Study CRO-13-111 - Sponsor code CB-17-03/01

Staining efficacy and safety of Methylene Blue enemas in patients undergoing flexible rectosigmoidoscopy

Single dose, open label, two arm, consecutive group, staining efficacy exploratory study

EudraCT Number: 2013-000452-18

Test products:	Methylene Blue enema 0.002% (Formulation A) Methylene Blue enema 0.02% (Formulation B)
Sponsor:	Cosmo Technologies Ltd., Riverside II, Sir John Rogerson's Quay, Dublin 2, Ireland Phone: +353.1.8170370 Fax: +353.1.8230718
Investigator:	Alessandro Repici, MD, Digestive Endoscopy Unit, Department of Gastroenterology, Istituto di Ricovero e Cura a Carattere Scientifico IRCCS, Istituto Clinico Humanitas, Via Alessandro Manzoni 56, I-20089 - Rozzano, Italy Phone: +39.02.822.42579 Fax: +39.02.82244590 Email: alessandro.repici@humanitas.it
Development phase:	Phase II
First subject first visit	29MAY13
Last subject last visit	25JUN14
Version and date:	Final version 1.0, 04APR16

This study was conducted in accordance with Good Clinical Practice (GCP), ICH topic E6

This document contains 48 pages plus appendices

REPORT APPROVAL

SPONSOR

Sponsor Representative
Richard Jones, Ph.D.
Cosmo Technologies Ltd., Ireland

18th April 2016 _____ _____
Date Signature

INVESTIGATORS

I declare to have conducted this trial in accordance with all the stipulations of the approved study protocol and in accordance with the ICH-GCP, "ICH Topic E6", July 1996 including post Step 4 errata, status September 1997 and post Step 5 errata (linguistic corrections), July 2002 reflected in the EU Note for Guidance on Good Clinical Practice CPMP/ICH/135/95, the Declaration of Helsinki, the applicable sections of the EU Directive 2001/20/EC, the EU Directive 2005/28/EC and all applicable regulatory requirements.

Principal Investigator
Alessandro Repici, MD
Digestive Endoscopy Unit, Istituto Clinico Humanitas, Rozzano, Italy

18 MAG 2016

Date

Signature

CRO
CROSS Research S.A. and CROSS Metrics S.A., sister companies

Coordination
Emanuela Terragni, Clinical Project Leader

15 APR 2016 — Emanuela Terragni
Date — Signature

Medical Writing
Andrea Di Stefano, Senior Medical Writer

15APR 2016 — Andrea Di Stefano
Date — Signature

Statistical Analysis
Andrea Vele, Biostatistician and Data Manager

15APR2016 — [signature]
Date — Signature

Quality Assurance Unit Representative
Maurizio Cuocolo, Quality Assurance

15 APR 2016 — [signature]
Date — Signature

2 SYNOPSIS

Name of Company: Cosmo Technologies Ltd.	**TABULAR FORMAT**		**(For National Authority Use only)**
Name of Finished Product: Methylene Blue enema	**REFERRING TO PART OF THE DOSSIER**	5.3	
Name of active substance: Methylene blue	**Volume:**		
	Page:		

Title of the study:
Staining efficacy and safety of Methylene Blue enemas in patients undergoing flexible rectosigmoidoscopy

Investigator:
Principal investigator: Alessandro Repici, MD

Study centre:
Digestive Endoscopy Unit, Department of Gastroenterology, Istituto Clinico Humanitas - Istituto di Ricovero e Cura a Carattere Scientifico IRCCS - Via Alessandro Manzoni, 56, I-20089 - Rozzano, Italy

Publication (reference):
--

Studied period (years):	**Date of first enrolment: 29MAY13**	**Phase of development:**
2013-2014	**Date last patient completed: 25JUN14**	**II**

Objectives:
Evaluation of mucosal staining efficacy after single rectal dose of the two Methylene Blue enema formulations containing, respectively, 0.002% methylene blue (Formulation A) and 0.02% methylene blue (Formulation B) in patients undergoing a rectosigmoidoscopy for various reasons.

Primary end-point:
Evaluation of the mucosal staining efficacy of the two test Methylene Blue enema Formulation A and Formulation B.

Secondary end-points:
Bowel cleansing quality evaluated according to a four-point scoring system after administration of Methylene Blue enema and of the cleansing enema.
To collect data about safety and tolerability of the two test Methylene Blue enema formulations.
Evaluation of the number of mucosal lesions detected with the two test Methylene Blue enema formulations and of the proportion of subjects with at least one mucosal lesion.

Methodology:
Single dose, open label, two arm, consecutive group, exploratory study of staining efficacy

Number of subjects (planned and analysed):
Efficacy of Methylene Blue enema in terms of mucosal staining quality was evaluated in the present study for the first time. Therefore, the planned sample size was not calculated by a statistical power analysis, but was regarded as sufficient to satisfy the exploratory purposes of the present study.
A total of 40 patients was planned to be included in the study (20 patients in each treatment group). Altogether, 5 patients were enrolled in the study. The study was terminated prematurely by the Sponsor due to slow enrolment rate.

Diagnosis and criteria for inclusion:
Inclusion criteria:
1. *Age:* ≥18 year old;
2. *Rectosigmoidoscopy:* out-patients with indication for rectosigmoidoscopy;
3. *Contraception (both males and females):* either sterile subjects or subjects practising at least one reliable method of contraception or females in post-menopausal status for at least 1 year;
4. *Informed Consent:* signed written informed consent prior to inclusion in the study.

Exclusion criteria:
1. *Pregnancy*: pregnant or lactating women or at a risk of becoming pregnant;
2. *Allergy*: known or suspected hypersensitivity to the active principle; history of anaphylaxis to drugs or allergic reactions in general;
3. *Diseases*: known or suspected gastrointestinal obstruction or perforation, toxic megacolon, major colonic resection, heart failure (Class III or IV), serious cardiovascular disease, severe liver failure, methaemoglobinemia, any other relevant disease that might interfere with the aim of the study;

SYNOPSIS (cont.)

Name of Company: Cosmo Technologies Ltd.	**TABULAR FORMAT**		**(For National Authority Use only)**
Name of Finished Product: Methylene Blue enema	**REFERRING TO PART OF THE DOSSIER**	5.3	
Name of active substance: Methylene blue	**Volume:**		
	Page:		

Diagnosis and criteria for inclusion (continued):

4. *Comprehension:* inability to comprehend the full nature and purpose of the study and unwillingness to co-operate with the investigator and to comply with the requirements of the entire study.

Test product, dose, mode of administration, batch N°:

Methylene Blue enema 0.002% (Formulation A). Batch: 6459/2; expiry: SEP13.
Methylene Blue enema 0.02% (Formulation B). Batch: 6508/1; expiry: DEC14.
The patients assigned to Formulation A took an entire single dose of bowel cleansing enema (Clisma-Lax, 133 mL) at home at about 4 h before the booked endoscopy. Afterwards, they took also the investigational product at about 2 h before the booked endoscopy according to the given instructions.
The patients assigned to Formulation B took only the investigational product at home at about 2 h before the booked endoscopy.
The first 20 patients were planned to receive one dose of Formulation A. The last 20 patients were planned to receive one dose of Formulation B.

Criteria for evaluation (efficacy):

The mucosal staining efficacy was evaluated scoring the observed staining as reported below for the explored regions (rectum and sigmoid colon).

