Department	:	Biostatistics and programming
Information Type	:	Statistical Analysis Plan (SAP)

Title	:	Randomized, Double-Blind, Single Center, Phase 2, Efficacy and Safety Study of autologous HB-adMSCs vs Placebo for the Treatment of Patients with Parkinson's Disease
Product	:	HB-adMSCs Hope Biosciences Adipose Derived Mesenchymal Stem Cells
Effective Date	:	29-Apr-2021

Description:

- The purpose of this SAP is to describe the planned analyses and output to be included in the Clinical Study Report for Protocol HBPD03.
- This SAP is intended to describe the planned safety, efficacy and tolerability analyses required for the study.
- This SAP is to convey the content of the complete statistical analysis deliverables.

1. INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to describe the planned analysis to be included in the Clinical Study Report for Protocol HBPD03.

2. SUMMARY OF KEY PROTOCOL INFORMATION

2.1. Study Objective(s) and Endpoint(s)

Objectives	Endpoints
Primary Objective	**Primary Endpoint**
• To investigate the safety and efficacy of intravenous infusions of HB-adMSCs vs Placebo in patients with Parkinson's disease as determined by improvements in Quality of Life, estimated by the MDS UPDRS assessment. (Time frame: Baseline to Weeks 4, 8, 16, 24, 32, 42 and 52). A battery of statistical tests will be applied, where suitable and applicable. Tests will include the report of parametric or non-parametric statistical tests, with the corresponding p-values, presented in pair-wise manner, as well as contingency tables, representing answers in their native form.	• Change from Baseline in Total MDS-UPDRS Part II, only at Week 52 • Number of treatment-emergent Adverse Event (TEAEs) during 52 week period • Number of serious Adverse Events (SAEs) during the period of 52 weeks • Number of AEs of special interest includes thromboembolic events, peripheral events defined as thromboembolism of the extremities, infections and hypersensitivities • Change from baseline in clinical parameters, measured at the end of the study (52 weeks): o Physical examination o Electrocardiogram (ECG) o Pulse o Systolic blood pressure o Diastolic blood pressure o Body weight o BMI • Change from baseline in laboratory assessments at the end of the study (52 weeks):

Objectives	Endpoints
	○ Biochemistry ○ Haematology ○ Urinalysis
Secondary Objectives	**Secondary Endpoints**
• To evaluate the safety and efficacy of intravenous infusions of HB-adMSCs vs Placebo in patients with Parkinson's disease based on changes in disease assessments and dosage of medications. (Time frame: Baseline to Weeks 4, 8, 16, 24, 32, 42 and 52).	• Change from Baseline in MDS-UPDRS Total score, at all the timepoints collected, during the period of study (52 Weeks) • Change from Baseline in Total MDS-UPDRS Part I, at all the timepoints collected during the period of study (52 Weeks) • Change from Baseline in Total MDS-UPDRS Part II, at all the timepoints collected during the period of study (52 Weeks) • Change from Baseline in Total MDS-UPDRS Part III at all the timepoints collected during the period of study (52 Weeks) • Change from Baseline in Total MDS-UPDRS Part IV, at all the timepoints collected during the period of study (52 Weeks) • Change from Baseline in Total Neuro-Quality of Life Assessments scores (Neuro-QOL), at all the timepoints collected during the period of study (52 Weeks) • Change from Baseline in Total Parkinson’s disease fatigue scale (PFS-16), at all the timepoints collected during the period of study (52 Weeks) • Change from Baseline in Total Parkinson’s disease Questionnaire (PDQ-39), at all the timepoints

Objectives	Endpoints
	collected during the period of study (52 Weeks) • Change from baseline in Total VAS score • Change from baseline in Muscle Spasm Scale VAS score at Week 52 • Change from baseline in Pain Scale VAS score at Week 52 • Number of subjects with changes in Daily Dosage of medications taken to treat Parkinson's disease at all the time point during the period of 52 weeks • Proportion of subjects requiring reinstatement of PD medication dose following reduction in dose during the study

