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: Supplementary SAP Version 1: 10-June-2021

SUPPLEMENTARY STATISTICAL ANALYSIS PLAN

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
Investigational Product:	GRF6021
Protocol Number, Version, and Date:	AKST6021-211, V3.0, 14NOV2019
Study Dates (Initiated to Completed):	12JUL2019 to 04MAR2021
Authors:	[redacted], PhD [redacted], PhD
Study Facility and Address:	Alkahest, Inc. 125 Shoreway Road, Suite D San Carlos, CA 94070
Supplementary Statistical Analysis Plan (SSAP) Number and Version:	#1, V1.0
Date:	09JUN2021

2 TABLE OF CONTENTS

2.1 List of Tables

2.2 List of Figures

3 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

3D-CAM	3-Minute Diagnostic Interview for Confusion
Ab	Antibody
arcsinh	Hyperbolic arcsine
ASA	American Society of Anesthesiologists
BDI-II	Beck Depression Index-II
Bnaive	Naïve b cells
CD	Cluster of differentiation
cMC	Classical monocytes
COVID-19	Coronavirus disease 2019
CREB	Cyclic AMP-responsive element-binding protein
CSR	Clinical study report
CyTOF	Mass cytometry
DNA	Deoxyribonucleic acid
EBL	Estimated blood loss
ECG	Electrocardiogram
EOS	End of study
ERK	Extracellular regulated kinase
iEN	Immunological elastic net
intMC	Intermediate monocyte
IV	Intravenous
MAPK	Mitogen-activated protein kinase
mDC	Myeloid dendritic cell
mem	Memory
M-MDSC	Monocytic myeloid-derived monocyte
ncMC	Non-classical monocyte
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	Natural killer
PACU	Post-anesthesia care unit
pDC	Plasmacytoid dendritic cell
PP	Per protocol
RFU	Relative fluorescence unit
SAP	Statistical Analytical Plan
SF-36	Short Form-36
SRS	Surgery Recovery Scale
SSAP	Supplementary Statistical Analysis Plan
STAT	Signal transducer and activator of transcription
THA	Total hip arthroplasty
TKA	Total knee arthroplasty
US	United States
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index

4 SIGNATURE PAGE

Prepared by:

DocuSigned by:
[redacted]
Signer Name: [redacted]
Signing Reason: I approve this document
Signing Time: 09-Jun-2021 | 2:18:19 PM PDT
[redacted]

[redacted], PhD
Biostatistician
Alkahest, Inc.

Prepared by:

DocuSigned by:
[redacted]
Signer Name: [redacted]
Signing Reason: I have reviewed this document
Signing Time: 10-Jun-2021 | 11:07:50 AM PDT
[redacted]

[redacted], PhD
[redacted] of Data Science
Alkahest, Inc.

Approved by:

DocuSigned by:
[redacted] MD
Signer Name: [redacted] MD
Signing Reason: I have reviewed this document
Signing Time: 09-Jun-2021 | 12:20:11 PM PDT
08C4D4FBFB384F8494A4FA1E5C440B16

[redacted], MD
[redacted], Clinical Development
Alkahest, Inc.

5 INTRODUCTION

This supplementary statistical analysis plan (SSAP) describes the planned statistical analyses for the primary endpoint and two (2) of the secondary endpoints (ActiGraph wearable device and plasma proteomics) for the study entitled "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" (V3.0, 14NOV2019). The statistical analysis plan (SAP) for the remaining secondary clinical efficacy and safety endpoints is a separate document (SAP 08OCT2020).

AKST6021-211 was designed to assess effects of GRF6021 on postoperative recovery following primary hip or knee arthroplasty by investigating the intracellular signaling cascades in blood leukocytes as determined by mass cytometry (CyTOF), a multiplexed, high-content immune profiling technology. In addition, the effects of GRF6021 on clinical recovery parameters and plasma proteomics, as well the safety and tolerability of GRF6021, will be assessed.

5.1 Background and Rationale

Worldwide, 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 United States (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 (Gaudillière 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.

6 STUDY OBJECTIVES AND ENDPOINTS

6.1 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 evaluation of 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.

6.2 Endpoints

- Primary Endpoint (analyses detailed in this SSAP):
  - Effect of GRF6021 on intracellular signaling cascades in blood leukocytes as determined by CyTOF.
- Secondary Endpoints (analyses detailed in this SSAP):
  - 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).
  - The effects of GRF6021 on plasma proteomics.
- Secondary Endpoints (analyses detailed in the SAP [08OCT2020]):
  - 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 (EOS) 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 EOS.
  - 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 from baseline in clinical laboratory parameters.
  - Change from baseline in vital sign measurements.
  - Change from baseline in electrocardiogram (ECG).