0	*no/poor staining* (unstained or irregularly stained mucosa)
1	*overstaining* (dark black stained mucosa and indiscernible mucosal pattern)
2	*good staining* (uniformly stained mucosa and well discernible mucosal pattern)
3	*excellent staining* (uniformly stained mucosa and very well discernible mucosal pattern)

- Mucosal staining score for rectum and sigma (SC).
- Total staining score (TSC).
- Number of stained colonic regions with staining score SC>2 (NSA).
- Number of detected mucosal lesions and proportion of subject with at least one detected lesion.
- Bowel cleansing preparation quality score.

Criteria for evaluation (safety):

Treatment-emergent adverse events; vital signs (blood pressure, heart rate, oxygen saturation in peripheral blood), physical examinations.

Statistical methods:

The statistical analysis was performed using SAS® version 9.3 (TS1M1). The data documented in this trial and the clinical parameters measured were described using classic descriptive statistics for quantitative variables and frequencies for qualitative variables.
The number of detected mucosal lesions was listed and described.

Results (efficacy):

No analysis could be conducted on the efficacy variables of the study due to premature study discontinuation. With formulation A, the mucosal staining was generally absent or poor. The only exception was one subject who took the enema 40 min before the endoscopy and had a good staining quality. With formulation B, the mucosal staining was good for the only subject who received it.
Cleansing of sigmoid colon and rectum was generally good. Only one subject, who received formulation A, showed a less good cleansing.

Results (safety):

No adverse events or serious adverse events occurred during the study. No discontinuation due to any treatment emergent adverse event occurred during the study.
No clinically meaningful effect of methylene blue on vital signs or subjects' health conditions was observed.

SYNOPSIS (cont.)

<table>
<tr><td rowspan="2">Name of Company:
Cosmo Technologies Ltd.</td><td colspan="3">TABULAR FORMAT</td><td rowspan="2">(For National Authority Use only)</td></tr>
<tr><td colspan="2">REFERRING TO PART OF THE DOSSIER</td><td>5.3</td></tr>
<tr><td rowspan="2">Name of active substance:
Methylene blue</td><td>Volume:</td><td colspan="2"></td><td rowspan="2"></td></tr>
<tr><td>Page:</td><td colspan="2"></td></tr>
<tr><td colspan="5">Conclusions:
Two Methylene Blue enema formulations, developed by Cosmo Technologies Ltd., Ireland, were investigated for their ability to stain the distal colonic mucosa in patients undergoing rectosigmoidoscopy. After enrolment of 5 out of the 40 planned patients, the study was prematurely discontinued due to slow enrolment rate. No analysis could be conducted on the efficacy data due to the small number of completed subjects.</td></tr>
<tr><td colspan="5">Date of the report: Final version 1.0, 04APR16</td></tr>
</table>

3 TABLE OF CONTENTS

Page

TABLES

FIGURES

APPENDICES

16.1 Study information
- 16.1.1 Protocol and protocol amendments
- 16.1.2 Sample case report form (unique pages only)
- 16.1.3 List of IEC's or IRB's (plus the name of the committee chair if required by the regulatory authority) and representative written information for volunteer/patient and sample consent forms
- 16.1.4 List and description of Investigators and other important participants in the study, including brief CV's or equivalent summaries of training and experience relevant to the performance of the clinical study
- 16.1.5 Signatures of principal or coordinating Investigator(s) or sponsor's responsible medical officer, depending on the regulatory authority's requirement
- 16.1.6 Listing of subjects receiving test drug(s)(investigational product(s) from specific batches, when more than one batch was used
- 16.1.7 Randomisation scheme and codes (subject identification and treatment assigned)
- 16.1.8 Audit certificates (if available)
- 16.1.9 Documentation of statistical methods
- 16.1.10 Documentation of inter-laboratory standardization methods and quality assurance procedures if used

16.2 Subject data listing
- 16.2.1 Discontinued subjects
- 16.2.2 Protocol deviations
- 16.2.3 Subjects excluded from the efficacy analysis
- 16.2.4 Demographic data
- 16.2.5 Compliance and/or concentration data
- 16.2.6 Individual efficacy response data
- 16.2.7 Adverse event listings (each subject)
- 16.2.8 Listing of individual laboratory measurements by subject, when required by regulatory authorities
- 16.2.9 Individual safety measurements
- 16.2.10 Other listings

16.3 Case Report Forms (CRF's)
- 16.3.1 CRFs for deaths, other serious adverse events and withdrawals for AE
- 16.3.2 Other CRFs submitted

16.4 Individual subject data listings

4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse Event
BP	Blood Pressure
BPM	Beat Per Minute
BW	Body Weight
C_0	Plasma concentration at the time of injection
C_{max}	Peak drug concentration
CRF	Case Report Form
CS	Clinically significant
CV	Coefficient of Variation
DBP	Diastolic Blood Pressure
EC	Ethics Committee
ECG	Electrocardiogram
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HR	Heart Rate
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
MedDRA	Medical dictionary for regulatory purposes
MW	Molecular Weight
NC	Not calculated
NCS	Not clinically significant
NSA	Number of Stained Areas with a SC>2
PK	Pharmacokinetics
PT	Preferred Term
PTAE	Pre-Treatment Adverse Event
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SC	Mucosal Staining Score
SD	Standard Deviation
SOC	System Organ Class
SOP	Standard Operating Procedure
SpO_2	Peripheral Oxygen Pressure
$t_{1/2}$	Half-life
TEAE	Treatment Emergent Adverse Event
T_{max}	Time to achieve C_{max}
TSC	Total Staining Score
WHODDE	World Health Organisation-Drug Dictionary Enhanced

5 ETHICS

5.1 Independent Ethics Committee (IEC)

The study protocol, the investigator's brochure and all other relevant documentation were reviewed and approved by an independent Ethics Committee (Comitato Etico Indipendente, Istituto Clinico Humanitas – IRCCS, Rozzano, Italy; Appendix 16.1.3) on 25FEB13. Ref. nr. CE ICH 31/13. The competent Health Authorities, Agenzia Italiana del Farmaco (AIFA) approved the study and assigned the reference number STDG/P/30199 to the study on 21MAR13. Protocol amendment 1 issued on 31JUL13 was reviewed and approved by the IEC on 16SEP13. Ref. nr. CE ICH 254/13.

5.2 Ethical conduct of the study

The study was performed in accordance with the relevant guidelines and the Declaration of Helsinki.

The present clinical trial was carried out according to the general principles of: "ICH Harmonised Tripartite Guidelines for Good Clinical Practice "ICH Topic E6, CPMP/ICH/135/95, July 1996 including post Step 4 errata, status September 1997 and post Step 5 errata (linguistic corrections), July 2002.

5.3 Subject information and consent

Before being admitted to the clinical study, subjects expressed their consent to participate. The investigator explained the nature, scope and possible consequences of the clinical study in an understandable form. Information was provided to the subjects in both oral and written form.

Each patient received a copy of the written informed consent form, signed by them and the investigator.

