Statistical Analysis Plan

A Multi-center, Double-blind, Randomized, Three-Arm, Parallel-Group, Placebo Controlled Study to Assess the Efficacy and Safety of NTRA-2112 on Intestinal Malabsorption in Preterm Infants

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DOCUMENT REVISION HISTORY

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V1.0	03-July-2017	[redacted]	
V1.1	27-Sept-2017	[redacted]	Revision after receiving FDA comments on V1.0 dated 3 July 2017
V1.2	11-Feb-2018	[redacted]	The reporting periods for AE reporting have been further defined in Section 2.3.2.1. Changed the hypothesis testing procedure for primary and key secondary endpoints. Re-calculated the sample size due to the testing procedure. The timing of sample size re-estimation relative to the futility analysis is further clarified. Added a clarification for the average scores to be used for tied observations in SAS one-way nonparametric procedure. [redacted] added a sensitivity analysis using the baseline enteral feeding volume to break the tied NFE ranking. [redacted] added a summary for number and percent of infants who achieve the complete and partial enteral feeding success for each treatment.

			Section 2.2.1.3.2 was modified to allow longer time, up to 180 days, to observe the actual time for discharge or being ready to be discharged.
V1.3	06APR2018	[redacted]	Section 3.1 Changed the conditional power from 20% to 35%. Based on the conditional power and the totality of the clinical data available at the futility analysis, the DMC will recommend to continue or discontinue one or both dose arms. Section 3.1 Added language that the sponsor will consider increasing the total sample size up to 450 patients based on the sample size re-estimation.

TABLE OF CONTENTS

ABBREVIATIONS

Abbreviation	Term
AE	Adverse event
ANCOVA	Analysis of Covariance
ATC	Anatomical Therapeutic Chemical Classification System
CRF	Case Report Form
CI	Confidence Interval
CT	Clinical trials
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
eCRF	Electronic Case Report Form
EN	Enteral nutrition
EOT	End of Treatment
FAS	Full Analysis Set
FEF	Full Enteral Feeding
GA	Gestational Age
ICF	Informed Consent Form
MAR	Missing at Random
MedDRA	Medical Dictionary for Regulatory Activities
ND	Not done
NEC	Necrotizing enterocolitis
NFE	Number of days to full enteral feeding
NGT	Nasogastric tube
NICU	Neonatal intensive care unit
NRD	Number of days to discharge from the hospital
OGT	Orogastric tube
OGTT	Oral glucose tolerance test
OMM	Own mothers milk
PDA	Patent ductus arteriosus
PE	Physical examination
PI	Principal Investigator
PN	Parenteral nutrition
PP	Per protocol
PT	Preferred Term
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
TEAE	Treatment Emergent Adverse Event
SAR	Serious Adverse Reaction
SUSAR	Suspected unexpected serious adverse reaction

Abbreviation	Term
TBV	Total Blood Volume
TMF	Trial Master File
TPN	Total parenteral nutrition
WHO	World Health Organization

1 INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to describe the procedures and the statistical methods that will be used to analyze and report results for Protocol FIT-04.

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

To assess the efficacy of 2 doses of NTRA-2112 as compared to placebo on intestinal malabsorption in preterm infants as measured by the time to full enteral feeding.

Achievement of enteral feeding will be regarded as the first of 3 consecutive days consumption of at least 150 ml/kg/day enteral feeding (EN).

The three treatment groups are

- Placebo
- NTRA – 2112 400 (µIU Insulin/ml)
- NTRA – 2112 2000 (µIU Insulin/ml)

1.1.2 Secondary Objective

Key secondary objective:
To assess the effect of 2 doses of NTRA-2112 compared to placebo on the number of days to achieve discharge from hospital or readiness for discharge, whichever occurs first.

Readiness for discharge from hospital is defined as achieving all of the below:
- Infant weight ≥ 1800g
- Stable body temperature

Capable of oral feeding (reached full enteral feeding and not dependent on parenteral nutrition [PN]).

Other secondary objectives:
To compare 2 doses of NTRA-2112 to placebo with respect to the following:

- Growth parameters (g/kg/day) at 6, 8, and 10 days, the last day of treatment (or Day 28), day of discharge, 3-month corrected follow-up, 12-month corrected follow-up, and 24-month corrected follow up
-
- Change in Z-score at 6, 8, and 10 days, the last day of treatment (or Day 28), day of discharge, 3-month corrected follow-up, 12-month corrected follow-up, and 24-month corrected follow up

- Gain in body weight at 6, 8, and 10 days, the last day of treatment (or Day 28), day of discharge, 3-month corrected follow-up, 12-month corrected follow-up, and 24-month corrected follow up
- Number and percentage of infants reaching full enteral feeding within 6, 8, and 10 days from initiation of treatment
- Total number of days receiving parenteral nutrition
- Number of days to 120Kcal/kg/day
- Number of days to wean-off PN
  (Wean off PN is defined as complete cessation of PN support.)

Exploratory secondary objective:
- Number of days to end gastric residuals over 2 ml/measurement according to the feeding protocol.

1.1.3 Safety Objective

To compare 2 doses of NTRA-2112 to placebo in preterm infants.

1.2 STUDY ENDPOINTS

1.2.1 Primary Efficacy Endpoint

Numbers of days to achieve full enteral feeding (NFE) is defined as: Number of Days to the first day of achieving enteral feeding of at least 150 ml/kg/day, which must be sustained for at least 3 consecutive days.
For subjects who are marked as fluid restricted, the cut-off value for reaching FEF is 10% less, thus 135 mL/kg/day.

1.2.2 Secondary Efficacy Endpoints

1.2.2.1 Key secondary endpoint:

Number of days to achieve discharge from hospital or readiness for discharge, whichever occurs first.

Readiness for discharge from hospital is defined as achieving all of the below:
- Infant weight ≥ 1800g
- Stable body temperature
- Capable of oral feeding (reached full enteral feeding and not dependent on PN)

Note: enteral feeding may also include tube feeds.

1.2.2.2 Other secondary endpoints:

- Growth velocity (g/kg/day) at 6, 8, and 10 days, the last day of treatment (or Day 28), day of discharge, 3-month corrected follow-up, 12-month corrected follow-up, and 24-month corrected follow up
- Change in Z-score at 6, 8 and 10 days, the last day of treatment (or Day 28), day of discharge, 3-month corrected follow-up, 12-month corrected follow-up, and 24-month corrected follow up from initiation of treatment
- Gain in body weight at 6, 8, and 10 days, the last day of treatment (or Day 28), day of discharge, 3-month corrected follow-up, 12-month corrected follow-up, and 24-month corrected follow up
- Number and percentage of infants reaching full enteral feeding within 6, 8, and 10 days from initiation of treatment.
- Total number of days receiving parenteral nutrition
- Number of days to 120Kcal/kg/day
- Number of days to wean-off PN

1.2.3 Exploratory Secondary Efficacy Endpoints

- Number of days to end gastric residuals over 2 ml
- Gain in length during the treatment period and follow up periods (3-month, 12-month, and 24-month corrected gestational age)
- Gain in head circumference during the treatment period and follow up periods (3-month, 12-month, and 24-month corrected gestation age)
- Percent enteral feedings from total nutrition
- Percent parenteral nutrition from total nutrition

1.2.4 Safety Endpoints

- Adverse events (AEs)
- Serious Adverse events (SAEs), including NEC and death
- Blood glucose level
- Complete blood count
- Anti-Insulin antibodies
- Frequency and consistency of stools
- Respiration status
- Lab measures
- Vital signs

1.2.5 Other Endpoints

The first three items below are measured at 24 months corrected follow-up only, the last two items are assessed at 12-month corrected follow-up and 24-month corrected follow-up.

