SIG-001-121 Protocol Version 5.0

A Phase 1/2 Open-Label, Dose-Escalation, Safety, Tolerability, and Efficacy Study of SIG-001 in Adult Patients with Severe or Moderately-Severe Haemophilia A Without Inhibitors (SIG-001-121)

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Approval Date: 04-Mar-2021

CLINICAL STUDY PROTOCOL

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Protocol Version	5.0
Protocol Date:	04 March 2021
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Sponsor:	Sigilon Therapeutics, Inc 100 Binney St, Suite 600 Cambridge, MA 02142

Previous Protocol Versions and Amendments:

Version 1.0	27 January 2020
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Version 2.0	23 April 2020
Version 3.0	29 April 2020
Version 4.0	04 May 2020

CONFIDENTIALITY & DISCLOSURE STATEMENT

1. ADMINISTRATIVE INFORMATION

1.1. Study Contacts

General advice on protocol procedures should be obtained through the clinical research associate (CRA) assigned to the study site. The following contacts are provided for this study.

CONTACT TYPE / ROLE	CONTACT
Sponsor Contact:	PPD Email: PPD Phone:
Medical Monitor (Sponsor):	PPD Email: PPD Phone
Medical Monitor (CRO):	PPD Email: PPD Phone:
Serious Adverse Event Reporting	PPD Email: PPD Fax: PPD

1.2. Corporate Identification

The Sponsor of this trial is Sigilon Therapeutics, Inc. (referred to as Sigilon or Sigilon Therapeutics, and its worldwide subsidiaries, as applicable).

SPONSOR SIGNATURE PAGE

Protocol Title: A Phase 1/2 Open-Label, Dose-Escalation, Safety, Tolerability, and Efficacy Study of SIG-001 in Adult Patients with Severe or Moderately-Severe Haemophilia A Without Inhibitors

This study will be conducted with the highest respect for the individual participants in accordance with the requirements of this clinical study protocol and also in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki
- International Council for Harmonisation E6 Good Clinical Practice Consolidated Guideline
- All applicable laws and regulations, including, without limitation, data privacy laws, clinical trial disclosure laws, and regulations

This clinical study protocol has been approved by Sigilon Therapeutics. The responsible Sigilon Therapeutics signatory is shown below. Electronic signatures are located on the last page of the document.

Signature: PPD Date: PPD

Name (print):

Title:

INVESTIGATOR AGREEMENT

Protocol Title: A Phase 1/2 Open-Label, Dose-Escalation, Safety, Tolerability, and Efficacy Study of SIG-001 in Adult Patients with Severe or Moderately-Severe Haemophilia A Without Inhibitors

I confirm that I have read and that I understand this Protocol. I agree to conduct this study in accordance with the requirements of this protocol and also to protect the rights, safety, privacy, and well-being of study subjects in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki
- International Council for Harmonisation, E6 Good Clinical Practice: Consolidated Guideline
- All applicable laws and regulations, including, without limitation, data privacy laws and regulations
- Regulatory requirements for reporting serious adverse events defined in this Protocol and described in the product's Investigator's Brochure
- Terms outlined in the Clinical Study Site Agreement

I further authorize that my personal information may be processed and transferred in accordance with the uses outlined in the Protocol and Clinical Study Site Agreement.

Signature: _____ Date: _____

Name (print): _____

Study Centre: _____

Table of Contents

List of Tables

List of Figures

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Table 1 Abbreviations

Abbreviation or Term	Expanded Term
Δ	delta, meaning "change from"
ABR	annualized bleeding rate
AE	adverse event
ALARP	as low as reasonably possible
ALT	alanine aminotransferase
aPTT	activated partial thromboplastin time
ARPE-19	allogeneic retinal pigment epithelial cell line
AST	aspartate aminotransferase
BDD-hFVIII	B-domain deleted human factor VIII
BMI	body mass index
BP	blood pressure
CFR	Code of Federal Regulations
CMO	Contract Manufacturing Organization
CRA	Clinical Research Associate
CRP	C-Reactive Protein
CRT	controlled room temperature
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
EC	ethics committee
ECG	electrocardiogram
EDC	electronic data capture
eCRF	electronic case report form
FBS	foetal bovine serum
EQ-5D-5L	5-level EQ-5D
FBR	foreign body response
FDA	Food and Drug Administration
FIH	first-in-human
FVIII	blood coagulation factor VIII
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practices
GTMP	gene therapy medicinal product
HA	Haemophilia A
Haem-A-QoL	Haemophilia Quality-of-Life Questionnaire for Adults
HBsAg	Hepatitis B virus "surface" antigen
HBeAg	Hepatitis B virus "extractable" antigen

Abbreviation or Term	Expanded Term
HBV	Hepatitis B virus
HCV	Hepatitis C virus
hFVIII	human factor VIII
HIV	Human Immunodeficiency Virus
HJHS	Haemophilia Joint Health Score assessment
IBD	inflammatory bowel disease
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	independent ethics committee
IND	investigational new drug
INR	international normalized ratio
IP	Intraperitoneal, or Investigational Product
IRB	institutional review board
ITI	immune tolerance induction
IV	intravenous(ly)
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic Resonance Imaging
NBU	Nijmegen Bethesda Units
PCR	polymerase chain reaction
PE	physical examination
PETG	polyethylene terephthalate glycol
PFO	pericapsular fibrotic overgrowth
PICC	peripherally inserted central catheter
PK	pharmacokinetic(s)
QP	Qualified Person
QTcF	QT interval corrected using Fridericia formula
RNA	ribonucleic acid
RPE	retinal pigment epithelial (cells)
rFVIII	recombinant FVIII
SAE	serious adverse event
SIG	Sigilon Therapeutics, Inc.
SRC	Safety Review Committee
TEAE	treatment emergent adverse events
ULN	upper limit of normal
US	United States
VWF	von Willebrand factor
WHO	World Health Organization
WHO-DDE	World Health Organization Drug Dictionary Enhanced

2. STUDY SYNOPSIS

Sponsor:	Sigilon Therapeutics, Inc.
Name of Investigational Treatment:	SIG-001 (BDD-hFVIII Producing Spheres)
Short Title:	Safety and Efficacy of Encapsulated Allogeneic FVIII Cell Therapy in Haemophilia A
Investigational Product: SIG-001 is comprised of an allogeneic human retinal pigment epithelial (ARPE-19) cell line genetically modified with a non-viral vector to express B-domain deleted human factor VIII (BDD-hFVIII). The cells are encapsulated within two-layered modified alginate spheres. The inner layer alginates are conjugated with a peptide to promote cell adhesion and the outer layer alginates are conjugated with a small molecule to protect from pericapsular fibrotic overgrowth and immune rejection	
Study Centre(s):	Approximately 10 study centres worldwide
Phase of Development:	Phase 1/2
Background and Rationale: SIG-001 is designed to enable sustained, long-lasting release of human FVIII. This study is being conducted as a first-in-human (FIH) trial to assess SIG-001 safety, tolerability, and preliminary efficacy	
Study Design: SIG-001-121 is a first-in-human (FIH), phase 1/2, multi-centre, open-label study in escalating dose cohorts to assess the safety, tolerability, and preliminary efficacy of SIG-001 in adults with severe or moderately-severe haemophilia A without inhibitors. Up to three dose cohorts are planned	
Number of Patients: Up to 18 patients are planned, including up to 3 initial and 3 additional (up to 6 total) patients per cohort	
Duration: Total study duration is a maximum of 63 months. This includes up to 90 days for screening and 5 years of follow-up after a single administration of SIG-001	
Route of Administration: Laparoscopic administration of a buffered suspension of SIG-001 into the greater sac of the peritoneal cavity	
Dose Levels: Three escalating dose cohorts are planned, with the starting dose (volume of SIG-001 spheres) targeted to achieve 10 IU/dL plasma level (equivalent to 10% of normal FVIII activity). The maximum dose evaluated in the trial will not exceed CCI spheres. • Cohort 1 (Dose 1): CCI spheres • Cohort 2 (Dose 2): CCI spheres • Cohort 3 (Dose 3): CCI spheres	

Cohorts and Dose Escalation Schema:
Patients will be enrolled in one of 3 escalating dose cohorts and followed for 5 years. Doses will be staggered by at least 8 weeks for the first 3 patients enrolled in the study. Thereafter, doses will be staggered by at least 4 weeks for the remaining patients in any dose cohort and between dose cohorts. For each staggered patient and each dose cohort, safety and efficacy parameters (FVIII activity) through at least the Week 8 visit (first 3 patients) and at least the Week 4 visit (for the remaining patients) will be reviewed by the Safety Review Committee (SRC) prior to dosing any additional patients. Decisions regarding dose escalation will be made by the SRC based on review of safety and FVIII activity data of the preceding patient(s) and/or cohort(s) and the Dose Escalation Decision Tree (Figure 3). Cohort expansions (up to 3 additional patients) may be triggered to collect additional information about safety and efficacy and do not require staggered enrolment. Cohort expansions can be initiated after one dose cohort (3 patients) has completed at least 8 weeks of follow-up or after all dose cohorts have completed at least 4 weeks of follow-up. Decisions regarding cohort expansions will be made by the SRC.

OBJECTIVES:	**ENDPOINTS:**
Primary Objective:	**Primary Endpoint:**
• To evaluate the safety and tolerability of up to 3 dose levels of SIG-001 administered laparoscopically into the greater sac of the peritoneal cavity	• Clinically significant changes from baseline in vital signs, clinical laboratory tests, and treatment emergent adverse events (TEAE)
Secondary Objectives:	**Secondary Endpoints:**
• To evaluate development of FVIII inhibitors after administration of SIG-001	• Factor VIII inhibitor titres (Nijmegen Bethesda Units [NBU])
• To characterize FVIII activity levels after administration of SIG-001	• FVIII activity levels (one-stage and chromogenic assays)
• To assess frequency of bleeding episodes following SIG-001 administration	• Bleeding rate (annualized; ABR)
• To assess effects of SIG-001 on usage of FVIII products	• Total use of factor VIII therapies (annualized)
Exploratory Objectives:	**Exploratory Endpoints:**
• To assess effects of SIG-001 on health and quality-of-life	• Summary responses to and change from baseline for EQ-5D-5L and Haem-A-QoL
• To assess the effects of SIG-001 on joint health	• Change from baseline in number of target joints and frequency of bleeding in target joints, and change from baseline in joint health using the Haemophilia Joint Health Score (HJHS) assessment

CRITERIA FOR EVALUATION:

Safety:

Safety will be assessed by monitoring clinically significant changes from baseline in vital signs, clinical laboratory tests, treatment-emergent AEs and SAEs. In addition, Factor VIII inhibitor titres (Nijmegen Bethesda assay) will be assessed.

Efficacy:

Efficacy will be measured by assessment of plasma FVIII activity using one-stage and chromogenic assays. Other efficacy measures will include the annualized number of bleeding episodes and usage of FVIII products during the study.

Quality-of-life and health will be assessed with the Haem-A-QoL and EQ-5D-5L questionnaires, while SIG-001 effects on target joints will be assessed as an exploratory endpoint evaluating the number of target joints, frequency of bleeding in target joints, and overall joint health scores using the HJHS tool.

ELIGIBILITY CRITERIA:

Inclusion Criteria:

1. Males aged 18 years or older
2. Diagnosis of Haemophilia A as follows:
   a) Severe (<1% FVIII activity) receiving FVIII prophylaxis or on-demand treatment; or
   b) Moderately-severe (≥1% - ≤2% FVIII activity) receiving FVIII prophylaxis
3. Greater than 150 exposure days to treatment with FVIII products
4. Fertile males (not surgically sterilized) who have sexual partners that are women of childbearing potential must be willing to use a barrier method for contraception (e.g. condoms) for at least 90 days post-SIG-001 administration
5. Normal levels of von Willebrand factor (VWF) antigen (≥50 IU/dL)
6. Able and willing to provide informed consent
7. Willing to withdraw from FVIII prophylaxis during specified periods in the study

Exclusion Criteria:

1. Morbid obesity defined as body mass index (BMI) ≥35
2. Current FVIII inhibitors (>0.6 NBU/mL) or prior Immune Tolerance Induction (ITI)
   Note: Patients with a history of low and/or transient inhibitors require Sponsor review and approval.
3. History of allergic reaction or anaphylaxis to recombinant FVIII products, alginate (including seaweed and algae), barium and barium products (including barium contrast agents), and/or products containing foetal bovine serum (FBS)
4. Evidence of any bleeding disorder in addition to haemophilia A
5. Abnormal laboratory values, as follows:
   a. Platelet count $<100 \times 10^9$/L
   b. Creatinine ≥1.5 mg/dL
   c. Haemoglobin <11 g/dL
   d. Elevated levels (i.e. greater than 2 × upper limit of normal (ULN) of aspartate aminotransferase (AST), alanine aminotransferase (ALT), or total bilirubin

   Note: Patients with a known history of Gilbert's disease and bilirubin levels above ULN are not excluded.
6. Active infection with Hepatitis B or Hepatitis C virus, defined as a positive HBsAg, HBeAg, HBV DNA, or HCV RNA results, or currently managed with antiviral medications for Hepatitis B or C
7. Uncontrolled HIV infection, defined as CD4+ counts ≤200/μL or by a viral load of >200 copies/mL

8. Active alcoholism or drug addiction during the 12 months before the screening visit
9. Active malignancy or history of malignancy in the 5 years prior to study entry, exclusive of surgically removed non-melanoma skin cancer
10. Participation in another investigational medicine or device study within 90 days of screening or 5 investigational product half-lives, whichever is longer
11. Prior administration of a gene therapy product
12. Treatment with emicizumab less than 6 months prior to screening

Note: Patients with emicizumab exposure may be eligible before the 6 months of prior exposure if FVIII activity assessed by one-stage assay is back to pre-emicizumab treatment levels.

13. Unable to tolerate general anaesthesia required for the laparoscopic procedure
14. History of:
    a. Abdominal adhesions due to prior large, median laparotomy(ies)
    Note: Uncomplicated prior laparoscopic procedures are not exclusionary (e.g. cholecystectomy, laparoscopic appendectomy or diagnostic laparoscopy)
    b. Septic peritonitis
    c. Intraperitoneal mesh
    d. Inflammatory Bowel Disease (IBD), including ulcerative colitis or Crohn's disease
15. Other comorbidities that in the opinion of the Investigator increase the risk of abdominal adhesions
16. Any disease or medical condition that in the Investigator's opinion is a contraindication for anaesthesia or the laparoscopic procedure (e.g. prior significant cardiovascular events)
17. In the Investigator's judgment, the patient is unlikely to complete all protocol-required study visits, procedures, or comply with the study requirements for participation

Summary of Patient Procedure(s):

Each patient will progress through 3 study periods, i.e. Screening, SIG-001 Administration, and Follow-up. Following completion of the consenting process, patients will undergo screening assessments as described in the Schedule of Assessments. Once eligibility is verified, patients will receive a single administration of SIG-001 on Day 1. Thereafter, patients will be followed for 5 years to assess safety and efficacy of SIG-001.

Prior to laparoscopic administration of SIG-001, patients will be dosed with a bolus of plasma-derived or recombinant FVIII (rFVIII), followed by maintenance FVIII administration either as repeat bolus administrations or as continuous infusion, per Investigator's and/or site's standard clinical practice. SIG-001 placement will proceed ONLY if:

a) pre-surgical FVIII activity levels reach ≥ 60 IU/dL (60% of normal) obtained within 1 hour of FVIII infusion
   OR
b) pre-surgical activated partial thromboplastin time (aPTT) is normal AND measured pre- and post- dosing FVIII levels (IU/dL) reach a difference of at least 60% during pharmacokinetic (PK) assessment

Prior to SIG-001 administration, patients will also receive a single dose of one or two different antibiotics (choice of antibiotic[s] to be as per the site's standard operating procedure) and will be placed under general anaesthesia. SIG-001 will be administered laparoscopically.

