STATISTICAL ANALYSIS PLAN

Protocol Title (Number):

Viable Allograft Supplemented Disc Regeneration in the Treatment of Patients with Low Back Pain with or without intervertebral Disc Herniation (VAST Trial)

VAST-001-017

Sponsor: Vivex Biologics, Inc.

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1 ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event
BBA	Boston Biomedical Associates
CRF	Case Report Forms
ITT	Intent-To-Treat Population
LOCF	Last Observation Carried Forward
MITT	Modified Intent-To-Treat Population
MRI	Magnetic Resonance Imaging
ODI	Oswestry Disability Index
PP	Per-Protocol Population
PT	Preferred Term
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOC	System Organ Class
SF-36	Short Form 36
VASPI	Visual Analogue Scale of Pain Intensity

2 SUMMARY

TITLE	Viable Allograft Supplemented Disc Regeneration in the Treatment of Patients with Low Back Pain with or without Intervertebral Disc Herniation
PREFACE	This Statistical Analysis Plan (SAP) describes the planned analysis and reporting For Vivex Biologics, Inc protocol VAST-001-017 (Viable Allograft Supplemented Disc Regeneration in the Treatment of Patients with Low Back Pain with or without Intervertebral Disc Herniation). This study is being completed to assess the safety and efficacy of viable allograft transplantation for the treatment of patients with symptomatic disc degeneration and tissue loss. The following documents were reviewed in preparation of this SAP: • Clinical Research Protocol VAST-001-017, issued 11MAY2018. • Case report forms (CRFs) issued 16APR2018 for Protocol VAST-001-017.
PURPOSE	The purpose of this SAP is to outline the planned analyses in support of the Clinical Study Report (CSR) for protocol VAST-001-017. Exploratory analyses not necessarily identified in this SAP may be performed to support the clinical development program. Any post-hoc, or unplanned, analyses not identified in this SAP will be clearly identified in the respective CSR.
STUDY OBJECTIVES	To evaluate the safety and efficacy of viable allograft transplantation for the treatment of patients with symptomatic disc degeneration and tissue loss.
STUDY DESIGN	A multi-center, prospective, randomized, parallel-arm study composed of a screening phase and an active phase. In the screening phase subjects will be assessed for study eligibility via X-ray and MRI and baseline SF-36, Oswestry Disability Index (ODI), and Visual Analogue Scale of Pain Intensity (VASPI) will be established. In the active phase, subjects who meet eligibility in the screening phase and meet Active Phase entry criteria will be randomized to receive viable allograft, placebo as saline, or be assigned to continue conservative care treatment. All subjects will return at 6 and 12 months for efficacy and safety assessments. Subjects randomized to the conservative care arm will also be evaluated at 3 months, during which they are eligible to receive viable allograft. The first 24 subjects randomized (at least 4 in each treatment group) will have an additional study visit at 1 month after treatment. All data from the 1-month study visit will be reviewed by the Steering Committee to confirm enrollment may continue.
ENDPOINTS	The study's **co-primary endpoints** are: • Improvement in the ODI at 12 months after treatment • Improvement in the VASPI at 12 months after treatment The study's **secondary endpoints** are: • Improvement in the ODI and VASPI at 6 months after treatment • Improvement in SF-36 at 6 and 12 months after treatment • MRI measurements at 6 and 12 months after treatment • Adverse Event (AE)/ Serious Adverse Events (SAE) rates after treatment • Hospitalization rate at 12 months after treatment • Re-operation rate at 12 months after treatment • Resource utilization following treatment at 6 and 12 months after treatment

INTERIM ANALYSES	For safety and efficacy reasons, interim analyses are planned to be performed after the first 24 subjects have completed the Month 1 assessment of the Active Phase. This analysis will be performed in a descriptive manner only and no un-blinding will occur unless a safety signal is evident. A Steering Committee will be convened to review the results of the interim analysis. A similar review will occur when they complete the Month 6 assessment of the Active Phase, where primary endpoints of pain and ODI, and randomized MRI will be available for analysis.
FINAL ANALYSES	All final analyses identified in this SAP will be completed after the last subject has completed the 12-month follow-up visit. The co-primary endpoints will be tested using the Kruskal-Wallis test to compare across the three treatment groups at a two-sided α=0.05 level. If the overall result is significant, the Dwass, Steel, Critchlow-Fligner method will be used to assess pairwise comparisons, while controlling for the overall familywise error level. Both of the co-primary endpoints need to meet statistical significance for the trial to be considered a success. Secondary outcomes will be analyzed in an exploratory way at a two-sided α=0.05 level, without controlling for multiple testing.