2.2. Study Design

Overview of Study Design and Key Features	
Randomized, double-blind, placebo-controlled, parallel group study	
Design Features	• Randomized, double-blind, Placebo controlled, parallel group study. • 24 Subjects • 2 Treatment groups ▪ Group 1 → HB-adMSCs ▪ Group 2 → Placebo • Duration of treatment: up to 32 weeks. The overall study includes screening (up to 4 weeks prior to randomisation), 6 treatment visits after randomisation scheduled according to the following schedule: Infusion 1 (Week 0), Infusion 2 (Week 4), Infusion 3 (Week 8), Infusion 4 (Week 16), Infusion 5 (Week 24) and Infusion 6 (Week 32). After all the treatments were administered to the patients, there will be two follow up visits, the first one the Week of 42, and the second one, at the end of study, at Week 52.
Dosing	• HB-adMSCs (Hope Biosciences adipose derived mesenchymal stem cells) will be administered as an IV infusion with the dose of 200 million cells • Placebo is to be administered as an IV infusion which contains saline solution (0.9%)
Treatment Assignment	• Participants will be randomised in 1:1 ratio, to receive either HB-adMSCs active treatment or Placebo.

2.3. Statistical Hypotheses

The null hypothesis states that there is no significant difference in primary efficacy endpoint, Change from Baseline in the total MDS-UPDRS Part II score, compared to the value at Week 52, between the HB-adMSCs and Placebo treatment groups. The alternative hypothesis is that there is a significant difference between the HB-adMSCs and Placebo treatment groups, meaning that the treatment was effective. The hypothesis will be tested using 5% significance level (two-sided).

All other secondary endpoints are tested as exploratory analysis. The criteria will be the same as for the primary endpoint, 5% significance level, two-sided tests, which is a standard in biology and medicine.

3. PLANNED ANALYSES

3.1. Final Analyses

The final planned primary analyses will be performed after the completion of the following sequential steps:

1. All participants have completed (or withdrawn from) the study as defined in the protocol.
2. All required database cleaning activities have been completed, final database was released, and database lock has been declared by Data Management.

4. ANALYSIS POPULATIONS

Population	Definition / Criteria	Analyses Evaluated
Safety analysis set	• All randomised subjects who received at least one dose of HB-adMSCs infusion or one dose of placebo. • If participants receive a treatment different to their randomized treatment, they will be analysed according to the treatment that was actually received.	• Safety • Study Population
Efficacy analysis set	• All randomized participants who received all 6 infusions of either HB-adMSCs or placebo. • Participants will be analysed according to their randomized treatment.	• Efficacy

5. CONSIDERATIONS FOR DATA ANALYSES AND DATA HANDLING CONVENTIONS

5.1 Study Treatment & Sub-group Display Descriptors

Treatment Group Descriptions		
Protocol treatment arm	**Data Displays for Reporting**	
Treatment Arm	**Treatment Arm**	**Order in TLF**
Arm 1	HB-adMSCs 200MM	1
Arm 2	Placebo	2

Treatment comparisons will be displayed as follows using the descriptors as specified in the statistical analysis:

HB-adMSCs 200MM vs. Placebo

5.2 Baseline Definitions

For all endpoints, the baseline value will be the value obtained at the latest pre-treatment assessment visit (a non-missing value), including those from unscheduled visits. For example, if an assessment has been made both at screening visit (Visit 1) and infusion 1 visit (listed as Visit 2, Week 0), the value from the later, "infusion 1 visit" is used as the baseline value, if the value is collected prior to the admission of infusion. If the value measured at the Week 0 visit is missing or if it was collected on/after the dose and the assessment has been made at screening/unscheduled visits (which is prior to Week 0 visit), then the screening/unscheduled visits value is used as the baseline value.

Unless otherwise stated, if baseline data is missing, no derivation will be performed, and baseline will be set to missing.

5.2.1 Change from Baseline

Definition	Reporting Details
Change from Baseline	= Post-Dose Visit Value – Baseline
% Change from Baseline	= 100 x [(Post-Dose Visit Value – Baseline) / Baseline]

NOTES:

- The baseline will be displayed along with Visit name on all summary displays.

5.3 Examination of Covariates, Other Strata and Subgroups

5.3.1 Covariates and Other Strata

Baseline will be included as a covariate in the efficacy analyses, wherever applicable.

5.3.2 Examination of subgroups

No subgroup analysis is planned.