Post-procedure, patients should continue to receive FVIII dosing to maintain trough levels ≥ 60% for the first 48 hours after surgery. Patients may be discharged from the hospital on Day 3 or later per the Investigator's discretion; FVIII should be continued at home until the Week 1 visit, to maintain minimal haemostatic trough levels of ≥ 30% FVIII activity. The post-procedure FVIII treatment regimen may involve twice daily dosing for standard half-life products or once daily dosing for extended half-life

products; adequate FVIII levels will be ensured during hospital and home dosing. As needed, patients may be discharged with peripherally inserted central catheter (PICC) lines. Per the Investigator's discretion, patients may remain longer in the hospital for observation during FVIII administration.

Patients will be withdrawn from FVIII therapy after the Week 1 post-op visit at the Investigator's discretion. Throughout the study, all patients will be allowed access to on-demand FVIII to treat bleeding episodes, as perioperative management for surgery or other medical procedures, or as preventative treatment prior to vigorous physical activity.

Throughout the study, FVIII levels will be assessed at regular intervals. Per the Investigator's discretion, exogenous FVIII use will be washed out for a minimum of 96 hours (4 days for standard half-life FVIII products) and at least 168 hours (7 days for prolonged half-life rFVIII products) to ensure that only FVIII secreted by SIG-001 is measured.

ANALYTICAL METHODS:

Sample Size justification:
The sample size for this Phase 1/2 study is not based on statistical considerations.

Study Populations:
Efficacy Analysis Set includes all patients who received the study product and had at least one post-baseline efficacy assessment.
Safety Analysis Set includes all patients who received study product.

General Statistical Methods:
No formal hypothesis testing is planned in the study. Instead, results will be analysed using descriptive statistics. Data will be summarized by cohort and overall, where appropriate.
Absolute values and changes from baseline (Δ baseline) will be descriptively summarized for vital signs, laboratory data and FVIII inhibitor titres (Nijmegen Bethesda assay).
Data on FVIII activity, annualized bleeding and FVIII usage, as well as Haem-A-QoL, EQ-5D-5L questionnaires, joint health, and physical activity will be summarized descriptively and provided in subject listings. Questionnaires will be scored according to the recommendations from the questionnaire authors. Haem-A-QoL, EQ-5D-5L and HJHS scale scores and sub-scale scores and changes from baseline will be calculated at planned timepoints for each dose cohort and overall.
Full methods of analysis will be presented in the *Statistical Analysis Plan*.

Preliminary Efficacy Analysis:
Efficacy analyses will include all data up to the end of Year 1. Additional analyses will be conducted including follow-up data in Years 2 to 5. Efficacy analyses (FVIII activity by one-stage and chromogenic assays; ABR and annualized use of FVIII replacement) will be analysed by dose cohort and overall for the Efficacy Analysis Set.

Safety Analysis:
Treatment-emergent AEs will be summarized for each dose cohort by severity and relationship to study product and study procedure. Serious AEs will be presented for each dose cohort by relationship to study product and relationship to study procedures. Summary tables will present incidence estimates and individual event rates by system organ class as well as within each system organ class. Severity will be summarized by CTCAE v5.0 grading. Titres of FVIII inhibitors (Nijmegen Bethesda assay) will be descriptively summarized for each dose cohort, and overall.

Table 2 Schedule of Assessments

Procedure	Screening		SIG-001 Administration				Follow-up [l]						
	Days -91 to -2						Year 1				Year 2	Years 3-5	
	Screening 1 45 Days	Screening 2 [b] / Rescreen 45 Days	Pre-Op Day -1	Dosing Day 1	Clinic Day 2	Discharge Day 3	Month 1 W1, 2, 4 (± 2d)	Month 2-6 W8, 12, 16, 24 (± 5d)	Month 9&12 W36, 52 (± 7d)	FVIII Assessment [a] W3 (± 2d) W6, 10, 20 (± 5d) W32, 44 (± 7d)	Every 3 months W65, 78, 91, 104 (± 14d)	Every 6 months (FVIII Assessment) [a] W117, 143, 169, 195, 221, 247 (± 14d)	Every 6 months (Office Visit) W130, 156, 182, 208, 234, 260 (± 14d)
Consent	X												
Demographics	X												
Medical history	X												
Physical exam	X	X	X	X	X	X	X	X	X		X		X
Joint Assessment[k]	X								X W52 only		X W104 only		X W156, 208, 260 only
Height	X												
Weight	X	X	X				X	X	X		X		X
ECG [c]	X								X W52 only				
Vitals [d]	X	X	X	X	X	X	X	X	X		X		X
Alcohol & Drug Screen	X												
Haematology, biochemistry	X	X	X			X	X W2, W4 only	X	X		X		X
Urinalysis	X		X				X W4 only	X W24 only	X W52 only		X W104 only		X W156, 208, 260 only
HIV, HBV, & HCV screen	X												
VWF Antigen	X												
ABO & Rh Type	X												
Genotyping [g]	X												
FVIII PK [h]	X (Local)			X[i] (Local)									
Non-contrast abdominal MRI [m]	X		X [m] (Only if not done at Screening)										

Procedure	Screening		SIG-001 Administration				Follow-up [l]						
	Days -91 to -2						Year 1				Year 2	Years 3-5	
	Screening 1 45 Days	Screening 2 [b] / Rescreen 45 Days	Pre-Op Day -1	Dosing Day 1	Clinic Day 2	Discharge Day 3	Month 1 W1, 2, 4 (± 2d)	Month 2-6 W8, 12, 16, 24 (± 5d)	Month 9&12 W36, 52 (± 7d)	FVIII Assessment [a] W3 (± 2d) W6, 10, 20 (± 5d) W32, 44 (± 7d)	Every 3 months W65, 78, 91, 104 (± 14d)	Every 6 months (FVIII Assessment) [a] W117, 143, 169, 195, 221, 247 (± 14d)	Every 6 months (Office Visit) W130, 156, 182, 208, 234, 260 (± 14d)
FVIII activity (chromogenic & one-stage assay)	X			X (Local)	X (Local)	X (Local)	X	X	X	X	X	X	X
FVIII inhibitors	X						X W2, W4 only	X	X		X		X
Blood draw [j]	X						X	X	X		X		X
FVIII usage [f]	X	X	X	X	X	X	←						→
Anaesthesia Evaluation	X												
Eligibility Review	X	X											
Distribution of patient diaries and training [e]	X	X	X										
Haem-A-QoL[e]	X						X W4 only	X W24 only	X W52 only		X W104 only		X W156, 208, 260 only
EQ-5D [e]	X						X W4 only	X W24 only	X W52 only		X W104 only		X W156, 208, 260 only
Physical Activity [e]							X W4 only	X W24 only	X W52 only		X W104 only		X W156, 208, 260 only
Patient Diary [e]	←												→
Hospital Check-in			X										
IP Administration				X									
FVIII prophylaxis for surgery				X	X	X	X						
Overnight Fasting			X										
Discharge						X							
Adverse events	←												→

Abbreviations: AE=adverse event; ECG=electrocardiogram; ICF=informed consent form; PK=pharmacokinetics; VWF=von Willebrand Factor; MRI= Magnetic Resonance Imaging

Notes for Table 2 (Schedule of Assessments):

a) FVIII Assessment Visits may occur at the study site, the patient's home or another approved alternative location. In addition to the blood draw, FVIII usage, adverse events, including bleeding episodes, and concomitant medications will be discussed with the patient.

b) Screening Visit 2 / Rescreening applies only to patients who have been determined to be eligible for the study but do not receive SIG-001 within the original screening window. Those subjects will be asked to return to the clinic for Screening Visit 2 / Rescreening to repeat select screening assessments. Patients must receive SIG-001 within 90 days of the original screening date. Subjects who fail screening assessments at Screening Visit 2 / Rescreening can rescreen once under a new subject ID.

c) Patients should lie down for at least 5 minutes before the ECG assessment is performed. ECG assessments should be performed before vital sign assessments if possible. If blood samples need to be collected first, the ECG and vitals should be performed at least 10 minutes after blood sample collection.

d) Vital signs include temperature, respiratory rate, seated blood pressure (BP), and pulse. Seated BP will be recorded after the patient has been sitting for at least 5 minutes. Three BP measurements will be recorded at screening, and the average will be calculated for eligibility assessment. After screening, a single BP measurement is sufficient.

e) Diary training and completion of the weekly diary should be initiated only after the patient has been confirmed to be eligible for the study. Patients may be brought back for a separate visit to be trained on how to complete the weekly diary.

f) FVIII usage history collected during screening will be based on medical records and patient recollection. During the hospital stay, FVIII usage will be recorded by hospital staff; otherwise, FVIII usage will be recorded by the patient in the electronic patient diary.

g) If historical records are available, F8 genotyping does not need to be conducted at Screening.

h) The PK assessment does not need to be conducted if historical records are available and the information can be obtained from the medical record. For standard half-life FVIII products, PK sampling may include 2 to 3 timepoints, with samples at least 12 hours apart in a 48-hour period after factor infusion; particularly informative is the 24-hour sample. For extended half-life FVIII products, an additional sample at 60-84 hours after factor infusion is advisable. A trough level may be added only if the patient is to be infused with factor at the clinic. The number and timing of samples are at the discretion of the Investigator. The actual collection times as well as FVIII administration time must be recorded.

i) FVIII PK will be sampled, if necessary, during the hospital stay but prior to SIG-001 placement, as described in Section 9.2.2.

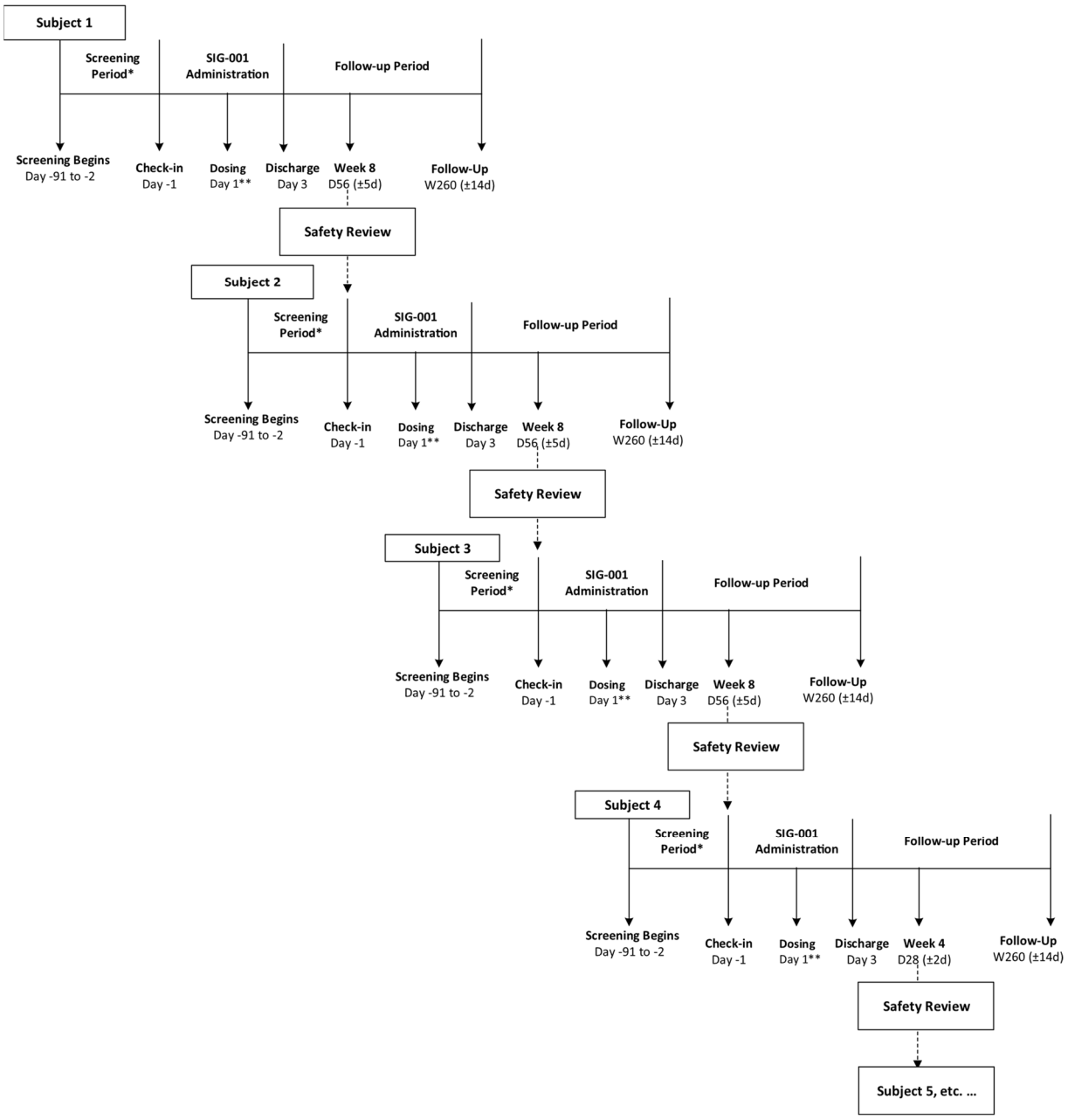
j) Blood samples will be collected for exploratory future analyses. Plasma samples will be stored as noted in the Schedule of Assessments, and may be used for future FVIII activity and SIG-001-specific inhibitor measurement or other assays that become available as they are developed. Serum samples will be stored at select timepoints only, i.e. Screening, Weeks 4, 8, 12, 24, and 52, and may be used for other testing related to SIG-001.

k) Target joints and frequency of bleeding in the target joints will be assessed. In addition, joint health will be assessed using the modified Haemophilia Joint Health Score (HJHS) tool, as described in Section 10.1.3

l) Remote visits are acceptable if an in-person visit cannot be scheduled. The Sponsor and the study team should be notified of the remote visit ahead of time to ensure remote blood sample collection can be achieved.

m) A single non-contrast abdominal MRI will be performed any time during the Screening period or on Day -1, i.e. prior to SIG-001 administration. If a patient is unable to undergo an MRI, an abdominal ultrasound will be done instead.

Figure 1 SIG-001-121 Study Schematic



Abbreviations: D = Day; W = Week

Note: Dosing will be staggered by at least 8 weeks for the first 3 patients enrolled in the study. Thereafter, doses will be staggered by at least 4 weeks for the remaining patients in any dose cohort and between dose cohorts.

* The screening window is 45 days. Eligible patients who do not receive SIG-001 within 45 days, will return to the clinic for Screening Visit 2 / Rescreening to repeat select screening assessments. If SIG-001 is not administered within 90 days of screening, patient can be rescreened under a new Subject ID.

** There is no Day 0.

3. INTRODUCTION

3.1. Disease Background

Defects in the F8 gene result in haemophilia A, an X-linked blood coagulation disorder. Haemophilia A is a rare disease occurring in approximately 173,000 patients worldwide requiring consumption of 0.875 IU Recombinant Factor VIII per capita annually, based on the most recent figures surveyed by the World Haemophilia Foundation (WHF) (WFO, 2019)

In healthy individuals, FVIII is produced in liver sinusoidal cells and in endothelial cells located outside of the liver throughout the body circulating in the bloodstream in an inactive form in a stable noncovalent complex bound to von Willebrand factor (vWF). F8 gene mutations may be inherited or occur spontaneously without previous family history (Ryu, Park, Yoo, Lee, & Choi, 2015). Various mutations causing haemophilia A result in different levels of factor VIII, which in turn influences bleeding tendency (Soucie et al., 2018).

