Official Title: A Randomized, Placebo-Controlled, Double-Blind Pilot Study to Evaluate the Effect of GRF6021 on Intracellular Signaling Cascades in Blood Leukocytes and Postoperative Recovery Following Primary Hip or Knee Arthroplasty

NCT Number: NCT03981419

Document Date: Protocol Version 3: 14-November-2019

A Randomized, Placebo-Controlled, Double-Blind Pilot Study to Evaluate the Effect of GRF6021 on Intracellular Signaling Cascades in Blood Leukocytes and Postoperative Recovery Following Primary Hip or Knee Arthroplasty

Protocol Number:	AKST6021-211
Clinical Phase:	2
Sponsor:	Alkahest, Inc. 125 Shoreway Road, Suite D San Carlos, CA 94070
Study Agent:	GRF6021
Indications:	Postoperative Recovery Following Primary Hip or Knee Arthroplasty
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Principal Investigator:	[redacted] MD [redacted] Department of Anesthesiology Stanford University 300 Pasteur Drive Stanford, CA 94305-5117 Telephone: [redacted]
Version Number:	V3.0
Version Date:	14NOV2019
Replaces Version(s):	V2.0, 15APR2019

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LIST OF ABBREVIATIONS

3D-CAM	3-Minute Diagnostic Interview for Confusion
AE	Adverse event
ALT	Alanine aminotransferase
ASA	American Society of Anesthesiologists
AST	Aspartate aminotransferase
BDI-II	Beck Depression Index-II
BP	Blood pressure
CBC	Complete blood count
CD68	Cluster of differentiation 68
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
CREB	Cyclic AMP-responsive element-binding protein
CRF	Case Report Form
CRO	Contract Research Organization
csEN	Cell signaling Elastic Net
CyTOF	Mass cytometry
EBL	Estimated blood loss
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transferase
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
HR	Heart rate
Iba1	Ionized calcium binding adaptor molecule 1
ICH	International Conference on Harmonization
ICH E6 R2	International Conference on Harmonization Guidance for Industry, Good Clinical Practice: Consolidated Guidance, Revision 2
IEC	Independent Ethics Committee
IgA	Immunoglobulin A
IRB	Investigational Review Board
ITT	Intent-to-treat
IV	Intravenous
LV	Left ventricular
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PACU	Post-anesthesia care unit
POMS	Profile of Mood States
PSS	Perceived Stress Scale

PT	Preferred Term
PT/INR	Prothrombin time/international normalized ratio
PTT	Partial thromboplastin time
RBC	Red blood cell
RR	Respiration rate
SAE	Serious adverse event
SAP	Statistical Analytical Plan
SF-36	Short Form-36
SOC	System Organ Class
SRS	Surgery Recovery Scale
STAT3	Signal transducer and activator of transcription 3
SUSAR	Suspected unexpected serious adverse reaction
THA	Total hip arthroplasty
TKA	Total knee arthroplasty
UPCR	Urine protein-to-creatinine ratio
US	United States
WOCBP	Women of childbearing potential
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index

PROTOCOL APPROVAL PAGE

Study Title:	A Randomized, Placebo-Controlled, Double-Blind Pilot Study to Evaluate the Effect of GRF6021 on Intracellular Signaling Cascades in Blood Leukocytes and Postoperative Recovery Following Primary Hip or Knee Arthroplasty
Protocol Number:	AKST6021-211
Version/Date:	V3.0_14NOV2019
Sponsor Name and Address:	Alkahest, Inc. 125 Shoreway Road, Suite D San Carlos, CA 94070

I, the undersigned, have read and approve this protocol and agree on its content. It is confirmed that the information and guidance given in this protocol complies with scientific principles, the guidelines of Good Clinical Practice, the Declaration of Helsinki in the latest relevant version, and applicable legal and regulatory requirements.

Approved by:

[redacted] MD, PhD	DocuSigned by: [redacted] Signer Name: [redacted] Signing Reason: I approve this document Signing Time: 14-Nov-2019 | 12:31:50 PM PST D45401DB10A04F25A1F5F77BBDD81AEC	14-Nov-2019
Sponsor Representative (print)	Signature	Date

STATEMENT OF COMPLIANCE

Protocol Title:	A Randomized, Placebo-Controlled, Double-Blind Pilot Study to Evaluate the Effect of GRF6021 on Intracellular Signaling Cascades in Blood Leukocytes and Postoperative Recovery Following Primary Hip or Knee Arthroplasty
Protocol Number:	AKST6021-211
Version/Date:	V3.0_14NOV2019

By my signature, I:

- Confirm that my staff and I have carefully read and understand this protocol or protocol amendment and are thoroughly familiar with the appropriate use of the investigational agent described herein.
- Agree to comply with the conduct and terms of the study specified herein and with any other study conduct procedures provided by the Sponsor, Alkahest, Inc., or their designee
- Agree to assume responsibility for the proper conduct of the study at this site, including complying with current relevant versions of the US Food and Drug Administration (FDA) regulations, the International Conference on Harmonization (ICH) GCP guidelines, the Declaration of Helsinki, and all applicable rules, regulations, and federal, state, and local laws relating to the conduct of clinical studies and the protection of human subjects.
- Agree not to implement deviations from or changes to the protocol or protocol amendments without agreement from the Sponsor and prior submission to and written approval (where required) from the Institutional Review Board (IRB) or Independent Ethics Committee (IEC), except when necessary to eliminate an immediate hazard to the subjects, or for administrative aspects of the study (where permitted by all applicable regulatory requirements).
- Agree to onsite monitoring of all source documents by Alkahest, Inc. or designee and to onsite inspection of source documents by appropriate regulatory authorities, including but not limited to the FDA, local governing regulatory bodies, and IRB inspectors.

_____ _____

Investigator's Signature Date

_____

Print Name

PROTOCOL SUMMARY

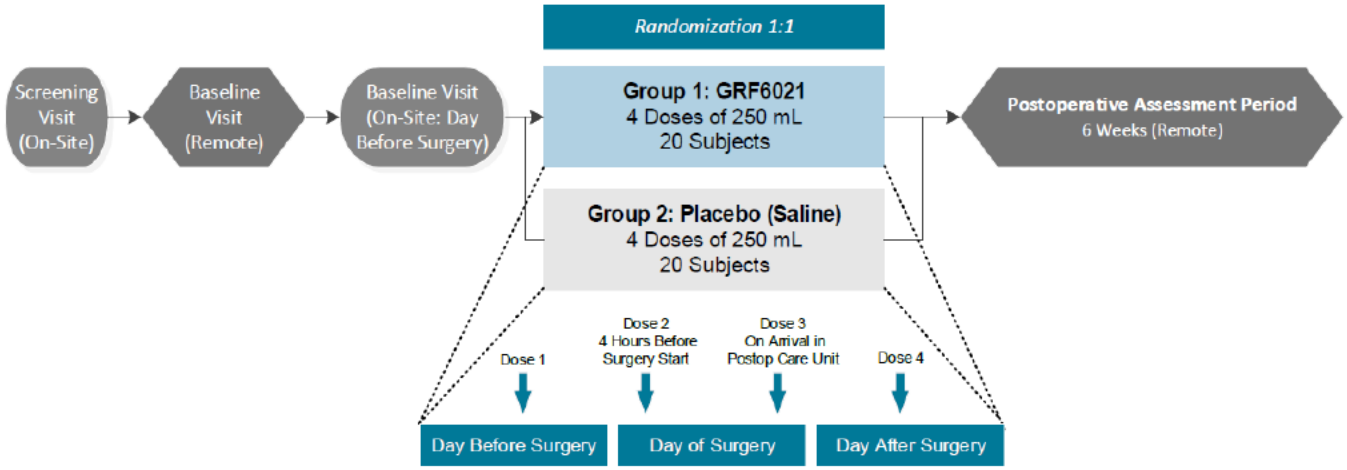
Title:	A Randomized, Placebo-Controlled, Double-Blind Pilot Study to Evaluate the Effect of GRF6021 on Intracellular Signaling Cascades in Blood Leukocytes and Postoperative Recovery Following Primary Hip or Knee Arthroplasty
Précis:	This is a randomized, placebo-controlled, double-blind pilot study to investigate the effects of GRF6021, a 5% human plasma protein fraction administered by intravenous (IV) infusion, on intracellular signaling cascades in blood leukocytes in subjects undergoing primary hip or knee arthroplasty. The study will enroll approximately 45 subjects with the aim of having 40 evaluable subjects randomized in a 1:1 ratio to active treatment or placebo. Each subject will receive 4 infusions of 250 mL GRF6021 or 4 infusions of 250 mL placebo (normal saline): 1) 1 infusion on the day before surgery 2) 1 infusion on the day of surgery within 4 hours before surgery start (first incision) 3) 1 infusion on the day of surgery upon arrival in the postoperative care unit (within 5 hours after the first incision) 4) 1 infusion on the day after surgery Each infusion will be administered over approximately 30 minutes according to Stanford Hospital procedures for colloid infusions, but infusion rates and infusion time will be adjusted according to tolerability as determined by the anesthesiologist and infusion nurse. The infusion procedure of active and placebo agents will be identical to maintain the blind. The following measures will be taken to ensure adequate blinding during infusions: blinding of subjects, study coordinators, physicians, and raters to treatment allocation; use of unblinded infusion nurses; and measures to block view of the vial and drip chamber throughout the infusion. Mass cytometry (CyTOF), a multiplexed, high-content immune profiling technology, will be used to provide high-resolution surveillance of circulating immune cells and the response to GRF6021 infusions on surgical recovery. Additional functional and quality of life assessments will be measured at baseline and approximately twice weekly for 6 weeks after hospital discharge.
Objectives:	The primary objective of the study is to investigate the effect of GRF6021 on intracellular signaling cascades in blood leukocytes as determined by CyTOF. Secondary objectives include the effect of GRF6021 on clinical recovery parameters in patients undergoing primary hip or knee arthroplasty, safety and tolerability of GRF6021, and the effects of GRF6021 on plasma proteomics.
Endpoints:	Primary Endpoint: • Effect of GRF6021 on intracellular signaling cascades in blood leukocytes as determined by CyTOF.

Secondary Endpoints:

- Change from baseline in 3-Minute Diagnostic Interview for Confusion Assessment Method (3D-CAM).
- Time to 50% recovery of baseline value on the Surgery Recovery Scale (SRS).
- End of study treatment comparison of the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)
- Time to a score of < 12/40 on a subset of questions from the WOMAC, Pain Subscale.
- Time to a score of < 18/60 on a subset of questions from the WOMAC, Physical Function Subscale.
- Change from baseline in the Short Form-36 (SF-36).
- Change from baseline in the Beck Depression Inventory-II (BDI-II).
- Opioid analgesic consumption during hospital stay and after discharge to end of study.
- Time to discharge.
- Perioperative outcomes (e.g., surgery duration, anesthesia duration, post-anesthesia care unit (PACU) stay duration, American Society of Anesthesiologists (ASA) status, estimated blood loss (EBL), type and amount of intraoperative fluids administered, type and amount of intraoperative blood products, dose and type of anesthesia (general, regional), and intraoperative opioid dose).
- Change in functional status using the ActiGraph wearable device providing measurements for physical activity/function and sleep (starting at least 3 days prior to surgery and continuing until the end of the study).
- Change from baseline in clinical laboratory parameters.
- Change from baseline in vital sign measurements.
- Change from baseline in electrocardiogram (ECG).
- The effects of GRF6021 on plasma proteomics.

Population:	The study will enroll approximately 45 subjects with the aim of having 40 evaluable subjects between 50 and 85 years of age undergoing primary hip or knee arthroplasty.
Phase:	2
Number of Sites:	Single site pilot study (Stanford University)
Description of Study Agent:	GRF6021: A 5% human plasma protein fraction for IV infusion
Study Duration:	Approximately 12 months

SCHEMATIC OF STUDY DESIGN



1 KEY ROLES

1.1 AUTHORIZED REPRESENTATIVE (SIGNATORY) / RESPONSIBLE PARTY

[redacted] MD, PhD
[redacted] Clinical Development
Alkahest, Inc.
125 Shoreway Road, Suite D
San Carlos, CA 94070
Telephone: [redacted]
E-mail: [redacted]

1.2 STUDY ORGANIZATION

The name and contact information of the responsible party and individuals involved with the study (e.g., investigator(s), Sponsor's medical expert and study monitor, Sponsor's representative(s), laboratories, steering committees, and oversight committees (including Independent Ethics Committees (IECs) and Institutional Review Boards (IRBs), as applicable) will be maintained by the Sponsor, or their designee, and provided to the investigator.

2 INTRODUCTION

2.1 BACKGROUND INFORMATION

Total knee arthroplasty (TKA) and total hip arthroplasty (THA) are effective treatments for end-stage osteoarthritis that significantly improve pain, mobility, function, and quality of life for most people who undergo them in both the short- and long-term (Mayer 2017, Martinez-Cano 2017). Given their success and the aging population, there has been a rise in the number of hip and knee arthroplasties performed in the US. By 2030, a study projects an estimated growth of 174% and 673% in the primary total hip and knee arthroplasties respectively (Kurtz 2007). Because TKA and THA are elective procedures, quality of life outcomes, including

physical, psychological, and social factors, inclusive of the return to normal activities, are important considerations (Martinez-Cano 2017, Fragiadakis 2015).

Convalescence after surgery is highly variable with inter-individual variability in fatigue, pain, and functional impairment (Gaudillière 2014, Kehlet 2003). Past studies have explored the association of preoperative psychological status with outcomes of total joint replacement surgery (Lingard 2007, Judge 2012, Faller 2003, Ayers 2004), and additional studies have demonstrated pain catastrophizing or negative preoperative emotional state is responsible for poor outcomes with resultant delays in recovery (Witvrouw 2009, Edwards 2008). In addition to psychological variables, physiological factors also play a vital role in determining duration of recovery. The physiologic stress response, characterized by endocrine and neuroimmune mechanisms, may also influence arthroplasty outcomes. Psychological stressors can elicit neuroimmune responses mimicking those initiated by physical trauma and/or inflammation (Deak 2017). Due to these factors, researchers have gained renewed interest in unravelling immune mechanisms in response to trauma that determine postoperative recovery (Gaudillière 2014, Fragiadakis 2015).

Surgical trauma triggers an intricate programmed immune response (Stoecklein 2012). Characteristics of this injury-mediated series of intracellular signaling cascades are associated with recovery from surgery. Specifically, changes in cyclic AMP-responsive element-binding protein 1 (CREB), signal transducer and activator of transcription 3 (STAT3), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signaling in monocytes were associated with postoperative functional hip recovery, fatigue, and pain, respectively (Gaudilliere 2014). Therefore, measuring the effect of GRF6021 on intracellular phosphorylation events in precisely phenotyped immune-cell subsets may increase our understanding of the immunomodulatory effects of GRF6021 and its potential to enhance postoperative recovery.

In 2011, data in parabiotic animal models suggested that differences in systemic chemokines, immune signaling molecules, and rejuvenating factors between young and old animals may explain the benefits in old animals of heterochronic parabiosis (Villeda 2011). Following these findings, the investigators performed a study to explore the therapeutic effects of systemic exposure of aged mice to young mouse plasma by intravenous injection (Villeda 2014). Systemic administration of young mouse plasma into aged mice improved age-related impairments.

Results from nonclinical studies conducted by Alkahest (see Section 2.2, Rationale) have repeatedly demonstrated beneficial effect of the plasma protein fraction GRF6021 in age-related decline and histopathological endpoints in mouse models, including a reduction in neuroinflammation. Specifically, treatment with GRF6021 – in aged mice and in mouse models characterized by neuroinflammation – reduces the number of microglia labeled with either ionized calcium binding adaptor molecule 1 (Iba1) or cluster of differentiation 68 (CD68). Since microglia derive from the same monocytic lineage as blood monocytes and tissue macrophages, these data demonstrate that GRF6021 has a specific effect on cells of the monocytic lineage. Given this, testing the effect of GRF6021 on intracellular signaling events in monocytes in the context of THA or TKA will provide important information about whether GRF6021 has the potential to improve postoperative recovery in patients undergoing hip and knee arthroplasty.

2.2 RATIONALE

Protocol AKST6021-211 will evaluate GRF6021 administered via intravenous (IV) infusion. GRF6021 is a purified 5% plasma protein fraction depleted of coagulation factors and gamma globulins that is manufactured by fractionation from pooled human plasma by Grifols Therapeutics, Inc. Additional processing steps with high pathogen clearance capacity are undertaken to reduce the potential for pathogen transmission. GRF6021 is

indicated for the treatment of hypovolemic shock due to burns, crushing injuries, abdominal emergencies, and any other condition where there is a predominant loss of plasma fluids. GRF6021 is also used more generally as a colloid for volume expansion. Fluid management is an essential part of surgical care as both inadequate and excessive fluid replacement can increase the stress on the circulatory system with deleterious consequences for tissue healing and recovery after surgery. Intraoperative and postoperative fluid management can involve crystalloids, colloids, or a combination thereof. Crystalloids (e.g. normal saline) are associated with interstitial and pulmonary edema, because up to 80% of the fluid volume can distribute to the interstitial (extravascular) space. Colloids (e.g. 5% albumin or 5% plasma protein fraction) provide better volume expansion with less interstitial edema; however, they are associated with potential hypersensitivity reactions (Kayilioglu 2015). There is controversy in the field as to which type of fluid replacement is more appropriate, and clinical care often depends on the preferences of the individual anesthesiologist and surgeon. Therefore, the use of either crystalloids and/or colloids (like GRF6021) is part of the intraoperative and postoperative standard of care in US hospitals.

