Statistical Analysis Plan

Study number: **SMOF-018-CP3**

Study title: **A Prospective, Randomized, Controlled, Double-Blind, Parallel-Group, Phase 3 Study to Compare Safety and Efficacy of Smoflipid 20% to Intralipid 20% in Hospitalized Neonates and Infants Requiring 28 Days of Parenteral Nutrition**

Study design: **Phase III**

Sponsor: **Fresenius Kabi (Deutschland GmbH)**

Author: [redacted]

Version: **2.0**

Date: **29 October 2020**

Signature Page

The undersigned have approved this Statistical Analysis Plan for use in this study.

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List of abbreviations and definition of terms

Abbreviation	Definition
AE	Adverse event
AUC	Area under the curve
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical Classification System
BDRM	Blinded Data Review Meeting
BUN	Blood urea nitrogen
CRF	Case report form
CRP	C-reactive protein
CS	Clinically significant
CTM	Clinical trial material
DSMB	Data safety monitoring board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transferase
GMP	Good Manufacturing Practice
HEENT	Head, eyes, ears, nose, and throat

Abbreviation	Definition
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IWRS	Interactive web response system
MedDRA	Medical Dictionary for Regulatory Activities
MGRS	Multicenter growth reference study
n	Number
NCS	not clinically significant
NEC	Necrotizing enterocolitis
PARP	Product accountability and randomization plan
PN	Parenteral nutrition
SAE	Serious adverse event
SAP	Statistical analysis plan
TEAE	Treatment-emergent adverse event
TG	Triglycerides
WHO	World Health Organization

1 INTRODUCTION

This Statistical Analysis Plan (SAP) describes the statistical methods and data presentations to be used in the summary and analysis of safety, and efficacy data from Protocol SMOF-018-CP3. This document reflects the amendments to the protocol and changes in statistical analysis after the interim analysis and consultancy with the Food and Drug Administration (FDA). Background information is provided for the overall study design and objectives. For details on study designs and data collection, the reader is referred to the study protocol, the interim analysis plan, the product accountability and randomization plan, and the case report form (CRF).

The content of this document and the Interim Analysis Plan are compliant with International Council for Harmonisation (ICH) guideline E9 - Statistical Principals for Clinical Trials. Any text copied from the study protocol has been italicized.

2 MODIFICATION HISTORY

2.1 Changes to the study protocol

This SAP reflects the amendments to the study protocol and changes in statistical analysis after the interim analysis and consultancy with the FDA.

2.2 Changes to previous SAP versions

This is version 2.0 of the SAP for the final analysis.

It is based on the following documents:

- Study Protocol SMOF-018-CP3 CSP final (Version 3.0, 5-October-2020)
- Statistical analysis plan (Version 1.0, 14-July-2015)
- Interim Analysis Plan Version 1.0, (14-February-2019)
- Product Accountability and Randomization Plan (Version 1.2, 31-March-2016)
- Case Report forms (e CRF, 26-July-2018)

The following major changes were included compared to SAP version 1.0:

Version	Date	Changes
2.0	29-Oct--2020	Chapter 2: Modification history added
		Chapter 3: Primary objective changed from confirmatory to exploratory
		Chapter 4: Condensed description of study design and procedures Requirement for figures in BDRM deleted Reference to BDRM on 28-Oct-2020 included
		Chapter 5: “Cumulative number of days of conjugated bilirubin levels > 1.5 mg/dL in patients” deleted from list of safety variables per study protocol Version 3.0

		Chapter 7: Statistical considerations adapted to changes in study design after interim analysis. Study week defined Details on imputation of missing data added, imputation of primary endpoint changed to “primary endpoint not met” if all post-baseline assessments missing (scenario C).
		Chapter 8: Presentation of protocol deviations according to CRF categories Presentation of medical history according to CRF categories instead of MedDRA codes Summary of prior and concomitant medication by ATC levels 2 and 4 instead of level 1 and preferred term Change of primary analysis from confirmatory to descriptive and exploratory analysis Line plot of mean Z-values replaced by box plot Cumulative risk analysis of time to enteral or oral feed amended Number of events added to AE summary tables AE summary tables by relationship and severity added Summary of TEAEs occurring with an incidence > 5% added Analysis of AEs of special interest in the following categories added: • all major neonatal morbidities • bronchopulmonary dysplasia • retinopathy of prematurity • intraventricular hemorrhage • periventricular leukomalacia • necrotizing enterocolitis • late-onset sepsis in premature and low birth weight neonates

		Additional AE tables for subgroups analysis by race and ethnicity added Cumulative risk analysis of discharge from hospital amended Analysis of caloric target removed as data not collected

3 STUDY OBJECTIVES

3.1 Primary Objective(s)

The primary objective of the study is to compare the safety of Smoflipid and Intralipid as measured by the number of study patients in each treatment group with conjugated bilirubin >2 mg/dL during the first 28 days of study treatment, confirmed by a second sample collected 7 days after the first sample.