A blank copy of the patient' consent form and written information sheet is presented in Appendix 16.1.3.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

6.1 Clinical centre

Digestive Endoscopy Unit, Department of Gastroenterology, Istituto Clinico Humanitas - Istituto di Ricovero e Cura a Carattere Scientifico IRCCS, Via Alessandro Manzoni, 56, I-20089 Rozzano, Italy
Phone: +39.02.822.42579
Fax: +39.02.822.44590
Email: alessandro.repici@humanitas.it

Principal Investigator
Alessandro Repici, MD

6.2 Sponsor

Cosmo Technologies Ltd., Riverside II, Sir John ROgerson's Quay, Dublin 2, Ireland
Phone: +353.1.8170370
Fax: +353.1.8230718
Email: rjones@cosmopharma.com

Sponsor representative
Dr Richard Jones, Research and Development Manager

6.3 CRO for co-ordination, data analysis & reporting

CROSS Research S.A. and CROSS Metrics S.A., Switzerland, sister companies, sharing the same quality assurance system.
Legal address: Via Francesco Antonio Giorgioli 14, CH-6864 - Arzo, Switzerland
Operative address: Via Luigi Lavizzari 18, CH-6850 - Mendrisio, Switzerland
Phone: +41.91.63.00.510
Fax: +41.91.63.00.511
Email: clinic@croalliance.com

Co-ordination
Emanuela Terragni, Clinical Project Leader

Medical Writing
Andrea Di Stefano, Senior Medical Writer

Statistical Analysis
Andrea Vele, Biostatistician and Data Manager

Quality assurance
Maurizio Cuocolo, Quality Assurance

6.4 Monitoring

Clinical Medical Services di Maria Pia Savorelli, Via Indipendenza 29A, CH-6883 Novazzano, Switzerland
Mobile: +41.79.827.27.67
Email: cmed@cmed.ch

A list of all investigators and other personnel involved in the study is provided in Appendix 16.1.4.

7 INTRODUCTION

7.1 Background

Currently, methylene blue (Figure below) is used in human medicine as an antidote for methaemoglobinemia and in various therapeutic and diagnostic procedures including use as an antiseptic and disinfectant of urinary tract, as a targeting agent for melanoma, as a tracer in parathyroid surgery and as a staining agent in bacteriology.

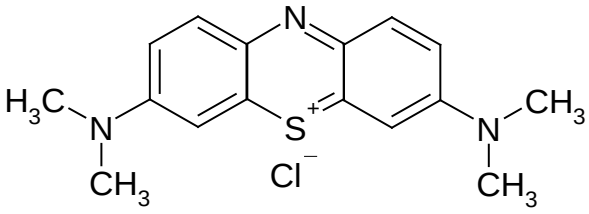


Figure 7.1.1 Methylene blue molecular structure

Methylene blue is a vital dye with the interesting property to stain the specialised columnar epithelium of intestine with high accuracy (1, 2, 3). Methylene blue has been used to screen for colonic neoplasia (4), to diagnose villous atrophy and to screen for areas of dysplasia and carcinoma. Usually a concentration of 0.5% of methylene blue is used as a spray solution. In the gastrointestinal epithelium the dysplastic epithelium areas and cancers are less absorptive of methylene blue. Thus, after staining with methylene blue, the portion of mucosa with these abnormalities appears as an area of either darker or lighter staining or as a heterogeneous staining pattern against a background of uniformly blue-stained mucosa (5).

7.1.1 Rectosigmoidoscopy

The rationale for rectosigmoidoscopy (also referred to as left colonoscopy if exploration is extended to the descending colon) is related to different frequency of colorectal cancer in the various colonic segments. According to literature data, the colorectal cancer occurs in the rectum and sigma in 55% of cases, in the descending colon in 6% of cases, in the transverse colon in 11% of cases, and in the caecum/ascending colon in 22% of cases (6).

In 2004, R. E. Carroll (7) published the results of his team's experiments on the addition of a rectosigmoidoscopy to a full colonoscopy for the diagnosis of aberrant crypt foci in the sigmoid and rectum. The author experimented a new procedure which included a full colonoscopy after mucosal staining with sprayed 0.25% methylene blue, then an infusion of a methylene blue 0.05% enema when maintaining the patients in a 20° Trendelenburg position and, finally, a magnification chromo-rectosigmoidoscopy. The authors concluded that the dye allows a magnification chromoendoscopy and that the new rapid method was comfortable for the patients.

7.2 Rationale and proposed indication

The Sponsor, Cosmo Technologies Ltd., developed two Methylene Blue enema formulations containing the conjectured minimum efficacious amounts of active ingredient, respectively, 0.002% methylene blue (Formulation A) and 0.02% methylene blue (Formulation B). These new formulations were tested in order to verify the ability to stain the sigmoidal and rectal (distal) colonic mucosa when administered through an enema cleansing preparation before a rectosigmoidoscopy. Formulation A, with the lower methylene blue concentration, was designed to be administered after the cleansing enema with the aim of staining the mucosa after the gut is cleansed through a previously administered cleansing enema. Formulation B combines both the cleansing and the staining properties and was designed to be administered alone, without any additional cleansing enema. The concentration of methylene blue in formulation B was increased by 10 times, because the affinity of methylene blue for faeces is higher than that for the intestinal mucosa. Since formulation B is administered when the gut lumen is not yet cleansed from faeces, the higher methylene blue concentration aimed at obtaining the same mucosal staining efficacy as with formulation A.

Methylene Blue enema is mainly proposed as local diagnostic help in the detection of inflammatory or neoplastic lesions (cancers, adenomas, polyps, dysplasiae and/or serrated lesions) of the distal colonic mucosa by taking advantage of site and method of administration.

The second end-point of the present study was to evaluate the bowel cleansing quality after Methylene Blue enema and to collect safety and tolerability data of the administration of the investigational product to patients undergoing rectosigmoidoscopy.

8 STUDY OBJECTIVES

The primary objective of the study was the evaluation of the mucosal staining efficacy after single dose of two Methylene Blue enema formulations containing, respectively, 0.002% methylene blue (Formulation A) and 0.02% methylene blue (Formulation B), in patients undergoing a rectosigmoidoscopy for various reasons.

8.1 Primary end-point

- Evaluation of the mucosal staining efficacy of the two test Methylene Blue enema Formulations, named A and B.

8.2 Secondary end-points

- Bowel cleansing efficacy evaluated according to a four-point scoring system after administration of Methylene Blue enema alone or in combination with the cleansing enema.
- To collect data about safety and tolerability of the two test Methylene Blue enema formulations.
- Evaluation of the number of mucosal lesions detected with the two test Methylene Blue enema formulations and of the proportion of subjects with at least one mucosal lesion.

9 INVESTIGATIONAL PLAN

9.1 Overall study design and plan

Single dose, open label, two arm, consecutive group, staining efficacy exploratory study.
A copy of the study protocol is given in Appendix 16.1.1. The study schedule of procedures is shown in § 9.5.1.5. Study procedures are summarised in Table 9.1.1.
From day -15 to day -1, out-patients scheduled for rectosigmoidoscopy were informed about the aims, procedures, benefits and possible risks of the study prior to sign the informed consent form for inclusion in the trial. Their medical history was evaluated and recorded. The subjects underwent a physical examination. The subjects were assigned a consecutive study number. The subjects received their individual clinical supply package, including the investigational medicinal product (IMP) for their treatment. A bowel cleansing enema was supplied to patients receiving formulation A. The patients assigned to Formulation A took the bowel cleansing preparation at home about 4 h before the booked endoscopy. Afterwards, they also took the IMP about 2 h before the booked endoscopy. The patients assigned to Formulation B took only the IMP about 2 h before the booked endoscopy. Afterwards, the patients returned to the clinic for the rectosigmoidoscopy. The investigator inquired the subjects about occurrence of any adverse event (AE), the intake of concomitant medications, compliance with the intake of the IMP and occurrence and timing of evacuation after the administration of the IMP. Discrete measures of vital signs (blood pressure - BP, heart rate - HR, peripheral oxygen saturation - SpO_2) were recorded prior to, during and after the end of the endoscopy. During the flexible rectosigmoidoscopy, the investigator scored and recorded the staining quality in the target regions, sigma and rectum, reported the number of detected mucosal lesions and rated the colon cleansing. Each endoscopy was videorecorded. After conclusion of the exam, the subjects definitely left the clinic.