7 STUDY OVERVIEW

7.1 Study Design and Treatment Groups

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, and the infusion procedure of active and placebo agents will be identical to maintain the blind.

In this study, 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. The Somalogic SomaScan platform (Somalogic, Boulder, Colorado, US), which is an affinity-based targeted proteomics approach, will be used to measure plasma protein levels. Blood samples for both CyTOF and proteomics will be collected at baseline (Visit 3) and 3 perioperative time points (Visits 4a, 4b, and 5).

At screening, subjects will be provided with an ActiGraph wearable device (ActiGraph 2021). Approximately -7 to -3 days prior to surgery, subjects will be instructed to wear the device which will then begin monitoring specific measurements of physical activity, mobility, and sleep. Data collection is ongoing, and subjects will wear the device until admission, except during Visits 4a and 4b when it is removed for surgery.

Additional functional and quality of life assessments will be measured at baseline and at various times after the surgery (detailed information regarding assessments can be found in the study protocol [V3.0, 14NOV2019]). Analysis of these secondary endpoints are detailed in the SAP (08OCT2020).

7.2 Statistical Hypothesis

The hypothesis is that active treatment with GRF6021 will modulate the immune response and/or proteomics profiles to surgery differently as compared to placebo, and will change the postoperative functional status measured by the ActiGraph wearable device. Machine learning approaches will be utilized to detect immune features (functional cellular attributes as well as

cell abundance) and/proteomics features, as well as ActiGraph measurements that separate the active and placebo groups.

It is also hypothesized that there are immunomodulatory changes significantly associated with postoperative recovery in this study (Gaudillière 2014).

7.3 Sample Size Justification

As detailed in the protocol, the goal is to enroll a total of approximately 45 subjects with the aim of having 40 evaluable subjects randomized in a 1:1 ratio to active treatment or placebo groups. Due to the coronavirus disease 2019 (COVID-19) pandemic, enrollment ended with 37 evaluable subjects. Evaluable subjects are those who received all 4 doses and had all blood draws for assessment of the primary endpoint. Previous work demonstrated that a sample size of approximately 40 subjects enabled the detection of clinically-relevant immunomodulatory changes, such as changes in signaling downstream of immune receptors, including interleukin-1 and toll-like receptor 4 (Aghaeepour 2017, Davis 2020).

7.4 Randomization and Blinding

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.

7.5 Interim Analysis

No interim analyses are planned.

7.6 Study Assessments and Time Points

The study design consists of 15 protocol-specified visits which will be assessed as nominal visits from an analysis perspective (Table 1, Figure 1):

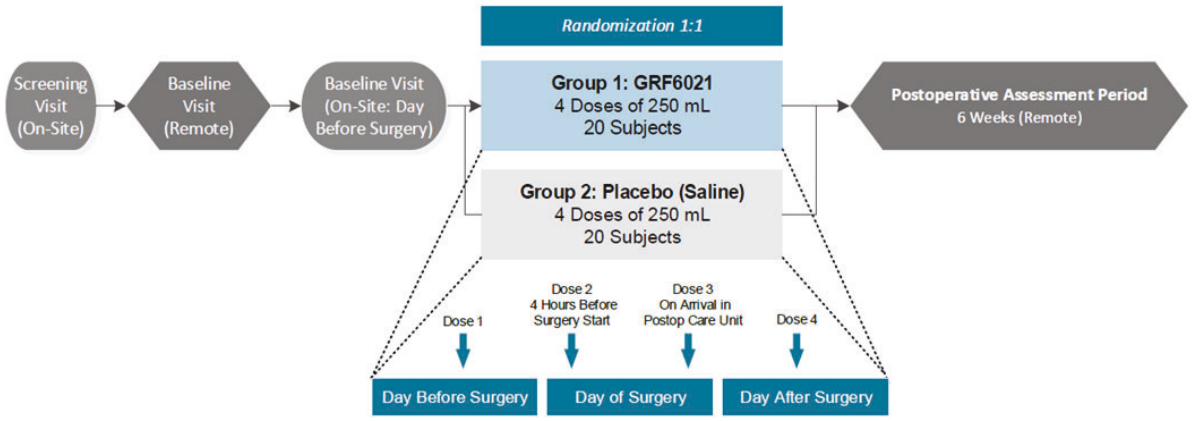
1) Visit 1 Screening (Day -42 to -3).
2) Visit 2 Baseline (Days -7 to -3): Subjects will be contacted by phone to obtain the remote Baseline Visit assessments. Subjects will be randomized after eligibility has been confirmed.
3) Visits 3 through 5, Treatment (Days 1-3): on-site Baseline assessments will 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.
4) Visits 6 through 14 (Day 4 through Day 37±2): these are the follow-up visits, which will be conducted remotely by phone and/or electronically.
5) Visit 15, EOS (Day 44±2): the EOS Visit will be conducted remotely by phone and/or electronically.
6) 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 EOS Visit (Visit 15) and complete the exit lab panel if the subject is still at the site and available and willing.

