basis throughout the trial, with a formal agenda designed to anticipate and address clinical, logistical, and practical problems that are encountered. Site staff will provide a site-specific report during each teleconference meeting, updating the Coordinating Center project manager on subject accrual, enrollment progress, data entry, and adherence strategies. During teleconference meetings, site staff will report any technical problems with ITI as applicable. Minutes from the weekly teleconferences will be distributed to each of the clinical sites.

Potential Benefits of the Proposed Research to Subjects and Others

Potential benefits to participants in this trial include the potential to achieve ITI success earlier and to have fewer bleeding episodes.

Importance of the Knowledge to be Gained

Inhibitors are associated with increased morbidity, mortality and associated costs. Determining an optimal ITI regimen has the potential to have an important impact.

Ethics

GCP and IRB Review

Compliance with GCP guidelines for the conduct and monitoring of this clinical trial will occur through observation of the ethical and regulatory requirements presented in ICH E6, Good Clinical Practice: Consolidated Guideline. By signing this protocol, the investigator agrees to adhere to these requirements. The study (protocol, informed consent, advertisements, product package insert, and subject-facing material) should be reviewed and approved by the IRB of Record for each site. Changes to the protocol will be also be reviewed and approved by the IRB of Record for each site. Subjects must sign written informed consent prior to being screened, before undergoing any study procedures.

The investigators and institutions affiliated with this study will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s) by providing direct access to source documents.

Informed Consent

A model informed consent will be prepared by the INITIATE Study Coordinating Center and subsequently provided to clinical centers for preparation of center-specific consent forms. A copy of each site's IRB approved consent form will be maintained at the Study Coordinating Center, and a new consent and IRB review will be required for any changes in the protocol. Families will be provided with a copy of their signed consent document. The peripheral clinical site should follow the sample informed consent as closely as possible. The informed consent can be modified to include IRB required language; the template language should not be deleted. Subjects will be provided with a copy of the informed consent and printed materials that further explain the purpose of the study, the medication(s) used in the study, procedures, and assessments. Subjects will also be provided with the telephone numbers of the Principal Investigator and qualified personnel who can assist with their questions and concerns.

Confidentiality

To ensure confidentiality, all enrolled subjects will be assigned a unique identification number that will be used in all correspondence between clinical centers and the Study Coordinating Center. The DSMC advises on issues such as study design, power estimates, stopping rules, interim analyses, and adverse event reporting. Members of the DSMC also assess the seriousness of unexpected adverse events. When the results of the trial are published, each subject's identity will remain confidential.

Disclosure of Data

The Principal Investigator, his or her staff and associates, and the appropriate regulatory agencies may use the information included in this protocol as necessary for the conduct of the trial and the safety of subjects. Data from the trial are confidential and may not be disclosed without the written permission of the Study Coordinating Center.

Publication of Research Findings

The results from this clinical trial have the potential for immediate public health applicability for the hemophilia community. A publications committee will be established to guide publications and authorship. Data analysis and manuscript preparation will occur during the last 6-12 months of the funding period. At the end of the trial, the main outcome paper will be submitted to a prestigious journal, such as the *New England Journal of Medicine*, *British Journal of Hematology*, or *Blood*. The trial results will be presented at the World Federation of Hemophilia meeting, the International Society of Haemostasis and Thrombosis (ISTH) meeting, the American Society of Pediatric Hematology/Oncology (ASPHO), and the American Society of Hematology (ASH). To educate allied health care professionals, the study results will be presented at Grand Rounds at the respective participating universities, plus the annual National Hemophilia Foundation Meeting. Other results generated from the trial that are not published in the main outcome manuscript will be submitted for publication in prestigious medical journals and presented at the scientific meetings listed above. ASPHO and ASH, organizations with which the investigators have a long-standing professional relationship, will be asked to assist in the educational effort of health care practitioners who care for subjects with hemophilia.