Table 9.1.1 Study schedule with procedures and activities

	Day	Procedures/Assessments
Enrolment - Visit 1	*From day -15 to day -1*	➢ Explanation to the subject of study aims, procedures and possible risks ➢ Informed consent signature ➢ Demographic data recording ➢ Medical/surgical history ➢ Previous/concomitant medications ➢ Full physical examination (BW, height, physical abnormalities) ➢ Inclusion/exclusion criteria evaluation ➢ Consecutive study number (001, 002, etc.) ➢ IMP dispensation ➢ AE monitoring
Day of endoscopy - Visit 2	*Day 1*	➢ Bowel cleansing preparation at home (*only subjects receiving formulation A*) ➢ IMP self administration at home ➢ Rectosigmoidoscopy ➢ Vital signs ➢ Staining quality evaluation ➢ Recording of detected mucosal lesions ➢ Bowel cleansing evaluation ➢ Physical examination ➢ Recording of AEs and concomitant medications

9.2 Discussion of study design, including the choice of control groups

This exploratory study aimed at evaluating and describing the colonic mucosa staining efficacy after use of the test investigational product, Methylene Blue enema, in two different formulations containing, respectively, 0.002% methylene blue (Formulation A) and 0.02% methylene blue (Formulation B).
In this trial, the IMP was administered to patients with the proposed indication (see § 7.2).

The dose regimen, the method of administration and the administration schedule were chosen as appropriate for the usual preparation for the rectosigmoidoscopy after the administration of the cleansing enema, i.e. after bowel evacuation, and reasonably before the planned endoscopy.

No randomisation took place.

The primary parameter, i.e. the colon mucosa staining efficacy, was evaluated using a subjective four-point scoring system and by subdividing the explored part of the colon into sigma and rectum.

The secondary parameter of bowel cleansing quality was evaluated basing on the reliable and validated Boston Bowel Preparation Scale. The same four-point scoring system was applied to the rectosigmoid (8).

9.3 Selection of study population

Out-patients of both sexes with indication for diagnostic rectosigmoidoscopy were the selected study population.

9.3.1 Inclusion criteria

1. *Age:* ≥18 year old;
2. *Rectosigmoidoscopy:* out-patients with indication for rectosigmoidoscopy;
3. *Contraception (both males and females):* either sterile subjects or subjects practising at least one reliable method of contraception or females in post-menopausal status for at least 1 year;
4. *Informed Consent:* signed written informed consent prior to inclusion in the study.

9.3.2 Exclusion criteria

1. *Pregnancy*: pregnant or lactating women or at a risk of becoming pregnant;
2. *Allergy*: known or suspected hypersensitivity to the active principle; history of anaphylaxis to drugs or allergic reactions in general;
3. *Diseases*: known or suspected gastrointestinal obstruction or perforation, toxic megacolon, major colonic resection, heart failure (Class III or IV), serious cardiovascular

disease, severe liver failure, methaemoglobinemia, any other relevant disease that might interfere with the aim of the study;

4. *Comprehension:* inability to comprehend the full nature and purpose of the study and unwillingness to co-operate with the investigator and to comply with the requirements of the entire study.

9.3.3 Removal of patients from therapy or assessment

Enrolled subjects could be withdrawn for the following reasons:

- voluntary subject's withdrawal for any reason;
- at the discretion of the investigator;
- if an adverse reaction (including a concomitant illness) developed, believed by the investigator incompatible with the continuation of the study;
- the necessary administration of any drug that was not permitted by the exclusion criteria;
- failure to comply with the requirements of the protocol.

For each withdrawn patient, a complete final examination had to be performed at the time of withdrawal to document the subject's health conditions. The reason for withdrawal had to be reported in the CRF and in the subject's medical records.

9.4 Treatments

9.4.1 Treatments administered

9.4.2 Identity of investigational product(s)

TEST product
(Formulation A)

Investigational medicinal product denomination	Methylene Blue enema
Active constituent	Methylene blue
Chemical name	3, 7-bis (dimethylamino-) phenothiazin-5-ium chloride
Chemical formula and MW	$C_{16}H_{18}ClN_3S$, CAS 61-73-4, MW 319.86
Manufacturer (Drug Substance)	Finorga SAS, France
Manufacturer (Drug Product)	Cosmo S.p.A., Italy
Pharmaceutical form	Enema
Dose	0.002% of methylene blue in 100 mL of product
Administration route	Rectal
Batch N.	6459/2
Expiry date	SEP13

TEST product **(Formulation B)**	
Investigational medicinal product denomination	Methylene Blue enema
Active constituent	Methylene blue
Chemical name	3, 7-bis (dimethylamino-) phenothiazin-5-ium chloride
Chemical formula and MW	$C_{16}H_{18}ClN_3S$, CAS 61-73-4, MW 319.86
Manufacturer (Drug Substance)	Finorga SAS, France
Manufacturer (Drug Product)	Cosmo S.p.A., Italy
Pharmaceutical form	Enema
Dose	0.02% of methylene blue in 100 mL of product
Administration route	Rectal
Batch N.	6508/1
Expiry date	DEC14

The analytical certificates are enclosed in Appendix 16.1.6.

9.4.2.1 Other product administered

The subjects, who received Formulation A, also received a commercial cleansing enema (Clisma-Lax 133 mL, by Sofar S.p.A., Italy; batch: R0330; expiry: MAY17) and took it at home following the instructions enclosed with the product.

The principal investigator dispensed the individual clinical supplies at the enrolment visit.

9.4.3 Method of assigning patients to treatment groups

This study was not randomised. The first 20 included patients were planned to receive one dose of Formulation A containing methylene blue at 0.002%. After collection and evaluation of the preliminary efficacy data of the first enrolled patients, Formulation A was no longer used in the study due to staining paucity and the patients were assigned to receive one dose of Formulation B containing methylene blue at 0.02% (see also § 9.8).

9.4.4 Selection of doses in the study

A total dose of 100 mL of Methylene Blue enema composition was selected for the present study. When administering formulation A, the dose contained methylene blue 0.002%. With formulation B, methylene blue was 0.02%. The selected doses are discussed and justified in § 9.2.

9.4.5 Selection and timing of dose for each patient

On study day 1, the patients assigned to Formulation A started to take the cleansing enema about 4 h before the booked endoscopy. The dispensed enema was taken by the rectal route according to the product instructions. Generally, the commercialised product instructions recommend patients to lie down on the left side and to possibly assume the genu-pectoral

position. Afterwards, the cannula of the product container must be gently inserted into the rectum and the content must be entirely discharged. After the administration, the patients remained lying until the stimulus to bowel evacuation occurred. After evacuation, the patients also took the test IMP about 2 h before the booked endoscopy. The product was taken by the rectal route following the same method of administration as for the cleansing enema.
The patients assigned to Formulation B took only the IMP enema composition about 2 h before the booked endoscopy according to the given instructions.