- Bayley scale of infant and toddler development
- Neurodevelopment disability composite
- Child behavior checklist total problem score
- Observer reported outcomes
- Co-morbidities related to Pre-maturity

1.3 SUMMARY OF THE STUDY DESIGN

1.3.1 General Study Design and Plan

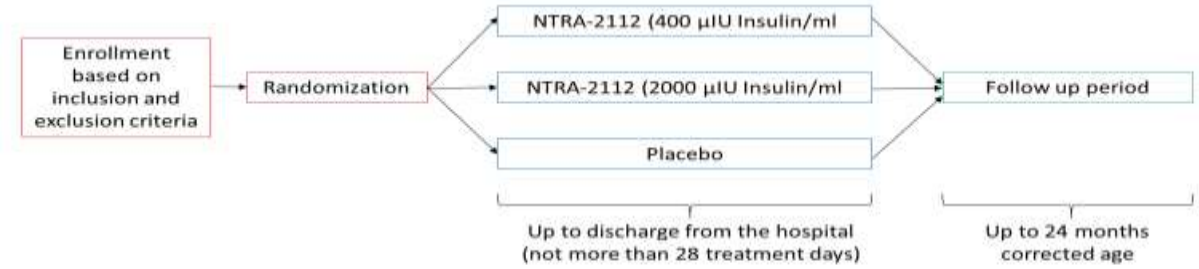
The study is a multi-center, double-blind, randomized, three-arm, parallel-group, placebo-controlled study to assess the efficacy and safety of NTRA-2112.

Infants will be enrolled within 5 days from birth (at least 6 hours post birth and up to 120 hours post birth) and will be treated for 28 days or until hospital discharge (whichever comes first). The study also includes a follow-up period up to 24 months corrected age.

Full enteral feeding is defined as reaching 3 consecutive days in which the infant consumed at least 150 ml/kg/day of enteral nutrition.

Enrolled infants will be under supervision at a NICU.

Figure 1: Study Flow Diagram



[redacted]

1.3.2 Randomization and Blinding

Following screening procedures eligible infants will be randomly assigned to one of the three treatment groups in a 1:1:1 ratio. Randomization, stratified by the gestation age group, should take place as close as possible to treatment initiation and within 24 hours of confirmation of eligibility.

Treatment should commence at postnatal age of up to and including Day 5 (up to 120 hours post birth).

Commencement of the treatment period for infants who are fed solely on own mother's milk should not begin within the first 72 hours post birth.

Randomization will be performed using random allocation generated by computer code. If both members of the twin pair are eligible to be included in the study at the same time, the firstborn will be randomized and the other sibling will be automatically allocated to the same treatment group as the firstborn sibling.

If siblings are determined to be eligible for the study at different times, the sibling who is determined to be eligible for the study first will be randomized first (regardless of birth order), and the other sibling will be automatically allocated to the same treatment group. The assigned medication numbers (kit number) will be entered in the electronic case report form (eCRF), and the corresponding medication kit administered to the infant.

It is assumed that in all cases when twins are both in the study, the first born will be the first one entered, and therefore the one who is randomized. However, to avoid ambiguity, any reference in this SAP to "first born" is intended to mean the first entered (and therefore randomized) into the study. Similarly, reference to "second born" is intended to mean the second twin entered (and who, therefore, is not randomized, but follows the treatment assignment of the twin who was first entered).

In the event that unblinding of treatment allocation is necessary for emergency treatment, it is required that the investigator contact the Medical Monitor for the treatment allocation of the subject. Following discussion on the urgency and requirement for knowing the exact treatment, the Medical Monitor will determine whether to unblind the allocation and provide the treatment assignment to the Investigator (PI). All unblinding events must be reported to the Sponsor and/or Contract Research Organization (CRO) within current approved protocol requirements by the Investigator, study coordinator (other designated study personnel), or by the clinical research associate.

1.3.3 Sample Size and Statistical Power Considerations

Presentation of sample size is based on the hypothesis that NFE in either treatment group is shorter than in the placebo group. Based on limited historical data (Phase 2), the

conservative mean is 8.0 days for the placebo group and 6.6 days for each of the 2 active dose groups. This assumes a standard deviation of 3.5 days for each group.

The analysis will be based on a nonparametric test. The NFEs for each infant in the FAS will be ranked from shortest time (the best outcome) to longest time. Infants who do not achieve full enteral feeding and also experience necrotizing enterocolitis (NEC) will be assigned the next set of ranks; all the infants experiencing NEC will be ranked in terms of their time to NEC (the earlier the worse) and will be assigned ranks one greater than the rank of the longest time to achieve NFEs. All infants who do not achieve full enteral feeding and die will be assigned the next set of ranks, again with the earliest deaths assigned the largest (i.e. worst) rank. Thus, each infant who has achieved EN or who has died or suffered NEC will have a recorded rank.

Randomization will be stratified by center and by gestational age group. The statistical analysis will stratify the infants by gestational age group and region (North America, Europe, and Israel), but not by center. Because the analysis will be based on ranks, a van Elteren test will be used to compare NTRA-2112 to placebo. To calculate sample size, an independent groups t-test for 85% power to account for the reduced power of the non-parametric test was used (i.e., expect that a t-test with 85% power to have at least 80% power when the van Elteren test is applied). A two-side 5% test will be used to compare each active dose to placebo. These calculations give N = 115 infants per group. To account for the reduction in power caused by potential missing data, the sample size was increased to 130 per group or a total sample size of 390

1.4 GENERAL CONSIDERATIONS

All descriptive statistics for continuous variables will be reported using mean, standard deviation (SD), median, minimum and maximum. Categorical variables will be summarized as number (percent) of subjects.

Confidence intervals (CIs) will be 95% and all tests will be two-sided, unless otherwise specified in the description of the analyses.

P-values will be rounded to three decimal places. If a P-value is less than 0.001 it will be reported as "<0.001." If a P-value is greater than 0.999 it will be reported as ">0.999."

For both fraternal and identical twins eligible for the study, the primary analysis will include only the first-twin (and only twin) to be randomized. If only one twin is eligible, then that twin (whether first to be randomized or second) will be included in the primary analysis. The same approach will be applied to the key secondary endpoint, the other secondary endpoints, the exploratory secondary endpoints, the other endpoints, and the analyses of the primary and key secondary endpoints on per protocol set, unless specifically specified which twin(s) will be included.