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Appendix I: Schedule of Events

Evaluation	Eligibility Screening		ITI							
Week	Week -8 to week -5	Consent to week -5	0	2 (±3 days)	4 (±3 days)	8 (±5 days)	12 (±5 days)	16 (±5 days)	20 (±5 days)	24 (±5 days)
Signed informed consent document	x		Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ
Clinical assessment*		x	x		x	x	x	x	x	x
Height and weight		x							x	
FVIII reactivity to Wilate®		x	^	^	^	^	^	^	^	^
Antibody epitopes on FVIII[C]		x								
Immunogenotyping/HLA genotyping[C]		x								
Factor VIII genetic testing[C]		x								
VWF activity/Ag		x	x	x	x	x	x	x	x	x
FVIII inhibitor titer		x	x	x	x	x	x	x	x	x[D]
FVIII Assay					ǂ	ǂ	ǂ	ǂ	ǂ	ǂ
FVIII trough level					ǂ	ǂ	ǂ	ǂ	ǂ	ǂ
FVIII recovery					ǂ	ǂ	ǂ	ǂ	ǂ	ǂ
Adherence assessment[A]					x	x	x	x	x	x
Dispense/collect diaries		x	x	x	x	x	x	x	x	x
HRQoL assessment[B]		x								

ITI=immune tolerance induction

ΔUpdate if participant turns 18 years old

*Clinical assessments must be done at clinical site every 8 weeks after the week 4 visit; telephone visits may be done 4 weeks ± 5 days after each in-person clinical site visit. All clinical assessments will include con med and adverse event follow-up.

^Repeat for participants who deplete originally assigned batch; should be done 3 weeks prior to lot depletion

ǂAssess FVIII recovery once FVIII inhibitor negative

ǂAssess FVIII 6 ± 2 hour and 22 ± 2 hour trough level once FVIII recovery at ≥66%

ǂ Perform FVIII assay as part of FVIII half-life and recovery assays

[A] Adherence assessments will be based on patient reports

[B] HRQOL assessment will be performed at the start of the study, at the first clinical evaluation after negative titer documented, and at study exit.

[C] Optional – collect only if consent is given by the subject

[D] Participants in the standard treatment arm who still have an inhibitor titer >0.6 may be switched to the alternative treatment arm.They will be treated as new participants who have failed prior ITI and a second individualized lot selection will be performed using the original plasma sample provided at baseline.

Schedule of Study Evaluations (cont)

Evaluation	ITI													Follow-up after Complete or Partial Success						
Week	28 (±5 days)	32 (±5 days)	36 (±5 days)	40 (±5 days)	44 (±5 days)	48 (±5 days)	52 (±5 days)	56 (±5 days)	60 (±5 days)	64 (±5 days)	68 (±5 days)	72 (±5 days)	76 (±5 days)	+8 (±5 days)	+16 (±5 days)	+24 (±5 days)	+32 (±5 days)	+40 (±5 days)	+48 (±5 days)	+52 (±5 days)
Signed informed consent document	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ
Clinical assessment*	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Height/weight							x						x							
FVIII reactivity to Wilate®	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^
VWF activity/Ag																				
FVIII Inhibitor titer	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
FVIII Assay	x	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ							
FVIII trough level	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ							
FVIII recovery	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ							
Adherence assessment[A]	x	x	x	x	x	x	x	x	x	x	x	x	x							
Dispense/collect diaries	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
HRQoL assessment[B]																				

ITI=immune tolerance induction

ΔUpdate if participant turns 18 years old

*Clinical assessments must be done at clinical site every 8 weeks after the week 4 visit; telephone visits may be done 4 weeks + 5 days after each in-person clinical site visit. All clinical assessments will include con med and adverse event follow-up.

^Repeat for participants who deplete originally assigned batch; should be done 3 weeks prior to lot depletion

ǂAssess FVIII recovery once FVIII inhibitor negative

ǂAssess FVIII 6 ± 2 hour and 22 ± 2 trough level once FVIII recovery at ≥66%ǂ Perform FVIII assay as part of FVIII half-life and recovery assays

[A] Adherence assessments will be based on patient reports

[B] HRQOL assessment will be performed at the start of the study, at the first clinical evaluation after negative titer documented, and at study exit.