Haemophilia A occurs in varying severities, defined in the World Federation of Haemophilia (WFH) *Guidelines for the Management of Haemophilia* 2nd Edition (2012) as:

- **Severe (factor levels <1 IU/dl or <1 % of normal)** represent approximately 60% of cases. People with severe haemophilia A experience bleeding following an injury and may have frequent spontaneous bleeding episodes, often into their joints and muscles.
- **Moderate (1-5 IU/dl or 1-5% of normal)** represent approximately 15% of cases. People with moderate haemophilia A tend to have bleeding episodes after injuries. Bleeds that occur without obvious cause are called spontaneous bleeding episodes.
- **Mild (5-40 IU/dl or 5 to <40% of normal)** represent approximately 25% of cases. People with mild haemophilia A generally experience bleeding only after serious injury, trauma or surgery.

The population in this study includes adult males with moderate to severe haemophilia A.

3.2. Treatment Background

Currently approved therapies for haemophilia A include a number of plasma-derived and recombinant human factor VIII replacement products and, more recently, non-factor therapies. Restoration of circulating FVIII to approximately 12% of normal levels has been shown to prevent most spontaneous joint bleeding episodes (den Uijl et al., 2011). FVIII replacement is administered as frequently as 1 to 3 times per week, but breakthrough bleeds and joint disease still occur due to suboptimal adherence, non-ideal factor kinetics, and FVIII inhibitor development (HEMLIBRA, 2017). More recently, a non-factor bispecific antibody (emicizumab) was also approved for the treatment of haemophilia A; potential risks include thrombotic microangiopathy, thrombotic events, and coagulation test interference (Aledort, 2019; Peters & Harris, 2018; Weyand & Pipe, 2019).

3.3. Need for New Haemophilia A Treatments

Standard care involves repeat prophylactic or on-demand FVIII products, which can impair quality-of-life and often fail to fully prevent long-term joint disease. In contrast to standard episodic (on-demand) or continuous prophylactic care with factor replacement and non-factor therapies that result in peaks and troughs of FVIII levels, SIG-001 offers the potential to achieve sustained, long-lasting therapeutic levels of FVIII with a laparoscopic procedure, avoiding the need for frequent infusions.

4. INVESTIGATIONAL MEDICINAL PRODUCTS USED IN THIS STUDY

This study involves the use of the investigational medicinal product SIG-001. No comparator or other treatments are being studied.

4.1. SIG-001 Description

SIG-001 is a genetically modified allogeneic cell therapy product designed to be administered via a single laparoscopic procedure into the greater sac of the peritoneal cavity. The primary mechanism of action is sustained secretion of BDD-hFVIII, specifically, replacement of the missing clotting FVIII that is needed for effective haemostasis.

SIG-001 BDD-hFVIII secreting spheres are comprised of a human epithelial cell line (ARPE-19 cells) genetically modified with a transposon-based non-viral vector to express B-domain deleted human factor VIII (BDD-hFVIII). The cells are encapsulated within two-layered modified alginate spheres, which are designed to form a physical barrier protecting cells and spheres from immune damage (e.g. immune rejection and/or pericapsular fibrotic overgrowth, PFO response). SIG-001 spheres consist primarily of alginate, a naturally occurring non-antigenic and biocompatible material with a well-stablished safety profile. The outer layer alginates are conjugated with a small molecule to protect from PFO, and internal compartment alginates are bonded with a peptide to promote cell adhesion.

Figure 2 Schematic Representation of SIG-001 Study Product

4.2. Prior Nonclinical Studies

Use of alginate hydrogels for cell encapsulation has been extensively studied *in vitro* and in animal models, demonstrating no concerning safety signals when used in genetically modified cell therapy systems (Moradali, 2018). Nonclinical studies of SIG-001 conducted by the Sponsor have demonstrated safety and preliminary efficacy (FVIII activity *in vivo*) in mouse and non-human primate (NHP) models without safety concerns. The results of these studies are described in greater detail in the *SIG-001 Investigator's Brochure*.

4.3. Prior Clinical Studies

This is the first-in-human (FIH) clinical study of SIG-001. However, the constituent sodium alginate cell encapsulation approach and genetically modified human ARPE-19 epithelial cell line have been studied clinically for over four decades (Basta et al., 2011; Calafiore et al., 2006; Jacobs-Tulleneers-Thevissen et al., 2013; S. Matsumoto et al., 2014; Tuch et al., 2009). Intraperitoneal administration of sodium alginate hydrogel spheres -without anti-fibrotic properties- containing encapsulated allogeneic pancreatic islets or porcine pancreatic islets in patients with Type 1 diabetes has resulted in transient improvement in mean blood glucose with no concerning safety issues (Basta et al., 2011; Calafiore et al., 2006; Tuch et al., 2009). Genetically modified human ARPE-19 cells are in clinical development for the treatment of ophthalmologic protein deficiencies as well as Alzheimer's disease; no concerning safety issues have been reported when administered to humans (Kauper et al., 2012; Mitra, Behbahani, & Eriksdotter, 2019).

4.4. Rationale for the Study

This FIH trial is designed to examine safety and preliminary efficacy of up to 3 doses of SIG-001 in humans. SIG-001 has been evaluated *in vitro* and in animal models to enable sustained secretion of FVIII *in vivo*.

4.5. Risk-Benefit Profile

This Phase 1/2 study has been designed to evaluate the potential risks and benefits associated with SIG-001 administration. Since this study is being conducted in haemophilia A patients, participation may benefit subjects because SIG-001 may result in sustained, long-lasting FVIII levels relative to the peaks and troughs that occur with repeated FVIII administration, but such benefit cannot be guaranteed.

Study participation requires subjects to have a laparoscopic procedure that they would not normally undergo with standard of care; however, this procedure is not expected to pose any risks greater than those of routine diagnostic laparoscopy, and may allow participants to avoid repeated FVIII infusions which inherently pose their own risks. All subjects will be followed for up to five years for safety. The information collected as part of this study is designed to help advance knowledge of SIG-001, which represents a new and potentially long-lasting first-in-class therapeutic option for patients with haemophilia A.

5. STUDY OBJECTIVES AND ENDPOINTS

5.1. Study Objectives

5.1.1. Primary Objective

- To evaluate the safety and tolerability of up to 3 dose levels of SIG-001 administered laparoscopically into the greater sac of the peritoneal cavity

5.1.2. Secondary Objectives

- To evaluate development of FVIII inhibitors after administration of SIG-001
- To characterize FVIII activity levels after administration of SIG-001
- To assess frequency of bleeding episodes following SIG-001 administration
- To assess effects of SIG-001 on usage of FVIII products

5.1.3. Exploratory Objectives

- To assess effects of SIG-001 on health and quality-of-life
- To assess the effects of SIG-001 on joint health

5.2. Endpoints

5.2.1. Primary Endpoint

- Clinically significant changes from baseline in vital signs, clinical laboratory tests, and treatment emergent adverse events (TEAEs)

5.2.2. Secondary Endpoints

- Factor VIII inhibitor titres (NBU)
- FVIII activity levels (one-stage and chromogenic assays)
- Bleeding rate (annualized; ABR)
- Total use of factor VIII therapies (annualized)

5.2.3. Exploratory Endpoints

- Summary responses to and change from baseline for EQ-5D-5L and Haem-A-QoL
- Change from baseline in the number of target joints and frequency of bleeding in target joints, as well as change from baseline in joint health using the HJHS assessment

6. STUDY DESIGN AND DESCRIPTION

6.1. Study Design

SIG-001-121 is a FIH, phase 1/2, multi-centre, open-label study in escalating dose cohorts to assess the safety, tolerability, and preliminary efficacy of SIG-001 in adults with severe or moderately-severe Haemophilia A without inhibitors. Up to three dose cohorts are planned.

6.2. Study Duration

Total study duration is a maximum of 63 months. This includes up to 90 days for screening and 5 years of follow-up after a single administration of SIG-001.

6.3. Cohorts and Grouping

Up to three (3) escalating dose cohorts are planned. Each cohort will initially include up to three patients and can be expanded to enrol up to three additional patients; thus, up to 6 patients may be enrolled at a single dose level.

- Cohort 1 (Dose 1): CCI of SIG-001 spheres
- Cohort 2 (Dose 2): CCI of SIG-001 spheres
- Cohort 3 (Dose 3): CCI of SIG-01 spheres

For a full description of the rationale for dose selection, refer to Section 6.5.1.

6.4. Dose Escalation Design

Patients will be enrolled in one of 3 escalating dose cohorts. Dosing will be staggered by at least 8 weeks for the first 3 patients enrolled in the study. Thereafter, doses will be staggered by at least 4 weeks for the remaining patients in each dose cohort and between dose cohorts. Cohort expansion does not require staggered enrolment Following SIG-001 administration, safety and efficacy parameters (FVIII activity) through at least the Week 8 visit (first 3 patients) and at least the Week 4 visit (for the remaining patients in any dose cohort and between dose cohorts) will be reviewed by the Safety Review Committee (SRC) prior to dosing any additional patients (Section 11.1).

Decisions regarding dose escalation will be made by the SRC based on review of safety and FVIII activity data of the preceding patient(s) and/or cohort(s) and the Dose Escalation Decision Tree (Figure 3). Dose escalation will occur only if no dose-limiting toxicities (DLTs) and/or clinically-relevant safety issues have been noted. FVIII activity will be reviewed to inform the dose selection (volume of spheres) for the next cohort.

After the first dose (Dose 1, CCI of SIG-001 spheres), the dose may be increased (e.g. planned Dose 2 & 3) or decreased/de-escalated (Dose -1). No dose increase will be >2-fold higher than a dose level previously found to be safe, and no dose will exceed the maximum dose of CCI

If any patient achieves FVIII activity >100 IU/dL (equivalent to >100% normal FVIII activity) no further dose escalation will occur. Dosing may proceed at the same level or at a lower level.

Cohort 1, Dose 1

If the starting dose in the first patient in Cohort 1, Dose 1 is safe and tolerated and FVIII activity is ≥ 5 IU/dL (equivalent to ≥5% normal FVIII activity) at Week 8, the next 2 patients will be administered the same dose and followed for safety and efficacy per the Schedule of Assessments (Table 2 Schedule of Assessments). SIG-001 administration for Patients 2 and 3 will be staggered to include a safety review at least 8 weeks after SIG-001 administration.

If no DLTs or clinically-relevant safety issues occur following safety review of Patient 1 Cohort 1 data but FVIII activity in Patient 1 Cohort 1 is <5 IU/dL at Week 8, dose escalation may occur, and the study will progress to Cohort 2, Dose 2.

Doses will be staggered by at least 8 weeks for the first 3 patients enrolled in the study. Thereafter, doses will be staggered by at least 4 weeks for the remaining patients in any dose cohort and between dose cohorts.

Cohort 2, Dose 2

If the starting dose in the first patient in Cohort 2, Dose 2 is safe and tolerated, the next 2 patients will be administered the same dose via a staggered approach with a safety review after SIG-001 administration, i.e. Dose 2, if:

1) FVIII activity in Cohort 2 Patient 1 is ≥5 IU/dL, or
2) FVIII activity in Cohort 2 Patient 1 is <5 IU/dL but FVIII activity in Cohort 1 Patient 2 or Cohort 1 Patient 3 is ≥5 IU/dL

The study will proceed to the highest dose, i.e. Cohort 3, Dose 3, if no DLT or clinically-relevant safety issues occur and:

1) FVIII activity in Cohort 1 Patient 1 is <5 IU/dL and FVIII activity in Cohort 2 Patient 1 is also <5 IU/dL, or
2) FVIII activity in Cohort 2 Patient 1 is <5 IU/dL, and FVIII activity in Cohort 1 Patients 2 and Patient 3 is <5 IU/dL.

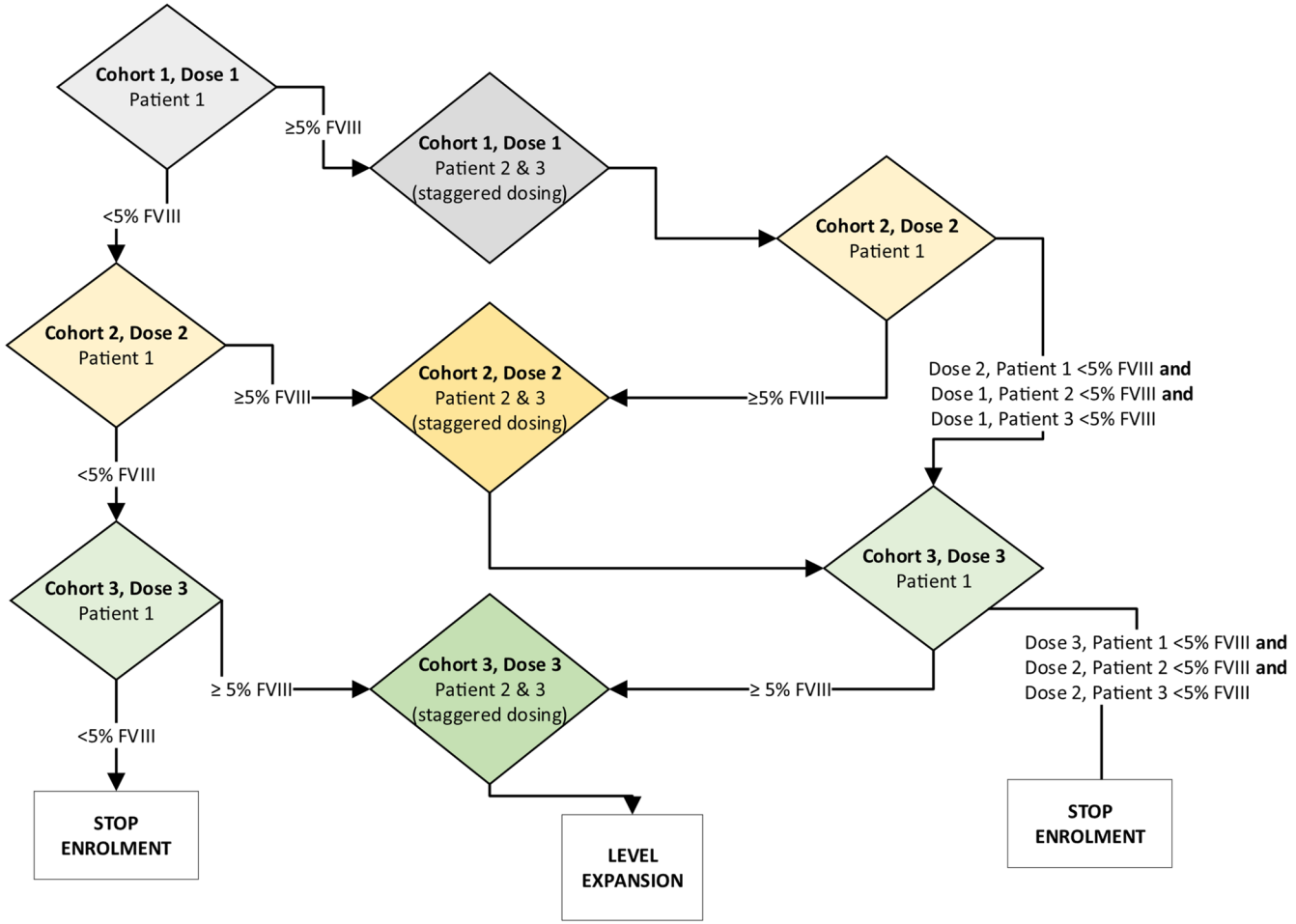
Cohort 3, Dose 3

If the starting dose in the first patient in Cohort 3, Dose 3 is safe and tolerated and no DLT or clinically-relevant safety issues occur, the next 2 patients will be administered the same dose via a staggered approach with a safety review after SIG-001 administration, i.e. Dose 3, if:

1) FVIII activity in Cohort 3 Patient 1 is ≥5 IU/dL, or
2) FVIII activity in Cohort 3 Patient 1 is <5 IU/dL but FVIII activity in Cohort 2 Patient 2 or Cohort 2 Patient 3 is ≥5 IU/dL.