5.4 Multiple Comparisons and Multiplicity

The multiplicity adjustment will be performed for key efficacy endpoints for the conclusion. The Bonferroni-Holm method is used for the multiplicity adjustment for the hypothesis tests performed for the efficacy endpoints. The multiplicity adjusted for the hypothesis tests for below endpoints,

- Change from Baseline in Total MDS-UPDRS Part I at Week 52
- Change from Baseline in Total MDS-UPDRS Part II at Week 52
- Change from Baseline in Total MDS-UPDRS Part III at Week 52
- Change from Baseline in Total MDS-UPDRS Part IV at Week 52
- Change from Baseline in Total Neuro-Quality of Life Assessments scores (Neuro-QOL) at Week 52
- Change from Baseline in Total Parkinson's disease fatigue scale (PFS-16) at Week 52
- Change from Baseline in Total Parkinson's disease Questionnaire (PDQ-39) at Week 52
- Change from baseline in Total VAS score at Week 52

Using the Bonferroni-Holm method the p-values will be ordered in increasing order and starts with testing the hypothesis with the lowest p-value on the 2.5/k% level (two-sided), where k is the number of hypotheses in the procedure. If significant, the testing proceeds to comparing the next lowest p-value with 2.5/(k-1)%. The testing will stop at the first non-significant result.

Due to high number of performed tests, the additional statistical corrections will be included in order to correct the p-value and avoid false positive results. As the observed phenomena are biological in nature, p-values will be also examined in such manner that the focus will be on the biological meaning. Both methods are providing more rigorous p-values and help avoiding the false positive interpretations.

5.5 Interim analysis

Interim analysis of primary and secondary efficacy data will be performed. Details of the planned displays for interim are provided in **Error! Reference source not found.**. Interim analysis will be conducted when at least 10 subjects from each group have completed Week 24.

For the interim analysis, subject-level data will be unblinded. The unblinded analysis of the interim outputs will be done by unblinded independent statistics and programming team, who have access to interim analysis data. An unblinded statistician, not otherwise associated with the project, will perform the interim analysis, which will include the displays shown in the **Error! Reference source not found.**, with delivery priory labelled as "INTER".

These results will be limited to the members of SPONSOR engaged in the discussion of interim analysis results, and will not be shared with the investigators.

5.6 General Considerations

Unless otherwise stated, all hypotheses will be tested at 0.95% confidence interval (p = 0.05, two-sided test, when applicable). In other words, statistical test will look for the difference (either better results or worse results), rather than focusing solely on efficacy. All continuous measurements will be summarised descriptively at each visit, followed by treatment, using observed data.

Summary of continuous variables will be presented using the total number of participants (N), Mean, Standard Error of mean (SE), Standard Deviation (SD), Median and Range (Minimum and Maximum). The categorical variables will be presented using the number and the percentage of subjects who have provided the defined categorical answer.

For measurements obtained during course of time, mean values will be plotted to explore the trends. Observed data will be used as the basis for plotting data along with standard error (SE) or Standard Deviation (SD) represented as bars, if not otherwise specified.

A non-parametric Mann-Whitney U Test (or Wilcoxon Rank-Sum Test) will be applied on primary and secondary efficacy endpoints to test the significance of the effects of the treatment by comparing median values.

For measurements performed over time, median values will be plotted to explore the trends. Observed data will be used as the basis for plotting the data along with Inter Quartile Range (IQR) used as bars, if not otherwise specified.

A parametric, repeated measurements ANOVA will be applied as a sensitive analysis to test the significance of the effects of the treatment.

Change from baseline safety Lab measurements will be analyzed using a repeated measurements ANOVA to test the significance of the effects of the treatment.

Presentation of results from a statistical analysis model will include the estimated means caused by treatment effects, using Adjusted Mean. HB-adMSCs 200MM vs Placebo group will be compared.

For all endpoints analyzed statistically, estimated mean treatment differences will be presented together with two-sided 95% confidence intervals and p-values.

All safety evaluations will be presented using Safety analysis set while the efficacy analysis will be presented using Efficacy analysis set.

Individual safety and efficacy parameters will be listed.

6. STUDY POPULATION ANALYSES

6.1. Overview of Planned Study Population Analyses

The study population analyses will be based on the Safety analysis set.

Study population analyses includes analyses of subject disposition, demographic, as well as characteristics compared to baseline, including medical history, prior and concomitant medications.

Details of the planned displays are presented in **Error! Reference source not found.**.

Categorical variables will be summarized by the number and percentage of subjects who provided a specific answer, and the continuous parameters will be summarized by N, mean, standard error of mean, median, sample standard deviation, minimum and maximum, unless otherwise specified.

Disposition summary includes subject screened and randomized, while the disposition at end of study, Week 52, along with reasons for withdrawals will be presented based on number of subjects and percentage. Subjects in different analysis sets also will be presented.