1.5 DEFINITIONS OF ANALYSIS SET

1.5.1 Full Analysis Set (Intention-to-treat)

The FAS will consist of all randomized singletons and, for twins who both participate in the study, both will be included.

In the situation where both twins are included in FAS, whether only the first twin to be randomized, or the second twin assigned to the same treatment group as the first randomized twin, or the both twins who will be included in the efficacy analyses on FAS will be explicitly stated in Section 2.2.

1.5.2 Per-Protocol Analysis Set

Per- protocol (PP) set consists of subjects in FAS who do not have an important protocol deviation. The categories of the importance protocol deviation that are thought to have a major impact on the interpretability of the overall interpretation of safety and efficacy of the trial are provided in Section 2.1.2.

The approach described in the protocol of using methods that separate the reasons for noncompliance from the effect of study drug (e.g. Robins, 1989) will not be followed. Instead, the more common approach of defining a PP set based on study compliance will be adopted. This is not considered as a major change although it will be mentioned in Section 4.0 – Summary of Major Changes in the Planned Analyses.

1.5.3 Safety Analysis Set

The safety analysis set will consist of all infants in whom any study drug (placebo or NTRA-2112) was initiated. If both members of a pair of twins are participating in the study, the safety analysis set will include both infants.

1.6 DEFINITIONS AND CONVENTIONS FOR DATA HANDLING

1.6.1 Baseline Definition

Baseline: for evaluations collected at multiple time points prior to initiation of study drug, the last assessment before the randomization day (Day 1), will be considered the "baseline" evaluation for analysis.

1.6.2 Study Day

Study Day will be calculated relative to the day of first dose (defined as Day 1), and will be used to show start/stop day of assessments and events.

If the assessment (or event) date falls on or after the date of first dose then

Study Day = Assessment Date – Date of First Dose + 1

If assessment date falls before the date of first dose then

Study Day = Assessment Date – Date of First Dose

In the case of adverse events or concomitant medications where the assessment date is partially or completely missing, Study Day and any corresponding durations will follow the conventions given in Appendix 6.1.

1.6.3 End of Study

The definition of end of study is the final study visit (24 months corrected age follow-up visit).

1.6.4 Analysis Visit Windows for Follow-up Visits

For follow-up endpoints at 3, 12 and 24 months, the variables will be summarized based on the closest scheduled visit for that variable based on "analysis-defined visit windows." These visit windows have been constructed so that every observation collected can be allocated to a unique visit. No visit windows will be defined for the screening visit.

The analysis-defined visit windows for assessments conducted at 3-month, 12-month, and 24-month are summarized in Table 2.

Table 2: Analysis - Defined Visit windows for measurements recorded for follow-up visits

Follow-up Visit	Scheduled Study Day	Maximum Windows
3-month	3-month corrected age	Day after the discharge until 7.5-month of corrected age
12-month	12-month corrected age	7.5-month + 1 day until 18-month of corrected age
24 -month	24-month corrected age	18-month + 1 day until 30-month corrected age

Note: 7.5-month is the mid-point between 3-month and 12-month, and 18-month is the mid-point between 12-month and 24-month. The exact mid-point will be determined as the day which has equal distance from two adjacent visits if there are odd number of days in between, or it is the earlier visit pluses a half number of days if there are event number of days in between,

If multiple readings are recorded within a single analysis-defined visit window, the following rules will apply:

- If there are 2 or more valid, non-missing observations within the same visit window, then the non-missing one closest to the scheduled visit day will be used in the analysis.
- If 2 valid observations are equidistant from the scheduled visit, then the non-missing observation with the earlier collection date will be used in the analysis.

- If 2 or more valid observations are collected on the same day, then the non-missing observation with the earlier collection time will be included in the analysis.

If a visit window does not contain any observations, then the data will remain missing.

1.6.5 Missing Data Handling Rules

The primary approach to handling missing ranks for the primary and the key secondary endpoints will be multiple imputation. The details are provided in Section 2.2.1.3.

No imputation will be made for other secondary or exploratory endpoints. No imputation will be made for any safety or lab measurements.

1.7 MULTIPLE COMPARISONS/MULTIPLICITY

This trial has one primary and one key secondary endpoint. The study is comparing each of two doses to placebo; a hierarchically ordered testing procedure will be used to test the primary endpoint, then secondary endpoint for the high dose first, then for the lower dose. Nutrinia believe that insulin as a growth factor will have effect with both doses. We expect that there is an opportunity for exploring higher dose that may yield a more favorable clinical effect than the lower dose that was used in the phase 2. Thus for that reason, a hierarchal approach is now being considered in the analysis. This procedure will preserve the type 1 error rate of 5% overall. The detailed testing procedure and the stopping rules are described below.

1. The NTRA – 2112 2000 µIU Insulin/ml group is compared with the placebo group for the primary endpoint using a two-sided test at the 0.05 significance level.
    - If the null hypothesis is not rejected, no further comparisons are made.
    - If the null hypothesis is rejected, comparisons continue in the next step.

2. The NTRA – 2112 2000 µIU Insulin/ml group is compared with the placebo group for the key secondary endpoint using a two-sided test at the 0.05 significance level.
    - If the null hypothesis is not rejected, no further comparisons are made.
    - If the null hypothesis is rejected, comparisons continue in the next step.

3. The NTRA – 2112 400 µIU Insulin/ml group is compared with the placebo group for the primary endpoint using a two-sided test at the 0.05 significance level.
    - If the null hypothesis is not rejected, the final comparison will not be made.
    - If the null hypothesis is rejected, the final comparison in the next step will be made.

4. The NTRA – 2112 400 µIU Insulin/ml group is compared with the placebo group for the key secondary endpoint using a two-sided test at the 0.05 significance level.

1.8 EXAMINATION OF SUBGROUPS

Descriptive statistics of effect sizes of treatment on the primary and key secondary endpoints will described in the following subgroups:

- Gestation age group
- Region (North America, Europe, and Israel)
- Birth weight (< 750g, ≥750g and < 1000g, ≥1000g and < 1500 g, ≥1500g)
- Gender
- Ethnicity
- Enteral feedings type:
    - Only own mother's milk
    - Only donor breast milk
    - Only human milk (combined)
    - Only Infant formula
    - Only own mother's milk and formula, in any combination of proportions
    - Only donor breast milk and formula, in any combination of proportions
    - Only human milk and infant formula, in any combination of proportions

For an infant to be in one of the above 7 categories, for example "Only own mother's milk", the infant had to be on own mother's milk for enteral feeding every day during the treatment period up to Day 28.

1.9 POOLING OF CENTERS

The study centers are not planned to be used as a stratification factor nor a covariate in the statistical modeling and testing, therefore it is not necessary to pool small centers together.