If FVIII activity in Cohort 3 Patient 1 is <5 IU/dL and all prior patients had FVIII activity <5 IU/dL, no further patients will be dosed and enrolment will be stopped. This dose escalation algorithm is intended to monitor patient safety while minimizing patient exposure to subtherapeutic SIG-001 doses. Cohort expansions (up to 3 additional patients) can be initiated after one dose cohort has completed at least 8 weeks of follow-up or after all dose cohorts have completed at least 4 weeks of follow-up. Cohort expansion may be triggered in order to collect additional information regarding safety and efficacy. Decisions regarding cohort expansions will be made by the SRC.

Figure 3 Dose Escalation Decision Tree



Note: Dosing will be staggered by at least 8 weeks for the first 3 patients enrolled in the study. Thereafter, doses will be staggered by at least 4 weeks for remaining patients in any dose cohort and between dose cohorts. If any patient achieves FVIII activity >100 IU/dL (equivalent to >100% normal FVIII activity) no further dose escalation will occur. Dosing may proceed at the same level or at a lower level.

6.5. Justification for Dose, Endpoints, and Mitigations

6.5.1. Dose

The starting and maximum SIG-001 doses (mL of spheres) were selected based on nonclinical safety and efficacy (pharmacokinetics). The initially planned doses selected for the study are CCI [redacted] As described in Section 4.2, nonclinical toxicology studies have not identified toxicity for SIG-001, and as described in Section 4.3, alginate spheres have been safely administered intraperitoneally to humans at doses known to exceed CCI and likely up to approximately CCI Additional details are described in the SIG-001 Investigator's Brochure.

The starting dose of CCI is expected to be of clinical benefit to participating patients, as supported by the prevention of most spontaneous joint bleeds in haemophilia A patients once FVIII activity rises above 12% (den Uijl et al., 2011). Furthermore, patients achieving the target steady-state FVIII activity for Cohort 1 may be able to discontinue prophylactic FVIII usage, or significantly decrease on-demand FVIII usage.

The starting dose (mL of spheres) for Cohort 1 (Dose 1) was extrapolated from a combination of in vitro and in vivo data to achieve a target steady-state FVIII activity in humans of 0.10 IU/mL (10% of normal hFVIII activity). More specifically, the starting dose volume (mL of spheres) is based on in vivo results of the bioavailability of FVIII produced by different doses of SIG-001 spheres administered IP in rodents, results of in vitro FVIII secretion by individual SIG-001 spheres, the ratio of FVIII secreted by SIG-001 spheres in vitro pre-administration vs. post-administration and the known clearance of a commercially available form of BDD-hFVIII (Moroctocog alfa or BDDrFVIII; trade names ReFacto®, XYNTHA®).

Three ascending dose levels are planned, with a starting dose (CCI of SIG-001 spheres) estimated to achieve approximately 0.1 IU/mL plasma level (equivalent to 10% of normal FVIII activity). Estimated steady-state FVIII plasma activity for the planned doses are described in Table 3 below.

Table 3 Estimated FVIII Plasma Activity for Planned Doses

Dose (mL)	Estimated FVIII Plasma Activity [a]
CCI [redacted]	6% to 22%
	11% to 44%
	17% to 65%

Note (a) CCI [redacted] Ranges for estimated plasma FVIII activity by SIG-001 dose volume are based on the estimated range of scenarios tested and physiologically reasonable.

Intraperitoneal administration of alginate spheres to humans in volumes of up to CCI (reported in the medical literature) and likely, up to CCI (calculated from reports in the medical literature) have been described; there were no reports of adverse events related to the administered alginate spheres. CCI [redacted]

The maximum volume of SIG-001 spheres to be administered in this study are approximately up

to CCI (for a 70 kg patient); this maximum volume is below the range of total volumes reported in published clinical experience with alginate spheres and below the nonclinical volumes tested with SIG-001 in 2 species. Additional details are described in the SIG-001 Investigator's Brochure (IB Section 2.1).

CCI The maximum volume of buffer corresponding to the highest dose of SIG-001 spheres (Dose 3, up to CCI) is CCI; this volume of buffer is expected to be fully absorbed from the peritoneal space in approximately 2.5 to 5 hours post-SIG-001 administration.

6.5.2. Endpoints

Endpoints in this phase 1/2 study examine the safety and preliminary efficacy resulting from up to 3 dose levels of SIG-001 administration in humans, which are not possible to extrapolate from studies in animal models.

The primary endpoint is any clinically significant change from baseline in vital signs, clinical laboratory tests, as well as TEAEs that arise. As this is the first time SIG-001 is being administered to humans, these basic safety considerations are of primary importance. Thus, changes from baseline in safety parameters will be monitored as the primary endpoint of this study.

Secondary endpoints include assessment of (1) FVIII inhibitor titres, (2) FVIII activity levels, (3) annualized bleeding rate, and (4) annualized FVIII therapy use.

Inhibitors limit the therapeutic efficacy of FVIII. There are no good animal models for assessment of inhibitor development. FVIII produced by SIG-001 has a human FVIII amino acid sequence and it is secreted by ARPE-19 cells, a human cell line. Human FVIII produced by SIG-001 is a xenogeneic setting when tested in nonclinical studies, and expectedly results in robust development of FVIII inhibitors in that context. It is unknown how secretion of human FVIII from SIG-001 will affect the inhibitor response to FVIII in humans, but the risk is considered low (Section 6.5.3). Inhibitor titres will be measured at various timepoints using a well-established method, the Nijmegen Bethesda assay.

The goal of SIG-001 administration is to achieve sustained therapeutic levels of FVIII and therefore decrease the risk for spontaneous bleeding episodes and preclude the need for frequent (prophylactic or on-demand) infusions of FVIII. FVIII activity is a well-characterized surrogate marker of clinical benefit and it is currently used in ongoing clinical trials of gene therapy in haemophilia A. As a FIH study, the relationship between SIG-001 dose and FVIII activity is unknown in humans. Therefore, FVIII activity will be evaluated by a central laboratory using two assays, a one-stage clotting assay and a chromogenic substrate assay. Both are standard measures of FVIII activity in a clinical setting.

Annualized bleeding rates and FVIII usage can also inform preliminary efficacy of SIG-001. Patients with haemophilia A with FVIII activity below 2% suffer frequent spontaneous joint bleeds, but annualized spontaneous joint bleeds are close to zero once sustained FVIII activity rises above 12% (den Uijl et al., 2011). A significant reduction in exogenous FVIII usage is

expected in patients reaching those FVIII levels. Therefore, data on bleeding rates and FVIII usage after SIG-001 administration will be assessed prospectively throughout the study.

Finally, exploratory endpoints include (1) change in health and quality-of-life as measured by the EQ-5D-5L and Haem-A-QoL instruments, respectively as well as (2) change in the number of target joints and frequency of bleeding episodes in target joints, as well as change in joint health scores as measured by the HJHS instrument. Low health-related quality-of-life is a long-recognized issue in haemophilia patients due to the physical, social, and emotional costs of the disease and the burden of lifelong treatment. It will be important to determine whether SIG-001 is able to impact this important facet of haemophilia patient care. In addition, poorly sustained FVIII levels often result in breakthrough bleeds and subsequent chronic joint disease. The effects of SIG-001 on health, quality-of-life and target joints and joint health will all be monitored as exploratory endpoints.

6.5.3. Mitigations

Risks have been mitigated to levels as low as reasonably possible (ALARP) for this trial. No prior clinical studies have been performed of SIG-001 to date; however, the constituent sodium alginate cell encapsulation approach and genetically modified ARPE-19 epithelial cells have been studied in humans for over four decades (Section 4.3) (Basta et al., 2011; Bochenek et al., 2018; Calafiore et al., 2006; Jacobs-Tulleneers-Thevissen et al., 2013; Shinichi Matsumoto, Abalovich, Wechsler, Wynyard, & Elliott, 2016; Tuch et al., 2009; Vegas et al., 2016). Although no concerning safety signals have been reported, immune response to allogeneic, non-encapsulated cells and a PFO response to the encapsulating materials have significantly limited their durability. SIG-001 design consists of an inner compartment made of peptide-modified alginates that promote cell adhesion, and an outer layer of modified alginates that acts as both a physical barrier to protect the genetically modified ARPE-19 cells from immune damage and to prevent a PFO response.

The study will not exceed CCI of SIG-001 spheres which is a volume approximately 3- to 10-fold lower than those administered in nonclinical studies of SIG-001.

Based on the dose modelling results discussed in Section 6.5.1, the volumes of SIG-001 to be administered in this study have a low risk for over-exposure to FVIII (e.g. FVIII levels well above 150% of normal), even at the highest SIG-001 dose of up to CCI (Cohort 3, Dose 3).

To decrease anaesthesia and laparoscopy procedure-related risks, only patients with a BMI of <35, with acceptable hematologic and blood chemistry values at baseline, who are deemed by the Investigator to be healthy to undergo the anaesthesia and laparoscopic procedure are eligible for this study. In addition, to decrease the risk of abdominal-related adverse events, patients with a medical history of adhesions, IBD or any other comorbidity potentially increasing the risk of abdominal adhesions are excluded.

Non-contrast abdominal MRI will be performed as part of this study. MRI involves non-ionising radiation, and study MRI exams will not require administration of contrast agents (e.g. gadolinium) or other medications. If a patient is unable to undergo an MRI, an abdominal ultrasound will be done instead. Study MRI and ultrasound exams are not expected to involve greater risk than routine MRI and ultrasound exams.

To further limit risks in this study, patients with known sensitivities to FVIII therapies, alginates (seaweed), barium and barium products (including barium contrast agents), and/or products containing FBS are excluded. FBS is predominantly removed using extensive washing during SIG-001 manufacturing. Only patients with haemophilia A who have achieved ≥150 exposures to FVIII and have no past or current titres of FVIII inhibitor are eligible to participate in this study. Treatment-naïve patients without known ability to tolerate FVIII therapies are excluded. Lastly, SIG-001 produces human FVIII with the same amino acid sequence of commercially available recombinant FVIII products that have a well-established safety profile (Moroctocog alfa or BDDrFVIII; trade names ReFacto®, XYNTHA®).

Throughout the study patients retain access to on-demand FVIII for any bleeding episodes, perioperative management for surgery or other medical procedures, or prior to vigorous physical activity. Criteria for re-starting prophylactic therapy after SIG-001 administration are described in Section 7.5.1.

6.6. Stopping Criteria

6.6.1. Individual Stopping Criteria

SIG-001 involves an intraperitoneal administration. In the event of an unforeseen safety event, the SRC will evaluate the issue. All patients who have already received SIG-001 will remain in the trial and continue to be followed for the duration of the study per the Schedule of Assessments (Table 2 Schedule of Assessments).

6.6.2. Dose Limiting Toxicities and Cohort and Study Stopping Rules

DLTs are defined as the AEs and SAEs listed below. DLTs will be recorded from first patient administration until 8 weeks after last patient administration of SIG-001. All AEs/SAEs will be graded and reported as per Section 12 Safety and Adverse Events.

Any occurrence of the DLTs listed below will trigger an ad hoc evaluation by the SRC and further dosing or dose escalation in the trial will be halted.

- Any death
- Any SAE that is considered possibly or probably related to SIG-001, which is deemed to pose unacceptable risk to study participants
- Any surgical SAE that is considered possibly or probably related to the anaesthesia or laparoscopic procedure, deemed to pose an unacceptable risk to study participants, and occurs in more than 2 patients at any dose level
- Any Grade III-IV toxicity assessed as possibly or probably related to the study product. Examples include, but are not limited to:
    - Inhibitor development with persistent titres >0.6 NBU/mL
    - Persistent abdominal pain
    - Persistently abnormal laboratory values, such as:
        - Alanine aminotransferase (ALT) elevations > 5 × upper limit of normal (ULN) in the absence of a concomitant bilirubin increase

  - ALT elevations > 3 × ULN in the presence of a total bilirubin increase > 2 × ULN or an international normalized ratio (INR) > 1.5 without findings of cholestasis or other alternate aetiology to explain the elevations
  - ALT elevations > 3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (> 5%)
- Any thrombotic event
- Any peritonitis
- Abdominal adhesions/fibrosis
- Supratherapeutic levels of FVIII activity (above 150 IU/dL, or >150% activity)
- An overall pattern of clinically significant changes in any safety parameter, considered to be potentially related to the study product that represents a safety concern.

The stopping criteria for this study is the occurrence of one or more of the DLTs listed above. The study will be halted if a stopping criterion is met. If the Sponsor suspends the trial, the Investigators, Ethics Committees (IEC/IRB) and Regulatory Authorities will be promptly notified in writing with the reason for suspension/termination. If, following a safety review by the SRC, it is considered appropriate to restart dosing in the trial, Regulatory Authorities and Ethics Committees will be notified accordingly. Any proposal to restart dosing in the trial will require Regulatory Authorities and Ethics Committees approval of a substantial protocol amendment prior to implementation.

7. STUDY POPULATION

7.1. Selection and Discontinuation/Withdrawal of Subjects

All entry criteria, including test results, need to be confirmed at Screening and Day -1.

7.2. Number of Subjects

Up to 18 patients will be enrolled in the study to receive SIG-001, including up to 3 initial and 3 additional patients per cohort (6 total) in 3 separate dose cohorts.

7.3. Inclusion Criteria

Patients must meet all inclusion criteria to be eligible for participation in the study:

1. Males aged 18 years or older
2. Diagnosis of Haemophilia A as follows:
   a) Severe (<1% FVIII activity) receiving FVIII prophylaxis or on-demand treatment; or
   b) Moderately-severe (≥1% - ≤2% FVIII activity) receiving FVIII prophylaxis
3. Greater than 150 exposure days to treatment with FVIII products
4. Fertile males (not surgically sterilized) who have sexual partners that are women of childbearing potential must be willing to use a barrier method of contraception (e.g. condoms) for at least 90 days post-SIG-001 administration
5. Normal levels of VWF antigen (≥50 IU/dL)

6. Able and willing to provide informed consent
7. Willing to withdraw from FVIII prophylaxis during specified periods in the study

7.4. Exclusion Criteria

Patients meeting any of the following exclusion criteria will be considered ineligible for participation and will not be enrolled in the study:

1. Morbid obesity defined as body mass index (BMI) ≥35
2. Current FVIII inhibitors (>0.6 NBU/mL) or prior Immune Tolerance Induction (ITI)
   Note: Patients with a history of low and/or transient inhibitors require Sponsor review and approval.
3. History of allergic reaction or anaphylaxis to recombinant FVIII products, alginate (including seaweed and algae), barium and barium products (including barium contrast agents), and/or products containing foetal bovine serum (FBS)
4. Evidence of any bleeding disorder in addition to haemophilia A
5. Abnormal laboratory values, as follows:
   a) Platelet count $<100 \times 10^9$/L
   b) Creatinine ≥1.5 mg/dL
   c) Haemoglobin <11 g/dL
   d) Elevated levels (i.e. greater than 2 × ULN of aspartate aminotransferase (AST), alanine aminotransferase (ALT), or total bilirubin

   Note: Patients with a known history of Gilbert's disease and bilirubin levels above ULN are not excluded.
6. Active infection with Hepatitis B or Hepatitis C virus, defined as a positive HBsAg, HBeAg, HBV DNA, or HCV RNA results, or currently managed with antiviral medications for Hepatitis B or C
7. Uncontrolled HIV infection, defined as CD4+ counts ≤200/µL or by a viral load of >200 copies/mL
8. Active alcoholism or drug addiction during the 12 months before the screening visit
9. Active malignancy or history of malignancy in the 5 years prior to study entry, exclusive of surgically removed non-melanoma skin cancer
10. Participation in another investigational medicine or device study within 90 days of screening or 5 investigational product half-lives, whichever is longer
11. Prior administration of a gene therapy product
12. Treatment with emicizumab less than 6 months prior to screening.
    Note: Patients with emicizumab exposure may be eligible before 6 months of prior exposure if FVIII activity assessed by one-stage assay is back to pre-emicizumab treatment levels.
13. Unable to tolerate general anaesthesia required for the laparoscopic procedure
14. History of:
    a) Abdominal adhesions due to prior large, median laparotomy(ies)
       Note: Uncomplicated prior laparoscopic procedures are not exclusionary (e.g. cholecystectomy, laparoscopic appendectomy or diagnostic laparoscopy).
    b) Septic peritonitis

c) Intraperitoneal mesh
d) Inflammatory Bowel Disease (IBD), including ulcerative colitis or Crohn's disease

15. Other comorbidities that in the opinion of the Investigator increase the risk of abdominal adhesions
16. Any disease or medical condition that in the Investigator's opinion is a contraindication for anaesthesia or the laparoscopic procedure (e.g. prior significant cardiovascular events)
17. In the Investigator's judgment, the patient is unlikely to complete all protocol-required study visits, procedures, or comply with the study requirements for participation

7.5. Permitted/Excluded Medications and Therapies

All prescription and over-the-counter medications taken by a patient for 30 days before Screening will be recorded on the designated eCRF. The Investigator may prescribe additional medications during the study as long as the prescribed medication is not prohibited by the protocol (Section 7.5.3). Any concomitant medications added or discontinued during the study should be recorded on the eCRF.

