Cooperative Clinical Drug Research and Development

1 Title Page

Clearance of the probiotic strain *Escherichia coli* Nissle 1917 in the gastrointestinal tract of healthy volunteers

Study Report

(1.0)

Author: Mark Montag Date: 13-Jul-2017

Project number: SYNB.NSL.01 (Sponsor) / CSL16001 (CRO)	**Clinical study phase**: 0
EudraCT number:	2016-001240-19
Sponsor: Synlogic 200 Sidney Street, Suite 320, Cambridge, MA 02139 USA Phone: +1-617-401-9975	**Project Leader**: Sarah Guilmain 200 Sidney Street, Suite 320 Cambridge, MA 02139 USA Phone: +1-617-500-6635 Ext 417 Email: sarahg@synlogictx.com
Study period: from 13-SEP-2016 to 19-APR-2017	**Early termination**: Yes: ☒ No: ☐
End of clinical trial: 19-APR-2017	
Principal Investigator: Ivo Bogdanov, MD, Bed space for short term stay at Diagnostic & Consultative Centre 'Ascendent' Ltd., 47 Bacho Kiro str., 1202 Sofia, Bulgaria	
Indication: No therapeutic indication in the current trial.	
Name of test drug/investigational product:	Mutaflor® capsules
Earlier reports from the same study: none	**Archiving**: Source data of volunteers: at principal investigator as specified in the ICH Topic E 6 (R1) guideline (chapter 8)
Medical Officer responsible for the medical content of this report	
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This study was conducted in compliance with Good Clinical Practice (GCP).

The report was produced on a word-processing system and bears no signatures.
The signatures of all persons responsible are filed separately in section 16.1.5

2 Synopsis

NAME OF COMPANY **Synlogic** **NAME OF FINISHED PRODUCT:** **Mutaflor® capsules** **NAME OF ACTIVE INGREDIENT(S):** ***Escherichia coli* strain Nissle 1917**	**INDIVIDUAL STUDY TABLE REFERRING TO CLINICAL DOCUMENTATION OF THE DOSSIER:** **Volume:** **Page:**	*(FOR NATIONAL AUTHORITY USE ONLY)*

Title of the study:
Clearance of the probiotic strain *Escherichia coli* Nissle 1917 in the gastrointestinal tract of healthy volunteers

Investigator(s):
Principal investigator: Ivo Bogdanov, MD

Study center:
Bed space for short term stay at
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Bulgaria
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Publication (reference):
the results are not yet published

Studied period: date of first enrolment: 13-SEP-2016 date of last completion: 19-APR-2017	**Phase of development:** Phase I

Objectives:
The objective of the present trial was to assess the clearance of *Escherichia coli* strain Nissle 1917 (EcN) in the gastrointestinal tract after administration of an oral test preparation containing 2.5-25 x 10^9 CFU (Test IMP: Mutaflor® capsules) after single or multiple dose administrations of one capsule three times daily for 28 days (48 volunteers) or for 1 day (10 volunteers) taken together with meals.

Methodology:
The study was conducted as a open, monocentric, single and multiple-dose trial in healthy volunteers.

Subjects (planned and analyzed):	planned for completion:	58
	enrolled (*study population*):	83
	screened only:	28
	drop-out(s) before IMP administration	0
	treated (*safety analysis population*):	45 (multiple dose group) 10 (single dose group)
	drop-out(s) after IMP administration:	2 (Rnd. Nos. 103, 108)
	completed:	53
	data set for statistical analysis (*per protocol set*):	53 (43 multiple dose group, 10 single dose group)
Diagnosis and main criteria for inclusion:	1. No pre-existing medical conditions based on medical history and physical examination 2. Non-smoking 3. Male and female volunteers, aged 18-55 years 4. Normal weight according to the BMI (accepted range 18.5 to 30.0 kg/m2) 5. Regular bowel habits including consistency, frequency (4-14 stools per week) 6. Willingness to commit to no major dietary changes to daily carbohydrate and protein intake during the trial and until 2 weeks after last administration 7. Negative pregnancy test for all females of childbearing potential at entry visit 8. Signed written informed consent by subject	

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Test product, dose and mode of administration, batch number:	name:	Mutaflor® capsules
	manufacturer:	Ardeypharm GmbH, Germany
	dosage form:	capsule
	unit dose:	2.5-25 x 10^9 CFU per capsule
	mode/route:	oral, 1 capsule together with meals
	regimen:	single or multiple dose
	batch no.:	542321
	expiry date:	09-Dec-2016

Duration of treatment:
The volunteers in the multiple dose group had to take one capsule three times daily for 28 days together with meals, and the volunteers in the single dose group had to take an oral single dose of 3 capsules for one day together with meals. The multiple-dose group was dosed first.