Appendix II. Dosing Chart

BW [kg]	Dose [IU/kg/day]			Total dose [IU]/day			Selected total dose [IU]	Actual dose [IU/kg]	Batch duration [months]
	150	Variable	200	Range					
5	150	180	200	750	900	1000	1000	200	56.7
6	150	180	200	900	1080	1200	1000	167	56.7
7	150	180	200	1050	1260	1400	1500	214	37.8
8	150	180	200	1200	1440	1600	1500	188	37.8
9	150	180	200	1350	1620	1800	1500	167	37.8
10	150	180	200	1500	1800	2000	2000	200	28.3
11	150	180	200	1650	1980	2200	2000	182	28.3
12	150	180	200	1800	2160	2400	2000	167	28.3
13	150	180	200	1950	2340	2600	2000	153	28.3
14	150	180	200	2100	2520	2800	3000	214	18.9
15	150	185	200	2250	2775	3000	3000	200	18.9
16	150	185	200	2400	2960	3200	3000	188	18.9
17	150	185	200	2550	3145	3400	3000	176	18.9
18	150	185	200	2700	3330	3600	3000	167	18.9
19	150	185	200	2850	3515	3800	4000	211	14.2
20	150	185	200	3000	3700	4000	4000	200	14.2
21	150	185	200	3150	3885	4200	4000	190	14.2
22	150	185	200	3300	4070	4400	4000	182	14.2
23	150	185	200	3450	4255	4600	4000	174	14.2
24	150	185	200	3600	4440	4800	4000	167	14.2
25	150	185	200	3750	4625	5000	5000	200	11.3
26	150	185	200	3900	4810	5200	5000	192	11.3
27	150	185	200	4050	4995	5400	5000	185	11.3
28	150	185	200	4200	5180	5600	5000	179	11.3
29	150	185	200	4350	5365	5800	5000	172	11.3
30	150	190	200	4500	5700	6000	6000	200	9.4
31	150	190	200	4650	5890	6200	6000	194	9.4
32	150	190	200	4800	6080	6400	6000	188	9.4
33	150	190	200	4950	6270	6600	6000	182	9.4
34	150	190	200	5100	6460	6800	6000	176	9.4
35	150	190	200	5250	6650	7000	7000	200	8.1
36	150	190	200	5400	6840	7200	7000	194	8.1
37	150	190	200	5550	7030	7400	7000	189	8.1
38	150	190	200	5700	7220	7600	7000	184	8.1
39	150	190	200	5850	7410	7800	7000	179	8.1
40	150	190	200	6000	7600	8000	8000	200	7.1
41	150	190	200	6150	7790	8200	8000	195	7.1