Nonclinical studies conducted by Alkahest have demonstrated that GRF6021 confers beneficial outcomes in cognitive decline, motor function, neuroinflammation, pain, and histopathological endpoints in mouse models (ALK-2015-R002, VIV-2016-R003, VIV-2016-R009, VIV-2016-R014, VIV-2016-R020, VIV-2016-R021, VIV-2016-R024, VIV-2017-R025, VIV-2017-R036, VIV-2017-R038, VIV-2018-R069, 2018-CET01, VIV-89).

Surgical trauma triggers an intricate programmed immune response (Stoecklein 2012). Characterization of this injury-mediated series of intracellular signaling cascades can provide insights about time to, and ease of, recovery from surgery. Therefore, measuring immune function responses at the single-cell level, and functional and phenotypic characterization of signals specific to immune cell subsets, may point to diagnostic signatures and potential therapeutic targets that could postoperatively improve patient recovery (Gaudillière 2014, Fragiadakis 2015).

In this randomized, placebo-controlled pilot study, approximately 45 patients will be enrolled (with the goal of obtaining 40 evaluable subjects) who are scheduled to undergo primary hip or knee arthroplasty without major co-morbidities. Subjects will be randomized 1:1 (active: placebo) to evaluate the effect of GRF6021 on intracellular signaling cascades in blood leukocytes and the relationship of these immunomodulatory effects with postoperative recovery. In addition, the effects of GRF6021 on plasma proteomics will be investigated. Four (4) doses of 250 mL of GRF6021 or placebo will be administered over 3 days. Each dose will be administered over approximately 30 minutes as per Stanford Hospital standard procedures, but infusion rates and infusion time may be adjusted according to tolerability or other factors as determined clinically by the anesthesiologist and infusion nurse. Assessments of surgical recovery will be measured at baseline, during the inpatient stay, and for 6 weeks after hospital discharge.

Human dose levels were selected to be consistent with the standard clinical use of GRF6021 as a colloid. In previous studies of patients undergoing THA at Stanford, the median volume of crystalloids for fluid management was 1,500 mL (interquartile range 1,000 to 2,000 mL) (Gaudilliere 2014). Given a conversion factor of 1.5-2 between colloids and crystalloids, 1,500 mL of crystalloid is the equivalent of 750-1,000 mL of colloid. The total volume of GRF6021 that will be administered in this study is 1,000 mL.

In mice, no safety concerns were observed using repeated daily (5-7) doses of 150 µL, which is the dosing regimen that produced robust anti-neuroinflammatory effects. Because GRF6021 contains a complex mixture of proteins with a molecular weight predominantly <100 kDa, and because the beneficial effects of these proteins on inflammation are believed to occur in the circulation, the concentration of these proteins per blood volume appears to be the scaling method most likely to accurately estimate the human potential effective dose.

Using isometric scaling based on blood volume, the mouse dose of 150 µL is equivalent to a human dose of 413 mL, as outlined in **Table 1**. The dose proposed in this study is 250 mL GRF6021 per infusion.

Table 1 Isometric Scaling Based on Blood Volume

	Mouse	Human Equivalent Dose (70 kg person)	Human Proposed Dose
GRF6021 Protein Content	5%	5%	5%
Dose Volume	150 µL	413 mL	250 mL
Protein per Dose	7.5 mg	20.7 g	12.5 g
Blood Volume	2 mL	5,500 mL	5,500 mL
Protein per Dose per mL of Blood	3.75 mg/mL	3.75 mg/mL	2.3 mg/mL

Given the long-standing clinical experience with GRF6021 in which doses of 250 mL to 1.5 L have been well tolerated, and the standard-of-care use of GRF6021 and similar colloids for fluid management, the investigational regimen of 4 doses of 250 mL of GRF6021 over a period of 3 days represents a reasonable and appropriate dosing regimen for evaluation in this clinical context (PSUR, PI, Bertrand 1959, Gahart 2019).

2.3 POTENTIAL RISKS AND BENEFITS

2.3.1 KNOWN POTENTIAL RISKS

GRF6021, a 5% human plasma protein fraction, is reduced in coagulation factors and gamma globulins and serves as a viable source of soluble, infusible plasma proteins from healthy male and female donors. GRF6021 has been marketed since 1958 in the US, is approved in other parts of the world, and has a well-established safety profile with a very low frequency of reported infusion reactions (0.002%) (PSUR). Based on the cumulative review of post-marketing safety surveillance data (PSUR), the benefit-risk balance for GRF6021 as an investigative product in individuals undergoing THA or TKA is favorable. Because it has been depleted of the majority of immunogenic proteins, GRF6021 is not expected to alter the blood typing characteristics of the recipient and should not sensitize humans to its subsequent administration (Bertrand 1959). ABO antigen typing is not required prior to administration. Finally, the additional steps taken during production to remove viral pathogens provide an increased margin of pathogen safety in comparison to fresh frozen plasma.

GRF6021 is not expected to significantly alter safety laboratory parameters, including urinalysis, coagulation times, prothrombin times, prothrombin consumption, platelet counts, or fibrinogen levels when given in quantities of up to 1000 mL (Bertrand 1959). Hypotension may occur, particularly following rapid infusion. The blood pressure may normalize spontaneously after the slowing or discontinuation of the infusion. Vasopressors will also correct the hypotension (PI). GRF6021 is contraindicated for use in patients on cardiopulmonary bypass as severe hypotension has been reported (Bland 1973). GRF6021 is contraindicated in patients with severe anemia, congestive heart failure, or increased blood volume (PI).

GRF6021 is made from human plasma and may carry a risk of transmitting infectious agents, e.g., viruses, the variant Creutzfeldt-Jakob disease (vCJD) agent, and, theoretically, the Creutzfeldt-Jakob disease (CJD) agent. The risk that GRF6021 can transmit an infectious agent has been reduced by screening plasma donors for prior exposure, testing donated plasma, and including manufacturing steps with the capacity to inactivate and/or remove pathogens.

2.3.2 KNOWN POTENTIAL BENEFITS

There is no known benefit of administering GRF6021 to patients undergoing THA or TKA.

3 OBJECTIVES AND PURPOSE

The primary objective of the study is to investigate the effect of GRF6021 on intracellular signaling cascades in blood leukocytes as determined by CyTOF. Secondary objectives include the investigation of GRF6021 on clinical recovery parameters in patients undergoing primary hip or knee arthroplasty, safety and tolerability of GRF6021, and the effects of GRF6021 on plasma proteomics.

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

This will be a randomized, placebo-controlled pilot study conducted at a single site, Stanford University (Stanford, California, US) to investigate the effects of GRF6021, a 5% human plasma protein fraction administered by intravenous (IV) infusion, on intracellular signaling cascades in blood leukocytes in subjects undergoing primary hip or knee arthroplasty.

Approximately 45 subjects will be enrolled with the aim of having 40 evaluable subjects randomized in a 1:1 ratio to active treatment or placebo. Each subject will receive 4 infusions of 250 mL of active GRF6021 or 4 infusions of 250 mL of placebo:

1) 1 infusion on the day before surgery
2) 1 infusion on the day of surgery within 4 hours before surgery start (first incision)
3) 1 infusion on the day of surgery upon arrival in the postoperative care unit (within 5 hours after the first incision)
4) 1 infusion on the day after surgery

Each infusion will be administered over approximately 30 minutes according to Stanford Hospital standard of care, but infusion rates and infusion time may be adjusted for tolerability or other clinical reasons, as determined by the anesthesiologist and infusion nurse.

CyTOF, a multiplexed, high-content immune profiling technology, will be utilized to provide high-resolution surveillance of circulating immune cells and the response to GRF6021 infusions on surgical recovery. Additional functional and quality of life assessments will be measured at baseline and for 6 weeks after hospital discharge. For a detailed overview of the timing of study assessments and interventions, please review Section 15 Schedule of Events.

The overall duration of the study/recruitment period is approximately 12 months from study initiation (i.e., following consent of first subject) to study completion (i.e., last subject, last visit). The subject participation period is approximately 7 to 12 weeks from screening through end of study, unless prematurely discontinued.

4.2 STUDY ENDPOINTS

4.2.1 PRIMARY ENDPOINT

Primary Endpoint:

- Effect of GRF6021 on intracellular signaling cascades in blood leukocytes as determined by CyTOF.

4.2.2 SECONDARY ENDPOINTS

This study is not powered to detect statistically significant differences in outcomes. Secondary endpoints will be summarized over the study period from baseline values using descriptive statistics.

Secondary Endpoints:

- Change from baseline in 3D-CAM (Inouye 2003)(Appendix 1).
- Time to 50% recovery of the baseline value in the SRS (Paddison 2011)(Appendix 2).
- End of study treatment comparison of the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) (Bellamy 1986, Bellamy 2002)(Appendix 3).
- Time to a score of < 12/40 on a subset of questions from the WOMAC, Pain Subscale (Bellamy 1986, Bellamy 2002)(Appendix 3).
- Time to a score of < 18/60 on a subset of questions from the WOMAC, Physical Function Subscale (Bellamy 1986, Bellamy 2002)(Appendix 3).
- Change from baseline in the SF-36 (Laucis 2015)(Appendix 4).
- Change from baseline in the BDI-II (Beck 1996)(Appendix 5).
- Opioid analgesic consumption during hospital stay and after discharge to end of study.
- Time to discharge.
- Perioperative outcomes (e.g., surgery duration, anesthesia duration, PACU stay duration, ASA status, EBL, type and amount of intraoperative fluids administered, type and amount of intraoperative blood products, dose and type of anesthesia (general, regional), and intraoperative opioid dose).
- Change in functional status using the ActiGraph wearable device (ActiGraph Corporation 2019) providing measurements for physical activity/function and sleep (starting at least 3 days prior to surgery and continuing until the end of the study).
- Change from baseline in clinical laboratory parameters.
- Change from baseline in vital sign measurements.
- Change from baseline in ECG.
- The effects of GRF6021 on plasma proteomics.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 INCLUSION CRITERIA

In order to be eligible for inclusion, all subjects must meet the following criteria:

1. Men and women scheduled to undergo primary THA or TKA.
2. Aged 50-85 years at time of enrollment, inclusive.
3. Renal function as defined by estimated glomerular filtration rate (eGFR) ≥ 45 mL/min/1.73 m^2 using the Modification of Diet in Renal Disease (MDRD) study equation.
4. Female subjects must not be pregnant or breastfeeding. Women of childbearing potential (WOCBP) must have a negative pregnancy test at screening. WOCBP must agree to use acceptable forms of contraception (Clinical Trial Facilitation Group) prior to study entry. A woman is considered of

childbearing potential following menarche and until becoming postmenopausal (no menses for at least 2 years without an alternative cause). Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in the study, she should inform her treating physician immediately.

5. The subject must understand and speak English and be able to understand and follow the study procedures, receive the treatment in the established timeframe, and continue during the follow-up interval.
6. Provide a signed and dated informed consent form in accordance with local regulations and/or IRB/IEC guidelines.

5.2 EXCLUSION CRITERIA

An individual will not be eligible for inclusion if any of the following criteria apply:

1. Blood coagulation disorders, including severe liver disease, vitamin K deficiency, von Willebrand disease, hemophilia A or B or other known deficiencies in clotting factors (fibrinogen, prothrombin, factor VII, X, XI, XII, or XIII) that in the opinion of the investigator could put the subject at risk.
2. Subjects who started chronic anticoagulant therapy (warfarin, heparin, low-molecular weight heparin, or Factor Xa inhibitors) in the last 6 months. Use of pre-, intra- and post-operative anticoagulation according to standard of care is permitted. Use of antiplatelet drugs (e.g., aspirin or clopidogrel) is permitted.
3. History of a known hypercoagulable state, including Factor V Leiden thrombophilia, prothrombin gene mutation, deficiencies in antithrombin, protein C or protein S, elevated levels of fibrinogen, or dysfibrinogenemia.
4. History of deep vein thrombosis or pulmonary embolism in the last 6 months and/or if it is associated with a known hypercoagulability disorder.
5. History of disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, or heparin-induced thrombocytopenia in the last 5 years.
6. Prior hypersensitivity reaction to any human blood product including plasma or a plasma protein fraction.
7. Treatment with any human blood product, including transfusions and IV immunoglobulin, during the 6 months prior to screening.
8. History of immunoglobulin A (IgA) or haptoglobin deficiency; at screening a test for IgA must be performed to confirm detectable levels of IgA.
9. History of complications of IV immunoglobulins, including stroke, anaphylaxis, or thromboembolic events.
10. History of major surgery, trauma, or injury in the last 3 months or minor surgery in the last 1 month.
11. History of unstable coronary heart disease (e.g. myocardial infarction or severe or unstable angina in the 6 months prior to dosing).
12. Moderate or severe congestive heart failure (New York Heart Association Class III or IV or metabolic equivalent < 4).
13. Uncontrolled high blood pressure (systolic blood pressure of 170 mmHg or higher and/or diastolic blood pressure of 110 mmHg or higher) despite adequate treatment during the 3 months prior to dosing.
14. Current treatment with 4 or more antihypertensive medications of different classes.
15. Clinically significant abnormalities on screening electrocardiogram (ECG) that in the opinion of the investigator could put the subject at risk, including QTc intervals (using Fridericia's correction formula) of ≥ 450 ms in men and ≥ 470 ms in women.
16. Current, active liver disease: > 3-fold elevation of liver enzymes (alanine aminotransferase (ALT)

and aspartate aminotransferase (AST) over upper limit of normal.
17. Clinically significant abnormalities in complete blood count (CBC), complete metabolic panel, serum albumin, or coagulation.
18. History of a major infection (e.g. bacterial pneumonia) in the last 3 months, or recent minor infection (e.g. influenza, urinary tract infection) or fever (measured body temperature above 100.5 degrees F on 2 consecutive days) that in the assessment of the Principal Investigator could interfere with the primary endpoint.
19. Severe anemia (hemoglobin < 8 g/dL).
20. Urine protein-to-creatinine ratio (UPCR) of > 1.5 grams of protein per gram of creatinine.
21. Inadequate venous access to allow IV drug delivery or multiple blood draws.
22. Concurrent participation in any other therapeutic treatment trial. If there was prior clinical trial participation, subject must have discontinued investigational agents for at least 30 days for small molecules, and 1 year for active or passive immunotherapies prior to screening.
23. Current diseases associated with significant alterations in immune function, including, but not limited to, autoimmune disease and cancer, except adequately-treated squamous or basal-cell carcinoma.
24. Ongoing immunosuppressant treatment or within 5 half-lives of the treatment.
25. History of substance abuse (alcohol, drugs of abuse) in the last 2 years.
26. Functional impairment of major joint or lower extremity other than joint undergoing surgery.
27. Any other condition and/or situation that the investigator believes may interfere with the safety of the subject, study conduct, or interpretation of study data.

5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

The Sponsor does not anticipate any specific challenges in enrolling 45 subjects with the goal of obtaining at least 40 evaluable subjects in this study. Subjects will be recruited continuously until the planned sample size is achieved. Subjects who withdraw or are withdrawn during screening, as well as subjects who discontinue, may be replaced (see Section 5.4.2).

The expected length of participation in the study of approximately 7 to 12 weeks is not expected to be challenging to subjects. A description of the study will be included in local clinical trial databases, as required.

5.4 SUBJECT WITHDRAWAL OR TERMINATION

5.4.1 REASONS FOR WITHDRAWAL OR TERMINATION

A subject may be withdrawn from the study for the following medical or administrative reasons:

- Occurrence of an adverse event (AE) that represents an unacceptable risk to the subject and when continued participation in the investigational study is not warranted, in the judgment of the investigator or Sponsor Medical Monitor. The investigator must follow the subject until the AE resolves or is stable, unless the subject is lost to follow up.
- Treatment with a prohibited concomitant medication other than the use of appropriate medications for the treatment of AEs under direction of the investigator.
- Subject noncompliance, defined as refusal or inability to adhere to the trial schedule or procedures.
- At the request of the subject (e.g., subject withdraws consent), investigator, Sponsor, or regulatory authority.
- Pregnancy.

5.4.2 HANDLING OF PARTICIPANT WITHDRAWALS OR TERMINATION

Subjects will be encouraged to complete the study and all assessments. Subjects may voluntarily withdraw at any time, and the investigator may discontinue individual subjects from the study at any time.

Approximately 45 subjects (1:1 ratio of GRF6021 to placebo) will be enrolled in the study with the aim of having 40 evaluable subjects. Evaluable subjects are those who received all 4 doses and had all blood draws for assessment of the primary endpoint. Subjects who discontinue or are unblinded may be replaced. Subjects who withdraw or are withdrawn during screening will be replaced.