3 STUDY OBJECTIVES AND ENDPOINTS

3.1 STUDY OBJECTIVE

The objective of this study is to evaluate the safety and efficacy of viable allograft transplantation for the treatment of patients with symptomatic disc degeneration and tissue loss.

3.2 STUDY ENDPOINTS

3.2.1 CO-PRIMARY ENDPOINTS

The study's **co-primary endpoints** are:

- Improvement in the ODI at 12 months after treatment
- Improvement in the VASPI at 12 months after treatment

3.2.2 SECONDARY ENDPOINTS

The study's **secondary endpoints** are:

- Improvement in ODI and VASPI at 6 months after treatment
- Improvement in the SF-36 at 6 and 12 months after treatment
- MRI measurements at 6 and 12 months after treatment
- Adverse Event (AE)/ Serious Adverse Events (SAE) rates after treatment
- Hospitalization rate at 12 months after treatment
- Re-operation rate at 12 months after treatment
- Resource utilization following treatment at 6 and 12 months after treatment
- Changes in laboratory tests

4 SAMPLE SIZE

The primary criteria will be analyzed using Wilcoxon rank-sum-test. Based on Fairbanks, 2000[1], Hudson-Cook, 1989[2] and Fritz, 2001[3] a common standard deviation of $\sigma = 15.5$ score points can be assumed.

Fritz, 2001, calculated a Minimum Clinically Important Difference of 6 score points. Reliability is reported in the literature in the range of 0.75 - 0.91. Worst case, using the standard deviation above results in a measurement error of 7.75 score points. Thus, we assume a clinically relevant difference of 9 score points. Under this assumption, the probability $p(x < y) = 0.341$ (x, y observations in the groups, respectively). A sample size of 40 in each group will have 80 % power to detect with a probability of 0.341 that an observation in one group is less than an observation in the other group using Wilcoxon rank-sum-test with a 0.05 two sided significance level (calculations were done using nquery 4.0).

Approximately 220 subjects randomized to viable allograft or placebo or control in 3.5:1:1 ratio:

Active Allograft = 140
Placebo = 40
Conservative Care = 40

5 SEQUENCE OF PLANNED ANALYSES

5.1 INTERIM ANALYSES

For safety and efficacy reasons, interim analyses are planned to be performed after the first 24 subjects have completed the Month 1 assessment of the Active Phase. This analysis will be performed in a descriptive manner only and no un-blinding will occur unless a safety signal is evident. A Steering Committee will be convened to review the results of the interim analysis. A similar review will occur when they complete the Month 6 assessment of the Active Phase, where primary endpoints of pain and ODI, as well as randomized MRI will be available for analysis.

There are no other planned interim analyses for this study.

5.2 FINAL ANALYSES AND REPORTING

All final, planned, analyses identified in the protocol and in this SAP will be performed only after the last subject has completed the 12-month visit after treatment. Key statistics and study results will be made available following database lock. Any post-hoc, exploratory analyses completed to support planned study analyses, which were not identified in this SAP, will be documented and reported as necessary. Any results from these unplanned analyses will also be clearly identified as post-hoc analyses.

6 ANALYSIS POPULATIONS

6.1 INTENT TO TREAT POPULATION (ITT)

The intent-to-treat (ITT) population for this study includes all enrolled subjects. Subjects are considered enrolled in the trial after they have signed the informed consent form, all screening procedures were

performed, and eligibility for the study was confirmed. Subjects are analyzed under the treatment to which they were randomized.

6.2 Modified Intent to Treat Population (mITT)

The modified intent-to-treat population (mITT) includes all ITT subjects who underwent treatment and had at least one valid post-treatment assessment of the ODI and VASPI. This mITT population is the primary analysis population for efficacy analyses. Subjects are analyzed under the treatment to which they were randomized.

6.3 Per-Protocol Population (PP)

The per-protocol population (PP) will include all mITT subjects who do not have a major protocol violation as described in section 7.4.