The reasons for screening failure will be presented based on number of subjects and percentage. The percentage will be calculated based on the total number of screened subjects.

7. EFFICACY ANALYSES

7.1. Primary Efficacy Analyses

7.1.1. Endpoint / Variables

The primary efficacy endpoint is difference between the Baseline value in Total MDS-UPDRS Part II score and the value measured at the end of the study (Week 52).

7.1.2. Summary Measure

Least Square Means using parametric RMA model.
Median values using Non-parametric method.

7.1.3. Population of Interest

The primary efficacy analyses will be based on the Efficacy analysis set population, unless otherwise specified.

7.1.4. Statistical Analyses / Methods

Details of the planned displays are provided in **Error! Reference source not found.**.

7.1.4.1. Statistical Methodology Specification

Primary endpoint will be analyzed using the Mann-Whitney U Test (or Wilcoxon Rank-Sum Test) for the comparison of HB-adMSCs 200MM vs. Placebo.

Sensitivity analysis

Sensitivity analysis will be performed on Efficacy analysis set using a repeated measurements ANOVA model to evaluate the robustness of using parametric method. If the normality of dataset shows significant violation from the presumption of normality, the parametric method will not be performed.

All primary endpoint efficacy measurements available at post-baseline at scheduled measurements will be analyzed using Repeated Measures ANOVA. The model will include treatment, visits as fixed factors. The within subject factor is visit and with 7 levels (Weeks 4, 8, 16, 24, 32, 42 and 52). The between-subjects factor is treatment (HB-adMSCs 200MM or Placebo).

7.2. Secondary Efficacy Analyses

7.2.1. Endpoints / Variables

Difference between the Baseline value and the value measured at all the timepoints collected, during the period of study (52 Weeks), at Weeks 4, 8, 16, 24, 32, 42 and 52 visits for below efficacy measures:

- MDS-UPDRS total score
- Total MDS-UPDRS score for Part I
- Total MDS-UPDRS score for Part II
- Total MDS-UPDRS Part III Score
- Total MDS-UPDRS Part IV Score
- Total Neuro-Quality of Life Assessments scores (Neuro-QOL)
- Total Parkinson's disease fatigue scale (PFS-16) Score
- Total Parkinson's disease Questionnaire (PDQ-39) Score
- Visual Analogue Scale (VAS) Score
- Muscle Spasm Scale VAS Score
- Pain Scale VAS score

- Daily dose of Medication to treat Parkinson's Disease

Proportion of subjects requiring reinstatement of PD medication dose following reduction in dose during the study.

7.2.2. Summary Measures

Adjusted Mean using parametric Repeated Measures ANOVA model
Median values using Non-parametric method.
Contingency tables, with the corresponding heatmaps.
Tables containing p-values, in pair-wise manner, with the corresponding heatmaps.

7.2.3. Population of Interest

The secondary efficacy analyses will be based on the Efficacy analysis set population, unless otherwise specified.

7.2.4. Statistical Analyses / Methods

Details of the planned displays are provided in **Error! Reference source not found.**.

7.2.4.1. Statistical Methodology Specification

Secondary endpoints will be analysed using the Mann-Whitney U Test (or Wilcoxon Rank-Sum Test), for the comparison of HB-adMSCs 200MM vs. Placebo.

Sensitivity analysis

Sensitivity analysis will be performed on Efficacy analysis set using a repeated measurements ANOVA model to evaluate the robustness of using parametric method. If the normality of dataset shows significant violation from the presumption of normality, the parametric method will not be performed.

All secondary endpoint efficacy measurements available at post-baseline at scheduled measurements will be analyzed using Repeated Measures ANOVA. The model will include treatment, visits as fixed factors. The within subject factor is visit and with 7 levels (Weeks 4, 8, 16, 24, 32, 42 and 52). The between-subjects factor is treatment (HB-adMSCs 200MM or Placebo).

For Levadopa, the comments will be made about the total number of patients have reduced the dose of medication.

Proportion of subjects requiring reinstatement of PD medication dose

Patients are defined as having reinstated with PD medication if, at any time during the study, their medication dose reinstated up to, or above, their baseline level after it has

been reduced. Number of subjects who have reinstated PD dose will be summarized using count and percentage.

Reinstatement is not applicable for patients who did not reduce their dose during the study.

8. SAFETY ANALYSES

The safety analyses will be based on the Safety analysis set, unless otherwise specified.