7.5.1. Permitted Medications and Therapies

Prior to the laparoscopic procedure, patients will receive a single dose of antibiotic (or a single dose of 2 different antibiotics), per the site's standard clinical practice.

Following the Week 1 visit, patients will be withdrawn from FVIII therapy per the Investigator's discretion. If FVIII therapy is continued after the Week 1 visit, the reason for continuation will be recorded.

On-demand FVIII use is allowed throughout the duration of the study for the treatment of bleeding episodes, perioperative management for surgery or other medical procedures, or preventative for vigorous physical activity. Each use of exogenous FVIII will be recorded and reported during the study.

7.5.2. Criteria for Re-Starting Prophylactic Therapy

Patients may restart prophylaxis if their FVIII level is <2% normal activity in two separate occasions done at least one week apart after 8 weeks following SIG-001 placement. If a patient needs to restart prophylaxis prior to Week 8, the Investigator should contact the Medical Monitor for approval.

7.5.3. Prohibited Medications and Therapies

Medications that increase the risk of bleeding or that are contraindicated for laparoscopic surgery per the site's standard of care will be prohibited. Examples may include use of aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs) in the 5 days period prior to, and following SIG-001 administration.

Emicizumab is prohibited any time during the trial unless the patient has already met the criteria to restart prophylaxis.

7.6. Discontinuation or Withdrawal of a Subject

Patients are free to withdraw from the study at any time and for any reason without prejudice to their future medical care by their physician or at the institution. The Investigator may discontinue

a subject's study participation at any time during the study. The Investigator may remove a patient from the study for medically indicated reasons, for non-compliance, or if, in the Investigator's opinion, it is not in the best interest of the patient to continue the study. When possible, the tests and evaluations listed for the last study visit should be carried out in the event of premature termination.

The primary reason for discontinuation or withdrawal of the subject from the study or study treatment should be recorded in the eCRF. The Investigator is responsible for ensuring all safety assessments and final follow up assessments, including follow up for unresolved AEs, are completed prior to subject discontinuation.

If a patient fails to return for scheduled visits, a documented effort must be made to determine the reason. If the patient cannot be reached by telephone, a certified letter should be mailed. This information should be recorded in the study records. For subjects withdrawn or lost to follow-up, the Investigator may continue to collect available long-term follow-up information where possible and permissible by local IRB/IEC policy, except where the subject has withdrawn consent for further information to be collected. The Sponsor must be notified of all patient withdrawals as soon as possible.

7.7. Discontinuation of the Study or Investigative Site

The Sponsor has the right to terminate the study at any time for clinical or administrative reasons and/or to discontinue participation of an individual Investigator or site for clinical or administrative reasons, including, but not limited to, poor enrolment or noncompliance with procedures of the protocol or GCP.

If the Sponsor suspends or terminates the trial or part of the trial, the Investigators and IEC/IRB will be promptly notified in writing with the reason for suspension/termination as per applicable regulatory requirements.

8. CLINICAL TRIAL INVESTIGATIONAL PRODUCT MANAGEMENT

This section contains information regarding all investigational product and materials provided directly by the Sponsor, and/or sourced by other means, that are required by the study protocol, including important sections describing the handling and management of the clinical trial investigational product (SIG-001).

8.1. Investigational Product

In this protocol, the term study drug or Investigational Product (IP) refers to:

- SIG-001 spheres in buffer, provided in trays (see the *SIG-001 Investigator's Brochure* for complete description)

No placebo or other active medicinal product is supplied. SIG-001 must be prepared, handled, and administered by qualified site staff who have been trained to the procedures for IP

preparation outlined in the *Laparoscopic Procedure Manual*. SIG-001 is to be dispensed only to patients enrolled in this study.

SIG-001 must be administered in accordance with the *Laparoscopic Procedure Manual* by a surgeon who has received study-specific training and must not be used after the expiry date and time.

8.1.1. Sponsor-Supplied Investigational Product

SIG-001 is supplied as a dose of spheres by volume suspended in buffer. SIG-001 is manufactured by Sigilon Therapeutics, Inc. via a GMP-compliant Contract Manufacturing Organizations (CMO).

SIG-001 is supplied in polyethylene terephthalate (PETG) trays with foil lids. Each SIG-001 tray is labelled in accordance with applicable laws and regulations (see the *Pharmacy Manual* for details on packaging and labelling).

8.1.2. Sponsor-Supplied Kits and Delivery Equipment

Study-specific clinical kits will be provided to sites prior to start of enrolment. These kits will contain supplies that are required for IP preparation and administration (e.g. a sterile drape, sterile tip syringes, administration buffer, catheter for intraperitoneal administration, 8mm disposable laparoscopic trocars).

These kits will be maintained in stock at clinical sites. Final content of the kit(s) and detailed instructions for preparation and use are defined in the *Laparoscopic Procedure Manual* and the *Pharmacy Manual*.

8.1.3. Storage

SIG-001 must be stored at 15°C - 25°C (59°F - 77°F). Brief temperature excursions during transit and/or storage may be acceptable. Any discrepancies or deviations from the above referenced temperature conditions must be reported to the Sponsor and resolved prior to IP administration. SIG-001 will be shipped in a qualified shipping container with a temperature monitor. Upon receipt of the product at the clinical site, the product batch number will be recorded, and the temperature monitor will be checked to ensure the product remained within the defined temperature conditions during transit.

Investigational product must be kept in a location with access limited to study staff until it is transferred to the surgical suite for preparation by the surgeon. Investigational product must be stored separate from normal hospital/practice inventory and be clearly identified from other investigational supplies or products. SIG-001 must be administered in accordance with the *Laparoscopic Procedure Manual* and must not be used after the expiry/use-by date and time. While all SIG-001 is expected to be administered to patients, SIG-001 not administered to a patient must be returned to the Sponsor or designee or destroyed per the site's standard procedures.

Investigational product must be stored under the conditions specified on the label, and must remain in the original container until used or destroyed. Sites should use caution to avoid temperature fluctuations or exposure to mechanical agitation, dropping, shaking, or stirring of the product.

The SIG-001 protective plastic pouch and foil lid should only be removed in the operating room,

immediately prior to preparation for surgery.

Additional details on storage, handling, and administration are included in the *Pharmacy Manual* and *Laparoscopic Procedure Manual*.

8.2. Product Assignment and Dispensing Procedures

8.2.1. Randomization and Blinding

No randomization or blinding is employed in this trial.

8.2.2. Accountability and Disposition Procedures

Sites must maintain an accurate study product Accountability Log throughout the study. While all SIG-001 is expected to be administered to patients, SIG-001 not administered to patients must be returned to the Sponsor or designee or destroyed per the site's standard procedures.

The Investigator or designee must ensure that the sponsor-supplied product and kits are used in accordance with the protocol and are dispensed only to subjects enrolled in the study. To document appropriate use of sponsor-supplied product and kits, the Investigator or designee must maintain records of all sponsor-supplied products and kits to the site, site inventory, dispensation and use by each subject, and return to the Sponsor or designee.

Proper product accountability includes, but is not limited to:

- Continuously monitoring expiration date/use-by date and time.
- Verifying that actual inventory matches documented inventory.
- Verifying that all containers used are accurately documented on the log.
- Verifying that required fields are completed accurately and legibly.

If any dispensing errors or discrepancies are discovered, the Sponsor must be notified immediately.

In the event of expiry date extension of supplies already at the study site, sponsor-supplied products may be relabelled with the new expiry date at that site. In such cases, Sigilon Therapeutics or its designee will prepare additional labels and all necessary documentation for completion of the procedure at the sites.

9. STUDY PLAN

9.1. Screening and Enrolment

9.1.1. Screening of Subjects

Following completion of the consenting process, patients will undergo screening assessments as described in the Schedule of Assessments (Table 2 Schedule of Assessments). Once eligibility is verified, the Investigator or site staff will work with the patient to schedule the SIG-001 placement procedure. If the laparoscopic procedure is scheduled outside of the original screening window, the patient will return to the clinic for Screening Visit 2 to repeat select screening assessments. The reason for completing Screening Visit 2 should be clearly documented. Patients must undergo SIG-001 placement within 90 days of consent.

All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. Screening assessments may be completed over several screening visits. The Investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.

9.1.2. Screen Failures

Screen failures are defined as patients who consent to participate in the clinical study but who do not subsequently receive the study product. A minimal amount of information is required to ensure transparent reporting of screen failure subjects in compliance with the Consolidated Standards of Reporting Trials requirements and to respond to queries from regulatory authorities. Minimal information includes reporting of demographics, screen failure details, eligibility criteria, and any AE/SAE. Screen failure data will be recorded within the electronic Case Report Form (eCRF).

9.1.3. Informed Consent Procedure

Informed consent must be obtained prior to subject enrolment in the study (as described in Section 14.1.3) and before any protocol-directed procedures are initiated. A unique subject identification number (subject ID = site number + subject number) will be assigned to each subject at the time that informed consent is obtained; this subject number will be used throughout the study.

Individuals who do not meet the eligibility criteria for participation in this study (i.e., screen failure) may be rescreened once. Rescreened subjects will be assigned a new screening number that is different from the number assigned at the initial screening.

9.2. SIG-001 Administration

9.2.1. Day -1

Patients will be admitted into the clinic on Day -1 (the day before the procedure) for baseline assessments and confirmation of eligibility. There is no Day 0 in this study. Patients are required to fast overnight or for at least 8 hours prior to SIG-001 administration.

9.2.2. Pre-Procedure Activities

Prior to laparoscopic administration of SIG-001, patients will be dosed with a bolus of plasma-derived or rFVIII (depending on the type of product the patient was receiving and hospital requirements), followed by maintenance FVIII administration either as repeat bolus administrations or as a continuous infusion, per Investigator's and/or site's standard clinical practice. The laparoscopic procedure will proceed ONLY if:

a) pre-surgical FVIII activity levels reach ≥ 60 IU/dL (60% of normal) obtained within 1 hour of FVIII infusion

   OR

b) pre-surgical aPTT is normal AND measured pre- and post- dosing FVIII levels (IU/dL) reach a difference of at least 60% during PK assessment. This FVIII level difference may have been determined during prior screening visits, per the Investigator's judgment and preference.

Prior to the laparoscopic procedure, patients will receive a single dose of one antibiotic (or a single dose of 2 different antibiotics), as per the site's standard clinical practice.

9.2.3. SIG-001 Procedure

These procedures should be carried out in the sterile field by trained study staff as described in the *Laparoscopic Procedure Manual*.

SIG-001 must be administered by a study surgeon who has been trained to the procedures for SIG-001 administration outlined in the *Laparoscopic Procedure Manual*. SIG-001 is placed into the greater sac of the peritoneal space via laparoscopy conducted under general anaesthesia. Each site will use their standard, clinically used laparoscopic methods and instrumentation to complete the surgery, supplemented with equipment, reagents, and IP provided by the Sponsor, including an 8mm trocar and a 24F catheter. Once in the peritoneal space, the catheter is inserted through a trocar, and syringes containing SIG-001 spheres and buffer at approximately 1:2 volume ratio are attached firmly to the catheter and the contents slowly expelled, including air volume. Once all syringes containing SIG-001 are administered, a syringe containing buffer alone will be administered to expel any remaining spheres from the catheter. Complete details regarding SIG-001 administration are provided in the *Laparoscopic Procedure Manual*.

9.2.4. Post-Procedure Activities

Following SIG-001 administration, patients will continue to receive FVIII to maintain trough levels ≥60% for the first 48 hours after surgery. Patients may be discharged from the hospital on Day 3 or later per the Investigator's discretion.

FVIII dosing will be continued at home until the 1-week visit, to maintain minimal haemostatic trough levels of ≥30% of FVIII activity. This post-procedure FVIII treatment regimen may involve twice daily dosing for standard half-life products or once daily dosing for extended half-life products. Patients may be discharged with a PICC line as needed. Per the Investigator's discretion, patients may remain in the hospital for longer observation during FVIII administration.

Patients will be withdrawn from FVIII therapy after the Week 1 (Day 7) visit, per the Investigator's discretion. If patients are not withdrawn from FVIII therapy at Week 1, the reason for continuing FVIII will be recorded.

9.3. Long Term Follow-up

Patients will be followed for approximately 5 years post SIG-001 administration for safety and efficacy per the Schedule of Assessments (Table 2 Schedule of Assessments).

FVIII levels will be measured at regular intervals throughout the study. Per the investigator's discretion, exogenous FVIII will be washed out for a minimum of 96 hours (4 days) and at least 168 hours (7 days) for prolonged half-life rFVIII products.

Throughout the study, all patients will have access to commercially available FVIII therapy, if needed, to treat bleeding episodes, for perioperative management of surgery or other medical procedures, or as preventative treatment prior to vigorous physical activity.

9.4. Unscheduled Visits

Unscheduled visits may be necessary for safety monitoring or to repeat a study assessment. Information regarding concomitant medications and adverse events will be collected at all unscheduled visits. Data collected during an unscheduled visit will be captured in the EDC.

9.5. End-of-Trial Date

The End-of-Trial date will be based on the final data collection date for the entire study, which is the date of the last Follow-up call/visit.

10. STUDY ASSESSMENTS

Unless otherwise indicated, all study-specific assessments will be performed by the Investigator or designated study personnel. The procedures to be performed throughout the study are outlined in the Schedule of Assessments (Table 2 Schedule of Assessments). The sections below provide additional details regarding certain procedures and assessments listed in the Schedule of Assessments.

10.1. Safety Assessments

10.1.1. Medical History

A complete medical history will be collected at Screening. Information to be documented includes demographic information, prior and ongoing medical illnesses and conditions, and previous surgical procedures. Medical history related to the patient's diagnosis of haemophilia A will be documented, including FVIII therapies and transfusion and bleeding history in the 12 months prior to screening.

10.1.2. Physical Examination

Physical examinations will be performed in accordance with the Schedule of Assessments (Table 2 Schedule of Assessments).

At Screening, Day -1, and M60, a complete physical examination will be performed, which will include a review of general appearance (head, eyes, ears, nose, and throat), as well as the cardiovascular, dermatologic, gastrointestinal, genitourinary, lymphatic, musculoskeletal, neurologic and respiratory systems.

At all other clinic visits, a directed physical examination will be completed. The directed physical examination will be based on the patient's clinical status and will include general appearance, cardiovascular, gastrointestinal, neurologic, and respiratory assessments. Particular attention will be given to signs of bleeding as well as possible hemarthroses.