6.4 Safety Population

The safety population includes all ITT subjects who underwent treatment. The safety population is the primary population for analyses of adverse events. Subjects are analyzed according to the treatment which they received, regardless of initial randomization.

7 General Issues for Statistical Analysis

Data will be presented using descriptive statistics. Categorical data will be presented using frequencies and percent of subjects in each category. Continuous data will be presented using mean, standard deviation, median, minimum, maximum, and sample size.

Results will be generated by treatment group.

7.1 Analysis Software

Analysis data sets, statistical analyses and associated output generated by Boston Biomedical Associates will be generated using SAS® Software version 9.4 or later.

7.2 Disposition of Subjects and Withdrawals

A CONSORT diagram will be presented based on the ITT population. All subjects who provide written informed consent will be accounted for. The frequency of subjects who completed each scheduled assessment will be presented. The number and percentage of ITT subjects prematurely withdrawing will be presented overall and by reason of discontinuation in a table.

7.3 Methods for Withdrawals and Missing Data

The primary endpoint analyses will be analyzed using all available data in the mITT population. An additional sensitivity analysis will be performed for the primary endpoints using last observation carried forward (LOCF) in the mITT population.

7.4 Protocol Violations

All protocol violations will be summarized in the ITT population, including the frequency and percent of subjects with each violation type. Prior to database lock, all protocol violations will be reviewed and subjects who had major violations will be noted and excluded from the per-protocol population.

7.5 Multiple Comparisons and Multiplicity

For the trial to be considered a success, both co-primary outcomes, the ODI and the VAS, must be statistically significant at an overall two-sided α-level of 0.05. Additionally, for each primary outcome, if the Kruskal-Wallis test is significant, pairwise comparisons will be analyzed using the Dwass, Steel, Critchlow-Fligner method, which controls the overall family-wise error rate.

No adjustments for multiple testing will occur for secondary endpoints, as the intention of these analyses is exploratory in nature.

8 Demographics and Other Baseline Characteristics

8.1 Demographics

Baseline demographics and subject characteristics will be summarized in the ITT and mITT populations including, but not limited to, age, gender, race, ethnicity, smoking history, history of endocrine or metabolic disorders, level of treatment (1 vs. 2), and number of years experiencing back pain.

8.2 Baseline Neurological Exam

Baseline neurological exam will be presented in the mITT population. Motor function, reflex exam, and sensory exam will be summarized by the left and right side.

8.3 Baseline vital signs

Baseline vital signs will be summarized in the mITT population including height, weight, body mass index, blood pressure, pulse rate, and temperature.

8.4 Baseline Physical Exam

Baseline physical exam will be summarized in the mITT population by frequency and percent of subjects in each category that are categorized as normal or abnormal. An additional listing will be provided for any abnormal physical exam findings.

8.5 Baseline Labs

Baseline hematology, clinical chemistry and coagulation labs will be summarized in the mITT population.

9 Efficacy Analyses

The co-primary efficacy variables will be analyzed in the mITT population (primary) and repeated in the PP population (sensitivity). The secondary variables will be analyzed in the mITT and PP populations.

9.1 Co-Primary Efficacy Endpoints

The co-primary endpoints are the change in ODI and VASPI at 12 months after treatment.

The hypothesis for the ODI is as follows:

H_0: $\tilde{x}_1 = \tilde{x}_2 = \tilde{x}_3$
H_a: at least one median is not equal

Where $\tilde{x}_1$ is the median pre-post difference in the active allograft group, $\tilde{x}_2$ is the median pre-post difference in the placebo group and $\tilde{x}_3$ is the median pre-post difference in the conservative care group.

The null hypothesis (H_0) for this endpoint states that there is no difference in change in ODI at 12 months between the three treatment groups (1) Active Allograft, (2) Placebo, and (3) Conservative Care. The alternative hypothesis (H_a) is that there is a difference in change in ODI at 12 months between the three treatment groups.

The hypothesis for the VASPI is as follows:

H_0: $\tilde{x}_1 = \tilde{x}_2 = \tilde{x}_3$
H_a: at least one median is not equal

Where $\tilde{x}_1$ is the median pre-post difference in the active allograft group, $\tilde{x}_2$ is the median pre-post difference in the placebo group and $\tilde{x}_3$ is the median pre-post difference in the conservative care group.