After administration of the IMP, the patients retained the medicated enema for at least 5 min, or as long as possible, before evacuation. The retention time of medicated enema was recorded in the CRF as reported by the patients on the day of endoscopy .

9.4.6 Blinding

This was an open trial and no masking procedure was applied.

9.4.7 Prior and concomitant medication and other constraints

No concomitant treatment with any drug listed on the drug safety alert published by FDA (9) was permitted. In particular, the selective serotonin reuptake inhibitors (paroxetine, fluvoxamine, sertraline, citalopram, etc.), the serotonin-norepinephrine reuptake inhibitors (venlafaxine, devenlafaxine, duloxetine), tricyclic antidepressants (amitriptyline, desipramine, clomipramine, imipramine, nortriptyline, protriptyline, doxepin, trimipramine) and other psychiatric drugs (amoxapine, maprotiline, nefazodone, trazodone, bupropion, buspirone, vilazodone, mirtazapine) were withdrawn at least 2 weeks before the study. Fluoxetine was withdrawn at least 5 weeks before the study.

9.4.8 Treatment compliance

The subjects took both Methylene Blue enema and the bowel cleansing enema at home. The investigator checked the treatment compliance by questioningthe subjects when they returned to the clinic and recorded on the CRF the compliance with the allocated treatment. Dispensation, intake and return of used and unused units were recorded. The investigator reported also if the subjects did not take the entire dose of Methylene Blue enema or of bowel cleansing enema.

9.5 Efficacy and safety variables

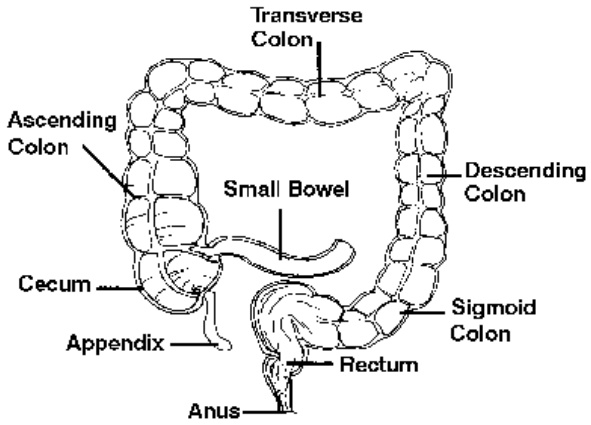
9.5.1 Efficacy and safety measurements assessed and flow chart

9.5.1.1 Rectosigmoidoscopy

During Visit 2 each patient underwent a flexible rectosigmoidoscopy. The investigator performed the endoscopy according to the standard procedure protocols of the clinic. The endoscopy was performed up to 30 cm from the anal verge. Explored area was the sigmoid and rectum (Figure 9.5.1.1).

Mucosal staining efficacy and colon cleansing efficacy were scored by the endoscopist during the rectosigmoidoscopy according to the scales mentioned below. The assigned scores were recorded in the CRFs.
Completeness and duration of the exam were recorded in the CRFs.
Each endoscopy was video-recorded and a pseudonymised copy of the video recordings was provided to the Sponsor for archive.

Figure 9.5.1.1 Target colonic regions of the study endoscopy were the rectum and the sigma



9.5.1.2 Mucosal staining quality evaluation

The staining efficacy was evaluated scoring the observed staining as reported below for the explored region (rectum and sigma).

0 *no/poor staining* (unstained or irregularly stained mucosa)
1 *overstaining* (dark black stained mucosa and indiscernible mucosal pattern)
2 *good staining* (uniformly stained mucosa and well discernible mucosal pattern)
3 *excellent staining* (uniformly stained mucosa and very well discernible mucosal pattern)

After scoring separately each colonic segment (SC), the total staining score (TSC) was calculated summing the SC of the 2 colonic segments and the number of regions with a SC>2 (NSA) was determined.

9.5.1.3 Colon cleansing score

The colon cleansing and the presence of fluid in the colon was rated by a four-point scoring system based upon the validated Boston bowel preparation scale (8).

0 unprepared colon segment with mucosa not seen due to solid stool that cannot be cleared
1 portion of mucosa of the colon segment seen, but other areas of the colon segment not well seen due to staining, residual stool and/or opaque liquid
2 minor amount of residual staining, small fragments of stool and/or opaque liquid, but mucosa of colon segment seen well
3 entire mucosa of colon segment seen well with no residual staining, small fragments of stool or opaque liquid

If the investigator aborted a procedure due to an inadequate preparation, then any non-visualised proximal segment was not scored and was rated as "not done" (8).

9.5.1.4 Safety variables

Safety and general tolerability of the drug were based on the following assessments:

Record of adverse events
AEs were assessed throughout the study.
Mucosal lesions of any kind detected during the endoscopy and not related to the examination procedure were not to be considered AEs, as it is believed that these lesions pre-existed the treatment with methylene blue and the endoscopic examination, and were not untoward. On the other hand, all injuries caused by the endoscopic procedure, or any untoward medical occurrence reported during or after the administration of the test IMP were to be considered AE.

Vital signs
Subjects blood pressure (BP), heart rate (HR) and peripheral oxygen pressure (SpO_2) assessed prior to, during and at the end of colonoscopy.

Physical examination
A physical examination was performed at the enrolment visit and on the day of colonoscopy. Body weight (BW) and height were recorded at the enrolment visit.
The medical and the surgical history were also recorded at Visit 1. With respect to the surgical history, only gastrointestinal therapeutic procedures, obstetric and gynaecological procedures and renal and urinary tract therapeutic procedures were recorded in the CRFs.

9.5.1.5 Flow chart

The schedule of measurements and activities of the study is given below:

ACTIVITIES	**Enrolment**	**Day of endoscopy**
Visit	**V1** **Days -15/-1**	**V2** **Day 1**
Informed consent	x	
Demography	x	
Medical and surgical history	x	
Physical examination	x	x
Height	x	
Weight	x	
Concomitant treatments	x	x
Inclusion/exclusion criteria	x	
Vital signs		x
Bowel cleansing enema		x[1]
Study drug administration		x
Tolerability		x
Endoscopy		x
Staining quality		x
Bowel cleansing evaluation		x
AEs monitoring	x	x

1: only the patients receiving test formulation A

9.5.2 Appropriateness of measurements

All parameters and measurements of the present study are reliable and accurate to accomplish study end-points.

9.5.3 Primary variables

- Mucosal staining score for rectum and sigma (SC);
- Total staining score (TSC).

9.5.3.1 Secondary variables

- Number of stained colonic regions with staining score SC>2 (NSA);
- Number of detected mucosal lesions and proportion of subject with at least one detected lesion;
- Bowel cleansing preparation quality;
- TEAEs;
- Vital signs (BP, HR, SpO_2).

9.5.4 Drug concentration measurements

No drug concentration was measured in the present study.

9.6 Data quality assurance

Monitoring visits were conducted by appropriate staff of Clinical Medical Services di Maria Pia Savorelli. The study was monitored by means of on-site visits and regular inspection of the CRF with sufficient frequency to ascertain the following:

- occurrence of AEs/serious adverse events (SAEs);
- subjects' enrolment;
- compliance with the protocol procedures;
- completeness and exactness of data entered in the CRF;
- verification against original source documents.

Data were verified by direct comparison with source documents, always giving due consideration to data protection and medical confidentiality.

All study documentation and results were reviewed according to the Quality Assurance procedures of CROSS Research S.A.

