Statistical Analysis Plan

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Statistical Analysis Plan

Title:

Discovering the mechanisms of action for the in-vivo protection of Aspirin-induced enteropathy by Bifidobacterium breve Bif195 in man - a randomized, double-blinded, placebo-controlled, cross-over trial in healthy volunteers

Trial Id:
HND-GI-036

Trial participants:

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List of Abbreviations

AE	Adverse Event
AOM	Acute Otitis Media
AUC	Areas under the curve
BMI	Body mass index
CFU	Colony Forming Units
CI	Confidence Interval
CRA	Clinical Research Associate
CRO	Contract Research Organization
CV	Coefficient of Variation
eCRF	Electronic Case Report Form
EFSA	European Food Safety Authority
ePRO	Electronic Patient Reported Outcome
FAO	Food and Agricultural Organization
FAS	Full- analysis set
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GI	Gastrointestinal
GP	General Practitioner
GRAS	Generally Regarded as Safe
Guardian	Parent or Legal Guardian
HR	Hearth rate
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
Mg	Milligram
LRTI	Lower Respiratory Tract Infections
Min	Minutes
mL	Milliliter
N	Sample Size
PI	Principle Investigator
PP	Per protocol
QC	Quality Control

QPS	Qualified Presumption of Safety
QQ	Quantiles-Quantiles
RA	Regulatory Authority
RTI	Respiratory Tract Infections
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Safety Analysis Set
SD	Standard Deviation
URTI	Upper Respiratory Tract Infections
WHO	World Health Organization

1 Introduction and Summary

1.1 Introduction

The present Statistical Analysis Plan (SAP) describes in detail and in accordance with ICH-GCP guidelines (1) and Signifikans Aps SOPs the statistical analyses to be carried out for the HND-GI-036 trial.

1.2 Summary

The table below provide an overview of all analyses performed on the primary, secondary and exploratory endpoints.

Primary and Secondary Outcome Variables

Variables	Time point	Endpoint	Analysis performed
Primary outcome variables	Week 2, 3 4 and 5	Lanza score	Generalized Estimating Equations (GEE) model including the following terms: Treatment (Bif195 or Placebo), Sequence (First Bif195 or Placebo), Period (1 and 2) and baseline (visit 2) value
Secondary outcome variables (safety and efficacy)	Week 2, 3 4 and 5	**Blood safety parameters:** Creatinin, Potasium, Sodium, ALAT, ASAT, HbA1c, Glucose (from HbA1c), CRP, Leucocytes, Thrombocytes **Blood efficacy parameters:** Erytrocytes, Haematocrit, Haemoglobin, INR	Each parameter will be analysed using a proc mixed model with patient as Random effect. The following terms will be including in the model: Treatment (Bif195 or Placebo), Sequence (First Bif195 or Placebo), Period (1 and 2) and baseline (visit 2) value

2 Trial Objective

2.1 Primary Objectives

Clinical evaluation of stomach and duodenum via the (5-scale) Lanza score.

0 = no visible lesions
1 = redness and hyperemia in mucosa
2 = 1 or 2 erosions or hemorrhaging lesions
3 = 3-10 erosions of hemorrhaging lesions
4 = >10 erosions or hemorrhaging lesions or an ulcer

Erosions = mucosal disruption denuded of villi with or w/o exudates or red color
Ulcer = large erosion with central area with exudates
Ref: Soylu et al. 2008, World J Gastroenterol, 14(43), 6704-6710