Clinically significant changes from baseline will be captured as AEs.

10.1.3. Target Joint Assessment

Target joints will be identified and assessed at Screening and yearly following SIG-001 administration. Target joints are defined as major joints (e.g. hip, elbow, wrist, shoulder, knee, ankle) into which repeated bleeding occurs and with symptoms of pre-existing target joint

involvement (e.g., synovitis, persistent swelling, effusion, limitation of range of motion). Repeated bleeding is defined as a frequency of 3 or more bleeding episodes into the same joint in a consecutive 12-week period (Ota et al., 2007).

The modified HJHS scoring tool will be utilized for joint health assessment (Feldman et al., 2011). Six joints (left ankle-LA, right ankle-RA, left elbow-LE, right elbow-RE, left knee-LK, right knee-RK) will be scored on a scale from 0 to 20. Gait will be scored on a scale from 0 to 4. The total score will be the sum of scores from all 6 joints plus the gait score (range from 0 to 124), with 0 being normal and 124 representing most severe disease.

10.1.4. Vital Signs

Collection of vital signs will be performed in accordance with the Schedule of Assessments (Table 2 Schedule of Assessments). Vital sign measurements include temperature, respiratory rate, seated BP, and pulse. Seated BP measurements will be recorded after the patient has been sitting for at least 5 minutes. Three BP measurements will be recorded at Screening, and the average will be calculated for eligibility assessment. After screening, a single BP measurement will be sufficient.

10.1.5. Prior and Concomitant Medications

All concomitant medications will be collected from the time of informed consent until the patient's last study visit. Please refer to Section 7.5 for permitted, prohibited and prophylactic medications.

10.1.6. Height and Weight

Height will be collected at the Screening Visit only. Weight will be collected as specified in the Schedule of Assessments (Table 2 Schedule of Assessments).

10.1.7. Electrocardiogram

Resting 12-lead electrocardiogram (ECG) will be recorded as specified in the Schedule of Assessments (Table 2 Schedule of Assessments).

ECG assessments should be obtained after the patient has been in a supine position for at least 5 minutes and recorded while the patient remains in that position. ECGs should be performed before vital sign assessments and blood sample collections. If this is not possible, the ECG must be performed at least 10 minutes after blood draws.

Mean QT interval corrected for heart rate will be calculated using the QT interval corrected by Fridericia formulae (QTcF).

Any clinically significant abnormalities detected require confirmation with triplicate ECG.

Electrocardiogram findings will be reviewed by the Investigator and assessed as "Normal," "Abnormal, Not Clinically Significant," or "Abnormal, Clinically Significant" in the corresponding eCRF. Any clinically significant findings will be reported on the corresponding AE page in the eCRF.

10.1.8. Clinical Laboratory Tests

Routine laboratory tests for haematology, serum chemistry, coagulation and urinalysis will be

performed at selected visits as specified in the Schedule of Assessments (Table 2 Schedule of Assessments). Additional clinical laboratory tests should be performed according to Investigator discretion as clinically indicated.

ABO blood typing, genotyping and screening for HIV, Hepatitis B, and Hepatitis C will be performed at the Screening visit.

Alcohol and illicit drug screening will take place at Screening.

At select timepoints (indicated in the Schedule of Assessments), additional blood samples will be collected and plasma and serum samples will be stored for exploratory future analyses. These analyses may include FVIII activity, SIG-001-specific inhibitor measurement, or other testing related to SIG-001. Additional details are included in the *Laboratory Manual*.

The Investigator (or designee) is responsible for reviewing available laboratory results for each patient to ensure that there are no laboratory abnormalities. Any out-of-range laboratory result that is considered clinically significant by the Investigator will be recorded as an AE and can be confirmed by repeat testing at the discretion of the Investigator. Clinically significant laboratory abnormalities at any visit will be followed to resolution (return to normal or to the baseline state) or until clinically stable as determined by the Investigator.

Instructions for obtaining and handling laboratory samples are provided in the *Laboratory Manual*.

A list of key clinical laboratory tests to be performed is presented in Table 4.

Table 4 Clinical Laboratory Tests

HEMATOLOGY	BIOCHEMISTRY	URINALYSIS DIPSTICK	SEROLOGY	DRUG SCREEN
Erythrocytes	Sodium	pH & gravity	HBs Ag (Hepatitis B)	Alcohol
Haemoglobin	Potassium	Clarity & Colour	Anti-HBc (Hepatitis B)	Amphetamines
Haematocrit	Chloride	Protein	HBV DNA (Hepatitis B)	Barbiturates
Platelets (incl. MCV, MCH, MCHC)	Calcium	Glucose	HCV RNA (Hepatitis C)	Benzodiazepines
Leukocytes	Magnesium	Leukocytes	HIV Ag/Ab	Cocaine
Neutrophils	Bicarbonate	Bilirubin	HBe Ag	Methadone
Lymphocytes	Protein, total	Ketones	HIV RNA	Oxycodone
Monocytes	Albumin	**URINALYSIS SEDIMENT**	T- and B-cell profile	Propoxyphene
Eosinophils	Creatinine	RBC		THC
Basophils	Uric acid	WBCC		**OTHER**
Neutrophils (abs.)	Blood urea nitrogen	Casts		ABO blood typing
Lymphocytes (abs.)	ALT	Casts (comment)		F8 genotype
Monocytes (abs.)	AST	Crystals		VWF Ag
Eosinophils (abs.)	Alkaline phosphatase	Crystals (comment)		FVIII inhibitors
Basophils (abs.)	Bilirubin, direct	Bacteria		FVIII activity (one-stage & chromogenic assay)
	Bilirubin, total			
	LDH			
	Lipase			
	Creatine kinase			
	eGFR			
	GGT			
	Phosphorus			
	Glucose			
	Triglycerides			
	C-Reactive Protein			

10.1.9. FVIII PK Assessment

FVIII PK assessment will be conducted prior to SIG-001 administration (i.e. during the screening period or on Day -1) to guide FVIII dosing during the perioperative period. For standard half-life FVIII products, PK sampling may include 2 to 3 timepoints, with samples at least 12 hours apart in a 48-hour period after factor infusion; particularly informative is the 24-hour sample. For extended half-life FVIII products, an additional sample at 60-84 hours after factor infusion is advisable. A trough level may be added only if the patient is to be infused with factor at the clinic (Iorio et al., 2017). The number and timing of samples are at the discretion of the Investigator. The actual collection times as well as FVIII administration time must be recorded. The assessment does not need to be conducted if previously completed and the information can be obtained from the medical records.

10.1.10. Patient Questionnaires

The following questionnaires will be administered at selected visits:

Quality-of-Life (Appendix 1)

The Haem-A-QoL Questionnaire is a haemophilia-specific quality-of-life assessment tool for patients who are 17 years old and above. It includes 46 questions pertaining to 10 dimensions of health-related quality-of-life (HRQoL) (Gringeri & Von Mackensen, 2008).

EQ-5D-5L (Appendix 2)

The EQ-5D-5L Questionnaire uses 5 questions to assess mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. It is a standardized instrument that was established by the EuroQol Group, providing a single index value for health status (Rabin, Oemar, & Oppe).

Physical Activity Questionnaire (Appendix 3)

Changes in physical activity level will be assessed at selected visits by asking subjects to evaluate whether the frequency and/or the intensity of their activity level has increased, decreased or remained unchanged.

10.1.11. Magnetic Resonance Imaging (MRI)

To establish a baseline imaging record for each patient, a non-contrast upper and lower abdominal MRI will be performed during the Screening Period or on Day -1 as specified in the Schedule of Assessments (Table 2 Schedule of Assessments). If a patient is unable to undergo an MRI, an abdominal ultrasound will be done instead. A follow-up MRI is not required per protocol; however, it may be performed, if clinically indicated or if recommended by the Investigator. Repeat imaging will be included in the study record.

10.2. Efficacy Assessments

Efficacy assessments will include serial measurement of FVIII activity. This activity will be assessed using both a one-stage clotting assay and a chromogenic substrate assay.

10.2.1. FVIII Activity

Throughout the study, FVIII activity levels will be assessed by one-stage and chromogenic assays performed by the central laboratory from samples obtained at regular intervals. Per the Investigator's discretion, exogenous FVIII use will be washed out for a minimum of 96 hours (4 days for standard half-life FVIII products) and at least 168 hours (7 days for prolonged half-life rFVIII products) to ensure that only FVIII secreted by SIG-001 is measured.

10.2.2. Bleeding Events and FVIII Usage

The number of bleeding events and quantity of FVIII replacement product used will be collected. At screening, the number of bleeding events and usage of FVIII replacement product will be documented for the past 12 months based on medical history and patient recollection. Bleeding events will be recorded by the patient as part of the patient diary. For each bleeding episode, the following parameters will be recorded: location (joint, muscle, internal organ vs other), type of bleed (spontaneous, traumatic, procedure-related), severity, and action taken/treatment.

Annualized bleeding rate will also be categorized by bleed location (joint, muscle, internal organ, other) and type of bleed (spontaneous, traumatic, procedure-related). Recombinant FVIII replacement therapy during the follow-up period will be recorded by the patient as part of the treatment diary. The following parameters will be recorded for each treatment event: reason for replacement therapy, name of product used, and dose.

10.3. Justification for Assessments

The clinical assessments and procedures used for the evaluation of efficacy and safety of SIG-001 are standard, widely used, and generally recognised as reliable, accurate, and relevant.

11. STUDY SPECIFIC COMMITTEES

11.1. Safety Review Committee (SRC)

A SRC composed of site Investigators, the Sponsor's representative and at least one independent individual with relevant experience in clinical research will be established. The SRC will review safety and efficacy on an ongoing basis. The SRC will review safety and efficacy parameters through at least the Week 8 visit for the first 3 patients enrolled in the study and at least the Week 4 visit for the remaining patients in any dose cohort, between dose cohorts, and for any cohort expansion. Additionally, the SRC will review all SAEs in an ongoing manner. The SRC may also meet on an ad hoc basis. Should a safety signal arise, the SRC will promptly convene for further assessment of subject safety. Notification of all SRC meetings and meeting outcomes will be sent to all participating sites. Additional information regarding membership and meeting frequency will be included in the *SRC Charter*.

12. SAFETY AND ADVERSE EVENTS

Throughout the course of the study, all AEs will be monitored and recorded in an AE section of the eCRF, including the AE's description, start and end date, seriousness, severity, action taken, and relationship to the Investigational Product. If AEs occur, the first concern will be the safety

of the study patients. All AEs will be followed until resolved or stable and the outcome documented in the eCRF.

12.1. Definitions and Criteria

12.1.1. Adverse Events

Per ICH E2A, an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, not necessarily having a causal relationship with this product. An AE can therefore be any unfavourable or unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Medical interventions such as surgeries, diagnostic procedures, and therapeutic procedures are not AEs in and of themselves. The medical condition requiring treatment should be captured as an AE/SAE as appropriate, while the action taken should be recorded as treatment of the AE.

12.1.2. Serious Adverse Events

A serious adverse event (SAE) or reaction is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event

The term “life-threatening” in the definition of “serious” refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an AE that hypothetically might have caused death if it were more severe.

Important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered serious when, based upon appropriate medical judgment, they may jeopardise the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation, but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

Seriousness (not severity) serves as a guide for defining regulatory reporting obligations. An event can be serious, but not necessarily severe (e.g., an event requiring overnight hospitalisation for a diagnostic procedure must be reported as an SAE even though the event may be mild in severity, or an event can meet the CTCAE definition of Grade 4 – life-threatening while not meeting the seriousness criterion of life-threatening potentially resulting in different reporting

timelines). Furthermore, a severe AE is not necessarily serious (e.g., nausea of several hours' duration may be rated as severe, but may not be considered serious).

Pregnancy of a female partner of a study patient is not considered an SAE.

12.1.3. Unexpected Adverse Drug Reactions

An unexpected adverse drug reaction is a reaction for which the nature or severity is not consistent with the applicable product information (i.e. Investigator's Brochure, package insert for marketed products). Until product information is amended, expedited reporting is required for additional occurrences of the reaction. Reports that add significant information on specificity or severity of a known, already documented SAE constitute unexpected events. For example, an event more specific or more severe than described in the Investigator's Brochure would be considered "unexpected". Specific examples would be (a) acute renal failure as a labelled adverse drug reaction with a subsequent new report of interstitial nephritis and (b) hepatitis with a first report of fulminant hepatitis.

Guidance on reporting AEs and SAEs is described in Section 12.2.

12.1.4. Abnormal Laboratory Values

Any abnormality in a laboratory value that is new in onset or that has worsened in severity or frequency from the baseline condition and meets one of the following criteria will be recorded in the AE section of the eCRF:

- Requires therapeutic intervention
- Leads to patient withdrawal prior to administration of the Investigational Product
- Has accompanying or inducing symptoms or signs
- Is judged by the Investigator as clinically significant

12.1.5. Assessing Severity and Relationship

All AEs will be assessed on two descriptive parameters, severity and relationship to the Investigational Product.

- Severity refers to the intensity of an event and references impact on a patient's functioning.
- Relationship refers to the likelihood that the event being assessed was caused by the Investigational Product.

12.1.6. Severity

- All AEs or SAEs will be assessed by the Investigator for severity, according to CTCAE v5.0.
- The following general scale may be used with Investigator discretion to assign severity:
- When changes in the intensity of an AE occur more frequently than once a day, the maximum intensity for the experience should be noted. If the intensity category changes over a number of days, those changes should be recorded separately (with distinct onset dates).

12.1.7. Relationship

Each AE will be assessed as to its relationship to the Investigational Product and its relationship to the laparoscopic procedure, based on the following criteria:

- Definitely Related: An AE, including laboratory test abnormality (if applicable), in which there is no uncertainty in its relationship
- Probably Related: There is strong medical evidence to suggest that the AE is related
- Possibly Related: There is medical evidence to suggest that there is a reasonable possibility that the AE may be related. However, other medical explanations cannot be excluded as a possible cause
- Unlikely Related: There is no medical evidence to suggest that the AE may be related, or there is another more probable medical explanation
- Not Related: The AE can be determined with certainty to have no relationship

All events assessed as unlikely related to the Investigational Product will be considered unrelated for the purpose of regulatory reporting. All events assessed as possibly or probably related to the Investigational Product will be considered related for the purpose of regulatory reporting.

The relationship assessment of possibly related will be used as the default assessment in the situation when the relationship assessment is omitted by the Investigator and until the relationship assessment is reported by the Investigator.

12.2. Reporting Procedures and Requirements

12.2.1. Adverse Events

Adverse events occurring from when the patient signs the ICF until the last study visit will be captured in the eCRF. Any medical conditions prior to the patient signing the ICF will be recorded in the medical history.

SAEs that occur in subjects that have signed the ICF, but are screen failures, will be captured in the Safety and Clinical databases.

The Investigator should report all AEs in the AE page(s) of the eCRF and source documents, regardless of seriousness, severity, and relationship. Whenever possible, an AE will be reported using a diagnostic term, (e.g., “common cold” or “upper respiratory infection” rather than “runny nose, cough, mild fever”) and should include the attributes described in Section 12.1.5.

12.2.2. Serious Adverse Events

Each AE will be assessed to determine whether it meets seriousness criteria (Section 12.1.2). Reports of SAEs require immediate reporting (within 24 hours of the site’s knowledge of the event) outlined below and to the IRB/IEC according to standard operating procedures whether or not the Investigator believes that the experience is related to the study product.