9.7 Statistical methods planned in the protocol and determination of sample size

9.7.1 Statistical and analytical plans

The statistical and analytical plan were detailed in the statistical analysis plan (SAP) document, final version 1.0, issued on 27OCT14 (Appendix 16.1.9). The data documented in this trial and the parameters measured were described using classic statistics, i.e. mean, SD, CV(%), minimum and maximum values for quantitative variables and frequencies for qualitative variables.
Data not available were evaluated as "missing values".
The statistical analysis on demography, efficacy and safety data was performed using SAS® for Windows Version 9.3 TS1M1 (10).

9.7.1.1 Unique subject identifier

All the subjects who signed the informed consent form for the present study were coded with "unique subject identifiers" when data were extracted from the study database into the domains of the CDISC SDTM model. The unique subject identifier consists of the sponsor study code (i.e. CB-17-03/01), the 3-digit site number (i.e. 001) and the 3-digit subject study number (e.g. 001, 002, etc.). Study code, site number and subject study number are separated by slashes ("/"). The last 3 digits of the unique subject identifier, corresponding to the subject study number, appear as subject identifier in the individual listings and figures of the clinical study report and are used to identify the subjects in in-text tables or wording (if applicable).

9.7.1.2 Analysis on demographic, baseline and background characteristics

Critical demographic characteristics were examined according to qualitative or quantitative data. Qualitative data (e.g. life style, race) were summarised by means of contingency tables. Quantitative data (e.g. age, BW) were summarised using quantitative descriptive statistics such as mean, standard deviation, minimum and maximum values.

9.7.1.3 Study analysis sets

The following analysis sets were defined:

- Enrolled set: all enrolled subjects. This analysis set was used for demographic, baseline and background characteristics.
- Safety set: all subjects who received at least one dose of the IMP. This analysis set was used for safety analyses.
- Efficacy set (Per Protocol set): the efficacy set was defined as all treated subjects who fulfilled the study protocol requirements in terms of IMP intake and efficacy measure collection, without major deviations that could affect the efficacy results. This analysis set was used for efficacy analysis.

Each subject was coded by the CRO Biometry Unit as valid or not valid for the safety set and the efficacy set. Subjects were evaluated according to the treatment they actually received.

9.7.1.4 Analysis of compliance

The administrations, the time of retention of the Methylene Blue enema and data of evacuation of the enema are listed. The dispensation of the Methylene Blue enema is listed and presented in frequency tables. The dispensation of the bowel cleansing enema is listed.

9.7.1.5 Analysis of efficacy parameters

Mucosal staining quality evaluation
SC, TSC and NSA were evaluated. The results are listed.

Cleansing score
Cleansing Score (CS) and Total Cleansing Score (TCS) were evaluated. Colon cleansing scores are listed.

Mucosal lesions
The number of detected mucosal lesions are listed.

Rectosigmoidoscopy data
The rectosigmoidoscopy date and duration are listed.

9.7.1.6 Analysis of safety parameters

Adverse events
AEs were coded by System Organ Class (SOC) and Preferred Term (PT), using the Medical Dictionary for Regulatory Activities (MedDRA).
AEs were classified as pre-treatment AEs (PTAEs) and TEAEs, according to the period of occurrence, as follows:

- PTAEs: all AEs occurring before the dose of the IMP and not worsening after the dose of the IMP;
- TEAEs: all AEs occurring or worsening after the dose of the IMP.

Individual PTAEs and TEAEs are listed.

Vital signs and body weight
Vital signs and BW values are listed and summarised.

9.7.2 Determination of sample size

Efficacy of Methylene Blue enema in terms of mucosal staining quality was evaluated in the present study for the first time. Therefore, the planned sample size was not calculated by a statistical power analysis, but was regarded as sufficient to satisfy the exploratory purposes of the present study.
A total of 40 patients were to be included in the study (20 patients in each treatment group). The total of 20 patients initially planned to receive one dose of test Formulation A was not completed due to paucity of mucosal staining recorded in the first patients. The study

continued with the enrolment of the 20 patients planned to receive Formulation B until premature termination by the Sponsor due to enrolment difficulties.

9.8 Changes in the conduct of the study or planned analyses

The investigator judged the colonic mucosa staining quality as unsatisfactory because no or poor staining was observed in the first patients included in the study and receiving one dose of Formulation A, as planned. On the basis of these preliminary results, the Sponsor and the investigator agreed not to complete the investigation of Formulation A and to continue the study with the enrolment of the following 20 patients planned to receive Formulation B. Protocol amendment 1, final version 1.0, 31JUL13 (Appendix 16.1.1) was issued to introduce these changes and to exclude the statistical comparison between the effects of the 2 tested formulations from the protocol.

The non-substantial protocol amendment 2, final version 1.0, 09MAR15, (Appendix 16.1.1) was issued due to replacement of the Sponsor representative.

On 09DEC14, the Sponsor decided to terminate prematurely the trial due to slow enrolment rate (5 enrolled patients from the site opening date: 22APR13).

The non-substantial protocol amendment 3, final version 1.0, 15MAR16, (Appendix 16.1.1) was issued to introduce the incorporation of CROSS S.A. into its associated company CROSS Research S.A. In detail, CROSS S.A., the CRO managing the clinical trial, ceased to operate and was incorporated into CROSS Research S.A., which has replaced CROSS S.A. in all its rights, obligations and agreements without interruption. This amendment was considered not substantial because it had no impact on the trial, which was terminated on 09DEC14, since CRO's policies, procedures, personnel and facilities remained unchanged.

On 06NOV15, the Sponsor moved to a new address: Riverside II, Sir John ROgerson's Quay, Dublin 2, Ireland.

No other changes in the study conduct or planned analysis occurred.

10 STUDY SUBJECTS

10.1 Disposition of subjects

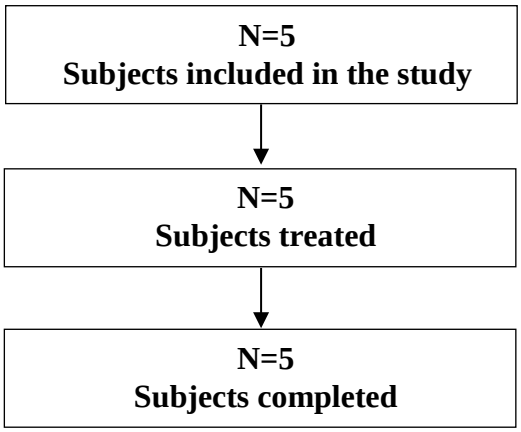


Figure 10.1.1 Subjects' disposition

The investigator included 5 subjects in the study. Disposition of subjects is summarised in Table 14.1.1.1. After inclusion, 5 subjects took the study treatment and completed the study as per protocol. Study dates are shown in Appendix 16.2.10, Listing 16.2.10.5.

10.2 Protocol deviations

Protocol deviations are presented in Table 14.1.1.3 and listed in Appendix 16.2.2, Listing 16.2.2.1.
In particular, a major deviation in the intake of study treatment was reported for subject 004. The subject took the bowel cleansing enema about 2 h before the scheduled endoscopy and the Methylene Blue enema about 40 min before the scheduled endoscopy instead of 4 and 2 h before, as planned.
In addition, other minor deviations from study procedures and in the visit schedule were reported for all the 5 subjects.

In addition to the other minor deviations, the endoscopy of subjects 002, 003 and 004 was not video-recorded.

There were no other protocol deviations.