2.2 Secondary Objectives

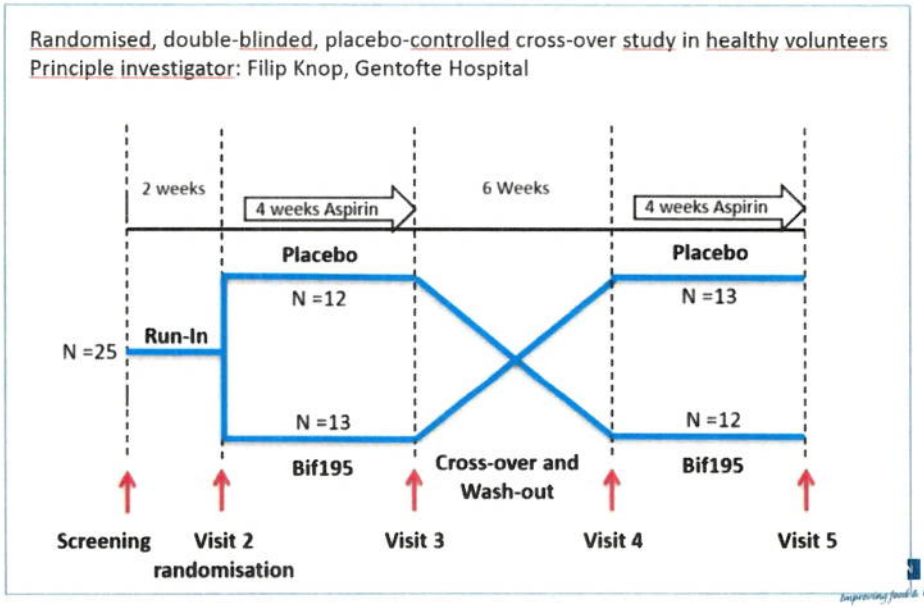
Safety blood parameters:
Blood safety parameters: Creatinin, Potasium, Sodium, ALAT, ASAT, HbA1c, Glucose (from HbA1c), CRP, Leucocytes, Thrombocytes

Efficacy blood parameters:
Erytrocytes, Haematocrit, Haemoglobin, INR

3 Trial Design

Study design (HND-GI-036)

- Aspirin 300 mg/day (1 capsule at breakfast)
- Placebo or 100 B CFU Bif195/day (1 capsule at breakfast, 1 capsule in the evening)



4 Trial Population

For the statistical analysis, the randomized subjects will be divided into the following datasets:

Full Analysis Set (FAS):	Subjects who are randomized and have consumed at least one dose of study product and for whom efficacy data is available.
No response to challenge (NC)	Subjects not responding on the Aspirin challenge response defined as having Lanza score=0 on all visits (visits 2, 3 4 and 5)
Modified FAS (mFAS)	FAS population minus NC population.
Per Protocol Set (PPS)	Subjects, who are randomized and have consumed at least one dose of study product, have available efficacy data and have no major protocol deviations

All endpoints will be analyzed on the FAS populations. For sensitivity analysis, selected analyses will also be performed on the mFAS and PPS.

5 Statistical Methodology

5.1 General statistical considerations

All statistical tests will be assessed using a nominal two-sided significance level of 5%.

The two treatment groups will be labelled as Bif195 and Placebo.

Weeks will be calculated as number of days divided by 7
Months will be calculated as number of days divided by 30.4375
Years will be calculated as number of days divided by 365.25

Number of days will be calculated using end date minus start date +1.

5.2 Subject disposition

An overall summary table of the subject disposition will be prepared with number of patients in the following categories (and sub-categories):

- Screened and randomized patients
- Analysis populations (FAS and mFAS)
- End of study reason

5.3 Handling of Missing Data and Dropouts

No imputation of data will be carried out in case of missing data, but all available data will be used.

5.4 Primary Efficacy Outcome Variable

Primary efficacy variable: Lanza score ranging from 0 (no visible lesions) to 4.

Change form baseline prior each period will be calculated and used in the analysis i.e. (visit 3 minus visit 2) (visit 5 minus visit 4).

This ordinal endpoint will be analysed using a Generalized Estimating Equations (GEE) model including the following terms:

- Treatment (Bif195 or Placebo)
- Sequence (first Bif195 or Placebo)
- Period (1 and 2)
- Baseline (visit 2) value

The model will take in to account the crossover design of the study with same patients having a result after both treatments.

1) Firstly, the analyse will include terms of Treatment, Sequence, period and baseline (visit 2) value
2) If no significant terms of either sequence or period, the factors will be removed from the model prior evaluating treatment effect

5.5 Secondary Safety Outcome Variables

Secondary safety outcome variables: Blood safety parameters: Creatinin, Potasium, Sodium, ALAT, ASAT, HbA1c, Glucose (from HbA1c), CRP, Leucocytes, Thrombocytes

Each parameter will be analysed using a proc mixed model with patient as Random effect.