3.2 Secondary Objective(s)

The secondary objectives are to

- compare efficacy of Smoflipid 20% to Intralipid 20% in neonates and infants reflected by specific nutritional endpoints
- evaluate overall safety of Smoflipid 20% in neonates

4 STUDY DESIGN

Number of centers:	15 (US)
Randomized:	Yes
Blinded:	Double-blind
Design:	Parallel two-stage group-sequential adaptive design, with interim analysis and sample size adjustment after stage 1
Dosing:	Target maximal lipid dose is 3.0 g/kg/day
Placebo controlled:	No
Strata:	Underlying Disease - (1) No Necrotizing Enterocolitis (No NEC) (2) NEC
Treatments:	A: Smoflipid B: Intralipid

4.1 Study Schedule

The schedule and sequence of procedures and assessments at screening, baseline, during treatment, the treatment extension phase and at the follow-up visit are summarized in the study schedule table shown below. Details of procedures and assessments are given in the Study Protocol.

Study Schedule

Initial Treatment Phase							Treatment Extension Phase								Follow-up Visit
Assessment / Records	Screening	Day 1 Baseline	Day 8	Day 15	Day 22	Day 29 / End of Treatment[a]	Day 36	Day 43	Day 50	Day 57	Day 64	Day 71	Day 78	Day 85 / End of Treatment[a]	7d after End of Treatment
Time Window (d)	Day 1 - 3d	0	±1	±1	±1	+1	±2	±2	±2	±2	±2	±2	±2	+1	±1
Parent Informed Consent	X														
In- / Exclusion Criteria	X	X													
Randomization		X													
Demographics incl. race and ethnicity	X														

Initial Treatment Phase							Treatment Extension Phase								Follow-up Visit
Assessment / Records	Screening	Day 1 Baseline	Day 8	Day 15	Day 22	Day 29 / End of Treatment[a]	Day 36	Day 43	Day 50	Day 57	Day 64	Day 71	Day 78	Day 85 / End of Treatment[a]	7d after End of Treatment
Medical History	X														
Prior Medication	X														
Physical Examination	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Body Length		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Head Circumference		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Time to Full Enteral or Oral Feeds						X								X	

Initial Treatment Phase							Treatment Extension Phase								Follow-up Visit
Assessment / Records	Screening	Day 1 Baseline	Day 8	Day 15	Day 22	Day 29 / End of Treatment[a]	Day 36	Day 43	Day 50	Day 57	Day 64	Day 71	Day 78	Day 85 / End of Treatment[a]	7d after End of Treatment
Length of Stay															X
Blood Sampling for Conjugated Bilirubin[b]	X		X	X	X	X		X		X		X		X	(X)[c]
Blood Sampling for Biochemistry, Hematology and Coagulation[d]	X		X	X	X	X		X		X		X		X	
Blood Sample for special analyses[e]		X				X				X				X	

[a]: End of treatment visit to be performed on the following day after receiving the last dose of study medication

[b]: If conjugated bilirubin exceeds 1.5 mg /dL it has to be assessed weekly, also after d29

[c]: If conjugated bilirubin is > 1.5 mg/dL at the end of treatment visit a blood sample for the assessment of conjugated bilirubin has to be drawn at the Follow-up Visit

[d]: Additional blood samples for assessment of triglycerides (TGs) and blood glucose need to be drawn during ramp up of lipid dose. Should TGs exceed 250 mg/dL at any time, lipids will be withheld until analysis of TGs in another blood sample the following morning.

[e]: Special analyses: Fatty acids, sterols, α-Tocopherol

4.2 Method of Assigning Patients to Treatment Groups/Randomization

Patients were identified using a sequential numbering system which combines a four-digit site number and a three-digit individual patient according to the sequence of entry into the study.

Patients were to be randomized at baseline in a 1:1 allocation ratio to receive either Smoflipid or Intralipid. Randomization was stratified according to underlying disease (Necrotizing Enterocolitis (NEC), and No NEC) and study site.

Randomization was implemented using TEMPO™ interactive web response system (IWRS). Random block sizes were used. Within each block, the number of patients allocated to each of the treatment groups was to be equal. The assigned treatment code was stored in TEMPO™.

Further details are described in the Study Protocol and the Product Accountability and Randomization Plan (PARP Version 1.2, March 31, 2016).

4.3 Blinding

As this is a double-blind study, the allocation to the treatment groups is blinded and will not be known by the Investigator, patient/ relatives, or members of the Fresenius Kabi Clinical Research Department until completion of the study. Blinded bags of study medication were dispensed to the blinded site personnel to preserve the blind for investigator and study patient. Each bag was labelled with a randomly assigned bag ID numbers from 00001 to 25000 to ensure that numbers are not indicative of study treatment. Emergency patient unblinding is also managed in TEMPO™.

4.4 Determination of Sample Size

Due to the lack of reliable data on the effect size of Smoflipid compared to Intralipid a two-stage group-sequential adaptive design according to Bauer and Köhne (Bauer, et al. 1994) was planned with an interim analysis after 100 patients (50 patients per treatment arm in the per protocol set) completed the first 28 days of study treatment (see Study Protocol and the Interim Analysis Plan for details). Based on the interim results, the study was to be stopped at interim or to be continued after sample size re-estimation.

A maximum study size of up to 200 patients per group appeared to be feasible for the scheduled recruitment period, based on recruitment estimates from the participating sites.

4.5 Null and Alternative Hypotheses

It was decided after the interim analysis and consultancy with the FDA to terminate the study and to evaluate all data descriptively and in an exploratory way. Point estimates of differences between treatment groups and corresponding two-sided 95%-confidence intervals will be presented for the primary and key secondary efficacy endpoints. Statistical hypotheses will not be tested and p-values will not be reported.

4.6 Blinded Review / Final Statistical Analysis Plan

The Sponsor will convene a Blinded Data Review Meeting (BDRM) after the data have been cleaned and before the study is unblinded. The review will be performed within the framework of the requirements of ICH E9.