Table 1. AKST6021-211 Study Visits

	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

Figure 1. Study Schematic



8 STATISTICAL METHODOLOGY

8.1 General Considerations

This section of the SSAP presents the analytical approaches that are anticipated for the high-dimensional data from AKST6021-211. Analysis of data from the clinical secondary endpoints from AKST6021-211 are detailed in the SAP (08OCT2020). These analytical approaches to the high-dimensional data may at times require modifications due to unanticipated features. Deviations from analyses summarized in this document will be noted in the Clinical Study Report (CSR).

All analysis dataset preparations and statistical analyses will be performed using R version 4.0.2 or higher.

The high-dimensional data results from AKST6021-211 will be analyzed using the immunological elastic net (iEN) algorithm for the primary endpoint and standard elastic net (EN) modeling for the ActiGraph and proteomics data. Models utilizing iEN or EN utilize a penalized regression method particularly adapted to the analysis of highly-correlated data, as it eliminates redundant parameters while retaining interrelated parameters (Zhou 2005, Culos 2020). All parameters will be set to default except for a = 0.5 (to limit the number of selected features, but account for the most important components of each intracorrelated module) and standardized as equal to FALSE to enable the specific modifications described in Section 8.4 Efficacy Analyses. A two-layer leave-one-out cross-validation strategy will be employed where the final model will have optimized the segregation of active versus placebo treatment groups. The internal layer will optimize the free parameters of the model, and the external layer will evaluate model performance. Data presentations will include a flow diagram of the progress of the 2 groups (GRF6021 and placebo) through enrollment, intervention allocation, follow-up, and data analysis. Additional multi-part visualizations (figures) for the primary endpoint (CyTOF) and select secondary endpoints (ActiGraph, proteomics) will be developed (see Appendix 1).

Unless otherwise specified, an alpha of 0.05 will be used for significance testing. For multiple comparisons due to the high dimensionality, the false discovery rate using the Benjamini-Hochberg method will be controlled and the corrected p-values will be reported as q-values.

8.2 Study Populations for Analysis

- Evaluable set: Subjects who received all 4 doses and in whom all blood samples were collected.
- Per Protocol set (PP): A subset of evaluable subjects who have completed all 4 doses of treatment and evaluations through Visit 5 and who follow the protocol without any major deviation(s). A subject who received Decadron will be excluded.

8.3 Methods for Handling Missing Data

In order to minimize missing data, every effort will be made to obtain required data for all randomized subjects at each scheduled evaluation. However, in the event that more than 5% of data are missing, imputation methods will be used for the analysis of the primary endpoint only.

8.4 Efficacy Analyses

8.4.1 Primary Endpoint

Determination of functional responses in precisely phenotyped cell subsets may be obtained utilizing CyTOF, a highly parameterized single-cell based method. Thus, CyTOF can comprehensively characterize phenotypic and functional alterations of the human immune system as they occur in vivo.

Serial blood samples collected at baseline and all 3 perioperative time points (Visits 3, 4a, 4b, and 5) will be processed using a standardized protocol for fixation (Smart Tube, San Carlos, CA), storage, and antibody (Ab) staining of whole-blood samples for CyTOF (Gaudillière 2014, Fragiadakis 2015, Gaudillière 2015). At each point, samples will be either left unstimulated (for quantification of cell frequency and endogenous intracellular activities) or stimulated with a series of extracellular ligands (for analysis of evoked intracellular signaling responses). Cell frequency and signaling activities will be quantified in all major innate and adaptive immune cell subsets (Table 2 [left]). In addition, the intracellular expression of specific signaling markers will be simultaneously quantified per single cell (Table 2 [right]).