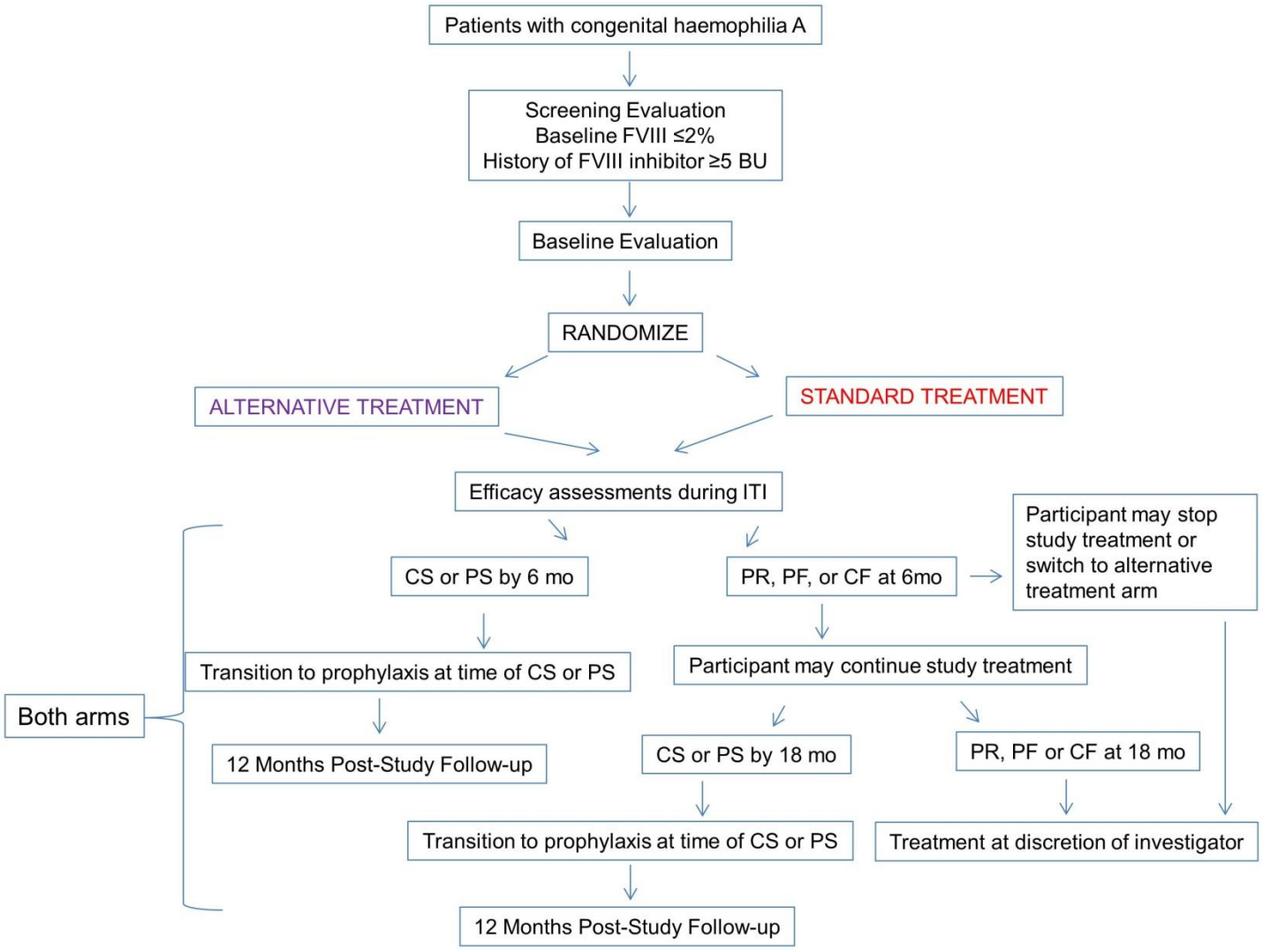
BW [kg]	Dose [IU/kg]			Total dose [IU]			Selected total dose [IU]	Actual dose [IU/kg]	Batch duration [months]
	150	Variable	200	Range					
42	150	190	200	6300	7980	8400	8000	190	7.1
43	150	190	200	6450	8170	8600	8000	186	7.1
44	150	190	200	6600	8360	8800	8000	182	7.1
45	150	190	200	6750	8550	9000	9000	200	6.3
46	150	190	200	6900	8740	9200	9000	196	6.3
47	150	190	200	7050	8930	9400	9000	191	6.3
48	150	190	200	7200	9120	9600	9000	188	6.3
49	150	190	200	7350	9310	9800	9000	184	6.3
50	150	190	200	7500	9500	10000	10000	200	5.7
51	150	190	200	7650	9690	10200	10000	196	5.7
52	150	190	200	7800	9880	10400	10000	192	5.7
53	150	190	200	7950	10070	10600	10000	189	5.7
54	150	190	200	8100	10260	10800	10000	185	5.7
55	150	190	200	8250	10450	11000	10000	182	5.7
56	150	190	200	8400	10640	11200	11000	196	5.2
57	150	190	200	8550	10830	11400	11000	193	5.2
58	150	190	200	8700	11020	11600	11000	190	5.2
59	150	190	200	8850	11210	11800	11000	186	5.2
60	150	195	200	9000	11700	12000	12000	200	4.7
61	150	195	200	9150	11895	12200	12000	197	4.7
62	150	195	200	9300	12090	12400	12000	194	4.7
63	150	195	200	9450	12285	12600	12000	190	4.7
64	150	195	200	9600	12480	12800	12000	188	4.7
65	150	195	200	9750	12675	13000	13000	200	4.4
66	150	195	200	9900	12870	13200	13000	197	4.4
67	150	195	200	10050	13065	13400	13000	194	4.4
68	150	195	200	10200	13260	13600	13000	191	4.4
69	150	195	200	10350	13455	13800	13000	188	4.4
70	150	195	200	10500	13650	14000	14000	200	4.0
71	150	195	200	10650	13845	14200	14000	197	4.0
72	150	195	200	10800	14040	14400	14000	194	4.0
73	150	195	200	10950	14235	14600	14000	192	4.0
74	150	195	200	11100	14430	14800	14000	189	4.0
75	150	195	200	11250	14625	15000	15000	200	3.8
76	150	195	200	11400	14820	15200	15000	197	3.8
77	150	195	200	11550	15015	15400	15000	195	3.8
78	150	195	200	11700	15210	15600	15000	192	3.8
79	150	195	200	11850	15405	15800	15000	190	3.8