2 STATISTICAL ANALYSES

Although the study includes follow-up visits up to 24 months corrected age, the primary analysis will be completed after the last subject has completed the 3 month corrected age follow-up visit. At that time, all data up to and including the 3 month follow-up visit will be cleaned and the study unblinded.

Subject Information

2.1.1 Disposition of Subjects

Subject disposition will be summarized based on all subjects who were screened for the study. The total number of subjects screened and the number (percent) of subjects who failed screening will be summarized, based on data reported on the eligibility assessment in the eCRF.

The distribution of the number of subjects enrolled by each country/region and site will be summarized for each treatment group and overall.

Study Completion: The number (percent) of randomized (including twins) and treated subjects who completed the treatment period, and 3-month follow-up visits as planned, and those who discontinued from the study (with reasons) will be summarized according to the end of treatment assessment in the eCRF.

A subject data listing of disposition of subjects and the reasons for screen failures will be provided.

2.1.2 Protocol Deviations

Important deviations will be those which are considered to potentially impact upon the interpretation of the primary endpoint in the study. Only important protocol deviations will be listed and tabulated in the CSR.

The following categories of protocol deviations will be reviewed by medical advisors and statisticians prior to unblinding to determine those which are considered important deviations.

- Subjects who do not meet the inclusion criteria
- Subjects who meet any of the exclusion criteria
- Concomitant use of disallowed medications (to be identified through programming)
- Subjects who developed withdrawal criteria during the study but were not withdrawn

Subjects for whom an important protocol deviation was recorded that impacts the interpretation of the overall study efficacy and/or safety outcomes will have a footnote added to applicable output to describe the deviation and its potential impact. Such subjects will be identified as part of the protocol deviation review process, prior to unblinding.

The number and percentage of subjects with important protocol deviations in the FAS will be presented by treatment group.

A listing of these cases will be provided for all important protocol deviations.

2.1.3 Demographics and Baseline Characteristics

Demographic data will be summarized for each treatment group using descriptive statistics on FAS. The following continuous and categorical demographic variables will be summarized and tabulated for each treatment group.

- Gestation age
- Gender
- Country

- Region (North America, Europe, and Israel)
- Race
- Ethnicity
- Whether on mother's milk

Vital signs (systolic and diastolic blood pressure, pulse rate, respiratory rate and body temperature), birth weight, birth body length and birth head circumference, will be summarized using descriptive statistics for each treatment group.

A subject data listing of demographic and other baseline characteristics at birth will be provided.

2.1.4 Maternal Pregnancy History

The maternal pregnancy history, previous and current, will be summarized for each treatment on FAS according to the maternal pregnancy history assessment in the eCRF.

A subject data listing of maternal pregnancy history will be provided.

2.1.5 Subjects Medical History

The medical history between birth and before the day of first study drug dose will be coded by MedDRA (March 1, 2017 or higher version). The number (percent) of subjects reporting a history of any medical condition, as recorded on the eCRF, will be summarized for each treatment group and overall on FAS.

A subject data listing of medical history will be provided.

2.1.6 Maternal Medication During Pregnancy

All investigator terms for medications recorded on the eCRF will be coded using the World Health Organization (WHO) Drug Dictionary (1 Quarter 2016). The number (percent) of subjects who took medications in the 30 days before the delivery will be summarized for each treatment group on FAS, using the Anatomical Therapeutic Chemical (ATC) Classification and WHO Drug preferred term.

2.1.7 Mother's Chronic Disease

Mother's chronic disease including diabetes, hypoglycemia, hypertension, and other will be summarized as percentage for each treatment group on FAS.

A subject data listing of mother's chronic disease will be provided.

2.1.8 Delivery Outcome

The delivery outcome and type, such as single birth or twin, dizygotic or monozygotic, and delivery route will be summarized as percentage for each treatment group on FAS. APGAR scores will be summarized using descriptive statistics at 1 min, 5 min, and 10 min for each treatment group.

A subject data listing of delivery outcome will be provided.

2.1.9 Breast Feeding

The volume of mother's milk (ml), total enteral feed, and percentage of mother's milk will be summarized using descriptive statistics by age day for the screening visit.

A subject data listing of breast feeding until treatment initiation will be provided on FAS.

2.1.10 Parenteral Nutrition Requirement per Day

Parenteral feeds per day will be summarized using descriptive statistics for each treatment group by day (from screening to discharge) on FAS for the following variables,

- Hours per day
- Volume of PN given per day
- Average number of days of PN per week
- Kcal per day
- Kcals/kg
- Total calories provided by PN per day
- Total volume of "feed" provided by PN per day (Volume of PN/ (volume of PN + volume of EN)

A subject data listing of parenteral feeding per day will be provided.

2.1.11 Total Parenteral Nutrition Intake

Subjects in the following categories will be summarized using descriptive statistics by day for each treatment group,

- Total daily volume (ml)
- Protein/Amino acids (ml)
- Carbohydrates/Dextrose (ml)
- Fat/Lipids (ml)
- Energy (kcal)

2.1.12 Treatment Discharge Assessment

Subjects in the following categories will be summarized using descriptive statistics for each treatment group on Day 28 or discharge day if earlier,

- Discharged to home
- Staying at the primary hospital
- Discharge to a secondary site/hospital

- Discharged to a secondary unit within this site/hospital

2.2 EFFICACY ANALYSES

2.2.1 Primary Efficacy Endpoint

2.2.1.1 Primary Analysis

The primary endpoint is the number of days to achieve enteral feeding of at least 150 ml/kg/day for at least 3 consecutive days (NFE). FAS will be used to test the primary efficacy endpoint. See Section 1.4 for explanation of how twins will be handled in this analysis.

For the primary endpoint, the following hypothesis will be tested:

H_0: The distribution of the number of days to full enteral feeding is the same in the treated and the placebo groups.

H_1: The distribution of the number of days to full enteral feeding differs in the treated and the placebo groups.

To maintain the overall study-wide two-sided Type I error rate at 0.05, a hierarchically ordered testing procedure, described in section 1.7, will be used.

Testing of the primary endpoint will be by the van Elteren test, stratified by gestational age group and region (North America, Europe, and Israel) with a threshold for statistical significance for each dose of P<0.05.

[redacted]

```
proc npar1way data=XXX;
  class treat;
  strata GA Region;
  var Days;
run;
```

The average rank will be used for tied NFE values in SAS procedure above. The unique NFE values will retain the ranks as there was no ties.

The Hodges–Lehmann estimator will be used to estimate the treatment differences (each NTRA-2112 dose minus placebo) in days, and their 95% confidence intervals.

2.2.1.2 Key Secondary Analysis

The key secondary endpoint is the number of days to achieve discharge from hospital or readiness for discharge (NRD), whichever occurs first.

The FAS will be used to test the key secondary efficacy endpoint. See Section 1.4 for explanation of how twins will be handled in this analysis.

For the key secondary endpoint, the following hypothesis will be tested:

H_0: The distribution of the number of days to NRD is the same in the treated and the placebo groups.