Table 2. Cell Frequency and Signaling Activities and Intracellular Expression of Signaling Markers per Single Cell to be Quantified

Cell Frequency and Signaling Activities	Intracellular Expression of Signaling Markers per Single Cell
1. Neutrophils	1. Phosphorylated-(p) signal transducer and activator of transcription 1 (STAT1)
2. Cluster of differentiation 27 (CD27)+ memory B (Bmem) cells	2. p(STAT3)
3. CD27- naïve B (Bnaive) cells	3. pSTAT5
4. CD56hiCD16-Natural Killer (NK) cells	4. pSTAT6
5. CD56loCD16+ NK cells	5. p nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)
6. CD4+CD45RA- T cells (CD4+ Tmem)	6. p mitogen-activated protein kinase 2 (MAPK2)
7. CD4+CD45RA+ T cells (CD4+ Tnaive)	7. pP38
8. CD4+Tbet+CD45RA-T cells (Th1)	8. prpS6
9. CD4+Tbet+CD45RA+ T cells	9. p extracellular regulated kinase (ERK)1/2
10. CD25+FoxP3+CD4+ T cells (Tregs)	10. p cyclic AMP response element-binding protein (CREB)
11. CD8+CD45RA- T cells (CD8+ Tmem)	11. total IκBα
12. CD8+CD45RA+ T cells (CD8+ Tnaive)	
13. CD8+Tbet+CD45RA- T cells	
14. CD8+Tbet+CD45RA+T cells	
15. TCRγδ T cells	
16. CD14+CD16- classical monocytes (cMCs)	
17. CD14-CD16+ non-classical monocytes (ncMCs)	
18. CD14+CD16+ intermediate monocytes (intMCs)	
19. Monocytic myeloid-derived suppressor cells (M-MDSCs)	
20. Myeloid dendritic cells (mDCs), and	
21. Plasmacytoid dendritic cells (pDCs)	

Measurement of CyTOF will be performed by Dr. [redacted] and Dr. [redacted] ([redacted]). Two (2) CyTOF datasets will be generated and analyzed separately:

- *CyTOF Dataset 1* (Effect on endogenous immune responses to surgery): Changes in cell frequency and endogenous intracellular signaling activities from Visit 3 to Visits 4b and 5.
- *CyTOF Dataset 2* (effect on immune response to *ex vivo* stimulation): Changes in cell frequency and evoked intracellular signaling responses to *ex vivo* stimulations from Visit 3 to Visit 4a.

Cell frequencies will be expressed as percentages of gated singlets in the case of granulocytes, and as percentages of mononuclear cells in the case of all other cell types. For each cell type,

frequency features will be calculated as the difference in cell frequency between Visit 3 and Visit 4b or Visit 5 (Dataset 1) or Visit 3 and Visit 4a (Dataset 2).

For each cell type, basal signaling activities will be expressed as the median signal intensity (arcsinh transformed value) of each signaling protein.

Endogenous signaling changes in response to surgery will be expressed as the difference in median signal intensity (hyperbolic arcsine [arcsinh] ratio) between post-operative time points (Visit 4b, Visit 5) and the day of surgery, preoperative time point (Visit 4a).

Evoked signaling responses to stimulations will be expressed as the difference in median signal intensity (arcsinh ratio) between the stimulated and unstimulated condition. Changes in evoked signaling responses will be expressed as differences in evoked signaling responses between the Visit 3 (baseline) time point and the Visit 4a time point.

The high-dimensional data results will be further analyzed and presented using the iEN algorithm which is a penalized regression method particularly adapted to the analysis of highly-correlated data, as it eliminates redundant parameters while retaining interrelated parameters (for further details see Section 8.1 General Considerations). Data presentations will include multi-part visualizations (figures) (see Appendix 1).

8.4.2 Select Secondary Endpoints (High-Dimensional Data)

8.4.2.1 Change in Functional Status using the ActiGraph Wearable Device

The ActiGraph wearable device is used to monitor subjects' physical activity, mobility and sleep (ActiGraph 2021). Functional status including heart rate and sleep time will be collected during the whole study period except perioperative times. Changes in functional statuses comparing to the baseline will be calculated. The complete list of 62 ActiGraph measurements can be found in the ActiLife 6 User's Manual (ActiGraph 2012).

The ActiGraph high-dimensional data will be analyzed and presented using the EN algorithm which is a penalized regression method particularly adapted to the analysis of highly-correlated data, as it eliminates redundant parameters while retaining interrelated parameters (for further details see Section 8.1 General Considerations). Data presentations will include multi-part visualizations (figures) (see Appendix 1).