The null hypothesis (H_0) for this endpoint states that there is no difference in change in VASPI at 12 months between the three treatment groups (1) Active Allograft, (2) Placebo, and (3) Conservative Care. The alternative hypothesis (H_a) is that there is a difference in change in VASPI at 12 months between the three treatment groups.

For both the ODI and the VASPI, the pre-post difference of the groups will be compared using the Kruskal-Wallis test at a two-sided α-level of 0.05. If the result for either test is significant, then the Dwass, Steel, Critchlow-Fligner Method will be used to assess all pairwise comparisons for that endpoint.

Both the ODI and the VASPI need to be statistically significant ($p<0.05$) for the trial to be a success.

9.2 Secondary Endpoints

All secondary variables will be analyzed in an exploratory way, with no adjustment for multiple testing. The secondary endpoints include:

- Improvement in ODI and VASPI at 6 months
- Improvement in ODI, SF-36, and VASPI at 6 and 12 months after treatment
- MRI measurements at 6 and 12 months after treatment
- Adverse Event (AE)/ Serious Adverse Events (SAE) rates after treatment
- Hospitalization rate at 12 months after treatment
- Re-operation rate at 12 months after treatment
- Resource utilization following treatment at 6 and 12 months after treatment
- Changes in laboratory tests

Improvement in ODI, VASPI, and SF-36 will be analyzed similar to the primary endpoint, using the Kruskal-Wallis test to test for an overall difference between the three treatment groups. If the overall test is significant, the Dwass, Steel, Critchlow-Fligner Method will be used to asses all pairwise comparisons.

Adverse event rates, serious adverse events rates, hospitalization rates, and re-operation rate will be summarized by treatment group and compared using the chi-square test of independence or Fisher's exact test, as appropriate.

MRI data will be summarized by treatment group as mean at each timepoint, and mean change from baseline. Analyses will be done focusing on the level treated, but additional analyses will be completed which examine all five levels to see if there are adjacent level effects. Group comparisons will be made using the Krusal-Wallis test. If the overall test is significant, the Dwass, Steel, Critchlow-Fligner Method will be used to assess the pairwise comparisons.

Resource utilization and changes in laboratory tests will be summarized by treatment group.

10 Adverse Events

All adverse events will be coded using the standardized Medical Dictionary for Regulatory Activities (MedDRA) central coding dictionary, version 22.1 or greater.

10.1 All Adverse Events

A summary of incidence rates of individual AEs by System Organ Class (SOC) and Preferred Term (PT) will be prepared. Because a subject may experience more than one AE, summaries will provide both the number of subjects experiencing at least one event and the number of events. Percentages provided will be the percent of subjects experiencing one or more adverse events.

A listing of all adverse events will be provided which includes subject number, AE number, days since procedure, AE SOC and PT, severity of AE, whether or not the AE is classified as serious (SAE), the relationship to investigational device, relationship to procedure, relationship to other etiology, the action taken, and the outcome.

10.2 Adverse Events Leading to Withdrawal

A summary of incidence rates of individual AEs leading to withdrawal by SOC and PT will be prepared. A data listing of AEs leading to withdrawal will also be provided, displaying details of the event(s) captured on the CRF.

10.3 Serious Adverse Events

A summary of incidence rates of individual SAEs by SOC and PT will be prepared. Because a subject may experience more than one SAE, summaries will provide both the number of subjects experiencing at least one event and the number of events. Percentages provided will be the percent of subjects experiencing one or more adverse events.

A listing of all serious adverse events will be provided which includes subject number, AE number, days since procedure, AE SOC and PT, severity of AE, whether or not the AE is classified as serious (SAE),

the relationship to investigational device, relationship to procedure, relationship to other etiology, the action taken, and the outcome.

10.4 Treatment or Procedure Related Adverse Events

A summary of incidence rates of individual treatment or procedure related AEs by SOC and PT will be prepared. Because a subject may experience more than one AE, summaries will provide both the number of subjects experiencing at least one event and the number of events. Percentages provided will be the percent of subjects experiencing one or more adverse events.

A listing of all treatment or procedure related adverse events will be provided which includes subject number, AE number, days since procedure, AE SOC and PT, severity of AE, whether or not the AE is classified as serious (SAE), the relationship to investigational device, relationship to procedure, relationship to other etiology, the action taken, and the outcome.