5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

The Sponsor reserves the right to terminate the study at any time. Should this be necessary, the Sponsor and/or their representatives will arrange discontinuation procedures and notify FDA and the IRB. In terminating the study, the Sponsor and the investigator will continue to protect the subjects' privacy and identity as required by relevant statutes and regulations.

Alkahest, Inc. has the right to terminate the study at any time. Reasons for study or site termination may include, but are not limited to:

- (Immediate) risk to subject safety.
- Unsatisfactory subject enrollment.
- Unacceptable Protocol Deviations as assessed by the Medical Monitor.
- Inaccurate or incomplete data entry and recording/fabricated data.
- Investigational site non-compliance with ICH/GCP.
- Unacceptable emergent safety profile.

6 STUDY AGENT

6.1 STUDY AGENT AND CONTROL DESCRIPTION

6.1.1 ACQUISITION

GRF6021 is manufactured by Grifols Therapeutics, Inc. (Clayton, North Carolina, US). The placebo control agent will be 0.9% sodium chloride for injection (normal saline) manufactured by Laboratorios Grifols, S.A. (Parets del Vallès, Barcelona, Spain). Both GRF6021 and placebo will be supplied to the site directly from a depot.

6.1.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

GRF6021 is prepared from large pools of human source plasma that has been depleted of coagulation factors and gamma globulins (PI). GRF6021 is iso-oncotic with normal human plasma and isotonic. Each 100 mL of GRF6021 contains 5 g selected plasma proteins buffered with sodium carbonate and stabilized with 0.004 M sodium caprylate and 0.004 M acetyltryptophan. The plasma proteins consist of approximately 88% normal human albumin, 12% alpha and beta globulins, and not more than 1% gamma globulin as determined by electrophoresis. The approximate concentrations of the main electrolytes in GRF6021 are: sodium 145

mEq/L, potassium 0.25 mEq/L, and chloride 100 mEq/L.

Both GRF6021 and placebo will be supplied in 250 mL glass vials labeled for investigational use according to the local regulatory requirements for clinical studies.

6.1.3 PRODUCT STORAGE AND STABILITY

The study agent and placebo should be stored at room temperature not exceeding 30°C (86°F) and not used after the expiration date. Solution that has been frozen should not be used. Solutions that are turbid should not be used. Administration of the study agent or placebo must begin within 4 hours of the container being entered. Vials that are cracked or have been previously entered or damaged should not be used, as this may have allowed the entry of microorganisms. Neither the study agent nor the placebo contain preservatives.

6.1.4 PREPARATION

GRF6021 vials should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. The study agent and placebo should be prepared in accordance with the GRF6021 Infusion Administration Manual that will be provided to the site (see Section 6.1.5 Dosing and Administration).

6.1.5 DOSING AND ADMINISTRATION

The study agent/placebo will be infused in accordance with the GRF6021 Infusion Administration Manual. The purpose of the GRF6021 Infusion Administration Manual is to promote safe administration of GRF6021 and to maintain appropriate blinding of staff and study subjects. The manual will include provisions for masking study agent/placebo and maintaining the staff and study subjects' blind during each infusion.

The study agent/placebo to be administered will be dispensed by an unblinded pharmacist (or other qualified personnel responsible for drug accountability) to blinded study personnel for transport to an unblinded infusion nurse. The study agent/placebo may also be dispensed directly to the unblinded infusion nurse. Administration of the study agent/placebo will be performed by the unblinded infusion nurse. Infusion nurses will be qualified by training and experience to administer infusions under the direction of the Principal Investigator. Only authorized infusion nurses who complete the training on the Infusion Administration Manual may administer the study agent/placebo, and only subjects enrolled in the study may receive the study agent/placebo in accordance with applicable regulatory requirements.

6.1.6 ROUTE OF ADMINISTRATION

The study agent and placebo will be administered by IV route only.

6.1.7 DOSING SCHEDULE

Subjects will be randomized to GRF6021 (Group 1: active dosing) or placebo (Group 2: placebo dosing). Subjects will receive 4 infusions of 250 mL per the following schedule:

1) On the day before surgery
2) On the day of surgery within 4 hours before surgery start (first incision)
3) On the day of surgery upon arrival in the postoperative care unit (within 5 hours after the first incision)

4) On the day after surgery

6.1.8 DURATION OF THERAPY

From screening to exit, the duration of study involvement for each subject is approximately 7 to 12 weeks. All subjects will receive 4 infusions over 3 days. The duration of therapy for a subject to be considered evaluable in the intent-to-treat population is 4 doses.

6.2 STUDY AGENT ACCOUNTABILITY

Under the supervision of the Principal Investigator, the study pharmacist or other qualified unblinded personnel are responsible for ensuring adequate accountability of all used and unused study agent and placebo control agent. This includes acknowledgment of receipt of each shipment of study agent and placebo (quantity and condition), subject dispensing records, and returned or destroyed study agent/placebo. Dispensing records will document quantities received and quantities dispensed to subjects including the date dispensed, intended subject's study identifier, initials of the individual responsible for dispensing, and initials of the infusion nurse administering the study agent. Drug accountability will be monitored by an unblinded Clinical Research Associate.

Accountability records must be maintained and readily available for inspection by representatives of Alkahest, Inc. or their designee and are open to inspection by regulatory authorities at any time. The accounts of any study agent/placebo accidentally wasted or intentionally disposed of must be maintained.

The disposal of used, partially used, or wasted study agent/placebo must be performed in accordance with the institution's drug disposal policy. At study initiation, the clinical study monitor will evaluate the site's standard operating procedure for study drug disposal/destruction in order to ensure that it complies with study requirements. At the end of the study, following final drug reconciliation by the monitor, the study site will be instructed by the Sponsor to return or destroy all unused study agent/placebo. A copy of the institution's drug disposal policy should be maintained or referenced in the investigator's study file.

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY PROCEDURES/EVALUATIONS

7.1.1 STUDY SPECIFIC PROCEDURES

7.1.1.1 Screening Procedures

During the screening period, the following information will be obtained:

- Medical history and comorbidities
- Demographics
- Review of medications
- Vital signs
- Physical examination
- Blood and urine collection for laboratory evaluations

Detailed descriptions of each of these procedures are provided in the sections immediately following. Some portions of information used to determine eligibility can be obtained from the subject directly or from an electronic medical record. Information pertaining to all study activities performed during screening, and the sequence of events, is provided in Section 7.3.1 Screening.

7.1.1.1.1 Medical History and Comorbidities

The investigator or designee will obtain pertinent medical history during screening that will include:

- Current/past illnesses and conditions, including a comprehensive review of comorbidities prior to surgery
- Current symptoms of any active medical condition
- Surgeries and procedures
- Allergies
- Social history (e.g. exercise, smoking, substance use) and current living situation

7.1.1.1.2 Demographics

Demographic information such as the subject's age, ethnicity, and race will be collected at screening.

7.1.1.1.3 Review of Medications

The investigator or designee should review the subject's current medications, including over-the-counter drugs, herbal supplements, and/or vitamins, as well as those taken by the subject in the past 1 month and any dose changes. In addition, any potential subjects who have had human blood product transfusions or immunosuppressants in the last 6 months will be excluded from the study. Assessment of eligibility should include a review of permitted and prohibited medications. Any additions, discontinuation, or dosage changes in medication during the course of the study will be recorded (see Section 7.1.1.2.2).

7.1.1.1.4 Vital Signs

Vital signs will include supine or seated systolic and diastolic blood pressure (BP) (mmHg), heart rate (HR) (beats per minute [bpm]), respiration rate (RR) (breaths per minute), and body temperature.

7.1.1.1.5 Physical Examination

A physical examination will be performed to assess the following organ systems: lungs/chest, heart, abdomen, neuro/spine. Height and weight will also be measured at screening. Should the subject have a physical exam, including height and weight measurements, as part of standard of care during the screening window, then the results may be obtained from the subject's medical record for the purposes of assessing eligibility.

7.1.1.1.6 Biological Specimen Collection

Biological samples will be analyzed at screening. Blood will be drawn by a qualified medical provider, and urine specimens will also be collected. The timing and frequency for specimen collection and laboratory evaluations to be performed are further described in Section 15 Schedule of Events.

The labs that will be tested at screening include:

- Pregnancy test.
- Alkaline phosphatase, ALT, AST, bicarbonate, bilirubin (total), ionized calcium, chloride, creatinine, gamma-glutamyl transferase (GGT), glucose (random), lactate dehydrogenase, magnesium, phosphate, potassium, protein total, sodium, blood urea nitrogen, albumin, partial thromboplastin time (PTT), and prothrombin time/international normalized ratio (PT/INR).
- CBC with differential (basophils, eosinophils, lymphocytes, monocytes, neutrophils).
- Urinalysis: blood, glucose, ketone, protein, pH, specific gravity, nitrite, leukocytes, urine creatinine, urine protein/creatinine ratio, (and reflex urine microscopy if indicated).
- Infectious serology (anti-human immunodeficiency virus (HIV) antibodies, anti-hepatitis B surface antigen, anti-hepatitis B core antigen, anti-hepatitis C antibodies), direct antiglobulin test, red blood

cell (RBC) antibody screen (including direct Coombs and anti-RBC antibody), serum IgA, and haptoglobin.
- Urine drug screen: cannabinoids, benzodiazepine, barbiturates, opiates, cocaine, amphetamines, phencyclidine.

7.1.1.2 Procedures to Assess Safety

Subjects enrolled in the trial will be monitored closely to assess safety and tolerability of the study agent and intervention. Study-specific procedures that will be used for this purpose are summarized below. Information regarding the timing and frequency of these procedures is provided in Section 7.3 Study Schedule.

- Review of AEs
- Review of medications
- Vital signs
- 12-Lead ECGs
- Targeted physical exams
- Blood draw and urine collection for laboratory evaluations

7.1.1.2.1 Review of Adverse Events

AEs will be reviewed, documented, and reported as required at each visit, beginning at Screening. For definitions, guidance, and additional information regarding AEs, refer to Section 8.

7.1.1.2.2 Review of Medications

The investigator or designee should review the subject's current medications, including over-the-counter drugs, herbal supplements and/or vitamins, as well as those taken by the subject since the last visit. Changes to the subject's list of medications should be reviewed and recorded. Review of medications should occur at every visit.

7.1.1.2.3 Vital Signs

Refer to Section 7.1.1.1.4 for a description of vital signs. Vital signs will be collected at every treatment visit within 1 hour prior to infusion start and after infusion start, at 15, 30, 45, and 60 minutes (for further details see Section 15 Schedule of Events). Vital signs may be collected ad hoc during or after the infusion.

7.1.1.2.4 12-Lead ECG

A 12-lead ECG will be performed after the subject has rested quietly for at least 5 minutes in a supine or sitting position. In some cases, it may be appropriate to repeat abnormal ECGs to rule out technical factors contributing to ECG artifacts or abnormality. It is important that leads are placed in the same positions each time for consistency. The overall conclusion with the interpretation of the ECGs will be recorded on the appropriate CRF. The interpretation of the ECGs will be recorded as normal, abnormal but not clinically significant, or abnormal and clinically significant. Corrected QTc intervals will be calculated using Fridericia's correction formula.

7.1.1.2.5 Targeted Physical Exams

A targeted physical exam, including auscultation of the heart and lungs and an assessment of peripheral edema, will be performed per the Study Schedule in Section 7.3. If performed as part of standard of care, then the results may be obtained from the subject's medical record for review and assessment.

7.1.1.2.6 Blood and Urine Collection for Laboratory Evaluations

In addition to screening evaluations (see Section 7.1.1.1.6), subjects will have blood and urine specimens collected for clinical evaluation (see Section 7.2.1 Clinical Laboratory Evaluations). Refer to Section 15 Schedule of Events for timing and frequency of blood and urine collection for laboratory evaluations.

The following labs will be performed within ~48 hours after the end of the infusion on Day 3 (exit lab panel):

- Infectious serology (anti-HIV antibodies, anti-hepatitis B surface antigen, anti-hepatitis B core antigen, anti-hepatitis C antibodies), direct antiglobulin test, RBC antibody screen (including direct Coombs and anti-RBC antibody).
- ALT, AST, bilirubin (total), ionized calcium, chloride, creatinine, potassium, protein total, sodium, blood urea nitrogen, PTT, PT/INR.
- CBC with differential (basophils, eosinophils, lymphocytes, monocytes, neutrophils).
- Urinalysis: blood, glucose, ketone, protein, pH, specific gravity, nitrite, leukocytes, urine creatinine, urine protein, urine protein/creatinine ratio, and reflex urine microscopy if indicated.
- Pregnancy test.

7.1.1.3 Procedures to Assess Efficacy

Procedures to assess efficacy include investigations of intracellular signaling cascades in blood leukocytes as determined by CyTOF following administration of GRF6021. In addition, the effects of GRF6021 on clinical recovery parameters in patients undergoing THA and TKA, and the effects of GRF6021 on plasma proteomics will be evaluated.

1. CyTOF
2. 3D-CAM
3. SRS
4. WOMAC
5. SF-36
6. BDI-II
7. POMS (covariate assessment)
8. PSS (covariate assessment)
9. Perioperative outcomes
10. ActiGraph assessments
11. Proteomic assessments

Descriptions of each assessment are provided below.

7.1.1.3.1 Mass Cytometry (CyTOF)

Surgical trauma triggers an intricate programmed immune response (Stoecklein 2012). This injury-mediated series of intracellular signaling cascades provides insights about time and ease of surgical recovery. Therefore, measuring immune function responses at the single-cell level, and functional and phenotypic characterization of signals specific to immune cell subsets, may point to diagnostic signatures and potential therapeutic targets that could postoperatively improve patient recovery (Gaudillière 2014, Fragiadakis 2015). CyTOF provides high-dimensional numerical and functional characterization of the immune response to surgical trauma and enables the detection of biological mechanisms critically associated with relevant health-associated outcomes (Gaudillière 2014).

7.1.1.3.2 3-Minute Diagnostic Interview for Confusion Assessment Method

The 3D-CAM (Inouye 2003)(Appendix 1) is a brief verbal assessment tool used to test patients for delirium. Each item in the instrument directly informs one of the 4 CAM features including acute onset of mental status change or fluctuating course of cognition, inattention, disorganized thinking, and altered level of consciousness. For all items on the assessment, the patient's answer is "incorrect," "correct," "no," or "yes."

7.1.1.3.3 Surgery Recovery Scale

The SRS (Paddington 2011)(Appendix 2) is a sensitive and simple tool for the assessment of functional recovery following major surgery. The scale contains 13 items. Impacts on daily activities are scored from 1 to 5 or 6 (1= not at all). It's considered the most broadly validated measure of surgical recovery available.

7.1.1.3.4 Western Ontario and McMaster Universities Osteoarthritis Index

The WOMAC (Pain and Physical Function Subscales) (Bellamy 1986, Bellamy 2002)(Appendix 3) was developed to assess pain, stiffness, and physical function in patients with knee and/or hip osteoarthritis, but it has been assessed among patients with many different conditions. The WOMAC is one of the most widely utilized self-report measures of lower extremity symptoms and function. It has been studied over a period of almost 30 years in many different contexts and patient populations, and there are abundant data regarding its utility and measurement properties. The Likert Scale version uses an 11-point scale anchored by the wording "no pain" and "extreme pain" for pain, and "no difficulty" and "extreme difficulty" for physical function. Higher scores on the WOMAC indicate worse pain and functional limitations.

7.1.1.3.5 Short Form-36

The SF-36 (Laucis 2015)(Appendix 4) is the most widely used health-related quality-of-life measure in research to date. The SF-36 yields 8 sub-scale scores and 2 summary scores for mental and physical health.

7.1.1.3.6 Beck Depression Inventory-II

The BDI-II (Beck 1996)(Appendix 5) was developed in 1996 and derived from the original BDI. It is a proprietary, 21-item self-administered survey scored on a scale of 0–3 in a list of four statements arranged in increasing severity about a particular symptom of depression. It is easy to complete, and relatively short compared to interview-based assessments. It was designed to assess mood within the most recent 2-week period, so comparison across assessments should reflect change over time.

7.1.1.3.7 Profile of Mood States

The POMS (Heuchert 2012)(Appendix 6) is widely used to assess affective traits, mood, and emotion. It contains 65 POMS items. It can be completed online or in a paper format in 3 to 5 minutes. For the purposes of this study, only 9 items in the "tension-anxiety" scale will be used as anxiety is an important covariate of postoperative pain.

7.1.1.3.8 Perceived Stress Scale

The PSS (Cohen 1983), is a widely used instrument consisting of 10 questions for measuring the degree to which situations in one's life are stressful during the last month. Items were also designed to tap how unpredictable, uncontrollable, and overloaded respondents find their lives. The scale includes a number of queries about current levels of experienced stress. Answers are given as 0=never, 1=almost never, 2=sometimes, 3=fairly often, and 4=very often. PSS scores are obtained by reversing responses (e.g., 0=4, 1=3, 2=2, 3=1, and 4=0) to the 4 positively stated items (items 4,5,7, and 8) and then summing across all scale items.

7.1.1.3.9 Perioperative Outcomes

Captured variables may include surgery duration, anesthesia duration, PACU stay duration, ASA status, EBL, type and amount of intraoperative fluids administered, type and amount of intraoperative blood products, dose and type of anesthesia (general, regional), and intraoperative opioid dose.