The SAE eCRF must be completed in the clinical database. The corresponding eCRFs requested on the SAE report must also be completed. Relevant medical records shall be provided at the time of the report. In the event that EDC is not accessible, the SAE must be reported by completing the paper SAE report form provided to the site. At a minimum, the initial report

should include the following information:

- Event term
- Patient number and age
- Investigational Product
- Reporter's name and contact information

In the case of a "minimum report" (one that solely comprises the information bulleted above), a more detailed follow-up report should be sent as soon as more information becomes available, but no later than 7 calendar days after the date of the initial report. Each SAE should be followed up until resolution or stabilisation and for reported deaths, the Investigator should supply the Sponsor or its delegate and the IRB/IEC with any additional requested information (e.g., autopsy reports and terminal medical reports).

The Sponsor or its representative will be responsible for determining and in turn, reporting SAEs to regulatory authorities according to the applicable regulatory requirements.

Serious AEs that are ongoing at the last study visit should be followed until they resolve or the Investigator determines the event to be chronic (stabilized for at least 30 days), or until the subject is considered lost to follow-up.

Any SAE reported by the subject to the Investigator that occurs after the last assessment, and determined by the Investigator to be reasonably associated with the use of the study product, should be reported to the Sponsor.

Adverse events and concomitant medications terms will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and the World Health Organization Drug Dictionary Enhanced (WHO-DDE), respectively.

12.3. Safety and Risk Mitigation

The clinical safety of SIG-001 is further described in the *SIG-001 Investigator's Brochure*.

12.3.1. Mitigation of Exposure Risks

SIG-001 consists of genetically modified human cells (allogeneic cells) encapsulated in alginate hydrogel spheres. The alginate sphere component of SIG-001 is sufficiently porous to allow macromolecules, such as FVIII, to be secreted and pass into the surrounding space within the body, but not to allow allogenic cell components to pass. Once in circulation, FVIII secreted by SIG-001 spheres is expected to have distribution similar to that observed for other recombinant FVIII products (ReFacto, 2007; Xyntha, 2008). This is supported by circulating levels of FVIII observed in testing of SIG-001 in cynomolgus monkeys (*SIG-001 Investigator's Brochure* Section 4.2).

Direct exposure of patients to allogenic cells is expected to be extremely rare. First, there is an inability of ARPE-19 cells to exit SIG-001 spheres based on pore size. Second, in multiple nonclinical studies SIG-001 spheres demonstrated to have excellent integrity *in vivo*, with more than 92% spheres unbroken even after invasive extraction and necroscopic examination in animals. Third, *in vitro* stability data demonstrate that spheres do not degrade following 18 months of simulated aging at temperatures up to 50ºC and 60ºC, well in excess of temperatures

encountered clinically. Fourth, vertebrates lack alginase, an enzyme necessary to break down alginate products. CCI [redacted] Thus, SIG-001 spheres are stable and sphere breakage in vivo has been demonstrated to be minimal. However, should any sphere breakage occur, ARPE-19 allogeneic cells are expected to be rapidly removed from the body by the immune system of the patient.

12.3.2. Mitigation of Contamination Risks

The SIG-001 shelf-life (96 hours) does not allow sufficient time for all microbiological contamination tests (lasting up to 14 days for bacteria and fungi and up to 35 days for mycoplasma) to be completed in full ahead of SIG-001 administration to a patient. However, multiple rapid microbial detection tests are completed prior to administration of SIG-001 to a patient (first stage certification criteria) and include negative results for gram stain, bacterial endotoxins and quantitative polymerase chain reaction (PCR) for mycoplasma, as well as no growth for 48 hours in the bacteria and fungi detection tests. Second stage certification criteria are completed after SIG-001 administration and include no growth for 14 days bacteria and fungi detection tests done via direct inoculation and no growth in the 35-day mycoplasma detection test. Both tests are read daily until completion. As such, there is a small risk that SIG-001 contamination may be identified after the laparoscopic administration is complete. In the unlikely event of detection of SIG-001 contamination after administration, the Sponsor will immediately notify the Investigator to ensure appropriate clinical management of the patient. Appropriate measures must be taken to ensure the safety and well-being of the patient. Such measures will be based on the sterility results (e.g. type of organism identified and antibiotic sensitivity), the patient health status/symptoms, and the Investigator's judgment. The clinical management of the patient may include additional visit(s) for physical examination, additional testing (e.g. complete blood counts, blood chemistry, inflammation markers such as C-reactive protein and blood cultures), and appropriate antibiotic treatment. A swab culture may be considered only if there is suspicion of inappropriate surgical site healing or presence of discharge from the surgical site. Investigators have the discretion to schedule additional visits and procedures as needed or decide to manage in-hospital. Surgical consultation and additional imaging, e.g. abdominal computed tomography (CT) with or without contrast may be needed to evaluate the patient progress and to inspect the abdominal cavity for fluid collections.

12.3.3. Potential Removal of SIG-001 Spheres

SIG-001 is intended to be permanently placed. In the event of unexpected serious safety issues related to SIG-001 that are not otherwise medically manageable (e.g. severe and persistent anaphylactic reaction), the Investigator may consider removal of the spheres. Preclinical data suggests that it is feasible to remove the majority of spheres after placement, which may require peritoneal lavage via laparoscopy or via laparotomy. Such procedures should be conducted at the discretion of the Investigator upon consultation with a surgery expert.

12.3.4. Mitigation of FVIII Inhibitor Development

New inhibitor formation in patients with haemophilia A and more than 150 exposure days to

FVIII products is rare (Kempton, 2010). In addition, patients at higher risk of developing inhibitors such as those with current FVIII inhibitor titres > 0.6 NBU/mL or prior ITI are excluded from this study. Lastly, SIG-001 produces human FVIII with the same amino acid sequence of commercially available recombinant FVIII products that have a well-established safety profile (Moroctocog alfa or BDDrFVIII; trade names ReFacto®, XYNTHA®), thus limiting the exposure of patients to neo-epitopes. As such, there is a small risk that SIG-001 administration may result in the development of FVIII inhibitors.

In the unlikely event of new development of FVIII inhibitors or persistently increased titres from baseline levels after SIG-001 administration, appropriate measures must be taken to ensure the safety and optimal care of the patient, including additional visit(s) for physical examination and frequent laboratory testing (including Bethesda assay for FVIII inhibitors). Clinical management will be instituted at the discretion of the Investigator and following established management guidelines (Carcao & Goudemand, 2018). Clinical management may range from monitoring alone to use of approved therapies to treat and prevent bleeds in patients with inhibitors, to institution of ITI. Should the Investigator consider that avoiding exposure to BDD-hFVIII produced by SIG-001 is necessary, removal of spheres may be considered (Section 12.3.3, Potential Removal of SIG-001 spheres).

12.3.5. Justification for Contraception Use

SIG-001 is locally administered, spheres (intact or broken) are not able to enter systemic circulation, and sphere components have been shown to be biocompatible, non-mutagenic, and non-genotoxic in nonclinical testing (*SIG-001 Investigator's Brochure* Section 4.3). This testing is conducted with consideration for ICH S2(R1) Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, as well as relevant regional guidance/guidelines for advanced therapy medicinal products. Due to the nature of the product, exposure to systemic circulation or seminal fluid is not expected for the spheres or the cellular components. Therefore, the use of barrier methods (condoms) is considered to be an appropriate form of contraception for fertile males with partners that are women of childbearing potential, without further contraception requirements for partners of study participants. This determination is in accordance with US 21 CFR and EU Directive 2001/20/EC, and follows recommendations that condoms are sufficient contraception for non-genotoxic products made by the Clinical Trial Facilitation Group "Recommendations related to contraception and pregnancy testing in clinical trials" and recognized by, among other Agencies, the UK Medicines and Healthcare Products Regulatory Agency (MHRA) (Heads of Medicines Agency, 2014; MHRA, 2018). In the event that a pregnancy occurs in the female partner of a study subject, the Investigator will be required to obtain a Partner Pregnancy Consent Form in order to collect information regarding the outcome of the pregnancy. The Investigator will notify the trial Safety Officers immediately following pregnancy confirmation by completing and sending a Pregnancy Report Form.

13. STATISTICAL ANALYSIS

13.1. Statistical Considerations

No formal hypothesis testing is planned in the study. All analyses will be descriptive in nature.

For each endpoint, detailed descriptions of the analyses will be provided in the *Statistical Analysis Plan* (SAP).

13.2. Determination of Sample Size

The sample size for this study is not based on statistical considerations, considering the limited number of available patients and the complexity of the study product. A maximum of 18 patients will be included in up to three dosing cohorts of a maximum size of six patients per cohort.

13.3. Conduct of Study Summaries

The number of patients who enrol, discontinue, or complete the study in each cohort will be summarized. Moreover, reasons for premature study withdrawal will be listed and summarized. Enrolment and major protocol deviations will be listed and evaluated for their potential effects on the interpretation of study results. The number of patients who reached the various stages of the study, how many dropped out and for what reasons will be described in the SAP.

13.4. Summaries of Demographic and Baseline Characteristics

Demographic and baseline characteristics (including age, sex, weight etc.) will be summarized using means, standard deviations, medians, and ranges for continuous variables and proportions for categorical variables, as appropriate. Summaries will be presented by dose cohort.

13.5. Efficacy Analysis

In general, efficacy analyses will be analysed by dose cohort and overall for the Efficacy Analysis Set, which will include all patients treated who have obtained at least one post-baseline efficacy assessment. The key efficacy analyses will include all data up to the end of Year 1 of follow-up. Additional analyses may be conducted including follow-up data in Years 2 to 5.

13.5.1. Development of FVIII Inhibitors

Testing for the development of FVIII inhibitors using the Nijmegen Bethesda inhibitor assay will be performed as described in the Schedule of Assessments (Table 2 Schedule of Assessments). The Nijmegen Bethesda inhibitor assay results will be summarized descriptively by visit as well as the number and proportion of patients with high titre values.

13.5.2. Factor VIII Activity Levels

Factor VIII activity (as measured by one-stage and chromogenic assays) will be summarized separately using summary statistics for each scheduled assessment, by dose cohort and overall. Factor VIII activity will be correlated to SIG-001 dose received.

13.5.3. Annualized Bleeding Rate (ABR)

The annualized number of bleeds per patient (annualized bleeding rate) will be calculated as the number of bleeding events divided by length of time on study product follow-up, in years. ABR will also be categorized by bleed location (joint, muscle, other) and type of bleed (spontaneous, traumatic, related to procedure).

13.5.4. Factor FVIII Product Usage

The annualized use of FVIII replacement products will be calculated for all patients and by dose

cohort as the total number of uses of replacement products divided by the length of time on study product follow-up.

13.6. Safety Analyses

All patients who received study product will be included in the Safety Analysis Set.

13.6.1. Adverse Events

Treatment-emergent AEs will be summarized for each dose cohort by severity and relationship to study product and relationship to the study procedure. SAEs will be presented for each dose cohort by relationship to study product and relationship to the study procedure. Summary tables will present incidence estimates and individual event rates by system organ class as well as within each system organ class. Severity will be summarized by CTCAE v5.0 grading. Patients experiencing an event more than once with varying severity will be counted only once, in the maximum grade within each system organ class and preferred term. For incidence of relationship to study product and study procedures, patients will be counted only once, in the category of the strongest relationship within each system organ class and preferred term.

13.6.2. Other Safety Assessments

For the absolute values and change from baseline summaries, for vital signs, laboratory data, and ECGs, the baseline value will be the latest result obtained prior to the start of study product. Standardized units will be calculated. The QTcF will be derived during creation of the reporting database using the reported ECG values (RR and QT) using Fridericia's formula:

$$\textit{Where, } \mathbf{QTcF = QT/RR^{(1/3)}} \textit{ where RR is in seconds.}$$

Summaries will be by dose cohort and overall.

Corrected calcium will be derived during creation of the reporting database using the following formula: Corrected calcium (mmol/L) = total calcium (mmol/L)+([40 – albumin (G / L)] x 0.02).

Laboratory toxicity data will be coded to CTCAE v5.0, where applicable, and summarized by shift tables of worst toxicity grade post-baseline compared with baseline toxicity grade.

13.6.3. Quality-of-Life Assessments and Physical Activity Level

Haem-A-QoL, EQ-5D scale scores and sub-scale scores will be calculated and summarized by planned timepoints, dose cohort, and overall. Change from baseline will also be summarized over the course of the entire study. Changes in physical activity level will also be summarized by planned timepoints, dose cohort, and overall.

14. DATA HANDLING AND RECORDKEEPING

14.1. Ethical Considerations

14.1.1. Regulations and Guidelines

The study will be performed in accordance with this Protocol, United States investigational new drug (IND) regulations (21 CFR 312), ICH guidelines for Good Clinical Practice, the General

Data Protection Regulation (EU) 2016/679 (GDPR) and applicable laws and regulations for the conduction of clinical trials in the participating countries.

US-based sites and laboratories or entities providing support for this study, must, where applicable, comply with the Health Insurance Portability and Accountability Act of 1996 (HIPAA). EU-based sites and laboratories or entities providing support for this study, must, where applicable, comply with the Data Protection Directive 95/46/EC (24 Oct 1995), EU data protection regulations No. 45/2001 (18 Dec 2001), and EU General Data Protection Regulation (GDPR) 2016/679 (27 Apr 2016).

14.1.2. Institutional Review Board (IRB) / Independent Ethics Committees (IEC)

The clinical trial authorisation granted by the competent authority and a favourable opinion from the relevant independent ethics committee (IEC)/institutional review board (IRB) will be obtained prior to the start of the study. The local authorities will be notified about the study as required by law.

The competent authority and the IEC/IRB will be notified about the end of the study and a report summarising the study results will be sent to the competent authority and the EC within 1 year after the end of the study. The statistical analysis and report will conform to the relevant best practices guidelines, e.g. GCP. If a patient withdraws from the study or is terminated early, the competent authority and the EC will be notified as applicable. IRB/IEC approval is required for the study Protocol, Investigator's Brochure, protocol amendments, ICFs, patient information sheets, and advertising materials. No Investigational Product will be shipped to a site until applicable Competent Authorities and IRB/IECs have provided favourable opinion to the Sponsor or its representative.

14.1.3. Informed Consent

The informed consent form (ICF) must be agreed to by the Sponsor and the IRB/IEC and must be in compliance with GCP, local regulatory requirements, and legal requirements. For each patient, a written ICF will be obtained before any protocol-related activities are initiated. As part of this procedure, the Investigator or a designated representative must explain orally and in writing the nature, duration, and purpose of the study, and the action of the Investigational Product in such a manner that the patient and (if applicable) appointed guardian are aware of the potential risks, inconveniences, or adverse effects that may occur. Patients should be informed that they may withdraw from the study at any time without any resulting disadvantage. They will receive all information that is required by local regulations and ICH guidelines.

14.1.4. Subject Confidentiality

The Sponsor and designees affirm and uphold the principle of the subject's right to protection against invasion of privacy, in accordance with applicable law and regulation. Throughout this study, a subject's source data will only be linked to the Sponsor's clinical study database or documentation via a unique identification number. As permitted by all applicable laws and regulations, limited subject attributes, such as sex, age, or date of birth, and subject initials may be used to verify the subject and accuracy of the subject's unique identification number.

To comply with ICH Guidelines for GCP and to verify compliance with this protocol, the Sponsor requires the Investigator to permit its monitor or designee's monitor, representatives

from any regulatory authority (eg, FDA, Medicines and Healthcare products Regulatory Agency, Pharmaceuticals and Medical Devices Agency), the Sponsor's designated auditors, and the IEC to review the subject's original medical records (source data or documents), including, but not limited to, laboratory test result reports, ECG reports, admission and discharge summaries for hospital admissions occurring during a subject's study participation, and autopsy reports. Access to a subject's original medical records requires the specific authorization of the subject as part of the informed consent process.

Copies of any subject source documents that are provided to the Sponsor must have certain personally identifiable information removed (ie, subject name, address, and other identifier fields not collected on the subject's eCRF).

14.2. Data Management and Quality Control

14.2.1. Electronic Case Report Forms (eCRF)

All clinical data will be captured via electronic data capture (EDC) using a web-based tool.