8.4.2.2 Effects of GRF6021 on Proteomics

Serial blood samples collected at baseline and all 3 perioperative time points (Visits 3, 4a, 4b, and 5) will be analyzed by the Somalogic SomaScan platform (Somalogic, Boulder, CO, USA), which provides a broad overview of proteomics changes by measuring approximately 7,000 proteins. It uses a highly multiplexed and high throughput aptamer based proteomic technology and deoxyribonucleic acid (DNA)-microarray-based detection. It provides concentration domain values as relative fluorescence units (RFUs). Details of sample collection and processing were included in the Laboratory Manual.

The proteomic high-dimensional data will be analyzed and presented using the EN algorithm which is a penalized regression method particularly adapted to the analysis of highly-correlated data, as it eliminates redundant parameters while retaining interrelated parameters (for further

details see Section 8.1 General Considerations). Data presentations will include multi-part visualizations (figures) (see Appendix 1).

CyTOF data and proteomics data may be combined for improving EN model performance. Additional exploratory analyses of CyTOF and proteomics data will be described in subsequent analysis plans.

9 REFERENCE LIST

ActiGraph Corporation. Medical Grade Activity Monitoring. Available at: CentrePoint Insight Watch | ActiGraph (actigraphcorp.com). Accessed May 26, 2021

ActiGraph Software Department. ActiLife 6 User's Manual; Release date: 04/03/2012. Available at: SFT12DOC13 - ActiLife 6 Users Manual (Rev A).pdf (theactigraph.com). Accessed May 26, 2021.

Aghaeepour N, Kin C, Ganio EA, Jenson KP, Gaudillière D, Tingle M, et al. Deep immune profiling of an arginine-enriched nutritional intervention in patients undergoing surgery. J Immunol. 2017;l1700421:1-10.

Ayers DC, Franklin PD, Trief PM, Ploutz-Snyder R, Freund D. Psychological attributes of preoperative total joint replacement patients: Implications for optimal physical outcome. J Arthroplasty. 2004;19:125-130.

Culos A, Tsai AS, Stanley N, Becker M, Ghaemi MS, McIlwain DR, et al. Integration of mechanistic immunological knowledge into a machine learning pipeline improves predictions. Nat Mach Intell. 2020; 2:619-628.

Davis KD, Aghaeepour N, Ahn AH, Angst MS, Borsook D, Brenton A, et al. Discovery and validation of biomarkers to aid the development of safe and effective pain therapeutics: challenges and opportunities. Nat Rev Neurol. 2020;16:381-400.

Deak T. A multispecies approach for understanding neuroimmune mechanisms of stress. Dialogues Clin Neurosci. 2017;19:37-53.

Edwards RR, Kronfli T, Haythornthwaite JA, Smith MT, Mcguire L, Page GG. Association of catastrophizing with interleukin-6 responses to acute pain. Pain. 2008;140:135-144.

Faller H, Kirschner S, König A. Psychological distress predicts functional outcomes at three and twelve months after total knee arthroplasty. Gen Hosp Psychiatry. 2003;25:372-373.

Fragiadakis GK, Gaudillière B, Ganio EA, Aghaeepour N, Tingle M, Nolan G, et al. Patient-specific immune states before surgery are strong correlates of surgical recovery. Anesthesiology. 2015;123:1241-1255.

Gaudillière B, Fragiadakis GK, Bruggner R V, Nicolau M, Finck R, Tingle M, et al. Clinical recovery from surgery correlates with single-cell immune signatures. Sci Transl Med. 2014;6:1-12.

Gaudillière B, Ganio EA, Tingle M, Lancero HL, Fragiadakis GK, Baca QJ, et al. Implementing mass cytometry at the bedside to study the immunological basis of human diseases: distinctive

immune features in patients with a history of term or preterm birth. Cytometry A. 2015;87:817-29.

Judge A, Arden NK, Cooper C, Javaid MK, Carr AJ, Field RE, et al. Predictors of outcomes of total knee replacement surgery. Rheumatology. 2012;51:1804-1813.

Kehlet H, Dahl JB. Anaesthesia, surgery, and challenges in postoperative recovery. Lancet. 2003;362:1921-1928.

Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg Am. 2007;89:780-785.

Lingard EA, Riddle DL. Impact of psychological distress on pain and function following knee arthroplasty. J Bone Joint Surg Am. 2007;89(6):1161-1169.

Martinez-Cano JP, Herrera-Escobar JP, Arango Gutierrez AS, Sanchez Vergel A, Martinez-Rondanelli A. Prospective quality of life assessment after hip and knee arthroplasty: short- and mid-term follow-up results. Arthroplast Today. 2017;3:125-130.