7.1.1.3.10 ActiGraph Wearable Device

At screening, subjects will be provided with an ActiGraph wearable device (ActiGraph Corporation 2019). Approximately -7 to -3 days prior to surgery, subjects will be instructed to wear the device which will then begin monitoring of physical activity, mobility, and sleep measures. Data collection is ongoing, and subjects will wear the device until admission, except during Visits 4a and 4b when it is removed for surgery.

7.1.1.3.11 Proteomic Assessments

Blood and plasma will be collected from subjects at multiple timepoints throughout the trial for proteomic assessments. Fasting samples are preferred. For information regarding the timing and procedures for sample collection and related requirements, refer to the study's laboratory manual and Section 15 Schedule of Events.

Plasma will be analyzed by proteomics using targeted approaches to assess the specific signature of proteins in subjects at baseline and to assess the changes in the proteome with repeated GRF6021 infusions. These methodologies will provide a broad overview of the proteins that are present in the plasma sample by assessing 1000-5000 analytes and allow generation of a proteomic signature. From this signature, it is hoped that key proteins that are drivers of postoperative recovery can be identified. Blood samples will be collected for analysis of emergent genetic markers. By understanding the composition and function of plasma samples from the trial, the goal is to identify the biomarkers relevant to further optimizing postoperative recovery in THA and TKA.

7.2 LABORATORY PROCEDURES/EVALUATIONS

7.2.1 CLINICAL LABORATORY EVALUATIONS

Biological samples (e.g. whole blood, serum/plasma, urine) will be collected for laboratory evaluations in accordance with the Schedule of Events. Clinical sample processing and laboratory evaluations will be conducted by the local lab at Stanford Hospital according to their standard processes. Refer to the study's laboratory manual for complete information regarding all exploratory biomarker (proteomics) samples to be collected.

The investigator is responsible for determining and documenting whether out of range laboratory values are clinically significant. All clinically significant values will be recorded as AEs in the CRF and followed until determined to be stable or resolved, unless the subject is lost to follow up. Once resolved, the appropriate CRF page(s) will be updated.

7.2.2 SPECIMEN PREPARATION, HANDLING, STORAGE, AND SHIPPING

For the biomarker (proteomics) samples, refer to the study's laboratory manual for specimen preparation, handling, storage, and shipping procedures. All other laboratory samples will be collected and processed according to standard policies at Stanford Hospital.

7.3 STUDY SCHEDULE

7.3.1 SCREENING

Visit 1, Screening Visit (Day -42 to -3)

- □ Informed consent of subject as documented by a signed and dated informed consent form that must be obtained prior to any study-related assessments.
- □ Collect demographic information.
- □ Review subject's current and prior medications, including opioid consumption assessment.
- □ Collect vital signs, height, and weight.
- □ Physical exam.
- □ Collect blood and urine samples required for Screening (including pregnancy test for WOCBP).
- □ Provide subject with ActiGraph wearable device and any training required for use of the device (the ActiGraph can also be mailed to the subject 1 week before the scheduled surgery).

7.3.2 BASELINE: REMOTE

Subjects will be contacted by phone to obtain the remote Baseline Visit assessments.

Visit 2, Baseline: Remote (Days -7 to -3)

- □ Review AEs and concomitant medications.
- □ SRS
- □ WOMAC
- □ SF-36
- □ BDI-II
- □ POMS
- □ PSS
- □ Subject will be instructed to begin wearing the ActiGraph device.

7.3.3 RANDOMIZATION

Subjects will be randomized after eligibility has been confirmed.

7.3.4 BASELINE: ON-SITE/TREATMENT

The on-site Baseline assessments will be gathered prior to the first infusion on Day 1. After the first infusion on Day 1, the subject will be discharged from the facility and come back the following morning (Day 2) for the inpatient period. The inpatient treatment period is 2 days during which each subject will receive 3 additional doses of 250 mL of their assigned treatment allocation (i.e., either GRF6021 or placebo).

Visits 3 through 5, Treatment (Days 1-3):

- □ Perform the following procedures on each day *prior* to administering (the first) study infusion:
  - Review AEs and concomitant medications.
  - 12-lead ECG (**Day 1 and 3 Only**).
  - Collect sample for CyTOF assessment (**Including second infusion on Day 2**).

- Obtain samples for proteomic assessments; fasting samples preferred (**Including second infusion on Day 2**).
- Collect vital signs (BP, HR, RR, and body temperature) (**Including second infusion on Day 2)**.
- Targeted physical exam (**Day 1 and 3 Only**).
- Pregnancy testing for WOCBP (**Day 1 Only**).
- Administer 3D-CAM, SRS, and WOMAC (**Day 1 and 3 Only**).
- ActiGraph device data collection (**Day 1 and 3 Only**).

□ Administer study treatment per the GRF6021 Infusion Administration Manual:
- On the day before surgery (**Day 1**).
- On the day of surgery within 4 hours before surgery start (first incision) (**Day 2**).
- On the day of surgery upon arrival in the PACU (within 5 hours after the first incision) (**Day 2**).
- On the day after surgery (**Day 3**).

□ Perform the following assessments following each infusion start:
- BP and HR at 15, 30, 45, and 60 min after infusion start.
- Body temperature and RR at 60 minutes.
- Collection of perioperative data (**Day 2 Only**, after second infusion).
- Opioid consumption assessment (**Days 2 and 3 Only**).
- 12-lead ECG (**Day 3+1 Only**, after end of last infusion).
- Exit lab panel including pregnancy test (**Day 3 Only**, after end of last infusion).
- Administer 3D-CAM (**Day 2 Only**, after second infusion).

7.3.5 FOLLOW-UP

All Follow-up Visits will be conducted remotely by phone or electronically.

Visits 6 through 14 (Day 4 through Day 37±2)

□ Review AEs and concomitant medications.

□ Perform the following assessments:
- SRS
- WOMAC
- Opioid consumption assessment.

□ Review AEs and concomitant medications.

□ Ongoing collections of data from wearable device.

7.3.6 END OF STUDY VISIT

The End of Study Visit will be conducted remotely by phone and/or electronically.

Visit 15, End of Study (Day 44 ± 2)

□ Review AEs and concomitant medications.

□ Perform the following assessments:
- SRS
- WOMAC
- SF-36
- BDI-II
- Opioid consumption assessment.

□ Subject will be instructed to return the ActiGraph wearable device.

7.3.7 EARLY TERMINATION

If a subject has received at least one infusion but is terminated or terminates from the study early, the site should try to perform all assessments scheduled at the End of Study Visit (Visit 15) and complete the exit lab panel if the subject is still at the site and available and willing.

7.3.8 SCHEDULE OF EVENTS TABLE

A tabular summary of all procedures/assessments at each study visit can be found in Section 15 Schedule of Events.

7.4 CONCOMITANT MEDICATIONS

All concomitant prescription medications taken during study participation will be recorded on the CRFs. For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the CRF are concomitant prescription medications, over-the-counter medications, and non-prescription medications. Medications that are considered standard use during surgery will not be collected in the CRF.

7.4.1 ANESTHESIA GUIDELINES

For the purposes of this trial, it is recommended to:

- Avoid adjuvant analgesic techniques that have immune-modulatory potential (e.g. ketamine or IV lidocaine infusions or bolus administration), unless clinically indicated.
- Avoid other medications with immune-modulatory potential such as steroids (e.g. decadron), unless clinically indicated.

7.5 PROHIBITED MEDICATIONS, TREATMENTS, AND PROCEDURES

The following concomitant medications are prohibited:

- Concurrent participation in any other therapeutic treatment trial. If there was prior clinical trial participation, subject must have discontinued investigational agents for at least 30 days for small molecules, and 1 year for active or passive immunotherapies prior to screening.
- Chronic use of anticoagulant therapy (e.g., heparin, warfarin, thrombin inhibitors, Factor Xa inhibitors). Anticoagulation used during surgery is permitted. Use of antiplatelet drugs (e.g., aspirin or clopidogrel) is acceptable.
- Any drugs of the interferon class.
- Systemic corticosteroids (e.g., hydrocortisone, cortisone, betamethasone, prednisone, prednisolone, triamcinolone, dexamethasone, fludrocortisone) for longer than 5 consecutive days. Ophthalmic, topical, intra-articular, and inhaled steroids are allowed.

8 ASSESSMENT OF SAFETY

Assessment of subject safety is the responsibility of the Investigator or medically qualified designee. In extraordinary circumstances where knowledge of whether GRF6021 or placebo was received by a subject is

essential, the Investigator may unblind. Any instances of unblinding will be managed as indicated in Section 10.6.3 Breaking the Study Blind/Subject Code.

8.1 SPECIFICATION OF SAFETY PARAMETERS

8.1.1 DEFINITION OF ADVERSE EVENTS

Per 21 CFR 312.32(a), an AE is any untoward (unfavorable, harmful, or pathologic) medical occurrence in a subject administered a pharmaceutical (investigational) product even if the event does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding that is deemed clinically significant), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

An AE does include any:

- Exacerbation of a pre-existing illness.
- Subjective or objective symptoms spontaneously offered by the subject and/or observed by the investigator or study staff.
- Increase in frequency or intensity of a pre-existing episodic event or condition.
- Condition detected or diagnosed after study drug administration even though it may have been present prior to the start of the study (unless it can be demonstrated by medical record review that the onset of the event preceded the date/time of Informed Consent).
- Continuous persistent disease or symptoms present at baseline that worsen following the start of the study.
- Symptoms associated with disease not previously reported by the subject.
- Untoward medical occurrences considered by the investigator to be related to study-mandated procedures.
- Abnormal assessments (e.g., change on physical examination, ECG findings), if they represent a clinically significant finding, that were not present at Baseline or worsened during the course of the study.
- Laboratory test abnormalities, if they represent a clinically significant finding, symptomatic or not, which were not present at Baseline or worsened during the course of the study.

An AE DOES NOT include a/an:

- Elective medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion).
- Pre-existing diseases or conditions present or detected at the start of the study that do not worsen.
- Situations where an untoward medical occurrence has not occurred (e.g., hospitalization for cosmetic elective surgery, social and/or convenience admissions).
- The disease or disorder being studied or sign or symptom associated with the disease or disorder unless more severe than expected for the subject's condition.
- Overdose of either study drug or concurrent medication without any signs or symptoms.

- Pregnancy.

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS

Note: if either the investigator or the Sponsor believes that the event is serious, the event must be considered serious and evaluated for expedited reporting.

Note: the terms "severe" and "serious" are not synonymous. Severity (or intensity) refers to the grade of an AE. "Serious" is a regulatory definition.

A serious adverse event (experience) or reaction is an untoward medical occurrence that, at any dose, fulfills one or more of the following criteria:

a. Results in death (i.e., the AE actually causes or leads to death).

b. Is life-threatening.
   - An AE is considered "life-threatening" if, in the view of either the investigator or Sponsor, its occurrence places the patient or subject at immediate risk of death; it does not include AEs which, had it occurred in a more severe form, might have caused death.

c. Results in inpatient hospitalization or prolongation of existing hospitalization.
   - Hospitalization for elective treatment of a pre-existing condition that did not worsen during the study is not considered an AE; hospitalization for participating in this study is not considered an AE.
   - Complications that occur during hospitalization are AEs; if a complication prolongs hospitalization, the event is an SAE.
   - "Inpatient" hospitalization means the subject has been formally admitted to a hospital for medical reasons that may or may not be overnight; it does not include presentation at a casualty or emergency room unless the event meets the definition of an Important Medical Event (in the opinion of the Investigator or Sponsor).

d. Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
   - The term 'disability' means a substantial disruption of a person's ability to conduct normal life functions; this definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, accidental trauma (i.e., sprained ankle) that may interfere or prevent everyday life functions but do not constitute a substantial disruption.

e. Results in a congenital anomaly in the offspring of a subject who received drug.

f. Results in an Important Medical Event. Important Medical Events are events that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the 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 used in deciding whether prompt reporting is appropriate in this situation.

8.2 CLASSIFICATION OF AN ADVERSE EVENT

8.2.1 SEVERITY OF EVENT

Each AE or suspected adverse reaction must be assessed for its seriousness and severity. Severity will be assessed by the investigator or designee using the following definitions:

SEVERITY	DEFINITION
MILD	Aware of sign or symptom, but easily tolerated
MODERATE	Discomfort enough to cause interference with usual activity
SEVERE	Incapacitating with inability to work or do usual activity

Outcome will be assessed using the following categories: recovered/resolved, not recovered/ not resolved, recovered/resolved with sequelae, fatal, or unknown.

8.2.2 RELATIONSHIP TO STUDY AGENT

Investigators are required to assess the causal relationship (i.e., whether there is reasonable possibility that the study drug caused the event) using the following definitions:

- Unrelated: another cause of the adverse event is more plausible; a temporal sequence cannot be established with the onset of the adverse event and administration of the study agent; or a causal relationship is considered biologically implausible.
- Possibly Related: There is a clinically plausible time sequence between onset of the adverse event and administration of the study agent, but the adverse event could also be attributed to concurrent or underlying disease, or the use of other drugs or procedures. Possibly related should be used when the study agent is one or several biologically plausible adverse event causes.
- Definitely Related: The adverse event is clearly related to use of the study agent.

If either the investigator or the Sponsor considers the event related, then the event will be considered related for reporting purposes.

8.2.3 EXPECTEDNESS

The Sponsor or designee will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the Reference Safety Information described in the Investigator's Brochure.

For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the Investigator's Brochure listed only cerebral vascular accidents. "Unexpected" as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the Investigator's Brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation. For example, although angioedema is anticipated to occur in some patients exposed to drugs in the angiotensin-converting enzyme (ACE) inhibitor class and angioedema would be described in the Investigator's Brochure as a class effect, the first case of angioedema observed with the drug under investigation should be considered unexpected for reporting

purposes (FDA 2012).

This definition of "unexpected" relies entirely on the Reference Safety Information in the Investigator's Brochure as the basis for determining if newly acquired information generated from clinical trials or reported from other sources is unexpected. The suspected adverse reactions listed in the Investigator's Brochure (i.e., "expected") are those observed with the investigational drug and for which a causal relationship between the event and the drug is suspected or confirmed.

Sponsor assessment of expectedness and relationship to study drug/causality will determine the need for expedited reporting of AEs.

8.3 TIME PERIOD/FREQUENCY FOR EVENT ASSESSMENT/FOLLOW-UP

At every clinic visit, subjects will be assessed for AEs and SAEs. After the subject has had an opportunity to spontaneously mention any problems, the investigator should inquire about AEs by asking a non-leading question such as the following:

1. "How are you feeling?"
2. "Have you had any changes since your last assessment/visit?"
3. "Have you taken any new medicines since your last assessment/visit?"

8.3.1 POST-STUDY AE AND SAE

The investigator is not obligated to actively seek SAE information in former study subjects, but the investigator is encouraged to notify Alkahest, Inc. or their designee of any AE or SAE occurring within 30 days after a subject completes the study (or has their last visit) that the investigator judges may be reasonably related to study treatment or study participation.

8.4 REPORTING PROCEDURES

8.4.1 ADVERSE EVENT REPORTING

All subjects who have given informed consent will be evaluated for AEs. All AEs that occur after the time of treatment with the study agent will be considered Treatment Emergent AEs. Subjects with Treatment-Emergent AEs must be followed until the AE is resolved or is stable, unless the subject is lost to follow up.

Each AE or suspected adverse reaction must be described as follows: the date of onset, date of resolution, severity (mild, moderate, severe), frequency of the event (single episode, intermittent, continuous), action taken with study treatment (no action taken, treatment held, treatment discontinued), outcome, causality* (unrelated, possibly related, definitely related), and seriousness criteria. Each AE or suspected adverse reaction must be recorded separately.

***Note**: Causality assessment will be made only when the AE occurs after the subject has initiated at least one infusion of the study agent. An AE occurring before the subject's exposure to study agent will always be labeled as "unrelated".

Any AE occurring during the study must be documented in the subject's medical records and as an AE in the

CRF. Any SAE occurring during the study must be documented in the subject's medical records and as an SAE in the CRF.

A separate set of SAE pages should be used for each SAE. However, if at the time of initial reporting, multiple SAEs are present that are temporally and/or clinically related, they may be reported on the same SAE page.

The investigator should attempt to establish a diagnosis of the event (that meets the definition of an AE or SAE) based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE and/or SAE and not the individual signs or symptoms. The diagnosis will become the basis for the verbatim term as reported by the investigator. If no diagnosis is known and clinical signs and symptoms are not present, the abnormal finding should be recorded.

In addition to the investigator's own description of the AE, each AE will be encoded according to the MedDRA.

The investigator will take all appropriate and necessary therapeutic measures required for resolution of the AE, whether or not the AE is considered related to the investigational product. Any medication necessary for the treatment of an AE must be recorded on the concomitant medication CRF.

The SAE pages of the CRF should be completed as thoroughly as possible and signed by the investigator or his/her designee before transmittal to the study Contract Research Organization (CRO). It is very important that the investigator provide his/her assessment of causality to study drug as well as an applicable diagnosis at the time of the initial SAE report.