H_1: The distribution of the number of days to NRD differs in the treated and the placebo groups.

where NRD = number of days to discharge (or readiness for discharge) from the hospital.

To maintain the overall study-wide two-sided Type I error rate at 0.05, a hierarchically ordered testing procedure, described in section 1.7, will be used. The testing of the key secondary endpoint will be by the van Elteren test, stratified by gestational age group and region (North America, Europe, and Israel) with a threshold for statistical significance of $P<0.05$. The average rank will be used for tied NRD values.

The Hodges–Lehmann estimator will be used to estimate the treatment differences (NTRA-2112 dose minus placebo) in days and their 95% confidence intervals.

2.2.1.3 Handling Missing Ranks for the Primary and Key Secondary Endpoints

[redacted]

[redacted]

2.2.1.3.2 Ranks for NEC and Death of Subjects

Infants who experience NEC (Necrotizing Enterocolitis) will be assigned ranks of NFE according to censoring times of 29 days and infants who die will be assigned ranks of NFE according to censoring times of 30 days. The individual ranks among subjects experiencing NEC and the individual ranks among subjects who die will be handled according to Section 1.3.3.

Since it can take significantly longer than 28 days to be discharged or being ready to be discharged, infants who experience NEC (Necrotizing Enterocolitis) will be assigned ranks of NRD according to censoring times of 181 days and infants who die will be assigned ranks of NRD according to censoring times of 182 days. The individual ranks among subjects experiencing NEC and the individual ranks among subjects who die will be handled according to Section 1.3.3.

2.2.1.4 Sensitivity Analysis: Missing Values

Some infants in the FAS are expected to drop out of the study or, for some other reason, not to have a ranking. As described in Section 2.2.1.3, multiple imputation will be used to include these infants in the analysis of the primary and key secondary endpoint.

The following sensitivity analyses will be conducted on the primary and key secondary endpoint to explore the effect of these missing values:

- **Worst reasonable case analysis**: Infants in an NTRA-2112 group with a missing rank will be assigned the median rank in the placebo group and infants in the placebo group with a missing rank will be assigned the median rank in the combined NTRA-2112 groups. The van Elteren test, stratified by gestation age group and region (North America, Europe, and Israel) will be performed.

- **Best reasonable case analysis**: Infants in an NTRA-2112 group with a missing rank will be assigned the median rank in the respective NTRA-2112 group and infants in the placebo group with a missing rank will be assigned the median rank in the

placebo group. The van Elteren test, stratified by gestation age group and region (North America, Europe, and Israel) will be performed.

Tipping point analysis: Infants in either of the NTRA-2112 groups with a missing rank will be assigned varying ranks to find the point at which statistical significance is lost. Specifically, if the outcome of the hypothesis test is in favor of a NTRA-2112 group over the placebo group (P-value <0.05), then one day will be added to each of the imputed values for the NTRA-2112 group in each copy of the imputed dataset, whilst the data for the placebo group will remain the same. The 5 imputed datasets will then be combined to run the van Elteren test results using PROC MI procedure in SAS. This "day shifting" will be repeated with one extra day at a time until the outcome of the hypothesis test is no longer in favor of the NTRA-2112 group over the placebo group (i.e. the P-value becomes greater than 0.05). As the overall ranks of NTRA-2112 group increase as the results of adding one day each time to each imputed missing rank, the highest rank for the imputed missing ranks will be truncated at Day 30 (implying that a missing data point is as bad as a death).

- **Survival ("time-to-event") analysis**: a stratified log rank test will be performed to compare time-to-FEF (the first day of Full External Feeding). In this analysis, infants who experience NEC will be assigned censoring times of 29 days and infants who die will be assigned censoring times of 30 days. Any other infants who withdraw from the study early will be considered censored at the day of their last participation in the study (unless they have already achieved FEF in which case they will not be censored).

  This time to event analysis will be reported for the time to FEF as the *first* of 3 consecutive days and again as the *third* of 3 consecutive days of enteral feeding.

2.2.1.5 Sensitivity Analysis: The Second (Born) Twin

The second twin is the one who is assigned to the same treatment group as the first (born) randomized twin.

The following analyses will be performed on FAS to include the second twin.

One analysis will repeat test the primary and key secondary endpoints replacing the first-randomized twin by the second twin.

Another analysis will be a hierarchical mixed model that includes both twins in a way that accounts for the possible correlation between them. For the mixed effects model, the dependent variable will be the number of days, the independent variable is the treatment group, with gestation age group and the region (North America, Europe, and Israel) as two class covariates. A twin effect will be included in the model as a random cluster effect. For each singleton, the cluster has one subject, and for each twin the cluster will have two subjects. This random cluster effect is used to model the correlation within each twin-pair under the compound symmetry covariance structure.

For infants who experience NEC (Necrotizing Enterocolitis) or death, their ranks will be assigned in the same way as described in Section 2.2.1.3.2.

2.2.1.6 Sensitivity Analysis: The Per Protocol Set

Per protocol set consists of subjects in FAS who do not have any important protocol deviations. The important protocol deviations will be determined before the database is unblinded. The analysis of the primary and the key secondary endpoint will be repeated on the per protocol set.

2.2.1.7 Sensitivity Analysis: Breaking Tied NFE Rankings

For the infants who have the same NFE value which lead to the tied NFE rankings, their baseline enteral feeding volume will be used to break the tie. Specifically, the infant with the lowest baseline enteral feeding volume will be ranked lowest among them, the infant with the second lowest enteral feeding volume will be ranked the second lowest, and so on. For infants who have the same NFE rank and the same baseline enteral feeding volume, their NFE ranks will remain to be tied. For subjects who have unique NFE rankings, they will retain their ranks if there were no ties. For infants who experience NEC (Necrotizing Enterocolitis) and infants who die, their NFE will be assigned to Day 29 and Day 30 without breaking ties by their baseline enteral feeding volume. The individual ranks among subjects experiencing NEC and the individual ranks among subjects who die will be handled according to Section 1.3.3. For subjects who didn't meet the enteral feeding criterion during the treatment period, their NFE will be censored on Day 28 without breaking by their baseline enteral feeding volume. The testing of such NFE ranking will be analyzed in the same way as described in 2.2.1.1 for the van Elteren test, stratified by gestational age group and region (North America, Europe, and Israel).

2.2.1.8 Supportive Analysis: Infants Who Achieved Partial Enteral Feeding

The number and percent of infants who achieve enteral feeding of at least 150 ml/kg/day for at least 3 consecutive days, only two consecutive days, and just one day will be summarized for each treatment group.

2.2.2 Other Secondary Endpoints

The following other secondary endpoints will be analyzed on FAS only.