BW [kg]	Dose [IU/kg]			Total dose [IU]			Selected total dose [IU]	Actual dose [IU/kg]	Batch duration [months]
	150	Variable	200	Range					
80	150	195	200	12000	15600	16000	16000	200	3.5
81	150	195	200	12150	15795	16200	16000	198	3.5
82	150	195	200	12300	15990	16400	16000	195	3.5
83	150	195	200	12450	16185	16600	16000	193	3.5
84	150	195	200	12600	16380	16800	16000	190	3.5
85	150	195	200	12750	16575	17000	17000	200	3.3
86	150	195	200	12900	16770	17200	17000	198	3.3
87	150	195	200	13050	16965	17400	17000	195	3.3
88	150	195	200	13200	17160	17600	17000	193	3.3
89	150	195	200	13350	17355	17800	17000	191	3.3
90	150	195	200	13500	17550	18000	18000	200	3.1
91	150	195	200	13650	17745	18200	18000	198	3.1
92	150	195	200	13800	17940	18400	18000	196	3.1
93	150	195	200	13950	18135	18600	18000	194	3.1
94	150	195	200	14100	18330	18800	18000	191	3.1
95	150	195	200	14250	18525	19000	19000	200	3.0
96	150	195	200	14400	18720	19200	19000	198	3.0
97	150	195	200	14550	18915	19400	19000	196	3.0
98	150	195	200	14700	19110	19600	19000	194	3.0
99	150	195	200	14850	19305	19800	19000	192	3.0
100	150	195	200	15000	19500	20000	20000	200	2.8
101	150	195	200	15150	19695	20200	20000	198	2.8
102	150	195	200	15300	19890	20400	20000	196	2.8
103	150	195	200	15450	20085	20600	20000	194	2.8
104	150	195	200	15600	20280	20800	20000	192	2.8
105	150	195	200	15750	20475	21000	20000	190	2.8
106	150	195	200	15900	20670	21200	20000	189	2.8
107	150	195	200	16050	20865	21400	20000	187	2.8
108	150	195	200	16200	21060	21600	20000	185	2.8
109	150	195	200	16350	21255	21800	20000	183	2.8
110	150	195	200	16500	21450	22000	20000	182	2.8
111	150	195	200	16650	21645	22200	20000	180	2.8
112	150	195	200	16800	21840	22400	20000	179	2.8
113	150	195	200	16950	22035	22600	20000	177	2.8
114	150	195	200	17100	22230	22800	20000	175	2.8
115	150	195	200	17250	22425	23000	20000	174	2.8
116	150	195	200	17400	22620	23200	20000	172	2.8
117	150	195	200	17550	22815	23400	20000	171	2.8
118	150	195	200	17700	23010	23600	20000	169	2.8

BW [kg]	Dose [IU/kg]			Total dose [IU]			Selected total dose [IU]	Actual dose [IU/kg]	Batch duration [months]
	150	Variable	200	Range					
119	150	195	200	17850	23205	23800	20000	168	2.8
120	150	195	200	18000	23400	24000	20000	167	2.8
121	150	195	200	18150	23595	24200	20000	165	2.8
122	150	195	200	18300	23790	24400	20000	164	2.8
123	150	195	200	18450	23985	24600	20000	163	2.8
124	150	195	200	18600	24180	24800	20000	161	2.8
125	150	195	200	18750	24375	25000	20000	160	2.8
							Max	214	56.7
							Min	153	2.8
							Mean	188	8.7
							Median	191	4.7

* New lot should be selected 8 weeks prior to end of current supply. Patients weighing more than 35 kg may need more than 1 lot during ITI and will need more samples collected at the beginning of the study