Mayer M, Naylor J, Harris I, Badge H, Adie S, Mills K, et al. Evidence base and practice variation in acute care processes for knee and hip arthroplasty surgeries. PLoS ONE 2017;12(7):e0180090.

Stoecklein VM, Osuka A, Lederer JA. Trauma equals danger—damage control by the immune system. J Leukoc Biol. 2012;92:539-551.

Witvrouw E, Pattyn E, Almqvist KF, Crombez G, Accoe C, Cambier D, et al. Catastrophic thinking about pain as a predictor of length of hospital stay after total knee arthroplasty: A prospective study. Knee Surg Sports Traumatol Arthrosc. 2009;17:1189-1194.

Zhou H, Hastie T. Regularization and variable selection via the elastic net. J Roy Stat Soc. 2005;67:301-320.

10 SUMMARY OF CHANGES

Not applicable as this is the initial version.

11 APPENDICES

11.1 Appendix 1: Figure Descriptions/Shells

Figure 14.1.1: Subject Disposition (CONSORT Diagram)

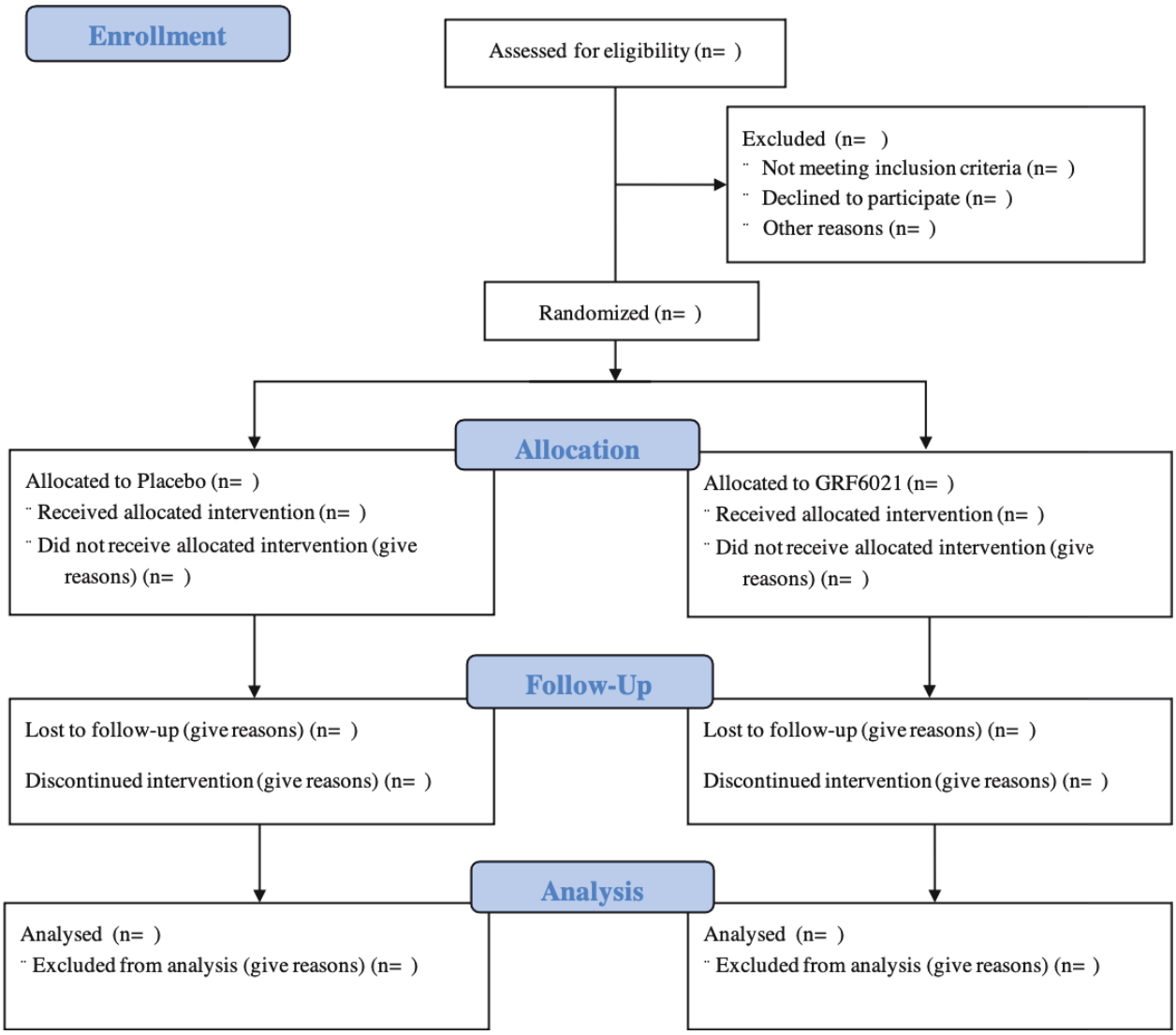
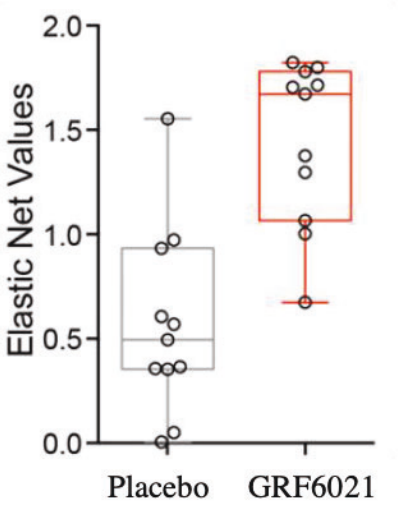