- Growth velocity (g/kg/day) at 6, 8, and 10 days, the last day of treatment (or Day 28), day of discharge, 3-month follow-up, 12-month follow-up, and 24-month follow up
- Change in Z-score at 6, 8 and 10 days, the last day of treatment (or Day 28), day of discharge, 3-month follow-up, 12-month follow-up, and 24-month follow up from initiation of treatment
- Gain in body weight at 6, 8, and 10 days, the last day of treatment (or Day 28), day of discharge, 3-month follow-up, 12-month follow-up, and 24-month follow up

- Number and percentage of infants reaching full enteral feeding within 6, 8, and 10 days from initiation of treatment.
- Total number of days receiving parenteral nutrition
- Number of days to 120Kcal/kg/day
- Number of days to wean-off PN

For the continuous variables (growth velocity, z-score, body weight), ANCOVA analysis will be performed at each time point of interest, including treatment, the stratification factors of gestation age and region (North America, Europe, and Israel) as class variables, and the baseline values as a continuous covariate, the estimated treatment differences (NTRA-2112 minus placebo), 95% confidence intervals, and P-values will be provided.

For the variable of number of day to a given event, the van Elteren test stratified for gestation age and region will be performed. The average score will be used for the ties. Infants who experience NEC (Necrotizing Enterocolitis) and infants who die will be assigned the second worst and the worst ranks. The individual ranks among subjects experiencing NEC and the individual ranks among subjects who die will be handled according to Section 1.3.3.

For percent of infants reaching full external feeding, Cochran-Mantel-Haenszel (CMH) test stratified for gestation age and region will be performed.

Statistical testing of these other secondary endpoints will be considered exploratory and will not control for Type I error rate inflation.

No imputation will be performed for the missing values for the other secondary endpoints.

2.2.3 Exploratory Secondary Efficacy Endpoints

The FAS will be used to analyze the exploratory secondary efficacy endpoints. The continuous variables will be analyzed using ANCOVA analysis at each time point of interest, including treatment, the stratification factors of gestation age group and region (North America, Europe, and Israel) as class variables, and the baseline values as a continuous covariate, the estimated treatment differences (NTRA-2112 minus placebo), 95% confidence intervals, and P-values will be provided.

For percent enteral feeding from total nutrition and percent parenteral nutrition from total nutrition, CMH test stratified for gestation age group and region (North America, Europe, and Israel) will be performed.

- Number of days to end gastric residuals over 2 ml
- Gain in length at 6, 8 and 10 days, the last day of treatment (or Day 28), day of discharge, 3-month follow-up, 12-month follow-up, and 24-month follow up from initiation of treatment

- Gain in head circumference during the treatment period and follow up period (long term follow-up period)
- Percent enteral feedings from total nutrition
- Percent parenteral nutrition from total nutrition

Graphical presentation of the average weight, height, and head circumference over time by treatment group will be provided.

2.2.4 Analysis of Predictor Variables

The effect of the following predictor variables on the primary and key secondary endpoints will be assessed by calculating descriptive statistics of the primary and key secondary endpoints in each category of predictor class variables on FAS.

- Gestation age group
- Region (North America, Europe, and Israel)
- Birth weight (< 750g, ≥750g and < 1000g, ≥1000g and < 1500 g, ≥1500g)
- Gender
- Ethnicity
- Enteral feedings type:
    - Only own mother's milk
    - Only donor breast milk
    - Only human milk (combined)
    - Only Infant formula
    - Only own mother's milk and formula, in any combination of proportions
    - Only donor breast milk and formula, in any combination of proportions
    - Only human milk and infant formula, in any combination of proportions

2.3 SAFETY ANALYSIS

All safety analyses will be performed on the Safety Analysis Set. Safety data presented by treatment group will be summarized on an "as treated" basis. Safety variables include the extent of exposure, treatment-emergent adverse events (TEAEs), clinical laboratory parameters, vital signs, and other relevant variables. Study Day 1 for all safety analyses is defined as the date of the first dose of study drug.

Both fraternal and identical twins enrolled in the study, will be included in the safety analyses.

2.3.1 Treatment Compliance

The compliance check will be based on the ratio of number of enteral feeds with study drug administered over the total number of feeds planned for the subject's treatment

duration (days). The following describes the calculation algorithm for individual subject compliance based on the information on eCRF.

1. If no study drug was missed for the day, then the daily compliance is 100%.
2. If the study drug was missed for the day, then based on how many feeds were missed and how many feeds were planned for the day, the daily compliance will be calculated as the ratio of number of feeds with study drug administered over the number of feeds planned x 100%.

For each subject, the daily treatment compliance will be calculated for each day while the subject was supposed to receive the treatment.

For an infant, the average of the daily compliance over the treatment period (up to Day 28) will be the overall treatment compliance for this infant.

The overall treatment compliance values will be summarized using descriptive statistics for each treatment group.

A subject data listing of treatment compliance will be provided.

2.3.2 Safety Outcome Variables

The following safety data will be collected: reported AEs (including SAEs), clinical chemistry, physical examination, vital signs, respiration status, complete blood count, glucose level, anti-insulin antibodies, and stool frequency and consistency.

All safety measurements will use all available data for analyses, including data from unscheduled visits and repeated measurements.

Change from baseline to each post-treatment time point where scheduled assessments were made will be calculated for relevant measurements.

No safety data will be imputed. The handling of partial/missing dates for AEs and prior/concomitant medications is detailed in Appendix 6.1.

2.3.2.1 Adverse Events

Adverse events (AEs) experienced by the subjects will be collected from initiation of dosing through the final study visit and will be coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA) per the Data Management Plan.

Adverse event data will be categorized according to their onset date into the following study periods:

- Treatment-emergent adverse events are the ones that they started during or after administration of the first dose of study medication up till the last dose date + 30 days. In addition, medical history events that worsen in severity after the start of dosing will be considered treatment-emergent adverse events.

- AEs during the 3 month corrected age follow up period : < last dose date + 30 days - <= Month 3 follow-up visit
- AEs during the 12 month corrected age follow up period : < Month 3 follow-up visit - <= Month 12 follow-up visit
- AEs during the 24 month corrected age follow up period : < Month 12 follow-up visit - <= Month 24 follow-up visit

If an AE has a missing onset date then unless the stop date of the AE indicates otherwise, this will be considered an ongoing AE. Similarly, if an AE has a partial onset date, then unless the partial onset date or the stop date indicates otherwise, this will be considered an ongoing AE.

Only those AEs that are treatment-emergent will be included in summary tables. All AEs, treatment-emergent or otherwise, will be presented in subject data listings.

TEAEs will be summarized by treatment group, separately for the four reporting periods above. The incidence of TEAEs will be reported as the number (percent) of subjects with TEAEs within SOC and PT. Subjects will be counted only once within a SOC and PT, even if the subject experienced more than one TEAE within a specific SOC and PT. The number (percent) of subjects with TEAEs will also be summarized by maximum severity of Common Terminology Criteria for Adverse Events (CTCAE) and the relationship to the study drug (unlikely, possible, probable).