Stool sampling:
Multiple dose group (MD):
Stool samples for qualitative PCR were collected at screening, before the start of treatment (day 0) as well as on days 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and at weeks 5, 6, 7, 8, 9, 12, 16, 24, 32, 40, and 48.
Stool samples for quantitative PCR were collected before the start of treatment (day 0) as well as on days 4, 6, 14, 20, and 28 and at weeks 9, 12, 16, 24, and 32.

Single dose group (SD):
Stool samples for qualitative PCR were collected at screening, before the start of treatment (day 0) as well as on days 1, 2, 3, 4, 5, 7, 14, 21, 28 and at weeks 5, 6, 7, 8, 9, 10, 11, and 12.
Stool samples for quantitative PCR were collected at screening, before the start of treatment (day 0) as well as on days 1, 2, 3, 4, 5, 7, 14, 21, 28 and at weeks 5, 6, 7, 8, 9, 10, 11, and 12.
Blood sampling

Blood sampling for microbiology:
Both dose groups:
Blood samples for microbiological cultures for *E. coli* were collected before the start of treatment (day 0), on days 4 and 14 as well as at week 16.

Analysis of *Escherichia coli* strain NISSLE 1917 in stool	Qualitative and quantitative PCR specific for *Escherichia coli* strain Nissle 1917
Analytical center for qualitative PCR analysis	MLM Medical Labs GmbH Dohrweg 63 41066 Moenchengladbach, Germany
Analytical center for quantitative PCR analysis	QPS LLC 3 Innovation Way, Suite 240 Newark, Delaware, USA 19711
Analytical center for microbiology (*E.coli* in blood)	RAMUS Private Medical and Diagnostic Laboratory region: Oborishte, 2-4 Angista Str. 1521 Sofia, Bulgaria

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Primary endpoints:

Descriptive statistical evaluation of

1. Percentage of volunteers with a stool sample positive for *Escherichia coli* strain Nissle 1917, 24 weeks after the start of treatment;
2. Time to no detection of EcN in the stool

Safety endpoints

1. Safety/tolerability (including nature and frequency of adverse events/reactions; laboratory assessments; blood cultures for *E. coli*)
2. Change in GI symptoms (including GI pain/discomfort, flatulence, bowel noises, stool pattern/consistency/frequency) over the course of treatment.

The changes in GI symptoms were evaluated by using the modified Gastrointestinal Symptom Rating Scale (GSRS).[1]

RESULTS

A total number of 55 volunteers were enrolled and treated with study medication. Fifty-three volunteers completed the trial according to the protocol. The samples of 53 study completers and the available samples of 2 drop-outs (Rnd. Nos. 103, 108) were analyzed. The statistical evaluation was based on the data of 53 study completers.

None of the subjects had a stool sample positive for *Escherichia coli* strain Nissle 1917, 24 weeks after the start of treatment.

In the multiple dose group was a median of 7 days (range: 0 to 142 days), and in the single dose group there was a median of 13 (range: 3 to 55 days) until no detection of EcN after study medication intake. Due to different post-last-dose sampling schedules, the time to clearance for single- and multiple-dose may be similar.

Safety:

A total number of 7 non-serious adverse events (AEs) were registered in 7 subjects of the MD group in the course of the trial. No AEs were registered in the SD group. Six subjects reported headache of moderate severity, for one subject elevated liver enzymes were reported. One headache event was judged by the PI as unlikely related, all other events were judged as not related to study medication. All events recovered/resolved within the same day, except one headache which recovered after 2 days.
There was one subject with elevated liver enzymes ALT and GGT at final examination assessed as implausible value and clinically relevant (control examination), but considered not related to study medication intake. Apart from that no clinically important laboratory changes or trends were observed during the present trial. The laboratory and clinical screening revealed no indications for adverse events or poor tolerability.

The blood cultures from samples taken before the start of treatment (day 0), on days 4 and 14 as well as at week 16 for *E. coli* did not show any positive results.

The evaluation of scores of the GSRS demonstrated minor changes in GI symptoms over the course of the trial. The differences of total scores between the daily ratings up to day 29 compared to baseline (day 0) are between -0.4 and 0.1 in mean (MD group). No differences were observed in the SD group.

CONCLUSIONS

- The intake of Mutaflor® capsules for 1 day and for 28 days was well tolerated.
- No safety concerns occurred based on the evaluation of adverse events, laboratory assessments, and gastrointestinal symptom ratings.
- Once treatment of subjects with Mutaflor® was ended it took a median of 7 days for clearing of the *E.coli* from the multi-dose subjects and a median of 13 days for the single dose group to clear.

Date of Study Report (1.0): **13-Jul-2017**