Figure 14.2.1: Boxplot Comparing the Elastic Net Values from a Cross-validated Model of CyTOF Features Between the Active GRF6021 Group and Placebo Group



Note: The above figure is for illustration purposes only. The figure does not represent the data of the study. Colors and symbols may change. Axis titles or values may also change.

In figure 14.2.1, y-axis is the calculated Elastic Net Values from cross-validated model of CyTOF features.

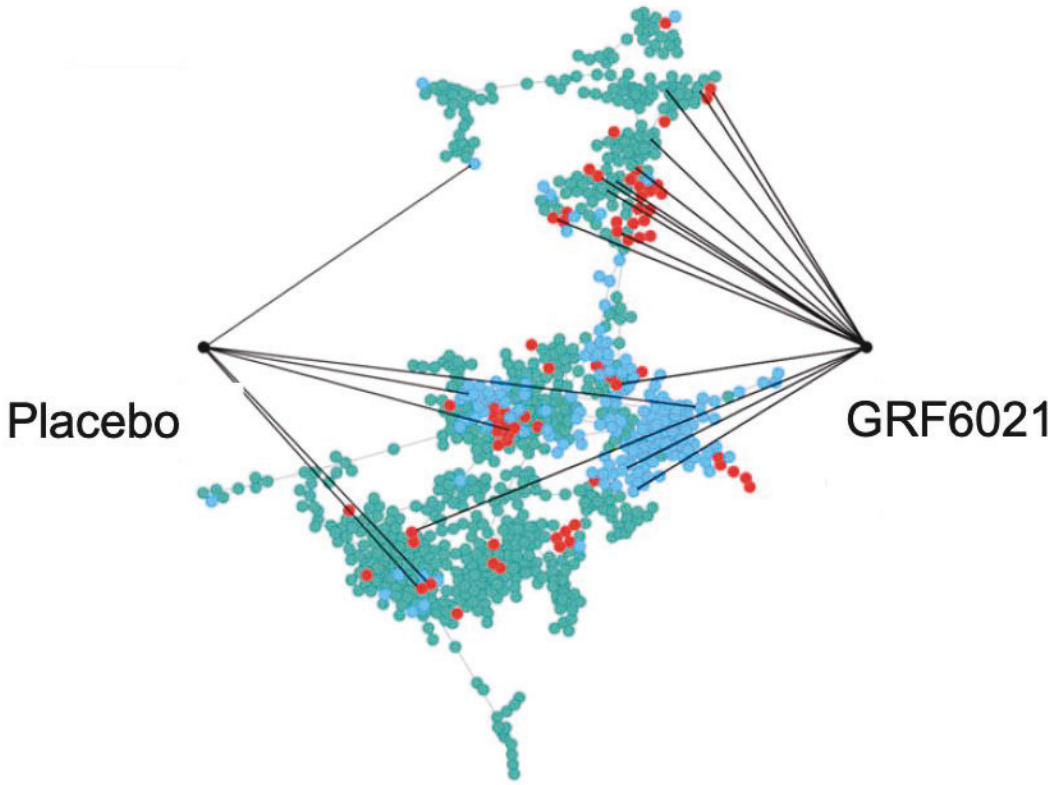
Similar figures:

Figure 14.2.2: Boxplot Comparing the Elastic Net Values from a Cross-validated Model of ActiGraph Features between the Active GRF6021 Group and the Placebo Group

Figure 14.2.3: Boxplot Comparing the Elastic Net Values from a Cross-validated Model of Proteomics Features (SomaScan platform) Between the Active GRF6021 Group and the Placebo Group

Figure 14.2.4: Boxplot Comparing the Elastic Net Values from a Cross-validated Model of Combined CyTOF Features and Proteomics Features (SomaScan platform) Between the Active GRF6021 Group and the Placebo Group

Figure 14.2.5: Correlation Network of CyTOF Features Represented by a Minimum Spanning Tree



Note: The above figure is for illustration purposes only. The figure does not represent the data of the study. Colors and symbols may change. Axis titles or values may also change.