2.3.2.2 Deaths, Serious and Other Significant Adverse Events

The number (percent) of subjects with TEAEs leading to death will be summarized by MedDRA SOC and PT for each treatment group separately for the on-study period and the long-term follow-up period. A subject data listing of all AEs leading to death will be provided.

The number (percent) of subjects with treatment-emergent serious adverse events (SAEs) will be summarized by MedDRA SOC and PT for each treatment group, separately for the on-study period and the long-term follow-up period. A subject data listing of all SAEs will be provided.

The number (percent) of subjects with TEAEs leading to discontinuation from study treatment will be summarized by MedDRA SOC and PT for each treatment group during the on-study period. A subject data listing of all AEs leading to discontinuation from study treatment will be provided.

2.3.3 Clinical Laboratory Parameters

In summaries, figures, and listings, lab results and normal ranges will be presented in International System (SI) units.

2.3.3.1 Blood Chemistry

For the purposes of clinical chemistry shift tables, baseline will be defined as the assessment prior to randomization and maximum or minimum value post-baseline will be calculated over the entire study period, up to the 3-month follow-up visit.

Changes in blood chemistry variables between baseline and post-baseline assessments will be calculated. The change from baseline is defined as the post-baseline visit value minus the baseline visit value. There will be no imputation for missing values. For values recorded with a leading greater than or less than symbol (“>”, “<”), the reported numeric value will be used for analysis and the value with the symbol will be included in the listings, unless otherwise specified. For example, a value of <0.01 will be analyzed as 0.01 and listed as <0.01.

Absolute values will be compared to the reference range and classified as normal, abnormal, or not performed. The abnormal values will be further classified as clinically significant or not clinically significant.

Data for subjects who have treatment-emergent changes outside laboratory reference ranges will be presented. This data presentation will include all visits for this subset of subjects.

Maximum post-baseline ALT and AST will be presented, expressed as multiples of ULN. AST and ALT will be presented in multiples of the following ULN ≤1, >1-3, >3-5, >5-10, >10.

2.3.3.2 Blood Glucose Level

Blood glucose level will be monitored twice/day for the first 4 days (96 hours), with one measurement approximately 1 hour after the study drug administration each day, and then starting at Day 4 every other day (at 07:00-10:00, before a meal).

The blood glucose level will be summarized using descriptive statistics for each treatment group by time and the day during the treatment period (Day 1 to Day 28), and the 3-month follow-up visit.

A shift table will be provided to summarize the changes from the baseline statuses to the post-baseline statuses.

Graphical presentation of the mean glucose level over time by treatment group will be provided.

A subject data listing of blood glucose level will be provided.

2.3.3.3 Respiration Status

Fraction of inspired oxygen will be summarized using descriptive statistics. The other categorical variables will be summarized as percentages for each treatment group.

A subject data listing of respiration status will be provided.

2.3.3.4 Complete Blood Count

A subject data listing of complete blood count will be listed.

2.3.3.5 Anti-Insulin Antibodies

A subject data listing of anti-insulin antibodies will be listed.

2.3.4 Vital Signs, Physical Examination Findings

2.3.4.1 Vital Signs

Descriptive statistics for vital signs (diastolic and systolic blood pressure, pulse rate, respiration rate, temperature, weight) and changes from baseline will be presented by visit and treatment group.

Baseline to maximum post-baseline and baseline to minimum post-baseline value shift tables will be generated, as applicable for each parameter and will include subjects with both baseline and post-baseline data.

A subject data listing of vital signs will be provided.

2.3.4.2 Physical Examinations

Physical examination results are collected at screening, each treatment day, the day of discharge, and 3-month corrected age follow-up visits. Each component of the physical examination will be recorded as normal, abnormal not clinically significant, or abnormal clinically significant.

Physical examination results will be summarized by treatment group in frequency and percentage at these visits.

A subject data listing of physical examination will be provided.

2.3.5 Other Safety Analyses

2.3.5.1 Frequency and Consistency of Stool

Data on stools are collected at screening, on treatment days (Days 1-28), the day of discharge, and 3-months follow-up visit for frequency per day, consistency (watery, soft, solid, meconium, or unknown consistency), and bloody stool.

The frequency will be summarized using descriptive statistics for each treatment group and by visit. The categorical responses will be summarized by the percentage for each treatment group by visit.

A shift table will be provided to summarize the changes from the baseline statuses to the

post-baseline statuses.

A subject data listing of stool frequency and consistency will be provided.

[redacted]

[redacted]

3 FUTILITY ANALYSIS AND DATA MONITORING COMMITTEE (DMC)

3.1 FUTILITY ANALYSIS

The study will include an interim futility analysis of the primary endpoint. The interim futility analysis will be performed when at least 150 subjects have been randomized. The date for data transfer, which includes the values for the primary endpoint and the key secondary endpoint, and gestation age group and region (North America, Europe, and Israel) will allow the randomized subjects to be followed for at least 28 days. The multiple imputation for the missing values due to drop-out will be implemented as described in Section 2.2.1.3.1. if more than 10% of values are missing due to early termination other than NEC or death for the primary endpoint. A partial data lock (snap shot) will be completed after the data transfer and the data and SAS program will be transferred to the unblinded statistician to complete the interim futility analysis.

To maintain adequate statistical power for the final analysis of the primary endpoint, a blinded sample size re-estimation will be carried out at the time of the interim futility analysis. The blinded team statistician will calculate the pooled standard deviation of the primary endpoint without distinguishing treatment groups. The sample size may be increased up to 450 patients (total) if the estimated standard deviation is at least 10% larger than that assumed in the sample size section of the protocol. In order to keep the sample size re-estimation independent of the futility analysis, the sample size re-estimation will be based on the blinded data used for the futility analysis to obtain the pooled standard deviation. The result of the blinded review will be documented and reported to the sponsor and DMC before the DMC meets to consider futility. The final sponsor decision on sample size will be provided to the DMC and will be the primary driver in the futility assessment. Because the analysis will be based on ranks, a van Elteren test will be used to compare NTRA-2112 to placebo. To calculate sample size, a 2-sample t-test for 85% power to account for the reduced power of the non-parametric test was used (i.e., expect that a t-test with 85% power to have at least 80% power when the van Elteren test is applied). With 144 evaluable patients per arm, assuming a standard deviation of 5.0 days, a 2-sample t-test will have 85% power to detect a mean difference of 1.77 days in the number of days to reach full enteral feeding between the treated and placebo arm. Similarly, assuming a standard deviation of 4.0 days, a 2-sample t-test will have 85% power to detect a mean difference of 1.42 days. Considering a dropout rate of 5%, an enrollment of 152 patients per arm will likely recruit 144 per arm evaluable

patients.

For the futility analysis, the conditional power of showing statistically significant ($P \leq 0.05$) evidence of benefit on the primary endpoint at the final analysis will be calculated for each dose group. Conditional power will be calculated from the z-value based on the interim analysis, treating the primary endpoint as a continuous variable, approximately following the normal distribution. The conditional power will be calculated based on the observed interim trend of effect size, not the original expectation in the sample size calculation. If the conditional power is less than 35% in either dose, continuing to enter infants in that dose group will be considered statistically futile.