The terms of reference of the BDRM shall include, but not be limited to:

- the determination of whether protocol deviations are 'major' or 'minor'.
- the allocation of patients to analysis sets
- a review of missing data and outliers, their assessment and definition of handling
- the distribution of the efficacy variables, considering any implications for the proposed methods for statistical analysis
- a review of whether additional covariates need to be included in the analyses
- the finalization of the SAP
- The listings and tables that will be prepared for the BDRM, should include, but not be limited to:
    - Listings
        - protocol deviations
        - missing pattern and imputation method
        - outliers
    - Tables
        - Summary of the primary endpoints by study stage and underlying disease
        - Summary of conjugated bilirubin

The BDRM Report will be finalized before the blind will be broken. Formal records shall be kept of when the statistical analysis plan was finalized as well as when the blind was subsequently broken.

5 STUDY VARIABLES

5.1 Primary Variable(s)

The primary study variable is the binary occurrence of conjugated bilirubin level > 2 mg/dL during the first 28 days of study treatment, confirmed by a second sample collected 7 days after the first sample.

5.2 Secondary Variables

5.2.1 Efficacy Variable

- Body weight (age-standardized*)
- Body length (age-standardized*)
- Head circumference (age-standardized*)
- Time to full enteral or oral feeds
- Fatty acids in plasma and red blood cell membranes
- Holman index

* Anthropometric data (weight, length, and head circumference) will be standardized by age and gender according to the WHO and Fenton standards.

5.2.2 Safety Variables

- Adverse events (AEs)
- Length of stay in hospital (Time to discharge from hospital)
- Ratio of number of independent bloodstream infections / number of days on study medication
- Ratio of the number of patients with 1 or more bloodstream infections / number of patients on study medication
- Completion of PN treatment without lipid minimization (Yes or No)
- Withdrawal from the study due to elevated conjugated bilirubin levels (Yes or No)
- Area under the curve (AUC) of conjugated bilirubin for time periods in which levels are > 1.5 mg/dL
- Cumulative number of days of PN without lipid minimization
- Number of days until conjugated bilirubin > 2 mg/dL confirmed by a second sample collected 7 days after the first sample
- Conjugated bilirubin

- Total bilirubin
- Serum triglycerides (TGs)
- Aspartate aminotransferase (AST)
- Alanine aminotransferase (ALT)
- Gamma-glutamyl transferase (GGT)
- Alkaline phosphatase (ALP)
- Blood glucose
- Blood urea nitrogen (BUN)
- Creatinine
- C-reactive protein (CRP)
- Sterols including Phytosterols
- α-tocopherol

6 ANALYSIS SETS

The allocation of patients to analysis sets will be performed during the blinded review before database close.

The decisions done at the final BDRM shall consider carefully previous decisions which were done at the BDRM before the interim analysis to maintain consistency of allocations to analysis sets and other decisions across both stages of the entire study.

6.1 Intention-to-Treat (ITT) Analysis Set

The ITT analysis set will consist of all randomized patients. Patients will be assigned as randomized. The analysis of the primary and secondary efficacy endpoints will be performed using the ITT analysis set.

6.2 Safety Analysis Set

The safety analysis set will consist of all randomized patients who received at least one dose of study medication. Patients will be assigned as actually treated. The analysis of secondary safety endpoints will be performed using the safety analysis set.

6.3 Per Protocol Set

The per protocol set includes all patients who received study drug for at least 14 days without major protocol violations. Patients will be assigned as treated. Major protocol deviations are those that may significantly impact the completeness, accuracy, and/or reliability of the study data. A patient may be excluded from the per protocol set for, but not limited to, any of the following conditions:

- Patient does not meet critical inclusion/exclusion criteria
- Patient is removed from the study prior to day 28 for other reasons than elevated conjugated bilirubin levels
- Incorrect administration of study product

Other deviations (protocol required evaluation not completed, investigational drug issues, anthropometric measurement issues) may be judged on an individual basis.

Protocol deviations in stage 1 patients were reviewed prior to the interim analysis according to the Data Review Plan (Version 1.0, 15 February 2019). Results of the interim BDRM were documented in the Protocol Deviation Listing dated 12 July 2019.

Protocol deviations in both study stages and exclusion of patients from the per-protocol set for the final analysis were reviewed in the BDRM before final analysis on October 28, 2020, and will be documented in the minutes of the BDRM and the Protocol Deviation Listing (date not yet available).

The analysis of the primary and secondary efficacy endpoints will be performed using the per protocol set.

7 STATISTICAL CONSIDERATIONS AND DEFINITIONS

All data will be evaluated descriptively and in an exploratory way. Point estimates of differences between treatment groups and corresponding two-sided 95%-confidence intervals will be presented for the primary and key secondary efficacy endpoints. Statistical hypotheses will not be tested and p-values will not be reported.

The analysis of the primary and secondary efficacy endpoints will be performed using both the ITT analysis set and the per protocol set. The analysis of the secondary safety endpoints will be performed using the safety set.

Descriptive statistics will be displayed to provide an overview of the efficacy and safety results by treatment group, stratum and overall. For categorical parameters, these will consist of the number and percentage of patients in each category. The denominator for percentages will be based on the number of patients in the corresponding analysis set, missing will be displayed as a category. For continuous parameters, descriptive statistics will include number of patients with number of non-missing values, mean, standard deviation, median, minimum, and maximum.