8.1. Adverse Events Analyses

AEs will be coded using the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA).

A treatment emergent adverse event (TEAE) is defined as an event that has onset date on the first day of exposure or after the first day of exposure to infusion treatment, and no later than 3 days after the last day of infusion treatment. AEs with missing start and/or stop dates will be assumed to be treatment emergent.

Treatment adverse events (TAE) are summarised descriptively, whereas non-TEAEs are presented in listings. TAE data will be displayed in terms of the number of subjects with at least one event (N), percentage of subjects with at least one event (%) and the number of events E.

Summaries of TAE and of serious AEs will be presented as an overview, including all AEs, AEs listed by severity, serious AEs, AEs by relation to treatment, action taken after AEs occurred and treatment advised, and the outcome of AEs.

Furthermore, summary tables based on system organ class and preferred terms are made for:

- All TAEs
- Serious AEs
- AEs leading to withdrawal of study

Individual adverse events will be listed.

The details of the planned displays are in **Error! Reference source not found.**.

8.2. Clinical Laboratory Analyses

Laboratory evaluations including the analyses of Biochemistry laboratory tests (includes Comprehensive Metabolic Profile), Hematology laboratory tests (Complete Blood Count

(CBC) and Coagulation Panel) and Urinalysis. The details of the planned displays are in **Error! Reference source not found.**.

All laboratory parameters, including numerical urine analysis parameters will be summarised descriptively. Categorical urine analysis results will be summarized using count and percentage based on subjects. Differences in safety Lab parameters after the treatment was applied are tested using the Mann-Whitney U (or Wilcoxon Rank-Sum) Test.

Distribution of laboratory test results will be presented using scattered box-plot.

Change from baseline laboratory measurements available at post-baseline at scheduled measurements will be analyzed using Repeated Measures ANOVA. The model will include treatment, visits as fixed factors. The within subject factor is visit and with 2 levels (Weeks 24 and 52). The between-subjects factor is treatment (HB-adMSCs 200MM or Placebo).

Results of urine pregnancy test will be listed only in the individual subject data listings.

Individual laboratory evaluations will be listed. In addition, a listing containing individual subject laboratory values outside the normal reference ranges will be provided separately.

Data recorded at unscheduled assessments will not be included in tables and figures but will be listed.

8.3. Other Safety Analyses

The analyses of non-laboratory safety test results, including physical examination and vital signs. The details of the planned displays are presented in **Error! Reference source not found.**.

Physical Examination and Vital signs will be summarized using count and percentage based on subjects. The vital signs based on visit and change from baseline will be summarized using descriptive statistics.

Individual Vital signs, Physical Examination evaluations will be listed.

9. REFERENCES

1. ICHE9 guideline
2. ICHE3 Guidelines

10. APPENDICES

10.1. Appendix 6: Derived and Transformed Data

10.1.1. General

Study Day

• Study Day 1 is defined as the day the first dose was taken.
• Study Day > 1 is calculated as the number of days that have passed since the date when the first dose was taken (Study Day 1):
  • Ref Date = Missing → Study Day = Missing
  • Ref Date < Date of Study Day 1 → Study Day = Ref Date – Date of Study Day 1
  • Ref Data ≥ Date of Study Day 1 → Study Day = Ref Date – (Date of Study Day 1) + 1

10.1.2. Handling of Missing Data

Element	Reporting Detail
General	• Missing data occurs when any requested data is not provided, leading to blank fields on the collection instrument: o If only some of the values within the test are missing, those fields will be marked as "blank". Otherwise, if all data for a specific visit are missing, the data will be completely excluded from the table. o Answers such as "Not applicable" and "Not evaluable" are not considered to be missing data and should be displayed as such.
Outliers	• Any participants with outlying results may be excluded in additional *ad hoc* summaries and/or statistical analyses. These will be documented along with the reason for exclusion in the clinical study report, but the primary conclusions will remain based on the full population sets.

10.1.2.1. Handling of Missing Data for Statistical Analysis

Element	Reporting Detail
efficacy endpoints	• For all analyses of the Efficacy analysis set, missing value imputation will not be performed and participants' data will be excluded from any statistical analyses due to missing data.

10.2.2.2 Multiple measurements at One Analysis Time point

Element	Reporting Detail
efficacy and safety endpoints	• For all analyses of the Efficacy and safety, last non-missing measurement will be flagged for summary and statistical analysis at any post-baseline visit. If listed, all data will be presented