11 EFFICACY EVALUATION

11.1 Data set analysed

No efficacy analysis could be conducted (§ 11.4.2).
All 5 enrolled subjects were considered in the safety analysis (Appendix 16.2.4, Listing 16.2.4.2).

11.2 Demographic and other baseline characteristics

Demographic data (mean, median and frequency data) are presented in Table 14.1.1.2 and summarised in the table below.

Table 11.2.1 Demographic and other baseline data (enrolled set – N=5)

Demographic data	Formulation A - N=4	Formulation B – N=1
Gender		
Female – n (%)	3 (75.0%)	0 (0%)
Male – n (%)	1 (25.0%)	1 (100%)
Age (years)		
Mean ± SD	62.5±9.3	46±NC
Median (range)	63.0 (51–73)	46 (46-46)
Body weight (kg)		
Mean ± SD	69.00±12.88	70.00±NC
Range	71.00 (52.0–82.0)	70 (70-70)
Height (cm)		
Mean ± SD	160.5±12.4	180.0±NC
Median (range)	159.5 (147–176)	180 (180-180)
Race		
White – n (%)	4 (100%)	1 (100%)

NC: Not calculated
Source: Table 14.1.1.2

All 5 subjects satisfied the study inclusion/exclusion criteria (Appendix 16.2.4, Listing 16.2.4.4).

At study entry, the medical and surgical histories were collected for all patients (Appendix 16.2.10, Listing 16.2.10.1). All patients underwent also a full physical examination. Outcome of individual examinations is listed in Listing 16.2.10.2.

Three (3) out of 5 subjects were under treatment with at least one previous medication at study entry. The list of subjects with any prior or concomitant treatment is presented in Listing 16.2.10.3. All previous treatments, which the subjects were taking against underlying diseases at the screening visit date, were continued during the study.

11.3 Measurement of treatment compliance

Treatment compliance was checked by the investigator upon patients' return to the clinic for endoscopy. The subjects returned the used and unused IMP units and orally reported about completion of intake of the bowel cleansing preparation, as applicable, and about the retention time of each enema before evacuation.

The frequency of subjects who took the dispensed methylene blue dose is presented in Table 14.3.5.2. The complete dose of Methylene Blue enema was taken by all the 5 enrolled subjects, whose compliance was 100%. Nevertheless, subject 004 took both the bowel cleansing enema and the methylene blue enema 2 h and 40 min before rectosigmoidoscopy instead of 4 and 2 h, respectively, as planned (§ 10.2).

After administration, subjects 001, 002 and 003, receiving formulation A, retained the Methylene Blue enema for 10 min and then evacuated it. Subjects 004, receiving formulation A, and 021, receiving formulation B, retained the enema for 15 min and then evacuated it (Appendix 16.2.5, Listing 16.2.5.1).

11.4 Efficacy results and tabulation of individual subject data

11.4.1 Analysis of efficacy data

11.4.1.1 Analysis of mucosal staining data

Individual values of the efficacy variables SC, NSA and TSC (§ 9.5.3) are listed in Appendix 16.2.6, Listing 16.2.6.1.

On average, the staining quality of the colonic mucosa was absent or poor with formulation A with the exception of the rectum of subject 004 where SC was 2 (good staining). Indeed, subject 004 took the methylene blue enema 40 min before the endoscopy. With formulation B (subject 021), the staining score was 2 (good staining) in both the sigma and the rectum. TSC of subject 021 was 4. NSA was 0 for all the subjects.

11.4.1.2 Analysis of detection rate of mucosal lesions

The rate of detection in the sigmoid colon and the rectum is listed individually in Appendix 16.2.6, Listing 16.2.6.3.

The investigator found up to one lesion per region in the subjects receiving formulation A, whereas he found one lesion in the sigmoid colon and 2 lesions in the rectum after use of formulation B.

11.4.1.3 Analysis of endoscopy completeness and duration

Results of endoscopy completeness and duration are listed in Appendix 16.2.6, Listing 16.2.6.4. The investigator completed the rectosigmoidoscopy of all 5 subjects. The duration of the endoscopy ranged from 5 to 15 min, considering all 5 enrolled subjects.

11.4.1.4 Analysis of colon cleansing

Individual cleansing scores are listed in Appendix 16.2.6, Listing 16.2.6.2. The investigator scored 3 (entire mucosa seen well) the cleansing of both sigmoid colon and rectum of 3 subjects receiving formulation A and of the subject receiving formulation B. One subject receiving formulation A was scored 2 (minor amounts of residues).

11.4.2 Statistical/analytical Issues

No statistical analysis could be conducted on the efficacy results due to premature termination of the study. Individual results are listed and commented in § 11.4.1.

11.4.3 Tabulation of individual response data

- Individual IMP administrations are listed in Appendix 16.2.5, Listing 16.2.5.1.
- Individual mucosal staining scores are listed in Appendix 16.2.6, Listing 16.2.6.1.
- Individual cleansing scores are listed in Appendix 16.2.6, Listing 16.2.6.2.
- Individual rate of detection of mucosal lesions is listed in Appendix 16.2.6, Listing 16.2.6.3.
- Individual data of duration and completeness of the endoscopy are listed in Appendix 16.2.6, Listing 16.2.6.4.

11.4.4 Drug dose, drug concentration, and relationships to response

The descriptive analysis on the relationship between Methylene Blue enemas and the quality of colon mucosal staining is presented in § 11.4.1.1. Conclusions are drawn below.

11.4.5 Drug-drug and drug-disease interactions

Not applicable.

11.4.6 By-subject displays

Individual listings of efficacy data are collected in Appendix 16.2.6.

11.4.7 Efficacy conclusions

- No analysis could be conducted on the efficacy variables of the study due to premature discontinuation;
- With formulation A, the mucosal staining was generally absent or poor. The only exception was one subject who took the enema 40 min before the endoscopy and had a good staining quality;
- With formulation B, the mucosal staining was good for the only tested patient, subject 021;
- Cleansing of sigmoid colon and rectum was generally good. Only one subject, who received formulation A, showed a less good cleansing.

12 SAFETY EVALUATION

12.1 Extent of exposure

The entire dose of Methylene Blue enema, according to the mode of administration illustrated in § 9.4.5, was taken by the 5 enrolled subjects. The whole cleansing enema was taken by the 4 subjects receiving formulation A. See also § 11.3.

12.2 Adverse events (AEs)

12.2.1 Brief summary of adverse events

No adverse events occurred during the study (Table 14.3.1.1).

12.2.2 Listing of adverse events by subject

No treatment emergent adverse event occurred during the study (Appendix 16.2.7, Listing 16.2.7.1).

12.3 Deaths, other serious adverse events, and other significant adverse events

No deaths or serious or other significant adverse events occurred during the study.

12.4 Clinical laboratory evaluation

No clinical laboratory evaluation was performed during the study.

12.5 Vital signs, physical findings, and other observations related to safety

12.5.1 Blood pressure and pulse rate

Individual SBP, DBP, HR and SpO_2 values measured at the screening visit and on the day of the endoscopy before, during and at the end of the endoscopy are summarised in Table 14.3.5.1. For most subjects, neither SBP nor DBP were measured. HR and SpO_2 were measured before and after the endoscopy for all the subjects. No CS abnormality was found by the investigator for any parameter. No significant effect of treatment on vital signs was detected during the study.

12.6 Safety conclusions

- No AEs or SAEs occurred during the study;
- No discontinuation due to any TEAE occurred during the study;
- No clinically meaningful effect of methylene blue on vital signs or subjects' health conditions was observed.