In Figure 14.2.5, each dot represented significantly associated features (after Bonferroni adjustment), different color indicated different feature types, such as cell frequency features and cell signaling features. Lines pointed the increased immune features in the GRF6021 group or in the placebo group.

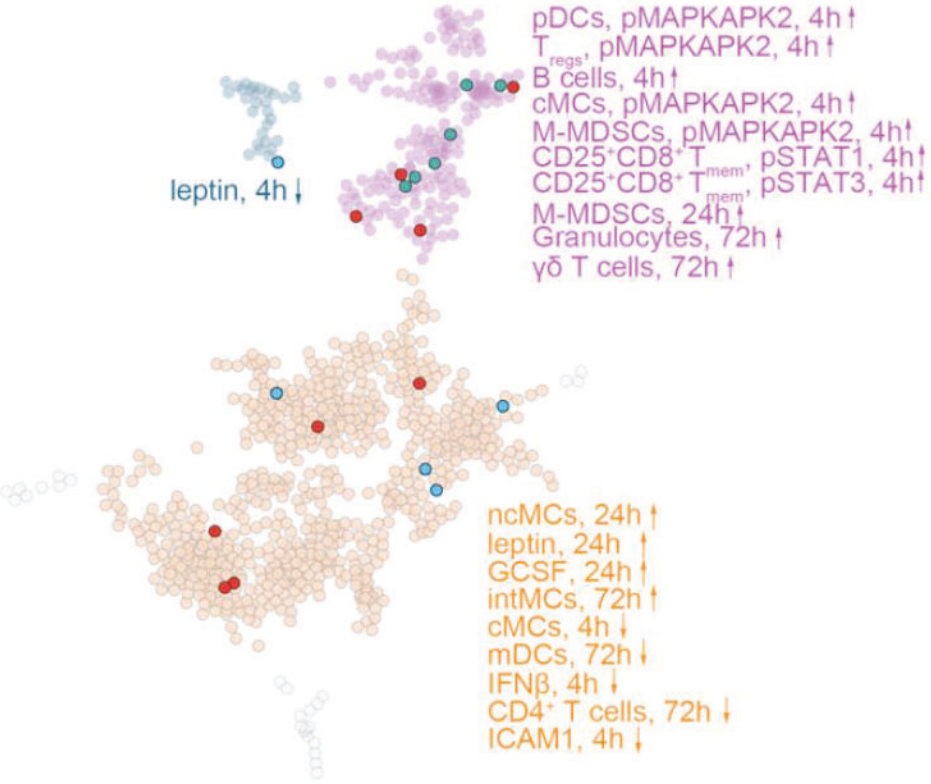
Similar figures:

Figure 14.2.6: Correlation Network of ActiGraph Features Represented by a Minimum Spanning Tree

Figure 14.2.7: Correlation Network of Proteomics Features Represented by a Minimum Spanning Tree

Figure 14.2.8: Correlation Network of CyTOF and Proteomics Features Represented by a Minimum Spanning Tree from Combined CyTOF and Proteomics Data

Figure 14.2.9: Identified CyTOF Features Showing on the Correlation Network Based on Elastic Net Regression Models



Note: The above figure is for illustration purposes only. The figure does not represent the data of the study. Colors and symbols may change. Axis titles or values may also change.

In Figure 14.2.9, identified CyTOF immune features from Elastic Net were listed and mapped on the correlation network, up-arrow indicated up-regulation in the GRF6021 group and down-arrow indicated down-regulation in the GRF6021 group.

Similar figures:

Figure 14.2.10: Identified ActiGraph Features Showing on the Correlation Network Based on Elastic Net Regression Models

Figure 14.2.11: Identified Proteomics Features Showing on the Correlation Network Based on Elastic Net Regression Models

Figure 14.2.12: Identified CyTOF and Proteomics Features Showing on the Correlation Network Based on Elastic Net Regression Models from Combined CyTOF and Proteomics Data