8.4.2 SERIOUS ADVERSE EVENT REPORTING

8.4.2.1 Timeframes for Reporting SAEs

Under 21 CFR 312.32(c), the Sponsor is required to notify FDA and all participating investigators in a safety report of potentially serious risks from clinical trials [i.e., Suspected Unexpected Serious Adverse Reactions (SUSARS)], as soon as possible after the Sponsor receives the safety information and determines that the information qualifies for reporting:

- No later than 7 calendar days for events that are life threatening (in the opinion of the investigator or the Sponsor) or that involve Death as an outcome.
- No later than 15 calendar days for all other SUSARS.

As such, prompt notification of the Sponsor, and/or the Sponsor's representatives, and promptly providing requested follow-up information regarding SAEs is essential so that ethical and regulatory responsibilities and legal obligations can be satisfied. Investigators are responsible for reporting SAEs according to the following timeframes:

- All SAEs occurring during the study should be reported immediately.
- The SAE Report Form and relevant source documents, if applicable, must be completed and emailed to drugsafety@inclin.com within 24 hours of observation or learning of the event.
- Follow-up information must be sent to the CRO within 24 hours of receipt of information by the investigational site.

SAEs will be followed until resolution, the condition stabilizes, the event is otherwise explained or is judged

by the investigator to be no longer clinically significant, or until the subject is lost to follow up.

8.4.2.2 SAE Information to Report

All information available regarding an SAE must be submitted in the timeframes indicated. At a minimum, SAE reports must contain the subject ID, the SAE verbatim term, onset date, relationship to study drug/causality, and a brief narrative of the event. Please note that **relationship to study drug/causality as well as the reported verbatim term are very important** and should be included in the initial report as it may impact expedited regulatory reporting requirements for the event. The date of SAE discovery by the site staff should be documented in the source documents.

The investigator must record all relevant information regarding an AE/SAE in the applicable sections of the CRF. It is not acceptable for the investigator to send photocopies of the subject's medical records in lieu of completion of the appropriate AE/SAE pages. However, there may be instances when copies of medical records for certain cases are requested by the CRO and/or the Sponsor. If medical records are submitted to the CRO then all subject personal identifiers must be completely and thoroughly redacted prior to submission.

A blank SAE Report Form and instructions for SAE reporting will be provided to the site and will be maintained in the investigator's study file. The SAE Report Form must be completed and emailed to drugsafety@inclin.com according to the timeframes specified in Section 8.4.2.1. The SAE Report Form should include copies of relevant source documents, if applicable. Reconciliation of any discrepancy noted during monitoring and amending the eCRF is required.

If new information about an SAE is received or corrections to data are needed, the investigator should complete a new SAE Report Form and check the "follow-up" box on the form. This follow-up SAE Report Form should be submitted within 24 hours of learning of the information, especially if the new information concerns seriousness, relatedness, or the event term of an AE.

The site, acting under their local IRB, should submit all applicable events, unanticipated problems, and safety reports to the site's local IRB, if applicable. All safety reporting deviations should also be submitted to their local IRB, if applicable.

8.4.3 ADVERSE EVENTS OF SPECIAL INTEREST

The following will be considered AEs of special interest (AESI):

- Clinically-significant peripheral edema or pulmonary edema.
- Reduced kidney function (eGFR < 45 mL/min/1.73 m^2).
- Suspected transmission of blood-borne infectious agents.

AESI occurring during the study should be reported within 48 hours of observation or learning of the event, unless the event is serious, in which case the event must be reported according to the timeframes specified in Section 8.4.2.1.

8.4.4 REPORTING OF PREGNANCY

While pregnancy itself is not considered an AE, pregnancy occurring in a clinical study must be followed to collect information regarding the experiences of gestation and pregnancy with study agent exposure. The investigator must report any pregnancy that occurs in a female study subject subsequent to first exposure to

the study agent until End of Study, or 3 months following a subject's last dose in the event of early termination. All pregnancies will be reported to the IRB, Sponsor, and CRO. In the event of a pregnancy, the subject will undergo continued safety follow-up through pregnancy outcome.

Any pregnancy must be followed by the investigator until delivery or to the end of pregnancy. Any anomalies, complications, abnormal outcomes, or birth defect(s) observed in the child must be reported as an SAE within 24 hours of the investigator or study personnel's first knowledge.

8.5 STUDY HALTING RULES

If any of the following safety events occur, a Safety Evaluation Meeting (defined below) will be triggered:

- Three or more SAEs in the same system/organ/class (SOC) that are assessed as possibly or definitely related to the study agent by the investigator and confirmed as such by the Sponsor (see Section 8.2.2 Relationship to Study Agent).
- Within or between any of the dosing groups: an overall pattern of symptomatic, clinical, or laboratory events associated with the study agent that the Sponsor's Program Physician or designee consider a serious potential safety concern (e.g., suspicious overall pattern).

Events that are more likely related to the infusion procedure, such as infiltration or hematoma, will not be considered "drug related" and will not contribute to the count of definitely-related SAEs that would trigger a Safety Evaluation Meeting.

Safety Evaluation Meeting

If safety events of potential concern occur during the trial (i.e., 3 related events in the same SOC or a suspicious overall pattern, as defined above) a Safety Evaluation Meeting may be triggered, and dosing may be temporarily halted based on the observations. The Sponsor will inform the FDA in the event of any temporary halt in dosing at any time during the conduct of the study. The purpose of the meeting is for the investigator, the Sponsor, and the CRO to discuss and evaluate the safety of the subjects using available aggregated safety data and without compromising study blinding, unless the Sponsor deems unblinding necessary for safety evaluation.

Attendants at the Safety Evaluation Meeting will include the Program Physician of Alkahest (or his/her designee), the CRO, and available active investigators participating in the trial. After sufficient data review the Sponsor will choose one of the following courses of action:

1. Continue dosing with no change to protocol.
2. Halt dosing in all groups and stop the study.
3. Continue with a modified protocol design and amend the protocol as appropriate.

8.6 SAFETY OVERSIGHT

Safety oversight will be provided by the Sponsor's Program Physician or his or her designee in concert with the site investigators. There will be no formal Data Safety Monitoring Board (DSMB) established. As needed, Safety Evaluation Meetings will be convened as described in Section 8.5 to monitor the ongoing safety of the study. The Sponsor's Program Physician or designee is the final authority for safety oversight in the study.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by the study CRO in accordance with the Clinical Monitoring Plan (CMP).
- A mix of on-site and centralized monitoring will be performed to ensure the safety of clinical subjects and the accuracy and completeness of study data.
- The Sponsor will be provided with copies of monitoring reports per the timelines specified within the CMP.
- Details of clinical site monitoring tasks and scope are documented in the study's CMP. The CMP describes in detail who will conduct monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.
- Independent audits may be conducted by the Sponsor in accordance with a quality oversight plan or equivalent to ensure monitoring practices are performed consistently and that monitors are following the CMP.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL DESIGN MODEL AND ANALYTICAL PLANS

Approximately 45 subjects (1:1 ratio of GRF6021 to placebo) will be enrolled in the study with the aim of having 40 evaluable subjects. Evaluable subjects are those who received all 4 doses and had all blood draws for assessment of the primary endpoint.

A Statistical Analysis Plan (SAP) with analytical details and assumptions will be developed and finalized before database lock and unblinding of the study data.

10.2 STATISTICAL HYPOTHESES

While we hypothesize that active treatment with GRF6021 will modulate the immune response to surgery, because this is an exploratory study, it is not designed to detect statistically significant differences between active and placebo on the primary endpoint. We will use a cell-signaling Elastic Net (csEN) approach to detect immune features (functional cellular attributes as well as cell abundance) that separate the active and placebo groups, and we hypothesize that active treatment will be associated with immunomodulatory changes that have previously been associated with improved clinical recovery. While a classical power calculation has not been performed, previous interventional studies from the Stanford group have demonstrated that a sample of 40 subjects is a pragmatic sample that enables the detection of clinically-relevant immunomodulatory changes, such as changes in signaling downstream of immune receptors, including interleukin-1 and Toll-like receptor 4.

For secondary endpoints, the statistical approach toward efficacy endpoints will be primarily descriptive; within-subject changes from baseline for each dosing group and among-group differences will be evaluated. The most important clinical outcomes will be the SRS (time to 50% recovery of the baseline score), WOMAC Pain (time to mild pain as indicated by a score <12/40), and WOMAC Physical Function (time to mild functional impairment as indicated by a score <18/60).

10.3 ANALYSIS DATASETS

Four analysis datasets are possible; however, analyses may not necessarily be conducted with all four:

- **Intention-to-Treat (ITT) Dataset**: all randomized participants.
- **Safety Dataset**: all subjects who received at least one dose of the study agent.
- **Evaluable Dataset:** all subjects who receive all 4 doses.
  - **Per Protocol Dataset:** a subset of the ITT Dataset. A detailed description of the reasons for exclusion from the PP population will be included in the SAP.

The presentation of baseline characteristics will be conducted on the ITT dataset. All safety analyses will be performed for the Safety Dataset. Analyses of the secondary endpoints will focus on the Evaluable and/or Per Protocol Datasets.

10.4 DESCRIPTION OF STATISTICAL METHODS

10.4.1 GENERAL APPROACH

Using the Evaluable and/or Per Protocol Datasets, all endpoints will be summarized serially over time using descriptive statistics to assess the within-subject changes and between-group differences. Overall baseline and demographic data will be summarized using descriptive statistics; between-groups testing will be used to evaluate the effectiveness of the randomization in producing homogeneous pre-treatment groups.

For analysis of the primary and secondary endpoints, the following will be considered:

- For endpoints that are continuous in nature:
  - Number of observations, mean, median, minimum, and maximum, and standard deviation (SD) values will be presented as descriptive summary
  - For inferential statistics:
    - If the Normality assumption is met, Paired t-test, or Analysis of Covariance (ANCOVA) using the baseline value as a covariate will be used
    - If the Normality assumption is not met, a rank –ANCOVA analysis i.e., an ANCOVA analysis on rank-transformed data or other non-parametric methods will be used
- For endpoints that are categorical in nature:
  - Frequency counts and percentages will be presented as descriptive summary
  - Chi-square test or Logit model will be used for inferential statistics

Subject disposition (e.g., the number of subjects randomized, completed, and discontinued) will be summarized, and medical history data will be listed. Prior and concomitant medications taken from screening and during the study will be categorized by World Health Organization classification for therapeutic class and drug name, listed and summarized by number and percentage of subjects.

Final analyses are not limited to the summaries described herein. As noted above, analytical details and assumptions will be fully presented in the SAP.

10.4.2 ANALYSIS OF THE PRIMARY ENDPOINT

The study is not powered to detect significant changes in the immune signatures; however, using available data from analysis of this endpoint, including changes from baseline, the primary interest is to detect

immune features (and proteomic features) that separate the two groups. Machine leaning approaches using cross-validation algorithms will be the primary approach for such analysis. The output of this analysis will produce a list of significant immune and/or proteomic features.

10.4.3 ANALYSIS OF THE SECONDARY ENDPOINTS

Using available data from analysis of the secondary endpoints, descriptive summaries will be developed. Of particular interest will be the within-subject changes from baseline and their distribution around a null value of zero and a comparison between groups to evaluate any trends in differences between subjects randomized to active and placebo agents.

For safety endpoints, actual values and changes from baseline in clinical laboratory measurements and vital signs will be assessed and summarized. Abnormal lab or vital sign values will be determined and flagged in the listings. Laboratory shift tables or graphics displaying the change (number of subjects) relative to the reference range from baseline to each study visit may also be presented for each test. The investigator should exercise his or her medical and scientific judgment in deciding and documenting whether an abnormal laboratory finding, or other abnormal assessment is clinically significant.

For safety endpoints that are continuous in nature (e.g. clinical laboratory parameters, systolic and diastolic blood pressure, heart rate, respiratory rate, body temperature) the mean, median, minimum, maximum, and standard deviation will be plotted over time.

For safety endpoints that are categorical in nature (e.g. targeted physical exam or ECG abnormalities), the frequency counts and percentages will be presented as a descriptive summary.

10.4.4 ADHERENCE AND RETENTION ANALYSES

Subject adherence with the study visit schedule, visit procedures, infusions, and subject retention will be assessed. Reasons for study discontinuation will be compared across groups and across other subgroups of subjects, as appropriate.

10.4.5 BASELINE DESCRIPTIVE STATISTICS

See Section 10.4.1.

10.4.6 PLANNED INTERIM ANALYSES

Safety will be monitored on an ongoing basis. If a Safety Evaluation Meeting is triggered (see Section 8.5), an ad hoc interim safety analysis will be performed. If such an ad hoc safety interim analysis is conducted, the treatment assignment will remain masked, unless unblinding is deemed necessary by the Sponsor for safety evaluation.

10.4.7 ADDITIONAL SUBGROUP ANALYSES

Not applicable.

10.4.8 MULTIPLE COMPARISON/MULTIPLICITY

No adjustments for multiplicity will be employed.

10.4.9 TABULATION OF INDIVIDUAL RESPONSE DATA

This will be further defined in the SAP.

10.4.10 EXPLORATORY ANALYSES

Not applicable.

10.5 SAMPLE SIZE

A total of approximately 45 subjects will be enrolled with the aim of having 40 evaluable subjects randomized in a 1:1 ratio to active treatment or placebo. The primary endpoint of this study (differences in immunological and/or proteomic signatures among patient groups), the principal computational approach (machine learning) used, and the exploratory nature of the study prevent a traditional power analysis. However, a sample size of 2 x 20 subjects was sufficient to detect relevant differences in previous studies examining interventions expected to have a similar effect size (Aghaeepour 2017).

10.6 MEASURES TO MINIMIZE BIAS

10.6.1 ENROLLMENT/RANDOMIZATION

To minimize the potential bias at the time of randomization, the study will be randomized in a 1:1 ratio (active: placebo), stratified by sex and surgical joint (hip, knee) to assure a balanced distribution of evaluable male and female subjects in both treatment groups.

10.6.2 EVALUATION OF SUCCESS OF BLINDING

Success of blinding will be assessed based on all occurrences (intentional or unintentional) of unblinding of blinded study subjects or study personnel (e.g. investigators, study coordinators and other blinded site staff, the Sponsor or their representatives). All intentional and unintentional unblinding will be documented and reported.

10.6.3 BREAKING THE STUDY BLIND/SUBJECT CODE

The study blind can be broken for safety reasons if the information is required for the management of SAEs or severe AEs. Any noted intentional or unintentional breaking of the blind should be reported to the Sponsor's Program Physician. If unintentional unblinding occurs during the study, root cause analysis will be evaluated, and corrective actions implemented.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The site will maintain appropriate medical and research records for this trial, in compliance with ICH E6 R2 and regulatory and institutional requirements for the protection of confidentiality of subjects. The site will permit authorized representatives of regulatory agencies, the IRB, the Sponsor, or the Sponsor's representatives to examine (and when permitted by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress, and data validity.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subject's memory aids or evaluation checklists, pharmacy dispensing records, recorded audio tapes of counseling sessions, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

It is not acceptable for the CRF to be the only record of a subject's participation in the study. This is to ensure that anyone who would access the subject's medical record has adequate knowledge that the subject is participating in a clinical trial. Source document templates will be developed for this study.

12 ETHICS/PROTECTION OF HUMAN SUBJECTS

12.1 ETHICAL STANDARD

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 42 CFR Part 11, 21 CFR Part 50, 21 CFR Part 54, 21 CFR Part 56, 21 CFR Part 312, ICH E6 R2, and the Declaration of Helsinki.

12.2 INSTITUTIONAL REVIEW BOARD

This protocol and any accompanying material to be provided to the subject (such as advertisements, subject information sheets, or descriptions of the study used to obtain informed consent) will be submitted by the investigator to an IRB. Approval from the IRB must be obtained before starting the study and should be documented in a letter to the investigator specifying the protocol number, protocol version, documents reviewed, and date on which the committee met and granted the approval.

All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented subjects need to be re-consented.

Any modifications or amendments to the protocol must also be submitted to the IRB for approval prior to implementation.

12.3 INFORMED CONSENT PROCESS

12.3.1 CONSENT FORMS

Consent forms describing in detail the study agent, study procedures, and risks are given to the subject, and written documentation of informed consent is required prior to any study-related procedures.

12.3.2 CONSENT PROCEDURES AND DOCUMENTATION

It is the responsibility of the investigator or designee to obtain written informed consent from each subject participating in this study after adequate explanation of the aims, methods, objectives, and potential hazards of the study and prior to undertaking any study-related procedures.

Subjects should have the opportunity to discuss the study with their family members or other advisors and the time to consider participation in the trial carefully. The subjects may withdraw consent at any time throughout the course of the trial. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

The investigator or designee must utilize an IRB-approved consent form that contains the elements required by ICH GCP and applicable regulatory requirements for documenting written informed consent. Each informed consent will be appropriately signed and dated by the subject and the person obtaining consent. A copy of the signed consent form will be provided to the subject. By signing the informed consent form, all parties agree they will complete the evaluations required by the study, unless they withdraw voluntarily or are terminated from the study for any reason.