Appendix III: Subject Flow Diagram



CS Complete Success PS Partial Success

PR Partial Response PF Partial Failure CF Complete Failure

Appendix IV: Dose Reduction for Participants on Hemlibra®

BW [kg]	Dose [50IU/kg QOD]	Selected Total Dose [IU]	Actual Dose [IU/kg]	Batch Duration [months]
5	250	500	100	267
6	300	500	83	267
7	350	500	71	267
8	400	500	63	267
9	450	500	56	267
10	500	500	50	267
11	550	500	45	267
12	600	500	42	267
13	650	1000	77	133
14	700	1000	71	133
15	750	1000	67	133
16	800	1000	63	133
17	850	1000	59	133
18	900	1000	56	133
19	950	1000	53	133
20	1000	1000	50	133
21	1050	1000	48	133
22	1100	1000	45	133
23	1150	1000	43	133
24	1200	1000	42	133
25	1250	1000	40	133
26	1300	2000	77	67
27	1350	2000	74	67
28	1400	2000	71	67
29	1450	2000	69	67
30	1500	2000	67	67
31	1550	2000	65	67
32	1600	2000	63	67
33	1650	2000	61	67
34	1700	2000	59	67
35	1750	2000	57	67
36	1800	2000	56	67
37	1850	2000	54	67
38	1900	2000	53	67
39	1950	2000	51	67
40	2000	2000	50	67

BW [kg]	Dose [50IU/kg QOD]	Selected Total Dose [IU]	Actual Dose [IU/kg]	Batch Duration [months]
		2000	49	
42	2100	2000	48	67
43	2150	2000	47	67
44	2200	3000	68	44
45	2250	3000	67	44
46	2300	3000	65	44
47	2350	3000	64	44
48	2400	3000	63	44
49	2450	3000	61	44
50	2500	3000	60	44
51	2550	3000	59	44
52	2600	3000	58	44
53	2650	3000	57	44
54	2700	3000	56	44
55	2750	3000	55	44
56	2800	3000	54	44
57	2850	3000	53	44
58	2900	3000	52	44
59	2950	3000	51	44
60	3000	3000	50	44
61	3050	3000	49	44
62	3100	3000	48	44
63	3150	3000	48	44
64	3200	4000	63	33
65	3250	4000	62	33
66	3300	4000	61	33
67	3350	4000	60	33
68	3400	4000	59	33
69	3450	4000	58	33
70	3500	4000	57	33
71	3550	4000	56	33
72	3600	4000	56	33
73	3650	4000	55	33
74	3700	4000	54	33
75	3750	4000	53	33
76	3800	4000	53	33
77	3850	4000	52	33
78	3900	4000	51	33
79	3950	4000	51	33

BW [kg]	Dose [50IU/kg QOD]	Selected Total Dose [IU]	Actual Dose [IU/kg]	Batch Duration [months]
80	4000	4000	50	33
81	4050	4000	49	33
82	4100	4000	49	33
83	4150	4000	48	33
84	4200	4000	48	33
85	4250	5000	59	27
86	4300	5000	58	27
87	4350	5000	57	27
88	4400	5000	57	27
89	4450	5000	56	27
90	4500	5000	56	27
91	4550	5000	55	27
92	4600	5000	54	27
93	4650	5000	54	27
94	4700	5000	53	27
95	4750	5000	53	27
96	4800	5000	52	27
97	4850	5000	52	27
98	4900	5000	51	27
99	4950	5000	51	27
100	5000	5000	50	27
101	5050	5000	50	27
102	5100	5000	49	27
103	5150	5000	49	27
104	5200	5000	48	27
105	5250	5000	48	27
106	5300	6000	57	22
107	5350	6000	56	22
108	5400	6000	56	22
109	5450	6000	55	22
110	5500	6000	55	22
111	5550	6000	54	22
112	5600	6000	54	22
113	5650	6000	53	22
114	5700	6000	53	22
115	5750	6000	52	22
116	5800	6000	52	22
117	5850	6000	51	22
118	5900	6000	51	22

BW [kg]	Dose [50IU/kg QOD]	Selected Total Dose [IU]	Actual Dose [IU/kg]	Batch Duration [months]
119	5950	6000	50	22
120	6000	6000	50	22
121	6050	6000	50	22
122	6100	6000	49	22
123	6150	6000	49	22
124	6200	6000	48	22
125	6250	6000	48	22
		Max	**100**	**267**
		Mean	**56**	**63**
		Min	**40**	**22**