The futility analysis results, including the calculated conditional power for each dose group and summaries of selected key safety parameters (adverse events, lab tests, physical exams, and vital signs), will be presented to the Data Monitoring Committee, who will review the results and make a final recommendation regarding the continuation or discontinuation of one or both dose arms. The recommendation will be based on the conditional power and the totality of the clinical data available.

3.2 DATA MONITORING COMMITTEE

An independent DMC is established and responsible for monitoring the trial to ensure the safety of patients in the trial. The DMC will review safety data and will make recommendations whether to continue or terminate the study. The DMC analyses and operations will be formally separated from the sponsor, the investigators, and the Steering Committee.

The DMC's responsibilities are to:

- Review safety and efficacy data during the study with consideration for the impact on patient safety, as well as the ethics of continued study conduct.
- Conduct review results of interim and final safety evaluations of the study, including an interim analysis for futility.
- Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on safety, scientific integrity, or the ethics of conducting the study.
- Review and evaluate ad hoc safety issues concerning the study that arise from any Serious Adverse Drug Reaction (an adverse event that might be related to the study drug, i.e. a causal relationship between exposure to the study drug and the serious adverse event is at least possible) or other safety matters at the request of the sponsor.
- Make recommendations to the sponsor concerning continuation/termination, or other modifications of the study based on the observed beneficial or adverse effects of the study drug.
- Operate according to the procedures described in the DMC charter.

A DMC Charter has been developed that defines the DMC organization, responsibilities, relationship with other trial entities, and the purpose and schedule of the DMC meetings.

The charter provides the procedures for ensuring confidentiality, proper communication, scopes of the interim data to be presented, and the statistical guidelines for interim futility analysis by the DMC.

4 SUMMARY OF MAJOR CHANGES IN THE PLANNED ANALYSES

The PP set is defined differently from the protocol, but the difference is not considered to be major. The approach described in the protocol of using methods that separate the reasons for noncompliance from the effect of study drug (e.g. Robins, 1989) will not be followed. Instead, the more common approach of defining a PP set based on study compliance will be adopted. PP analysis will only be supportive of the primary intention-to-treat analysis based on the FAS.

The treatment difference between two NTRA-2112 doses will be estimated for the primary endpoint, key secondary endpoint, exploratory secondary endpoint, and other endpoints.

The Bonferroni correction was described in the protocol for testing the primary and the key secondary endpoints simultaneously for each dose level. Nutrinia believe that insulin as a growth factor will have effect with both doses. We expect that there is an opportunity for exploring higher dose that may yield a more favorable clinical effect than the lower dose that was used in the phase 2. Thus for that reason, a hierarchical approach is now being implemented in the analysis. This procedure is described in Section 1.7 of this SAP to allow testing the primary endpoint, then secondary endpoint for the high dose first, then for the low dose while maintaining the type 1 error rate of 5% overall.

The sample size was re-calculated in Section 1.3.3 due to the change in the testing procedure stated above.

Section 2.2.1.3.2 was modified to allow longer time, up to 180 days, to observe the actual time for discharge or being ready to be discharged (NRD).

Section 2.2.1.7 was added for a sensitivity analysis using the baseline enteral feeding volume to break the tied NFE ranking.

Section 2.2.1.8 was added for a summary of number and percent of infants who achieve the complete and partial enteral feeding success for each treatment.

The reporting periods for AE reporting have been further defined in Section 2.3.2.1.

Section 3.1. The timing of the sample size re-estimation relevant to the futility analysis is further clarified. The conditional power is raised from 20% to 35%. Based on the conditional power and the totality of the clinical data available at the futility analysis, the DMC will recommend to continue or discontinue one or both dose arms. The sponsor will consider increasing the total sample size up to 450 patients based on the sample size

re-estimation.

5 REFERENCES

van Elteren P H (1960) On the combination of independent two-sample tests of Wilcoxon. *Bulletin of the International Statistical Institute*, 37: 351-361.

Hodges, J. L.; Lehmann, E. L. (1963). Estimation of location based on ranks. *Annals of Mathematical Statistics*. 34 (2): 598-611.

Lachin JM (2005). A review of methods for futility stopping based on conditional power" *Statistics in Medicine*, 24: 2747-2764.

Robins J. (1989). "The Control of Confounding by Intermediate Variables" *Statistics in Medicine*, 8: 679-701.

6 APPENDIX

6.1 PARTIAL DATES FOR ADVERSE EVENTS AND PRIOR/CONCOMITANT MEDICATION

Dates missing the day or both the day and month of the year will adhere to the following conventions to classify treatment-emergent AEs and to classify prior/concomitant medications. When the conventions are applied to infants, if an imputed date is before infant's birthday, the date should be set on the birthday. For example, if a missing date of AE is set on January 1, but January 1 is before infant's birth, then the date of AE should be set on the birthday.

Adverse events

The missing day of *onset* of an AE will be set to:

> First day of the month that the event occurred, if the onset YYYY-MM is after the YYYY-MM of first study treatment
>
> The day of the first study treatment, if the onset YYYY-MM is the same as YYYY-MM of the first study treatment
>
> The date of informed consent, if the onset YYYY-MM is before the YYYY-MM of the first treatment.

The missing day of *resolution* of an AE will be set to:

> The last day of the month of the occurrence. If the subject died in the same month, then set the imputed date as the death date.

If the *onset* date of an AE is *missing* both the *day and month,* the onset date will be set to:

> January 1 of the year of onset, if the onset year is after the year of the first study treatment
>
> The date of the first treatment, if the onset year is the same as the year of the first study treatment
>
> The date of informed consent, if the onset year is before the year of the first treatment

If the *resolution* date of an AE is missing both the *day and month,* the date will be set to:

> December 31 of the year of occurrence. If the subject died in the same year, then set the imputed date as the death date.

Prior/concomitant medication

The missing day of *start date of a therapy* will be set to the first day of the month or the infant's birthday, whichever is later, that the event occurred.

The missing day of *end date of a therapy* will be set to the last day of the month of the occurrence.

If the *start date of a therapy* is missing both the *day and month*, the onset date will be set to January 1 of the year of onset.

If the *end date of a therapy* is missing both the *day and month*, the date will be set to December 31 of the year of occurrence.

If the *start date of a therapy* is missing and the *end date* is not a complete date then the start date will be set to the date of the first study visit.

If the *start date of a therapy* is missing and the *end date* is a complete date
and the end date is after the date of the first study visit then the start date will be set to the date of the first study visit.

otherwise the start date will be set to the end date of the therapy.

If the *end date of a therapy* is missing and the *start date* is not a complete date then the end date will be set to the date of the last study visit.

If the *end date of a therapy* is missing and the *start date* is a complete date
and the start date is prior to the date of the last study visit then the end date will be set to the date of the last study visit.

otherwise, the end date will be set to the start date of the therapy.