Investigators will be expected to maintain a screening log of all potential study candidates that includes limited information about the potential candidate (e.g., date of screening and reason for screen failure if appropriate).

All subjects who provide consent will be assigned a unique study number. This number will be used to identify the subject throughout the clinical study and must be used on all study documentation related to the study subject. Once a number is assigned to a subject, that number will remain with that study subject and will not be reused.

If an individual's medical chart or results of diagnostic tests performed as part of an individual's regular medical care are going to be used for screening, written informed consent must be obtained prior to review of that information in accordance with HIPAA.

12.4 PARTICIPANT AND DATA CONFIDENTIALITY

Subject confidentiality is held in strict trust by the participating investigators, their staff, the Sponsor, and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to subjects. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or data will be released to any unauthorized third party without prior written approval of the Sponsor.

The study monitor, other authorized representatives of the Sponsor, representatives of the IRB, or government regulatory agencies may inspect documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

The investigator must assure that subjects' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Only subject initials and an identification code (i.e., not names) should be recorded on non-local lab samples, requisitions, and any documents submitted to the CRO, Sponsor, and/or IRB. The investigator must keep a subject log showing codes, names, and addresses for all subjects screened and for all

subjects enrolled in the trial. The study subject's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

12.5 FUTURE USE OF STORED SPECIMENS

With the subject's approval, and as approved by local IRB, de-identified biological samples may be stored at Alkahest, or designee, for future use. These samples could be used for research. Alkahest will also maintain a code link that will allow linking of the biological samples to specific data from each subject, while maintaining masking of the identity of the study subject. Subjects will be consented, and this consent documented, to allow the Sponsor to store and use samples for further research. An individual subject can choose to withdraw consent to have biological specimens stored and used for future research.

When the study is completed, access to study data and/or samples will be managed by Alkahest. In the event Alkahest transfers ownership to another commercial Sponsor, ownership of the samples may be transferred as well.

13 DATA HANDLING AND RECORD KEEPING

13.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, and timeliness of the data reported.

Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the investigator's site. Data entered in the eCRFs that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL. The investigator may need to request previous medical records or transfer records, depending on the trial; also, current medical records must be available.

For each subject who receives the study agent, the eCRF must be completed in a timely manner. The investigator will review and approve the eCRF for each study subject after all data have been entered, the eCRFs have been source document verified, and all queries have been resolved. This also applies to records for those subjects who fail to complete the study. If a subject withdraws from the study, the reason must be noted on the eCRF. If a subject is withdrawn from the study because of an AE, thorough efforts should be made to clearly document the outcome.

All data collection and recordkeeping procedures must be compliant with applicable ICH GCP.

13.1.1 INVESTIGATOR RESPONSIBILITIES

The investigator will comply with the protocol (which has been approved/given favorable opinion by an IRB), ICH GCP, and applicable regulatory requirements. The investigator is ultimately responsible for the conduct of all aspects of the study at the study site and verifies by signature the integrity of all data transmitted to the

Sponsor. The term "investigator" as used in this protocol as well as in other study documents, refers to the investigator or authorized study personnel that the investigator has designated to perform certain duties. Sub-investigators or other authorized study personnel are eligible to sign for the investigator, except where the investigator's signature is specifically required.

13.1.2 STUDY FILES

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two separate categories (although not limited to) the following: (1) investigator's study file, and (2) subject clinical source documents.

The investigator's study file will contain the protocol/amendments, eCRF, IRB approval with correspondence, informed consents, drug records, staff curriculum vitae and authorization forms, and other appropriate documents and study-specific manuals (e.g., lab manual).

Subject clinical source documents would include (although are not limited to) the following: subject hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, radiologic imaging, X-ray, pathology and special assessment reports, consultant letters, screening and enrollment log, etc.

13.2 STUDY RECORDS RETENTION

Before the investigator destroys any material related to the clinical study, he/she must obtain approval in writing from the Sponsor. According to ICH (E6), essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. The Investigator must retain protocols; amendments; IRB approvals; copies of the Form FDA 1572 or equivalent; completed, signed, dated consent forms; patient medical records and original source documents; CRFs; drug accountability records; all study-related correspondence; and any other documents pertaining to the conduct of the study. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the Sponsor. It is the responsibility of the Sponsor to inform the Investigator/institution as to when these documents no longer need to be retained.

The investigator should keep a file where the full name and address of each subject and all signed informed consents are included for at least 15 years after completion of the trial. Any original study-related information that permits verification of inclusion and exclusion criteria, including clinical history, a copy of all data collection logs, and documents on the use of the study agent, must be stored for as long a time period as permitted by the center.

Should the investigator wish to move study records to another location, arrangements must be made to store these in sealed containers so that they can be returned sealed to the investigator in case of a regulatory audit. Where source documents are required for the continued care of the subject, appropriate copies should be made for storage outside of the site.

13.3 PROTOCOL DEVIATIONS

A Protocol Deviation is any noncompliance with the clinical trial protocol or with GCP. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. When deviations occur, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2

Protocol Deviations will be categorized as either Major or Minor and will be defined in the study-specific Protocol Deviation Guidance Plan, or equivalent.

> **Major Protocol Deviations** are departures from the approved protocol relating to the conduct of the study which may significantly impact the completeness, accuracy, and/or reliability of the study data or that may significantly affect the rights, safety or wellbeing of study participants. Major Protocol Deviations may result in data that are not deemed evaluable for the *per protocol* analysis and/or may require that subjects are discontinued from the study.

Minor Protocol Deviations are departures from the approved protocol relating to the conduct of a study that does not affect the rights, safety, and/or wellbeing of study participants or the study outcomes or data quality. Minor Protocol Deviations do not require review by the Medical Monitor. Minor Protocol Deviations would not generally preclude subject data from the *per protocol* analysis population.
All deviations will be logged and tracked by the site and CRO. Periodic review of Protocol Deviations will serve an indicator of site performance.

It is the responsibility of the site to use continuous vigilance to identify and report deviations promptly to the study CRO and/or Sponsor. All deviations must be addressed in study source documents. Notification of Protocol Deviations must be sent to the local IRB/IEC per their guidelines. The investigator/study staff is responsible for knowing and adhering to their IRB/IEC requirements.

13.4 PUBLICATION AND DATA SHARING POLICY

In compliance with The International Committee of Medical Journal Editors (ICMJE) clinical trials registration policy and Section 801 of the Food and Drug Administration Amendments Act of 2007, this study will be registered by the Sponsor in ClinicalTrials.gov, a public trials registry which is sponsored by the National Library of Medicine.

Notwithstanding the Sponsor's requirements for registration and data sharing in ClinicalTrials.gov, any formal presentation or publication of data collected as a direct or indirect result of this trial will be considered as a joint publication by the investigator(s) and the Sponsor. The resulting publication will name investigators according to the policy of the chosen journal. Where it is not permitted for all investigators to be included as authors, the publication will name all investigators within the publication.

The investigator will provide the Sponsor with copies of collaborative written publications (including abstracts and posters) at least 60 days in advance of submission. This will permit the Sponsor to review the communication for accuracy (thus avoiding potential discrepancies with submissions to regulatory authorities), to verify that

confidential information is not inadvertently divulged (including patent protection), to allow adequate input or supplementary information that may not have been available to the investigator, and to allow establishment of co-authorship.

Data will be reviewed by all participating investigators prior to publication. The study Sponsor will have 90 days to review all definitive publications, such as manuscripts and book chapters, and a minimum of 30 days to review all abstracts.

14 FINANCIAL DISCLOSURE AND CONFLICT OF INTEREST POLICY

A separate financial disclosure agreement will be made between each Principal Investigator and Alkahest, Inc. or its authorized representative before the study agent is shipped. Each investigator will notify Alkahest, Inc. or its authorized representative of any relevant changes during the conduct of the study and for 1 year after the study has been completed. Alkahest and the study CRO will evaluate any disclosed conflicts of interest and will establish a mechanism for their management.

15 SCHEDULE OF EVENTS

15.1 SCHEDULE OF EVENTS TABLE

	Screening (On-site)	Baseline Visit (Remote)	Baseline Visit (On-site)/ Treatment (On-site)				Follow-up/End of Study (EOS) (Remote)									
Visit	1	2	3	4[a]	4[b]	5	6	7	8	9	10	11	12	13	14	15
Infusion Number			1	2	3	4										
Postoperative day			-1	0	0	1	2	4	7	10	14	17	21	28	35	42
Day	Day -42 to -3	Day - 7 to -3	1	2	2	3	4	6 ± 1	9 ± 1	12 ± 1	16 ± 1	19 ± 1	23 ± 2	30 ± 2	37 ± 2	44 ± 2
Week	-6 to -1	-1	1				1	1	1	2	2	3	3	4	5	6 EOS
Informed Consent	X															
Medical History	X															
Demographics	X															
Vital Signs	X		X[1,2]	X[1,2]	X[1,2]	X[1,2]										
Physical Exam/Height and Weight	X															
12-lead ECG[i]			X[1]			X[1,3]										
Randomization			X[1]													
Targeted Physical Exam			X[1]			X[1]										
Concomitant Medication Review	X	X	X[1]	X[1]		X[1]	X	X	X	X	X	X	X	X	X	X
Adverse Event Review		X	X[1]	X[1]		X[1]	X	X	X	X	X	X	X	X	X	X
GRF6021/Placebo Infusion			**X**	**X**	**X**	**X**										
Blood/Urine Sampling																
Screening Lab Panel[c]	X															
Exit Lab Panel[d]						X										

	Screening (On-site)	Baseline Visit (Remote)	Baseline Visit (On-site)/ Treatment (On-site)				Follow-up/End of Study (EOS) (Remote)									
Visit	1	2	3	4[a]	4[b]	5	6	7	8	9	10	11	12	13	14	15
Infusion Number			1	2	3	4										
Postoperative day			-1	0	0	1	2	4	7	10	14	17	21	28	35	42
Day	Day -42 to -3	Day - 7 to -3	1	2	2	3	4	6 ± 1	9 ± 1	12 ± 1	16 ± 1	19 ± 1	23 ± 2	30 ± 2	37 ± 2	44 ± 2
Week	-6 to -1	-1	1				1	1	1	2	2	3	3	4	5	6 EOS
Pregnancy Testing[e]	X		X[1]			X										
Proteomic Assessments[f]			X[1]	X[1]	X[1]	X[1]										
Efficacy Assessments																
CyTOF			X[1]	X[1]	X[1]	X[1]										
3D-CAM			X[1]		X	X[1]										
SRS		X	X[1]			X[1]	X	X	X	X	X	X	X	X	X	X
Full WOMAC		X														X
Subset of WOMAC questions			X[1]			X[1]	X	X	X	X	X	X	X	X	X	
SF-36		X														X
BDI-II		X														X
POMS		X														
PSS		X														
Opioid Consumption Assessment[g]	X				X	X	X	X	X	X	X	X	X	X	X	X
Perioperative Assessments					X											
ActiGraph[h]	(Provide Device)	(Place Device)	X			X	X	X	X	X	X	X	X	X	X	X

Notes	X[1]: To be performed prior to infusion start (on Day 2, this is prior to the start of the first infusion).
	X[2]: To be performed: Prior to each infusion, within 1 hour of infusion start: BP, HR, RR, and body temperature. After the start of each infusion, at 15, 30, and 45 minutes: BP and HR. At 60 minutes after infusion start: BP, HR, RR, and body temperature.
	X[3]: To be performed following final infusion on Day 3.
	a: Preoperative infusion on the day of surgery.
	b: Postoperative infusion on the day of surgery.
	c: Screening lab panel: Alkaline phosphatase, ALT, AST, bicarbonate, bilirubin (total), ionized calcium, chloride, creatinine, GGT, glucose (random), lactate dehydrogenase, magnesium, phosphate, potassium, protein total, sodium, blood urea nitrogen, albumin, PTT, PT/INR. CBC with differential (basophils, eosinophils, lymphocytes, monocytes, neutrophils). Urinalysis: blood, glucose, ketone, protein, pH, specific gravity, nitrite, leukocytes, urine creatinine, urine protein/creatinine ratio, (and reflex urine microscopy if indicated). Infectious serology (anti-HIV antibodies, anti-hepatitis B surface antigen, anti-hepatitis B core antigen, anti-hepatitis C antibodies), direct antiglobulin test, RBC antibody screen (including direct Coombs and anti-RBC antibody), serum IgA, and haptoglobin. Urine drug screen: cannabinoids, benzodiazepine, barbiturates, opiates, cocaine, amphetamines, phencyclidine.
	d: Exit lab panel performed within ~48 hours after the last infusion (Day 3): Infectious serology (anti-HIV antibodies, anti-hepatitis B surface antigen, anti-hepatitis B core antigen, anti-hepatitis C antibodies), direct antiglobulin test, RBC antibody screen (including direct Coombs and anti-RBC antibody), ALT, AST, bilirubin (total), ionized calcium, chloride, creatinine, potassium, protein total, sodium, blood urea nitrogen, PTT, PT/INR. CBC with differential (basophils, eosinophils, lymphocytes, monocytes, neutrophils). Urinalysis: blood, glucose, ketone, protein, pH, specific gravity, nitrite, leukocytes, urine creatinine, urine protein, urine protein/creatinine ratio, and reflex urine microscopy if indicated.
	e: Serum pregnancy test required at screening. Serum and/or urine pregnancy test may be used at subsequent timepoints.
	f: For proteomic assessments, fasting samples are preferred.
	g: Baseline opioid consumption assessment will be included at screening during medication history. Subsequent opioid measurements will begin following surgery on Day 2 (Visit 4b).
	h: The ActiGraph wearable device will be given to the subject at the screening visit. Between -7 and -3 days before surgery, the subject will be told by phone to put the ActiGraph on and wear it until admission. The ActiGraph will be removed for the surgery on Day 2 (Visits 4a and 4b).
	i: On Day 3, second 12-lead ECG to be performed within +1 day

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VIV-2018-R069. Effects of plasma protein fraction (human), 5% on cognition and neurogenesis in aged, C57BL/6J mice.

17 APPENDICES

The assessments in this section and associated information are provided as EXAMPLES ONLY. The actual assessments, related source documents, and instructions for administration and scoring are included in the associated guides if available.

Appendix 1. 3-Minute Diagnostic Interview for Confusion Assessment Method

The 3D-CAM (Inouye 2003) is a brief verbal assessment tool used to test patients for delirium. Each item in the instrument directly informs one of the 4 CAM features. For all items, the patient's answer is "incorrect" or "yes."

3D CAM ASSESSMENT			CAM Feature			
Coding Instructions: Incorrect also includes "I don't know", and No response/non-sensical responses. For any 'Incorrect' or 'Yes' responses, check the box in the final column designating which feature is present.						
READ: **I have some questions about your thinking and memory....**			1	2	3	4
1. Can you tell me the year we are in right now?	☐ Incorrect	☐ Correct	■	■		■
2. Can you tell me the day of the week?	☐ Incorrect	☐ Correct	■	■		■
3. Can you tell me what type of place is this? [hospital]	☐ Incorrect	☐ Correct	■	■		■
4. **I am going to read some numbers. I want you to repeat them in backwards order from the way I read them to you. For instance, if I say "5 – 2", you would say "2 -5". OK? The first one is "8-2-5" (5-2-8).**	☐ Incorrect	☐ Correct	■		■	■
5. **The second is "3-1-9-4" (4-9-1-3).**	☐ Incorrect	☐ Correct	■		■	■
6. **Can you tell me the days of the week backwards, starting with Saturday?** [S,F,T,W,T,M,S] *may prompt with "what is day before ...." for up to 2 prompts.*	☐ Incorrect	☐ Correct	■		■	■
7. **Can you tell me the months of the year backwards, starting with December?** [D,N,O,S,A,J,J,M,A,M,F,J] *may prompt with "what is month before ...." for up to 2 prompts.*	☐ Incorrect	☐ Correct	■		■	■
8. **During the past day have you felt confused?**	☐ Yes	☐ No		■	■	■
9. [IF Q3 is "Incorrect", do not ask and check "Yes", otherwise, ASK:] **During the past day did you think that you were not really in the hospital?**	☐ Yes	☐ No		■	■	■
10. **During the past day did you see things that were not really there?**	☐ Yes	☐ No		■	■	■
Observer Ratings: To be completed after asking the patient questions 1-10 above.			■	■	■	■
11. Was the patient sleepy, stuporous, or comatose during the interview?	☐ Yes	☐ No	■	■	■	
12. Did the patient show excessive absorption with ordinary objects in the environment (hypervigilant)?	☐ Yes	☐ No	■	■	■	
13. Was the patient's flow of ideas unclear or illogical, for example tell a story unrelated to the interview (tangential)?	☐ Yes	☐ No	■	■		■
14. Was the patient's conversation rambling, for example did he/she give inappropriately verbose and off target responses?	☐ Yes	☐ No	■	■		■
15. Was the patient's speech unusually limited or sparse? (e.g. yes/no answers)	☐ Yes	☐ No	■	■		■
16. Did the patient have trouble keeping track of what was being said during the interview?	☐ Yes	☐ No	■		■	■
17. Did the patient appear inappropriately distracted by environmental stimuli?	☐ Yes	☐ No	■		■	■
18. Did the patient's level of consciousness fluctuate during the interview, for example, start to respond appropriately and then drift off?	☐ Yes	☐ No		■	■	■
19. Did the patient's level of attention fluctuate during the interview, e.g., did the patient's focus on the interview or performance on the attention tasks vary significantly?	☐ Yes	☐ No		■	■	■
20. Did the patient's speech/thinking fluctuate during the interview, for example, patient spoke slowly, then spoke very fast?	☐ Yes	☐ No		■	■	■
OPTIONAL QUESTIONS: COMPLETE ONLY IF FEATURE 1 IS NOT CHECKED AND FEATURE 2 IS CHECKED AND EITHER FEATURE 3 OR 4 IS CHECKED			■	■	■	■
21. Contact a family member, friend, or health care provider who knows the patient well and ask: "Is there evidence of an acute change in mental status (memory or thinking) from the patient's baseline?"	☐ Yes	☐ No		■	■	■
22. IF SECOND DAY OF HOSPITALIZATION OR LATER AND PREVIOUS 3D-CAM RATINGS ARE AVAILABLE: Review previous 3D-CAM assessments and determine if there has been an acute change in performance, based on ANY new "positive" items	☐ Yes	☐ No		■	■	■
CAM Summary: Check if Feature Present in column above			1	2	3	4
DELIRIUM REQUIRES FEATURE 1 AND 2 and EITHER 3 OR 4: _____ Present _____Not Present						

Appendix 2. Surgery Recovery Scale

The SRS (Paddison 2011) is a sensitive and simple tool for the assessment of functional recovery following major surgery. The scale contains 13 items. Impacts on daily activities are scored from 1 to 5 (1= not at all). It's considered the most broadly validated measure of surgical recovery available.