7.1 Changes in the Conduct of the Trial or Planned Analysis

The study was terminated early after the interim analysis and consultancy with the FDA. Changes in statistical analysis are described in this document and the Study Protocol Version 3.0.

7.2 Statistical Software

The statistical analysis will be performed using SAS version 9.4.

7.3 Protocol Deviations/Data Review

After all queries issued and answered to the extent possible, all medical codes are approved, all SAEs are reconciled, and prior to locking and unblinding the database for the final analysis, the sponsor will convene a BDRM.

The primary purpose of the BDRM will be to resolve outstanding data issues, to access and define the handling of missing data, to examine and classify major and minor protocol deviations, to define the analysis sets, and to finalize other statistically related issues. The final allocation of patients to analysis sets will be performed during the blinded review as described in Sections 4.6 and 6.

7.4 Handling of Drop-outs

Dropouts (not early dropouts, see Section 7.5) are patients who are withdrawn after randomization and receiving any study drug. Dropouts were not to be replaced. The number of dropouts and reason of dropout will be tabulated by treatment group.

7.5 Procedures for Replacement of Patients

Patients withdrawn from study after randomization but before receiving any study drug will be counted as “early drop-outs”. These patients will be replaced and will not have any additional assessments.

7.6 Adjustment for Covariates / Strata

The randomization is stratified by underlying disease (No NEC vs. NEC). The analysis of the primary endpoint will be stratified by underlying disease.

7.7 Multi-center Trials

The randomization was stratified by study site. The primary endpoint will be summarized descriptively by treatment group and site.

7.8 Multiple Comparison

As all analyses are descriptive and exploratory and no statistical hypotheses will be formally tested, multiplicity will not be taken into account.

7.9 General Calculation Rules

Unless stated otherwise, the term “descriptive statistics” refers to the total number of patients in the analysis set, the number of non-missing values, mean, median, standard deviation, minimum, and maximum for continuous data and frequencies and percentages for categorical data. Minimum and maximum values will be rounded to the precision of the original value, means and medians will be rounded to 1 decimal place greater than the precision of the original value, and standard deviations will be rounded to 2 decimal places greater than the precision of the original value. Precision of AUC will be four significant digits (e.g., 123.2 or 1.456 depending on the range of the values). Percentages will be rounded to one decimal place. For categorical variables, categories with no patients or all patients should be indicated by 0% and 100%. Unless otherwise stated confidence intervals will be two-sided with 95 % confidence level and will be presented with the same precision of the estimated parameter.

Unless otherwise noted, all data collected during the study will be included in data listings and will be sorted by treatment group, site and patient number and then by date/time for each patient number.

Baseline values for efficacy and safety variables will be based on the last non-missing data collected prior to the first administration of clinical trial material (CTM), unless otherwise noted.

Study days on or after the initial dose of CTM will be computed as
Study Day = Date – Study Drug Start Date + 1.

Study week is the sequence of 7 consecutive study days 1-7, 8-14, 15-21, 21-28, 29-35 … starting at Study Day 1 (baseline).

For pre-dosing dates, study days will be computed as Study Day = Date – Study Drug Start Date.

Age will be calculated from the date of informed consent.

7.10 Handling of Missing Data and Outliers

Missing data imputation is planned for both intention-to-treat and per protocol analysis. The distribution of missing value pattern, the appropriateness of imputation rules as well as the potential introduction of bias will be investigated at the BDRM. Also, the impact and handling of outliers will be discussed at the BDRM.

The following table shows the anticipated missing data patterns and the pre-specified imputation approach for the primary endpoint:

Missing Data Patterns and Imputation Approach for the Primary Endpoint

Missing Data Pattern of Conjugated Bilirubin	**Imputation Approach for Primary Endpoint**
A) Some missing value(s) and at least one value > 2mg/dL	
1. Value > 2mg/dL is confirmed	Primary endpoint met.
2. Value > 2mg/dL but not confirmed due to missing value	Primary endpoint met.
B) Some missing value(s) and all non-missing value(s) are <= 2mg/dL	
3. Value available on D28	Primary endpoint not met.
4. Latest value available on D22	Primary endpoint met if value > 1.5 mg/dL on D22. Otherwise, the primary endpoint will not be considered met.
5. Latest value available on D15	Primary endpoint not met if latest value <=1.5 mg/dL. Otherwise, imputation will depend on - Reason for dropout or missing data (adverse event/withdrew consent/weaned off before D28/lost to follow-up/death) - Conjugated bilirubin level(s) and trend
6. Latest value available only before D15 or no data available	Primary endpoint not met if latest value <=1.5 mg/dL. Otherwise, imputation will depend on - Reason for dropout or missing data (adverse event/withdrew consent/weaned off before D28/lost to follow-up/death) - Conjugated bilirubin level(s) and trend
C) All values missing	Primary endpoint not met.

In particular, for cases A2, B3, B4, and C information on missing conjugated bilirubin values (e.g. timing) and conjugated bilirubin levels over time as well as the reason for dropout or missing data will be reviewed for each patient during the BDRM and an individual decision for imputation will be taken.

7.10.1 Missing Severityor Causality of AEs

Missing severity will not be imputed but counted as a separate category. AEs which are not rated as "not related" will be reported as related according to the study protocol and sponsor standards.