10.5 Deaths

Should any subjects die during the course of the VAST trial, relevant information will be supplied in a data listing.

11 Other Planned Analyses

11.1 Primary Analysis According to Treatment Received

A sensitivity analysis will be performed for the primary endpoints according to treatment subjects received, regardless of their initial randomization.

11.2 Responder Analyses

A responder analysis will be performed for both primary endpoints. For the ODI, a subject with at least a 15-point reduction will be considered a responder. For the VAS, a subject with at least a 50% reduction will be considered a responder. The chi-square test of independence will be used to compare the percent of subjects considered a responder in each treatment group.

Additionally, regressions will be used to determine whether any characteristics are associated with responder status.

11.3 Planned Subgroup Analyses

The primary endpoints will be assessed in the following sub-groups:

- Age (< Median vs. ≥ Median)
- Gender (Male vs. Female)
- Level of Treatment (1 vs. 2)

12 Reporting Conventions

All reporting will meet the standards of BBA SOP BS002 and its associated work instructions.

13 CHANGES IN THE PLANNED ANALYSIS

Section	Description	Justification
3.2.1 Co-primary endpoints	The primary endpoint is now a co-primary endpoint of the ODI and VASPI at 12 months.	The protocol inconsistently stated what the primary endpoint would be. It stated the ODI and VASPI at 6 and 12 months, but the primary analyses and powering was for the ODI at 12 months. At the time this SAP was written it was decided it would be a co-primary endpoint and both endpoints would need to be met for the trial to be considered a success.
3.2.2 Secondary endpoints	The ODI and VASPI at 12 months was removed as a secondary endpoint.	The protocol repeated the primary endpoints in the secondary endpoint section. The primary endpoints were removed from the secondary endpoint section.
6 Analysis Populations	The mITT population will be the primary analysis population for efficacy and safety.	The protocol summary stated the main analysis would occur in the mITT population. The body of the protocol stated analyses would occur in the ITT and PP population. At the time the SAP was written it was decided the mITT population would be used for the primary analyses and endpoints would also be run in the PP population.
7.3 Methods for Withdrawals, Missing Data, and Outliers	The primary analysis will be completed using available data. A sensitivity analysis will be performed using LOCF.	The protocol summary did not indicate LOCF, but the body of the protocol noted the use of LOCF. At the time this SAP was written it was decided the primary analysis would occur in those with available data, and LOCF would be utilized in a sensitivity analysis in the mITT population.
9.1 Primary Efficacy Endpoints	The primary efficacy analysis will be in the ODI and the VASPI at 12 months. The Kruskal-Wallis test will be used in place of the Wilcoxon Rank Sum test. If the result is significant, the Dwass, Steel, Critchlow-Fligner Method will be used to assess all pairwise comparisons. A responder analysis that was initially described in the protocol was removed.	The Kruskal-Wallis test is being used in place of the Wilcoxon Rank Sum test as it is the appropriate test for a three-group comparison, where the Wilcoxon Rank Sum test is the equivalent test for a two-group comparison. The addition of the Dwass, Steel, Critchlow-Fligner method for pairwise comparisons is needed to evaluate which comparisons are significantly different. The originally proposed responder analysis was removed since it had the potential for results to be clinically incompatible due to the use of the Bonferroni-Holm procedure (e.g. a difference of 1 could be significant, but a difference of a higher value may not be).
3.2.2 Secondary Endpoints and 9.2 Efficacy Endpoints	X-ray data was removed from the secondary endpoints.	X-ray data was listed with the MRI data in the protocol. X-ray and MRI data was collected, but X-ray data was only used to aid in the interpretation of the MRI data by the core lab. Therefore, there is no x-ray data to analyze.

14 REFERENCES

1. Fairbank JC.; Pynsent PB.: The Oswestry Disability Index. Spine 25 (2000) 2940 - 2953

2. Hudson-Cook N.; Tomes-Nicholson K.; Breen A: A revised Oswestry Disability Questionnaire. Back Pain: New approaches to rehabilitation and education (1989) 187 – 204

3. Fritz JM.; Irrgang JJ.: A Comparison of a Modified Oswestry Low Back Pain Disability Questionnaire and the Quebec Back Pain Disability Scale. Phys Ther 81 (2001) 776 – 788

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