Surgery Recovery Scale
During the last two days:

- I have been feeling energetic
- I have been feeling worn out
- I have been feeling vigorous
- I have been forgetful
- I have achieved very little with the day
- I have been feeling fatigued
- Physically, I have felt tired
- I have had to restrict how much I try to do in a day
- I have been feeling lively

During the last two days, I have had enough energy to:

- Read a newspaper/book or watch TV
- Dress
- Do household chores
- Engage in leisure or recreational activities
- Shop or do errands

Appendix 3. Western Ontario and McMaster Universities Osteoarthritis Index

The WOMAC (Bellamy 1986, Bellamy 2002) was developed to assess pain, stiffness, and physical function in patients with knee and/or hip osteoarthritis, but it has been used among patients with many different conditions. The WOMAC is one of the most widely utilized self-report measures of lower extremity symptoms and function. It has been studied over a period of almost 30 years in many different contexts and patient populations, and there are abundant data regarding its utility and measurement properties. The Likert Scale version uses an 11-point scale anchored by "no pain" and "extreme pain" for pain, and "no difficulty" and "extreme difficulty" for physical function. Higher scores on the WOMAC indicate worse pain and functional limitations.

Appendix 4. Short Form-36

The SF-36 (Laucis 2015) is the most widely used health-related quality-of-life measure in research to date. The SF-36 yields 8 scale scores and 2 summary scores. The physical component summary and mental component summary scores.

SF-36 QUESTIONNAIRE

Name:_____ **Ref. Dr:**_____ **Date:** _____

ID#: _____ **Age:** _____ **Gender: M / F**

Please answer the 36 questions of the **Health Survey** completely, honestly, and without interruptions.

GENERAL HEALTH:
In general, would you say your health is:
◯ Excellent ◯ Very Good ◯ Good ◯ Fair ◯ Poor

Compared to one year ago, how would you rate your health in general now?
◯ Much better now than one year ago
◯ Somewhat better now than one year ago
◯ About the same
◯ Somewhat worse now than one year ago
◯ Much worse than one year ago

LIMITATIONS OF ACTIVITIES:
The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.
◯ Yes, Limited a lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Lifting or carrying groceries
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Climbing several flights of stairs
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Climbing one flight of stairs
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Bending, kneeling, or stooping
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

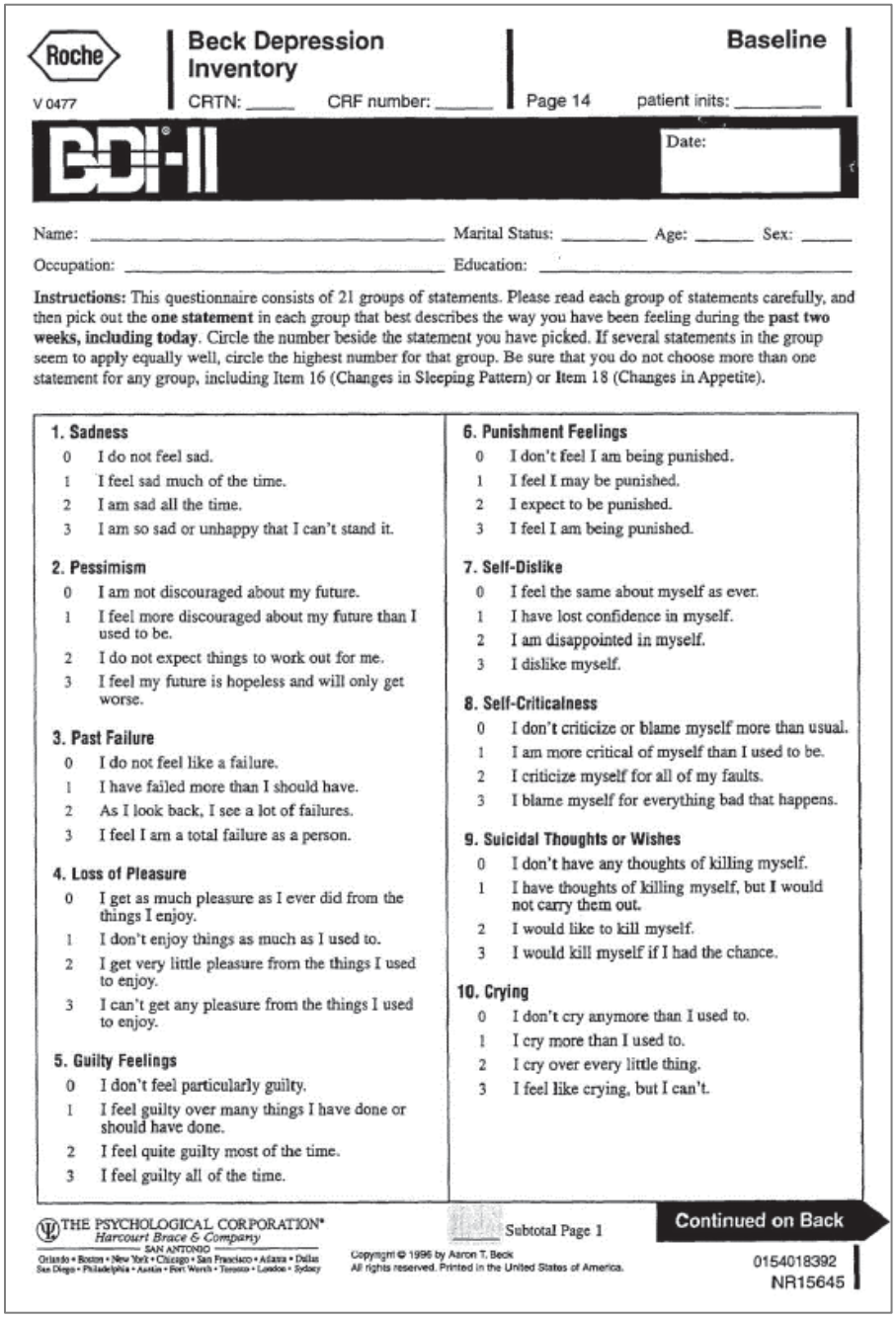
Walking more than a mile
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Walking several blocks
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Walking one block
◯ Yes, Limited a Lot ◯ Yes, Limited a Little ◯ No, Not Limited at all

Appendix 5. Beck Depression Inventory-II

The BDI-II (Beck 1996) is a 21-item self-administered survey is scored on a scale of 0–3 in a list of four statements arranged in increasing severity about a particular symptom of depression.



Roche

V 0477

Beck Depression Inventory

CRTN: _____ CRF number: _____

Page 14 patient inits: _____

Baseline

BDI-II

Date:

Name: _____ Marital Status: _____ Age: _____ Sex: _____

Occupation: _____ Education: _____

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the **one statement** in each group that best describes the way you have been feeling during the **past two weeks, including today**. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness
0 I do not feel sad.
1 I feel sad much of the time.
2 I am sad all the time.
3 I am so sad or unhappy that I can't stand it.

2. Pessimism
0 I am not discouraged about my future.
1 I feel more discouraged about my future than I used to be.
2 I do not expect things to work out for me.
3 I feel my future is hopeless and will only get worse.

3. Past Failure
0 I do not feel like a failure.
1 I have failed more than I should have.
2 As I look back, I see a lot of failures.
3 I feel I am a total failure as a person.

4. Loss of Pleasure
0 I get as much pleasure as I ever did from the things I enjoy.
1 I don't enjoy things as much as I used to.
2 I get very little pleasure from the things I used to enjoy.
3 I can't get any pleasure from the things I used to enjoy.

5. Guilty Feelings
0 I don't feel particularly guilty.
1 I feel guilty over many things I have done or should have done.
2 I feel quite guilty most of the time.
3 I feel guilty all of the time.

6. Punishment Feelings
0 I don't feel I am being punished.
1 I feel I may be punished.
2 I expect to be punished.
3 I feel I am being punished.

7. Self-Dislike
0 I feel the same about myself as ever.
1 I have lost confidence in myself.
2 I am disappointed in myself.
3 I dislike myself.

8. Self-Criticalness
0 I don't criticize or blame myself more than usual.
1 I am more critical of myself than I used to be.
2 I criticize myself for all of my faults.
3 I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes
0 I don't have any thoughts of killing myself.
1 I have thoughts of killing myself, but I would not carry them out.
2 I would like to kill myself.
3 I would kill myself if I had the chance.

10. Crying
0 I don't cry anymore than I used to.
1 I cry more than I used to.
2 I cry over every little thing.
3 I feel like crying, but I can't.

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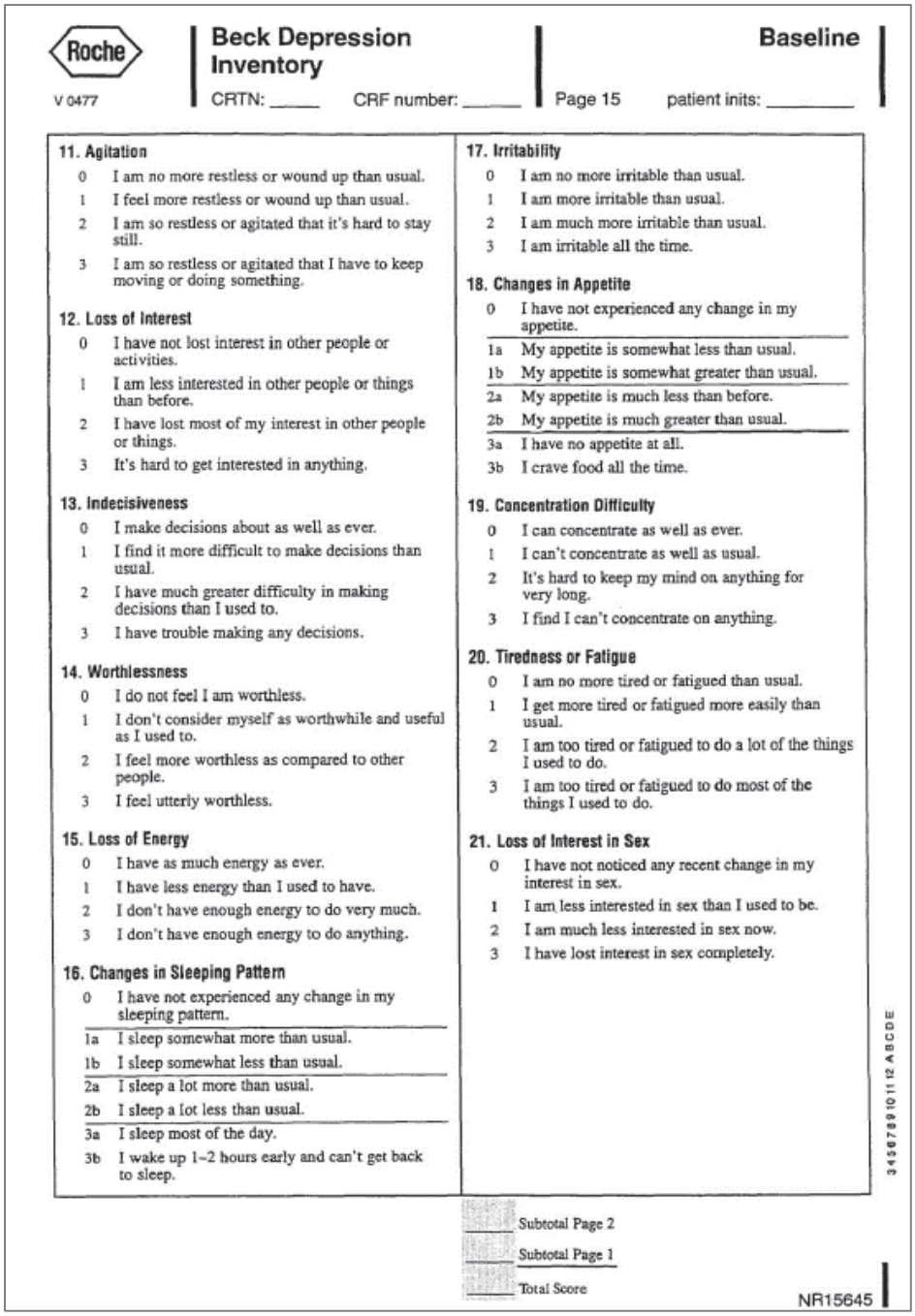

Roche

V 0477

Beck Depression Inventory

CRTN: _____ CRF number: _____ Page 15 patient inits: _____

Baseline

11. Agitation

0 I am no more restless or wound up than usual.
1 I feel more restless or wound up than usual.
2 I am so restless or agitated that it's hard to stay still.
3 I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest

0 I have not lost interest in other people or activities.
1 I am less interested in other people or things than before.
2 I have lost most of my interest in other people or things.
3 It's hard to get interested in anything.

13. Indecisiveness

0 I make decisions about as well as ever.
1 I find it more difficult to make decisions than usual.
2 I have much greater difficulty in making decisions than I used to.
3 I have trouble making any decisions.

14. Worthlessness

0 I do not feel I am worthless.
1 I don't consider myself as worthwhile and useful as I used to.
2 I feel more worthless as compared to other people.
3 I feel utterly worthless.

15. Loss of Energy

0 I have as much energy as ever.
1 I have less energy than I used to have.
2 I don't have enough energy to do very much.
3 I don't have enough energy to do anything.

16. Changes in Sleeping Pattern

0 I have not experienced any change in my sleeping pattern.
1a I sleep somewhat more than usual.
1b I sleep somewhat less than usual.
2a I sleep a lot more than usual.
2b I sleep a lot less than usual.
3a I sleep most of the day.
3b I wake up 1–2 hours early and can't get back to sleep.

17. Irritability

0 I am no more irritable than usual.
1 I am more irritable than usual.
2 I am much more irritable than usual.
3 I am irritable all the time.

18. Changes in Appetite

0 I have not experienced any change in my appetite.
1a My appetite is somewhat less than usual.
1b My appetite is somewhat greater than usual.
2a My appetite is much less than before.
2b My appetite is much greater than usual.
3a I have no appetite at all.
3b I crave food all the time.

19. Concentration Difficulty

0 I can concentrate as well as ever.
1 I can't concentrate as well as usual.
2 It's hard to keep my mind on anything for very long.
3 I find I can't concentrate on anything.

20. Tiredness or Fatigue

0 I am no more tired or fatigued than usual.
1 I get more tired or fatigued more easily than usual.
2 I am too tired or fatigued to do a lot of the things I used to do.
3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex

0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I am much less interested in sex now.
3 I have lost interest in sex completely.

3 4 5 6 7 8 9 10 11 12 A B C D E

_____ Subtotal Page 2
_____ Subtotal Page 1
_____ Total Score

NR15645

Appendix 6. Profile of Mood States

The POMS (Heuchert 2012) is widely used to assess affective traits, mood, and emotion. It contains 65 POMS items. It can be completed online or in a paper format in 3 to 5 minutes. The subscale measurements include anger-hostility, confusion-bewilderment, depression-dejection, fatigue-inertia, tension-anxiety, and vigor-activity. For the purposes of this study, only the 9 items in the "tension-anxiety" scale will be used.