7.10.2 Date Values

In cases of incomplete dates (e.g., pertaining to AE, concomitant medication, medical history, etc.), the missing component(s) will be assumed as the most conservative value(s) possible.

For example, if the start date has a missing day value and year and month are available, the first day of the month but not before first intake of study medication will be imputed for study day computations (i.e., treatment-emergent status, etc.). The start date will be set to the first day of the year but not before first intake of study medication if month and day are missing and year is available.

If day is missing for an end date, the last day of the month will be imputed. Similar logic will be assumed for missing month and year components.

Date imputation will only be used for computational purposes e.g., treatment-emergent status, etc. Actual data values as they appear in the original CRFs will be shown in the data listings.

7.10.3 Non-date Values

Every effort will be made to obtain the protocol-required data for all study assessments that are scheduled for each scheduled visit for all patients who have been enrolled.

Missing values will be reviewed and methods how to handle these missing data in the analysis will be discussed in the BDRM.

7.10.4 Handling of Outliers

Potential outliers will be clarified during the data cleaning process.

7.11 Visit Windows

Study visits will be windowed as given in Section 4.1. Data values will be presented for all scheduled study visits according to the nominal day obtained from the CRF (e.g., data value obtained on Day 9 within visit window Day 8+/-1).

If an unscheduled visit falls into a visit window with an existing scheduled assessment, the scheduled assessment will be used for summary presentation. Otherwise, if no scheduled assessment exists for a visit window but unscheduled visits are available within the same visit window, the unscheduled

assessment obtained closest to the midpoint of the visit window will be used for summary presentation. If there is no scheduled visit assessment for a visit window but unscheduled assessments outside of this visit window, then the unscheduled assessment obtained closest to the midpoint of the visit window but not beyond +/-3 days from the midpoint will be used for summary presentation. In case of ties, the latest assessment will be used.

All scheduled and unscheduled values will be included in the data listings.

8 STATISTICAL ANALYSIS

As only a small number of patients was enrolled after the interim analysis in stage 2 of the study, all data other than the primary endpoint will be presented for stages 1 and 2 combined and analyses will not be stratified by study stage.

8.1 Disposition

Patient disposition will be presented by underlying disease (No NEC / NEC) and treatment arm for all patients in stage 1 and stage 2 of the group-sequential design combined and for each stage separately. The composition of the analysis sets and those who completed or discontinued from the study will be presented by underlying disease and treatment arm and overall by absolute numbers and percentages. The reasons for early discontinuation will be listed and summarized descriptively.

8.2 Protocol Deviations

- Number and percentage of protocol deviations will be presented overall and by deviation type (major, minor), deviation review category (intent-to-treat, per protocol), and by the following deviation categories:
- inclusion/exclusion criteria
- informed consent issue
- out-of-window visit
- protocol required evaluation not completed
- physician decision
- parent / legal guardian decision
- investigational drug issue
- anthropometric measurement issue
- other

for each underlying disease group and treatment arm.

A listing of all events (sorted by criterion patient) will be included.

8.3 Demographic Data and Baseline Characteristics

Demographic and baseline characteristic data include postmenstrual and chronological (“post-natal”) age, gender, race, ethnicity, body weight, length, head circumference (low for age, normal, high for age, section 8.11.1), and underlying disease at screening. In addition, liver laboratory values including bilirubin, triglycerides will be summarized by underlying disease and treatment at baseline.

8.4 Medical History

Medical history will be summarized by the following medical history categories as reported in the CRF for each treatment group:

- Allergy
- Head, ear, eyes, nose and throat (HEENT)
- Respiratory
- Cardiovascular
- Gastrointestinal
- Hepatic
- Genitourinary
- Immunologic
- Hematologic/Lymphatic
- Neurologic
- Endocrine/Metabolic
- Musculoskeletal
- Dermatologic
- Infectious Disease
- Other

A comprehensive data listing will also be included.

8.5 Prior and Concomitant Medication

Prior and concomitant medications are any medications, vitamins, dietary supplements and vaccinations received by the patient prior to and since signing Informed Consent.

Prior medications are medications started and ended before start of study medication, concomitant at baseline are medications started before or at baseline visit and ongoing during study treatment, and concomitant medications are medications given after baseline.

Prior and concomitant medications will be coded with the World Health Organization (WHO) drug dictionary and number and percentage of patients with the event will be summarized with descriptive statistics by level 2 (therapeutic / pharmacological subgroup) and level 4 (chemical / therapeutic / pharmacological subgroup) of the Anatomical Therapeutic Chemical Classification System (ATC) by treatment arm. A data listing will be included that shows all medications sorted by generic name and verbatim name. In addition, prior and concomitant medications will be listed for each patient.

8.6 Treatment Exposure and Compliance

The duration of exposure (days) to a study drug, total amount of study drug administered and average daily dosage of study drug (per kg body weight) administrated will be summarized with descriptive statistics by underlying disease and treatment arm. For patients with conjugated bilirubin levels > 2 mg/dL, the cumulative dose administrated before the primary endpoint is reached (bilirubin >2 mg/dL) will be summarized by underlying disease and treatment arm.

Duration of exposure will be calculated as the difference between the date of last infusion of a study drug and the date of first infusion with a study drug plus 1 day. A data listing for treatment exposure will be included.

The duration, dose, and type of pre-treatment will be extracted from prior and concomitant medication form and summarized with descriptive statistics by underlying disease and treatment group. The frequency and percentage of patients who received PN for pre-treatment will be summarized by treatment group.