Sequence and period effect will be evaluated prior evaluating the treatment effect in a similar way as the primary efficacy outcome variable.

For statistical analysis of data measured on an interval scale model check is assessed using QQ residual plots together with Kolmogorov-Smirnov test for normality.

In case for normality not reached using untransformed data nor transformed data using the log2-scale, appropriate non-parametric statistical methods will be used instead.

5.6 Secondary Efficacy Outcome Variables

Secondary Efficacy outcome variables: Erytrocytes, Haematocrit, Haemoglobin, INR. All variables are analysed like the secondary safety outcome variable analysis.

5.7 Sensitivity analysis on secondary variables

Percent change from baseline on the above secondary variables will also be analysed, where baseline value prior each period (visit 2 and visit 4) is set to index=100.

Subject 1, visit 2 = 1.2 (index=100)

Subject 1, visit 3 = 3.4 (index=283.3, calculated as 100*3.4/1.2)

Subject 1, visit 4 = 2.3 (index=100)

Subject 1, visit 5 = 1.0 (index=43.5, calculated as 100*1.0/2.3)

5.8 Data structure prior primary and secondary analysis

Below is the required data structure for using the statistical models (A and B are the two treatment groups).

subject	sequence	period	tmt	result	baseline
1	AB	1	A	3	0
1	AB	2	B	5	1
2	AB	1	B	4	4
2	AB	2	A	5	3
3	AB	1	B	4	0
3	AB	2	A	3	0
4	AB	1	B	0	2
4	AB	2	A	3	0
5	AB	1	A	4	0
5	AB	2	B	1	0
6	BA	1	A	3	0
6	BA	2	B	4	1
7	BA	1	B	3	0
7	BA	2	A	2	0
8	BA	1	B	4	0
8	BA	2	A	4	0
9	BA	1	A	1	1
9	BA	2	B	2	0
10	BA	1	A	2	1
10	BA	2	B	4	2

5.9 Challenge analysis

The challenge model will be analysed comparing baseline (visit 2 and 4) with "after" results (visit 3 and visit 5) using similar model as the primary and secondary outcome analysis with the following terms in the model:

- Treatment (Bif195 or Placebo)
- Sequence (first Bif195 or Placebo)
- Period (1 and 2)
- Before_after (baseline and "after")

The challenge model will thereby be analysed adjusted for period, sequence and treatment.

The data will be structured as seen below:

subject	sequence	period	tmt	Before_after	result
1	AB	1	A	before	1
1	AB	1	A	after	2
1	AB	2	B	before	1
1	AB	2	B	after	0
2	AB	1	B	before	4
2	AB	1	B	after	5
2	AB	2	A	before	4
2	AB	2	A	after	5
3	AB	1	B	before	4
3	AB	1	B	after	3
3	AB	2	A	before	4
3	AB	2	A	after	3
4	AB	1	B	before	0
4	AB	1	B	after	3
4	AB	2	A	before	0
4	AB	2	A	after	3
5	AB	1	A	before	4
5	AB	1	A	after	1
5	AB	2	B	before	4
5	AB	2	B	after	1
6	BA	1	A	before	3
6	BA	1	A	after	4
6	BA	2	B	before	3
6	BA	2	B	after	4
7	BA	1	B	before	3
7	BA	1	B	after	2
7	BA	2	A	before	3
7	BA	2	A	after	2

5.10 Other Efficacy Outcome Variable

All biopsies will be collected and analysed post unblinding of the study. Data will be analysed descriptively.

5.11 Control of Multiplicity for the Primary and secondary Efficacy Analysis

No adjustment for multiplicity is done.

5.12 Subject Disposition, Demographics and Other Characteristics

Subject disposition demographics and other characteristics will be reported descriptively.

6 Software

All statistical analyses will be computed using the statistical software SAS®, Release 9.3 or later versions.

7 References

- Slides set: 2021-01-12 Strain X MoA study.pptx
- Bif195 study group meeting_29Jan2020_NL_genskabt.pptx