Descriptions of POMS Scales

Scales	Descriptions
Anger-Hostility (AH)	The feeling of anguish and hostility described by words of "Annoyed" or "Resentful."
Confusion-Bewilderment (CB)	The mood reflecting anxiety labeled with bewilderment and cognitive inefficiency.
Depression-Dejection (DD)	The scale indicating depression or self-inadequacy hallmarked by sadness, loneliness, guilt, worthlessness, and hopelessness.
Fatigue-Inertia (FI)	The factor reflecting weariness, inertia, and low energy.
Tension-Anxiety (TA)	The state of intensified physical tension caused by anxiety or impatience, which is usually expressed through observable and nonobservable behaviors.
Vigor-Activity (VA)	The cheerful mood referring to high, positive energy contrasts to other scales of the POMS 2 above.
Friendliness (F)	The positive factor reflecting interpersonal influences on mood.
Total Mood Disturbance (TMD)	A global indicator of emotional disturbance, psychological distress, or subjective well-being. [TMD = (AH + CB + DD + FI + TA) − VA]

18 REVISION HISTORY

18.1 SUMMARY OF CHANGES

Protocol Version 3.0 dated 14NOV2019
Replaces: Protocol Version 2.0 dated 15APR2019

In this table, changes from Version 3.0 dated 14NOV2019 are described and the rationale for each revision is provided.

Location	Description	Purpose
Throughout	***Previously read:*** V2.0_15APR2019 ***Now reads:*** V3.0_14NOV2019	Version Control
Throughout	Removed mention of Ethics Committee(s).	This study is only taking place in the United States with one Investigational Review Board.
Throughout	***Previously read***: Secondary efficacy endpoints ***Now reads***: Secondary endpoints	Secondary endpoints include assessments for safety as well as efficacy.
Section 4.2.2.	***Added***: End of study treatment comparison of the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) (Bellamy 1986, Bellamy 2002)(Appendix 3). Clarified time to score of WOMAC is only for a subset of questions.	Updated per revised timepoints in Section 15.1.
Section 7.1.1.3.4 and 17	Revised for the full WOMAC to be completed at Baseline (Visit 2) and End of Study (Visit 15). A subset of questions from the WOMAC to be completed during interim visits per Section 15.1.	Updated per the revised timepoints in Section 15.1.
Section 5.2	***Previously read:*** Exclusion Criteria #1 Blood coagulation disorders, including severe liver disease, vitamin K deficiency, von Willebrand disease, hemophilia A or B or other known deficiencies in clotting factor (fibrinogen, prothrombin, factor VII, X, XI, XII, or XIII). ***Now reads***: Exclusion Criteria #1 Blood coagulation disorders, including severe liver disease, vitamin K deficiency, von Willebrand disease, hemophilia A or B or other known deficiencies in clotting factors (fibrinogen, prothrombin, factor VII, X, XI, XII, or XIII) that in the opinion of the investigator could put the subject at risk.	GRF6021 does not contain coagulation factors, and the volume of GRF6021 given to subjects in this trial will not cause hemodilution and secondary coagulopathy. Thus, the sentence was modified to clarify that the PI should use his/her clinical judgement to assess whether a coagulation disorder may put the subject at risk.

Section 5.2	***Previously read***: Exclusion Criteria #2 Subjects on chronic anticoagulant therapy (warfarin, heparin, low-molecular weight heparin, or Factor Xa inhibitors). Use of pre-, intra- and post-operative anticoagulation according to standard of care is permitted. Chronic use of antiplatelet drugs (e.g., aspirin or clopidogrel) is permitted. ***Now read*s**: Exclusion Criteria #2 Subjects who started chronic anticoagulant therapy (warfarin, heparin, low-molecular weight heparin, or Factor Xa inhibitors) in the last 6 months. Use of pre-, intra- and post-operative anticoagulation according to standard of care is permitted. Use of antiplatelet drugs (e.g., aspirin or clopidogrel) is permitted.	GRF6021 does not contain coagulation factors, and the volume of GRF6021 given to subjects in this trial will not cause hemodilution and secondary coagulopathy. Thus, the sentence was modified to clarify that subjects who have been off anticoagulation therapy for 6 months or longer may be eligible for enrollment. Subjects who had a recent medical event that warranted anticoagulation therapy will be excluded.
Section 5.2	Split Exclusion Criteria #3 into Exclusion Criteria #3 and #4, and revised length of time for Exclusion Criteria #4. ***Previously read***: Exclusion Criteria #3 History of a known hypercoagulable state, including Factor V Leiden thrombophilia, prothrombin gene mutation, deficiencies in antithrombin, protein C or protein S, elevated levels of fibrinogen, or dysfibrinogenemia. A history of deep vein thrombosis or pulmonary embolism is only exclusionary if it occurred in the last 24 months and/or if it is associated with a known hypercoagulability disorder. ***Now reads***: Exclusion Criteria #3 History of a known hypercoagulable state, including Factor V Leiden thrombophilia, prothrombin gene mutation, deficiencies in antithrombin, protein C or protein S, elevated levels of fibrinogen, or dysfibrinogenemia. Exclusion Criteria #4 History of deep vein thrombosis or pulmonary embolism in the last 6 months and/or if it is associated with a known hypercoagulability disorder.	Human immunoglobulin concentrates for IV infusion (IVIg) are known to be associated with a risk of thromboembolic events; however, because the concentration of IgG in GRF6021 is very low in comparison with IVIg, there is no known risk of thromboembolic events associated with GRF6021.
Section 5.2	***Removed****:* Exclusion Criteria #14 History of Torsades de Pointes dysrhythmia.	GRF6021 is not associated with any known effects on ECG or electrolytes or any cardiac risk. Thus, The PI should use his/her clinical judgement to determine whether an ECG abnormality would warrant the exclusion of the subject.
Section 5.2	***Previously read***: Exclusion Criteria #15 Clinically significant abnormalities on screening electrocardiogram (ECG) including QTc intervals (using Fridericia's	GRF6021 is not associated with any known effects on ECG or electrolytes or any cardiac risk.

	correction formula) of ≥ 450 ms in men and ≥ 470 ms in women. ***Now reads***: Exclusion Criteria #15 Clinically significant abnormalities on screening electrocardiogram (ECG) that in the opinion of the investigator could put the subject at risk, including QTc intervals (using Fridericia's correction formula) of ≥ 450 ms in men and ≥ 470 ms in women.	Thus, The PI should use his/her clinical judgement to determine whether an ECG abnormality would warrant the exclusion of the subject.
Section 5.2	***Previously read***: Exclusion Criteria #19 History of a major infection (e.g. bacterial pneumonia) in the last 3 months, or minor infection (e.g. influenza, urinary tract infection) or fever (measured body temperature above 100.5 degrees F on 2 consecutive days) in the last 1 month. ***Now reads:*** Exclusion Criteria #18 History of a major infection (e.g. bacterial pneumonia) in the last 3 months, or recent minor infection (e.g. influenza, urinary tract infection) or fever (measured body temperature above 100.5 degrees F on 2 consecutive days) that in the assessment of the Principal Investigator could interfere with the primary endpoint.	The PI should use his/her clinical judgment to determine whether the infectious or inflammatory process was systemic, in which case there may be effects on the primary endpoints.
Section 5.2	***Previously read***: Exclusion Criteria #20 Hemoglobin < 9 g/dL in women and < 10 g/dL in men. ***Now reads:*** Exclusion Criteria #19 Severe anemia (hemoglobin < 8 g/dL).	GRF6021 is contraindicated in severe anemia because of the risk of hemodilution; however, there is no known risk in mild or moderate anemia.
Section 5.2	***Previously read***: Exclusion Criteria #25 Immunosuppressant treatment in the last 6 months. ***Now reads***: Exclusion Criteria #24 Ongoing immunosuppressant treatment or within 5 half-lives of the treatment.	Sentence was modified to allow for subjects who stopped taking an immunosuppressant but whose immune function has returned to normal.
Section 6.1.5 and throughout	Clarified study personnel and administration of the study agent/placebo.	Updated language per the current Infusion Administration Manual.
Section 7.1.1.1.1.	***Removed****:* Cumulative Illness Rating Scale	The scale was determined not to be required for medical history, as it is being obtained from the electronic medical record.
Section 7.1.1.1.5	***Previously read***: A full physical examination will be performed to assess the following organ systems: lungs/chest, heart, abdomen, neuro/spine. Height and weight will also be measured at screening.	The original intent was for the physical exam that is performed as part of presurgical clinical care to be used to assess eligibility. Added sentence to clarify original intent.

	Now reads: A physical examination will be performed to assess the following organ systems: lungs/chest, heart, abdomen, neuro/spine. Height and weight will also be measured at screening. Should the subject have a physical exam, including height and weight measurements, as part of standard of care during the screening window, then the results may be obtained from the subject's medical record for the purposes of assessing eligibility.	
Section 7.1.1.2.6	Added window of ~48 hours for completion of the exit lab panel.	To aid trial feasibility.
Section 7.1.1.3.4 and 17	Revised summary of the WOMAC.	To align with the licensed scale and Section 15.1.
Section 7.1.1.3.7 and 17	Removed Profile of Mood States (POMS) Short Form and replaced with full-length POMS.	The 9 items in the "tension-anxiety" scale are present in the full-length POMS but are not all present in the Short Form.
Section 7.3.4	Under the section for "Perform the following procedures on each day prior to administering (the first) study infusion" • ***Added***: "Including second infusion on Day 2" to bullets 3, 4, and 5. • ***Removed***: Bullets 8, 9, and 11. Under the section for "Perform the following assessments following each infusion start" • ***Added***: Administer 3D-CAM (Day 2 Only, after second infusion).	Corrected and aligned section with Section 15.1 (Schedule of Events).
Section 7.3.7	Added exit lab panel to End of Study/Early Termination visit for subjects who have received at least one infusion but terminate from the study prior to completing the last infusion.	To ensure samples for the primary endpoint and safety endpoints are collected should a subject discontinue participation early.
Section 7.4.1	***Added***: For the purposes of this trial, it is recommended to: • Avoid adjuvant analgesic techniques that have immune-modulatory potential (e.g. ketamine or IV lidocaine infusions or bolus administration), unless clinically indicated. • Avoid other medications with immune-modulatory potential such as steroids (e.g. decadron), unless clinically indicated.	Recommended guidelines added to avoid anesthetics and other medications that may have effects on the primary endpoint, unless these are clinically indicated.
Section 8.4.3	***Removed****:* Systolic blood pressure (BP) <80 or > 180 mm Hg and/or diastolic BP <50 or >110 mm Hg, or a change of >30% from baseline in systolic and/or diastolic BP.	Based on the evaluation of aggregate safety data from ongoing trials with this and similar plasma fractions, only

		changes in blood pressure that result in an SAE need to be promptly communicated to the Sponsor, in which case per the timeframes in Section 8.4.2.1.
Section 10.3	Updated definition of the Per Protocol dataset.	Updated definition for standardization with current Alkahest statistical standards.
Section 10.6.1	Added stratification of surgical joint (hip, knee).	Additional stratification for surgical joint added to minimize potential bias at the time of randomization.
Section 13.3	Updated definitions of Major and Minor Protocol Deviations.	Updated definition for standardization with current Alkahest SOPs.
Section 15.1	Revised WOMAC timepoints for full scale and subset of scale.	WOMAC timepoints updated for feasibility of implementation.
Section 15.1	Added footnote "h" for ActiGraph.	Corrected and aligned section with Section 7.3.4 (Study Procedures).
Section 15.1	Added footnote "i": 12-lead ECG to be performed within +1 day from Day 3.	To aid trial feasibility.
Section 16.1	Added reference for Perceived Stress Scale (Cohen 1983). Replaced reference of POMS-SF (Curran 1995) with full-length POMS (Heuchert 2012).	Added missing reference for PSS, and updated POMS reference for the full-length version.

Protocol Version 2.0 dated 15APR2019
Replaces: Protocol Version 1.1 dated 11MAR2019

In this table, changes from Version 1.1 dated 11MAR2019 are described and the rationale for each revision is provided.

Location	Description	Purpose
Throughout	***Protocol version previously read:*** V1.1_11MAR2019 ***Now reads:*** V2.0_15APR2019	Version Control
Throughout	Laboratory previously included as activated partial thromboplastin time (aPTT) will now be partial thromboplastin time (PTT).	Investigator confirmed these labs are equivalent at the study site.

Section 5.3	***Content removed:*** During the study, financial support for meal and miscellaneous expenses will be available, as appropriate, consistent with local regulations and guidelines. Use of visit transport services may also be incorporated into the trial to support the subject in maintaining study visit compliance.	Subjects will receive a moderate stipend for successful completion of the study and for incurred hotel expenses only.
Section 5.4.1	***Previously read:*** Occurrence of an adverse event (AE) that represents an unacceptable risk to the subject and when continued participation in the investigational study is not warranted, in the judgment of the investigator, Sponsor, or medical monitor. ***Now reads:*** Occurrence of an adverse event (AE) that represents an unacceptable risk to the subject and when continued participation in the investigational study is not warranted, in the judgment of the investigator or Sponsor Medical Monitor.	The Sponsor Program Physician will be acting as Medical Monitor for the study; sentence updated to correctly reflect Sponsor's responsibility.
Section 7.1.1.1.5	***Previously read:*** A full physical examination will be performed to assess the following organ systems: skin, ENT (ears, nose, and throat), head, eyes, lungs/chest, heart, abdomen, musculoskeletal, extremities, neurologic and lymphatic systems. Height and weight will also be measured at screening. ***Now reads:*** A full physical examination will be performed to assess the following organ systems: lungs/chest, heart, abdomen, neuro/spine. Height and weight will also be measured at screening.	Physical examination modified to reflect requirements for this study.
Section 7.1.1.1.6	***Content removed:*** Samples that remain after study screening is complete will be stored at the study site in the event additional testing (e.g., further evaluation of an AE or assessment of effect) is required. Samples will be stored in a deidentified coded form.	There will be no samples remaining following screening; therefore, storage will not be required.
Sections 7.1.1.1.6 and 15.1	Removed methadone from urine drug screen.	Evaluation not required for this study.
Section 7.1.1.3.10	***Previously read:*** Approximately -7 to -3 days prior to surgery, subjects will be instructed to wear the device which will then begin remote monitoring of physical activity, mobility, and sleep measures. ***Now reads:*** Approximately -7 to -3 days prior to surgery, subjects will be instructed to wear the device which will then begin monitoring of physical activity, mobility, and sleep measures.	Removed word "remote" as the device does not provide data transmitted remotely.
Sections 7.3 and 15.1	The following assessments were revised to occur as follows: • CyTOF: V3, V4a, V4b, V5 • 3D-CAM: V3, V4b, V5	Revision to assessment dates and frequency to conform with original study design.

	• SRS/WOMAC: V2, V3, V5-V15	
Section 7.3.1	***Bullet 3 previously read:*** Review subject's current and prior medications. ***Bullet 3 now reads:*** Review subject's current and prior medications, including opioid consumption assessment. ***Bullet 6 previously read:*** Collect blood and urine samples required for Screening. ***Now reads:*** Collect blood and urine samples required for Screening (including pregnancy test for WOCBP).	Opioid consumption assessment will now be captured at screening (Visit 1) during review of medications. Pregnancy testing required at screening for WOCBP.
Section 7.3.2	Removed "Opioid consumption assessment."	Assessment will now be captured at screening (Visit 1) during review of medications.
Section 7.3.4	Added bullet1/sub-bullet 7: "Pregnancy testing for WOCBP (**Day 1 Only**)."	Pregnancy testing should occur in WOCBP prior to the first infusion on Day 1.
Section 8.4.4	***Previously read:*** The investigator must report any pregnancy that occurs in a female study subject or female partner of a male subject subsequent to first exposure to the study agent until End of Study, or 3 months following a subject's last dose in the event of early termination. All pregnancies will be reported to the IRB, Sponsor, and CRO. In the event of a pregnancy, treatment will be discontinued, and the subject will undergo continued safety follow-up through pregnancy outcome. ***Now reads:*** The investigator must report any pregnancy that occurs in a female study subject subsequent to first exposure to the study agent until End of Study, or 3 months following a subject's last dose in the event of early termination. All pregnancies will be reported to the IRB, Sponsor, and CRO. In the event of a pregnancy, the subject will undergo continued safety follow-up through pregnancy outcome.	Content updated to reflect appropriate precautions for this study design.
Sections 8.5 and 8.6	Term "CRO medical monitor(s)" was changed to "CRO."	The Sponsor Program Physician will be acting as Medical Monitor for the study; terms updated to correctly reflect CRO responsibility.
Section 15.1	• Added footnote: "X^3: To be performed following final infusion on Day3." • Notes did not contain "e" footnote (previously deleted); alpha listing revised.	• Clarification of 12-lead ECG timing. • Notes were revised for continuity of alpha listing.

	• Added footnote: "g: Baseline opioid consumption assessment will be included at screening during medication history. Subsequent opioid measurements will begin following surgery on Day 2 (Visit 4b)."	• Clarification of opioid consumption assessment and timing.

Protocol Version 1.1 dated 11MAR2019
Replaces: Protocol Version 1.0 dated 27FEB2019

In this table, changes from Version 1.0 dated 27FEB2019 are described and the rationale for each revision is provided.

Location	Description	Purpose
Throughout	***Protocol version previously read:*** V1.0_27FEB2019 ***Now reads:*** V1.1_11MAR2019	Version Control
Section 6.1.2	The following sentence was removed: "The label will clearly identify if it is the study agent or placebo control agent."	The same study label is used for both the study agent and placebo. The placebo also maintains its primary drug label that specifies its contents.