Compliance will be assessed by

- the ratio of the actual number of study drug infusions over the expected number of study drug infusions expressed as percent
- the ratio of the actual cumulative dose over the expected cumulative dose

evaluated separately over the initial and extension phase, resp., and summarized descriptively by treatment group and study phase.

Infusion of study drug will be expected on a given Study Day over the treatment period (Day 1 to Day 28 for initial treatment phase and Day 29 to Day 85 for treatment extension phase). Treatment with a study drug on a given Study Day will be considered an actual study drug infusion, provided any interruption of study drug infusion does not exceed 4 hours. Any interruption of the infusion of study drug longer than 4 hours will be documented and explained in the eCRF.

Compliance data will be provided in a data listing by patient.

Dose interruption (dose delay and modification) will be summarized with descriptive statistics (frequency and percentage) by treatment group.

8.7 Subgroup Analysis

The primary endpoint (using the ITT analysis set and the per protocol set) as well as adverse events will be summarized by treatment group for the subgroups of ethnicity (Hispanic/non-Hispanic) and the following race categories:

- Caucasian
- Black or African American
- Asian
- Native Hawaiian or Other Pacific Islander
- American Indian or Alaska Native
- Other

In addition, the primary endpoint will be summarized by treatment group and site, and by underlying disease.

8.8 Interim Analysis

An unblinded interim analysis was performed for testing of the primary hypotheses. A detailed Interim Analysis Plan had been provided in a separate document (Interim analysis Plan Version 1.0, February 14, 2019).

152 randomized patients were included in the interim analysis. Interim results will not be disclosed before final database close and unblinding.

8.9 Data Monitoring

In this trial, an independent Data Safety Monitoring Board (DSMB) was involved to monitor patient safety.

The working principles were defined in a DSMB charter describing the composition of the DSMB, responsibilities, organization, flow of information, evaluation and decision principles, and interactions.

8.10 Analysis of the Primary Endpoint

The primary study variable is the number of patients in each treatment group with conjugated bilirubin levels > 2 mg/dL during the first 28 days of study treatment, confirmed by a second sample collected 7 days after the first sample.

Number and incidence of patients with conjugated bilirubin levels > 2 mg/dL during the first 28 days of study treatment will be presented overall and stratified by the patients' underlying disease (No NEC / NEC) for each stage of the study and the combined stages. Differences between treatment groups will be quantified using the estimates of the common relative risk (Mantel-Haenszel estimate) - controlling for stratum - and the Cochran-Mantel-Haenszel statistics of general association for the combined strata, and corresponding 95% confidence intervals will be presented.

Data from each stage without imputation will be summarized with descriptive statistics, combined data from both stages with and without imputation will be summarized with descriptive statistics using the ITT analysis set and the per protocol set.

8.11 Analysis of Secondary Efficacy Variables

The secondary outcome parameters for efficacy will be summarized descriptively for the ITT analysis set and the per protocol set from both stages combined.

8.11.1 Age-Standardized Growth – Body Weight, Length, and Head Circumference

Growth and development of infants will be evaluated based on the parameters body weight, body length/height, and head circumference. Data will be age-standardized using growth charts as suggested by Fenton (Fenton et al., 2013) and the World Health Organization (WHO) Multicenter Growth Reference Study (MGRS; WHO 2006, 2007). Term infants (i.e., born at ≥ 37 completed weeks of gestational age) will be evaluated solely using WHO charts. For preterm infants, growth data collected at ≤ 50 weeks of completed gestational age will be standardized with Fenton charts. Otherwise, WHO charts will be applied.

Fenton charts at 50 weeks gestational age are equivalent to the WHO standard at 10 weeks chronological age. Thus, a preterm infant with growth data as of > 50 weeks of gestational age needs to be evaluated along the x-axis of the WHO charts at gestational age subtracted by 40 weeks.

Standardization according to the WHO standard will be derived for all growth parameters using the SAS macros provided by WHO (WHO, Anthropometry version 3.2.2, 2011).

The authors of the Fenton charts published various Z-score and percentile calculators for download. Age-standardized Z-scores of the standard normal distribution will be derived using a publicly available Microsoft Excel calculation tool (Actual Age Calculator v7, 2015).

Z-scores will be assigned to any of the three categories: normal, low for age, high for age. The following cut-off values for categorization will be used (Wang & Chen, 2011; WHO Child Growth Standards, 2006):

Categorization of Z-Scores on Fenton and WHO Growth Charts

Z-score cut-off	Category	Corresponding p%-percentile
Z ≤ -1.88	Low for age	p ≤ 3 %
-1.88 < z < 1.88	Normal	3% < p < 97%
Z ≥ 1.88	High for age	p ≥ 97%

Categorization of age-standardized Z-score will be performed for the end of the initial treatment phase and the end of the extended treatment phase. The number and percentage of patients with low, normal and high weight for age will be presented by time point (including end of treatment and end of study) and treatment group.

Anthropometric data will be listed and summarized by time point and treatment group, and Spaghetti plots and box plots over time will be displayed by treatment group.

Patient listings of measured anthropometric data (body weight [g], height/length [cm], head circumference [cm], corresponding Z-scores after standardization for age, and allocated categories will be provided.