The Investigator site staff will enter and edit the data via a secure network, with secure access features (username and password). A complete electronic audit trail will be maintained. The Investigator will approve the data using an electronic signature (21 CFR 11), and this approval is used to confirm the accuracy of the data recorded. Electronic CRFs will be used for all patients. Subject data at each Investigator site will be accessible to the Sponsor and representative CRO throughout the duration of the study. The eCRF must be kept current to reflect patient status at each phase during the course of the study participation. The eCRF will not capture personalised data. The Investigator must make a separate confidential record of personalised details (name and initials) on the patient identification and enrolment log.

It is the responsibility of the Principal Investigator of the respective site to ensure that all patient discontinuations or changes entered in the EDC system are reflected in the patient's medical records.

14.2.2. Study Monitoring and Quality Assurance

This study will be monitored for quality assurance at all stages of its development by the clinical research personnel employed by the Sponsor or its representative. Monitoring will include personal visits and telephone communication to assure that the investigation is conducted according to the protocol, standard operating procedures, GCP guidelines, and applicable regulatory requirements. Quality control procedures will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly. On-site review of eCRFs will include a review of forms for completeness and clarity, and consistency with source documents available for each patient. Note that a variety of original documents, data, and records will be considered as source documents in this study. The eCRF itself is not to be used as a source document under any circumstances.

Medical advisors and clinical research associates or assistants may request to witness patient evaluations occurring as part of this protocol. The Investigator and appropriate personnel will be periodically requested to attend meetings/workshops organized by the Sponsor to assure acceptable protocol execution. The study may be audited by the Sponsor or by regulatory authorities. If such an audit occurs, the Investigator must agree to allow access to required

patient records. By signing this protocol, the Investigator grants permission to personnel from the Sponsor, its representatives, and appropriate regulatory authorities, for on-site monitoring of all appropriate study documentation, as well as on-site review of the procedures employed in eCRF generation, where clinically appropriate.

14.3. Retention of Records

The Investigator must arrange for retention of study records at the site. The nature of the records and the duration of the retention period must meet the requirements of the relevant regulatory authority. In addition, because this is an international study, the retention period must meet the requirements of the most stringent authority. The Investigator should take measures to prevent accidental or premature destruction of these documents.

The Investigator will allow representatives of Sigilon's monitoring team, the governing IRB/IEC, the FDA or other applicable local authorities to inspect all study records, eCRFs, and corresponding portions of the patient's study site and/or hospital medical records at regular intervals throughout the study. These inspections are for the purpose of verifying adherence to the protocol, completeness and accuracy of the data being entered onto the eCRF, and compliance with FDA or other local authority regulations.

14.4. Use of Study Findings

By signing the study Protocol, the Investigator agrees to the use of results of the study for the purposes of national and international registration. If necessary, the authorities will be notified of the Investigator's name, address, qualifications, and extent of involvement. Reports covering clinical and biometric aspects of the study will be prepared by the Sponsor or its representative.

14.5. Publication and Disclosure

The data and information generated in this study are the exclusive property of Sigilon Therapeutics, Inc. and should be considered confidential, except where agreed otherwise in writing and authorization by Sigilon Therapeutics. Please refer to the clinical trial agreement for additional information on publication and disclosures.

14.6. Clinical Trial Registration

In order to ensure that information on clinical trials reaches the public in a timely manner and to comply with applicable laws, regulations, and guidance, Sigilon Therapeutics will, at a minimum register interventional clinical trials it sponsors anywhere in the world on ClinicalTrials.gov or other publicly accessible websites before start of study.

APPENDIX 1: HAEM-A-QOL QUESTIONNAIRE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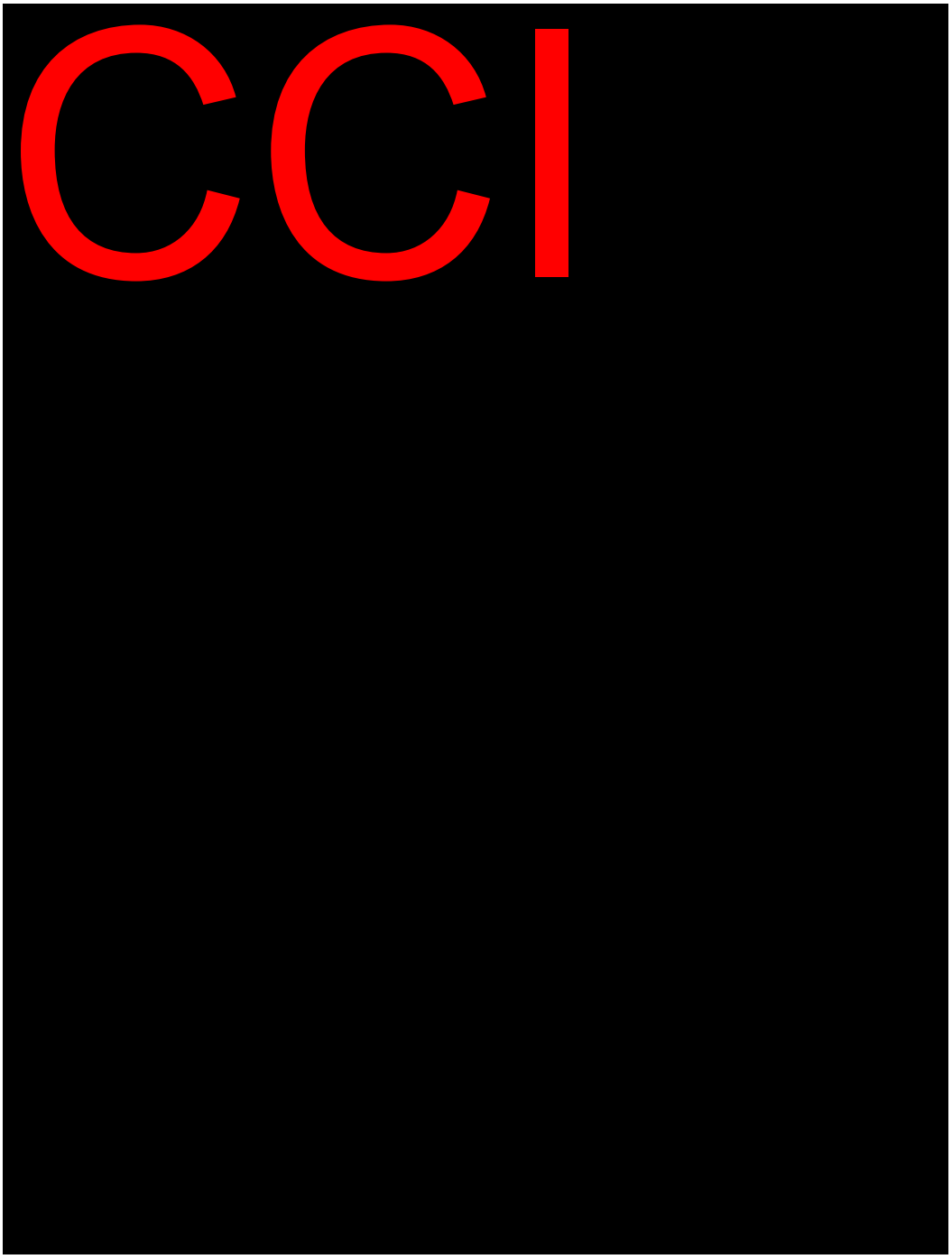
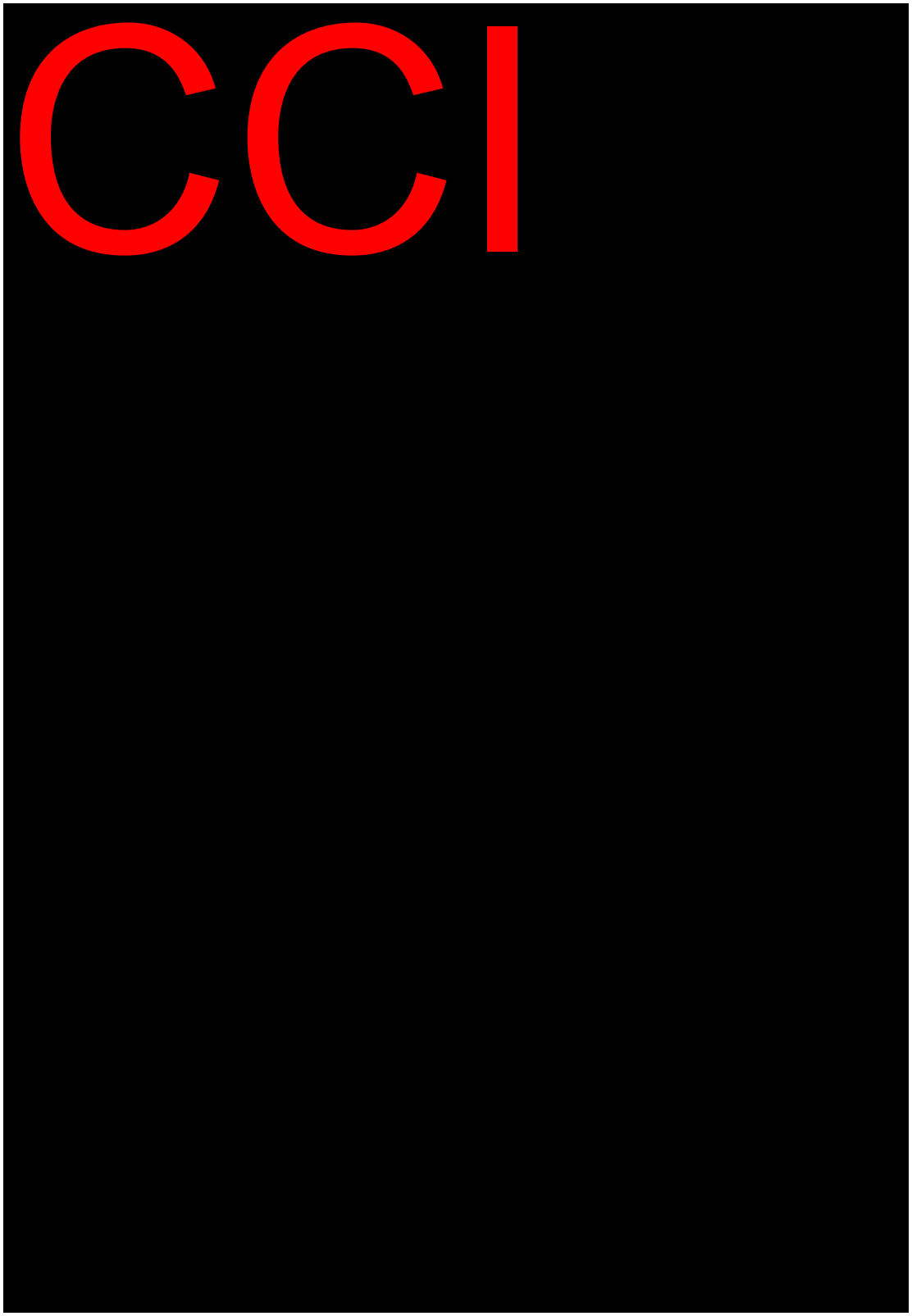
APPENDIX 2: EQ-5D-5L

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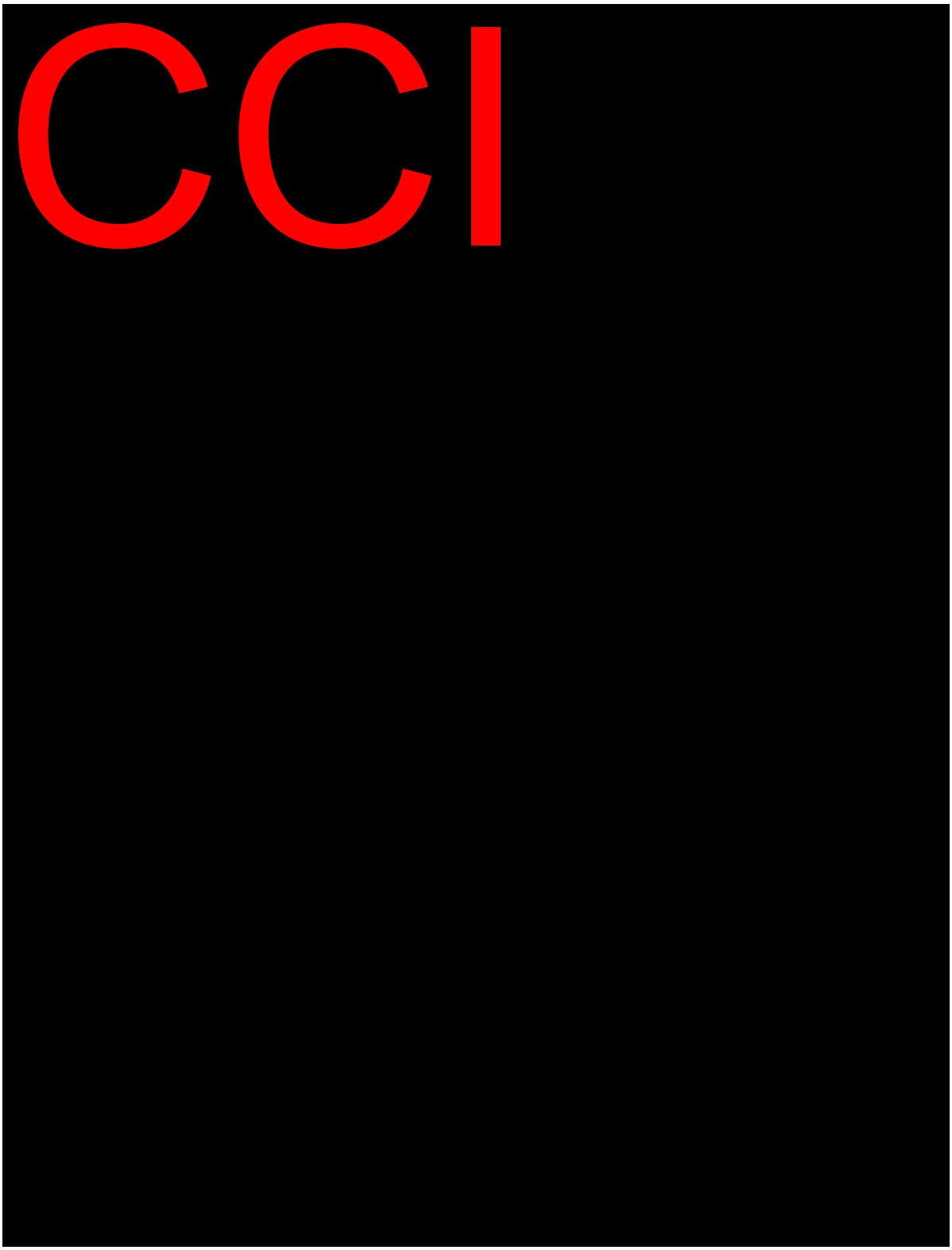
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APPENDIX 3: PHYSICAL ACTIVITY

APPENDIX 4: SUMMARY OF CHANGES

Separate *Summary of Changes* documents have been prepared to detail the protocol revisions made in each protocol version. Refer to the documents listed below for a detailed summary of protocol revisions, rationale and a detailed table of changes:

- Summary of Changes for Protocol Amendment 1: SIG-001-121, version 1.1 (dated 06 February 2020)
- Summary of Changes for Protocol Amendment 2: SIG-001-121, version 2.0 (dated 23 April 2020)
- Summary of Changes for Protocol Amendment 3: SIG-001-121, Version 3.0 (dated 29 April 2020)
- Summary of Changes for Protocol Amendment 4: SIG-001-121, Version 4.0 (dated 04 May 2020)
- Summary of Changes for Protocol Amendment 5: SIG-001-121, Version 5.0 (dated 04 March 2021)

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