8.11.2 Time to Full Enteral or Oral Feeds

Time to full enteral or oral feeds (i.e. PN weaning) is the time from the randomization date to the date of the first full enteral or oral feeds. Patients alive who were not weaned from PN will be censored at the last follow up date (either in the initial treatment phase or in the treatment extension phase) and patients who died will be considered a competing risk. The cumulative incidence function for time to full enteral or oral feeds will be estimated using the non-parametric Aalen estimator (Aalen, 1978) and displayed graphically over time by treatment group.

The cause-specific hazard will be estimated using the subdistribution hazard model according to Fine and Gray with factors treatment, underlying disease group (No NEC /NEC) and the interaction term (Fine & Gray, 1999).

The number of patients with full enteral or oral feeds, the number of patients who died, the number of patients censored, the median time to event determined from the cumulative incidence functions

by underlying disease group and treatment group and the hazard ratio and its 95% confidence interval determined from the Fine and Gray-model will be presented in summary table.

8.11.3 Fatty Acids in Plasma and Red Blood Cell Membranes

Fatty acids to be assessed include linoleic acid, α-linolenic acid, Mead acid, eicosapentaenoic acid, arachidonic acid, docosahexaenoic acid. The fatty acids and absolute and relative change from baseline will be summarized descriptively by underlying disease group and treatment group.

The incidence of essential fatty acid deficiency and shift from baseline will be summarized with descriptive statistics by treatment group. Essential fatty acid deficiency is based on the ratio of Mead acid and Arachidonic acid (also called the triene/tetraene ratio or Holman index; Holman, 1960). A ratio of > 0.2 will be considered abnormal (=essential fatty acid deficiency; Holman et al., 1979). The Holman index (= triene/tetraene ratio) will be calculated by statistical programming.

8.12 Analysis of Secondary Safety Variables

The analysis of the secondary safety endpoints will be performed using the safety analysis set. All analysis of the secondary safety endpoints will be descriptive, no statistical hypothesis testing will be performed. Unless otherwise stated, safety endpoints will be presented using data from the initial and extension phase combined. For all safety endpoints data from both stages of the group-sequential design will be combined.

8.12.1 Time to Event Variables

All time to event variables will be summarized with descriptive statistics using competing risk analysis. A detailed data listing for each time to event variable will be included.

8.12.1.1 Time to discharge from hospital

Length of stay in hospital (time from randomization to discharge) will be calculated using the Worksheet of the Vermont Oxford Network (see Annex 6 to the protocol). Death during hospitalization will be considered a competing risk. Patients alive who are not reported to be discharged during the study will be censored at the last follow-up date.

The cumulative incidence function for time to discharge from hospital will be estimated using the Aalen estimator (Aalen, 1978). The cause-specific hazard will be estimated using the subdistribution hazard model according to Fine and Gray with factors treatment, underlying disease group (No NEC / NEC) and the interaction term (Fine & Gray, 1999).

The number of patients discharged from hospital, the number of patients who died, the number of patients censored, the median time to discharge from hospital determined from the cumulative incidence function and the hazard ratio and its 95% confidence interval estimated determined from the Fine and Gray-model will be presented in a summary table.

8.12.1.2 Number of days until conjugated bilirubin > 2 mg/dL

Time (in days) to when conjugated bilirubin levels > 2 mg/dL is the time from the randomization date to the date of first occurrence of conjugated bilirubin levels > 2 mg/dL (confirmed by a second sample collected 7 days after the first sample). Death during hospitalization will be considered a competing risk. Patients alive will be censored if bilirubin levels > 2 mg/dL are not observed during the study using the last follow-up date as the censoring time.

The cumulative incidence function until conjugated bilirubin > 2 mg/dL will be estimated using the Aalen estimator (Aalen, 1978). The cause-specific hazard will be estimated using the subdistribution hazard model according to Fine and Gray with factors treatment, underlying disease group (No NEC / NEC) and the interaction term (Fine & Gray, 1999).

The number of patients with conjugated bilirubin levels > 2 mg/dL, the number of patients who died, the number of patients censored, the median time to event determined from the cumulative incidence function and the hazard ratio and its 95% confidence interval estimated determined from the Fine and Gray-model will be presented in a summary table.

8.12.2 Other Secondary Safety Variables

For each patient, the ratio of the number of independent bloodstream infections and the number of days on study medication will be computed. The ratio, as well as the number and incidence of patients with at least one bloodstream infection will be summarized descriptively by underlying disease group, treatment arm and study phase. A data listing of number of bloodstream infections and number of days on study medication will be included.

The number and incidence of patients who complete PN treatment without lipid minimization, as well as the cumulative number of days of PN administration without lipid minimization will be summarized descriptively statistics by underlying disease, treatment arm and study phase. A corresponding data listing will be included.

The number of patients who need to be withdrawn from the study due to elevated conjugated bilirubin levels will be summarized with descriptive statistics by underlying disease, treatment arm and study phase.

8.12.3 Area under the curve of conjugated bilirubin for time period in which levels are > 1.5 mg/dL

The area under the curve ($AUC_{>1.5}$) is defined as the area between conjugated bilirubin concentrations > 1.5 mg/dL and the horizontal line at 1.5 mg/dL, restricted by the time point of study withdrawal, if applicable. The $AUC_{>1.5}$ will be calculated using the Riemann method[10], which is the same as the trapezoidal rule. An AUC value will only be used if there was no more than one conjugated bilirubin value missing. Descriptive summary statistics by treatment group and study phase and a detailed data listing will be presented.

8.12.4 Adverse Events, Serious Adverse Events, Deaths

All reported terms (investigator descriptions) for AEs will be coded using the version of the Medical Dictionary for Regulatory Activities (MedDRA) at database closure and will be summarized descriptively.

A treatment-emergent adverse event (TEAE) is an event that emerges during treatment, having been absent pre-treatment, or worsens relative to the pre-treatment state.

For all AE summaries, events will be counted only once for a given patient by primary system organ class and preferred term. When an AE occurs more than once for a patient, the maximum severity and causality will be used. Missing severity will not be imputed but counted as a separate category. AEs which are not rated as "not related" will be reported as related according to the study protocol and sponsor standards.

The following summaries will be presented with numbers and percentage of patients with the event and number of reported events for each system organ classification and preferred term by underlying disease group and treatment group:

- All AEs
- All TEAEs
- TEAEs leading to discontinuation from the study
- TEAEs by relation to study procedure
- TEAEs by relation to study drug (relationship was reported as "related" or "unrelated").
- TEAEs by CTCAE severity
- TEAEs by relation to study drug and CTCAE severity
- TEAEs occurring with an incidence > 5 % within any treatment arm
- All serious TEAEs (see Section 13.1.2 of the protocol)
- Serious TEAEs by relation to study drug

- TEAEs with fatal outcome

In addition, the number and percentage of patients with the event of the following TEAEs of special interest (syndromes reported as AEs) will be presented by treatment group:

- all major neonatal morbidities
- bronchopulmonary dysplasia
- retinopathy of prematurity
- intraventricular hemorrhage
- periventricular leukomalacia
- necrotizing enterocolitis
- late-onset sepsis in premature and low birth weight neonates.

The following AEs will also be presented by treatment group for each subgroup of ethnicity and race category, respectively:

- All AEs
- All TEAEs
- TEAEs leading to discontinuation from the study
- TEAEs by relation to study procedure
- TEAEs by relation to study drug
- TEAEs by CTCAE severity
- All serious TEAEs (see Section 13.1.2 of the protocol)
- TEAEs with fatal outcome
- TEAEs of special interest, as defined above

All AEs will be presented in data listings by patient. Separate data listings will be created for deaths, serious adverse events (SAEs, death excluded), AEs leading to discontinuation from the study, and AEs of special interest. The Listings of AEs of special interest will also include birth weight.

8.12.5 Clinical Laboratory

Laboratory assessments and change from baseline will be summarized with descriptive statistics by test panel, individual laboratory test, underlying disease and group treatment group, and time point. Additionally, abnormal results including normal, abnormal clinically significant (abnormal CS), abnormal not clinically significant (abnormal NCS) captured by the eCRF will be summarized with

frequencies and percentages by clinical significance, panel, test, underlying disease group and treatment group, and time point. The denominators for percentages will be the number of patients in the analysis set for the treatment group summarized. Missing values will be presented in the summary tables. Counts and percentages of patients with shifts for the period from baseline to the end of the initial treatment phase will be summarized by analyte, underlying disease group and treatment group:

- Normal -> abnormal CS: from normal at Baseline (Day 0 or Day 1) to abnormal CS at the end of the initial treatment phase
- Normal -> abnormal NCS: from normal at Baseline to abnormal NCS at the end of the initial treatment phase
- Abnormal NCS -> abnormal CS: from abnormal NCS at Baseline to abnormal CS at the end of the initial treatment phase
- In addition, counts and percentages of patients with shifts for the period from baseline to the end of the extended treatment phase will be summarized by analyte and treatment group as described above. In this table, the denominator will be number of patients still in the study at the end of the initial treatment phase.

A data listing will display all laboratory test results and findings. A listing of lab data (including all visits) for patients who had at least one abnormal result will be provided.

8.12.6 Vital Signs

Vital sign measurements and change from baseline of vital signs (including heart rate, temperature, systolic and diastolic blood pressure) will be summarized with descriptive statistics by underlying disease group, treatment group, and time point. A data listing of all data will also be included.

8.12.7 Physical Examinations

Physical examinations will be summarized by assessment, body system, underlying disease group and treatment group using number and percentage of patients in each assessment category (i.e., normal, abnormal NCS, and abnormal CS). Denominators for the percentages will be based on the number of patients within the treatment group for the assessment summarized. In addition, counts and percentages of patients with shifts will be summarized by body system and treatment group (see laboratory data above). Denominators for the percentages will be based on the number of patients with a body system evaluation at both baseline and end of study in each treatment group.

A data listing of physical examination data will be generated by patient.

8.13 Analysis of Nutritional Intake

The calories per kg of body weight and day and the percentage of calories provided as enteral, oral, parenteral (including study drug) and study drug relative to total calories will be summarized by underlying disease and treatment group for each study week and the overall treatment period.

For enteral and parenteral nutrition components, the protein, fat and carbohydrate intake will be calculated based on the product information. For example, the protein intake from enteral nutrition (EN) for a patient will be computed according to

$$\text{Protein intake [g/kg/day]} = \frac{\text{EN intake [kcal/kg/day]} \cdot \text{ g Protein per 100 kcal}^{1}}{100}$$

Calculations for fat and carbohydrates and PN products will be performed analogously.

All information regarding the exposure to other nutrition, (i.e., oral, enteral and non-nutritional sources) will be listed. This includes the item/product, dosage/amount and (estimated) calories.

[1] Protein contents per 100 kcal are based on product information.

9 CONTENTS OF STATISTICAL TABLES AND FIGURES

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