13 DISCUSSION AND OVERALL CONCLUSIONS

13.1 Discussion

The Sponsor, Cosmo Technologies Ltd., developed two Methylene Blue enema formulations. These new formulations were tested in order to verify the ability to stain the distal (rectum and sigma) colonic mucosa when administered before a rectosigmoidoscopy.

A total of 40 patients was planned to be included in the study (20 patients in each treatment group). Altogether, a total of 5 patients were enrolled in the study. After completion of the 5 enrolled patients, the study was terminated prematurely by the Sponsor due to slow enrolment rate (5 patients in 21 months).

Four (4) out of the enrolled patients received 0.002% methylene blue enema (Formulation A) and one subject received 0.02% methylene blue enema (Formulation B).

With formulation A, the mucosal staining was generally absent or poor. Therefore, the study was amended to stop the investigation of formulation A and to continue the study with formulation B only. With formulation B, the mucosal staining was good for the only subject who received it. No analysis could be conducted on the efficacy variables of the study due to premature discontinuation.

Safety data did not evidence any untoward effect of methylene blue enema.

13.2 Conclusions

Two Methylene Blue enema formulations, developed by Cosmo Technologies Ltd., Ireland, were investigated in their ability to stain the distal colonic mucosa in patients undergoing rectosigmoidoscopy. After enrolment of 5 out of the 40 planned patients, the study was prematurely discontinued due to slow enrolment rate. No analysis could be conducted on the efficacy data due to the small number of completed subjects.

14 TABLES AND FIGURES REFERRED TO BUT NOT INCLUDED IN THE TEXT

14.1 Demographic data and other baseline characteristics

14.1.1 Demography and other baseline characteristics

Table 14.1.1.1 Subjects' disposition (Enrolled set)

	Methylene Blue enema 0.002% N=4 n (%)	**Methylene Blue enema 0.02% N=1 n (%)**
Enrolled	4 (100.0)	1 (100.0)
Treated	4 (100.0)	1 (100.0)
Completed	4 (100.0)	1 (100.0)

Note: The denominator for calculating the percentages is the number of enrolled subjects of each IMP group

Source: Listing 16.2.4.1 - Subjects' disposition

Program: Tables\c111-ds-tbl.sas

Table 14.1.1.2 Demography (Enrolled set, Safety set)

		Statistics	Enrolled set		Safety set	
			Methylene Blue enema 0.002% N=4	**Methylene Blue enema 0.02% N=1**	**Methylene Blue enema 0.002% N=4**	**Methylene Blue enema 0.02% N=1**
Sex	Female	n (%)	3 (75.0)	0 (0.0)	3 (75.0)	0 (0.0)
	Male	n (%)	1 (25.0)	1 (100.0)	1 (25.0)	1 (100.0)
Race	White	n (%)	4 (100.0)	1 (100.0)	4 (100.0)	1 (100.0)
Age (years)		N	4	1	4	1
		Mean	62.5	46.0	62.5	46.0
		SD	9.3	NC	9.3	NC
		CV%	14.9	NC	14.9	NC
		Min	51	46	51	46
		Median	63.0	46.0	63.0	46.0
		Max	73	46	73	46
Height (cm)		N	4	1	4	1
		Mean	160.5	180.0	160.5	180.0
		SD	12.4	NC	12.4	NC
		CV%	7.8	NC	7.8	NC
		Min	147	180	147	180
		Median	159.5	180.0	159.5	180.0
		Max	176	180	176	180
Body Weight (kg)		N	4	1	4	1
		Mean	69.00	70.00	69.00	70.00

Table 14.1.1.2 Demography (Enrolled set, Safety set)

Statistics	Enrolled set		Safety set	
	Methylene Blue enema 0.002% N=4	**Methylene Blue enema 0.02% N=1**	**Methylene Blue enema 0.002% N=4**	**Methylene Blue enema 0.02% N=1**
SD	12.88	NC	12.88	NC
CV%	18.67	NC	18.67	NC
Min	52.0	70.0	52.0	70.0
Median	71.00	70.00	71.00	70.00
Max	82.0	70.0	82.0	70.0

Note: The denominator for calculating the percentages is the number of subjects of the set of each IMP group

Source: Listing 16.2.4.3 - Demography

Program: Tables\c111-dm-tbl.sas

Table 14.1.1.3 Protocol deviations (Enrolled set, Safety set)

	Enrolled set		Safety set	
	Methylene Blue enema 0.002% N=4 n (%)	**Methylene Blue enema 0.02% N=1 n (%)**	**Methylene Blue enema 0.002% N=4 n (%)**	**Methylene Blue enema 0.02% N=1 n (%)**
Number of subjects with any protocol deviation	4 (100.0)	1 (100.0)	4 (100.0)	1 (100.0)
Major	1 (25.0)	0 (0.0)	1 (25.0)	0 (0.0)
Deviation from IMP administration	1 (25.0)	0 (0.0)	1 (25.0)	0 (0.0)
Minor	4 (100.0)	1 (100.0)	4 (100.0)	1 (100.0)
Deviation from study procedures	4 (100.0)	1 (100.0)	4 (100.0)	1 (100.0)
Deviation from study visit schedule	1 (25.0)	0 (0.0)	1 (25.0)	0 (0.0)

Note: The denominator for calculating the percentages is the number of subjects of the set of each IMP group

Source: Listing 16.2.2.1 - Protocol deviations

Program: Tables\c111-dv-tbl.sas

14.2 Efficacy data

Individual compliance data in Appendix 16.2.5 and individual efficacy data in Appendix 16.2.6

14.3 Safety data

14.3.1 Displays of adverse events

Table 14.3.1.1 Global incidence of treatment emergent adverse events (Safety set)

No treatment emergent adverse event occurred during the study

Source: Listing 16.2.7.1 - Treatment emergent adverse events
Program: Tables\c111-ae-tbl.sas

14.3.2 Listing of deaths, other serious and significant adverse events

There were no deaths or other serious adverse events.

14.3.3 Narrative of deaths, other serious and significant adverse events

There were no deaths or other serious adverse events.

14.3.4 Abnormal laboratory value listing (each subject)

Not applicable.

14.3.5 Vital signs, electrocardiograms and other safety results

Table 14.3.5.1 Vital signs (Safety set)

Systolic blood pressure (mmHg)

	Statistics	Methylene Blue enema 0.002% N=4	Methylene Blue enema 0.02% N=1
Prior to rectosigmoidoscopy	N		1
	Mean		130.0
	SD		NC
	CV%		NC
	Min		130
	Median		130.0
	Max		130
After the end of rectosigmoidoscopy	N	1	1
	Mean	110.0	120.0
	SD	NC	NC
	CV%	NC	NC
	Min	110	120
	Median	110.0	120.0
	Max	110	120

Table 14.3.5.1 Vital signs (Safety set)

Systolic blood pressure (mmHg)

Statistics	Methylene Blue enema 0.002% N=4	Methylene Blue enema 0.02% N=1

Note: Subjects are summarised according to the IMP they actually received

No assessment was performed during rectosigmoidoscopy

Source: Listing 16.2.9.1 - Vital signs

Program: Tables\c111-vs-tbl.sas

Table 14.3.5.1 Vital signs (Safety set)

Diastolic blood pressure (mmHg)

	Statistics	Methylene Blue enema 0.002% N=4	Methylene Blue enema 0.02% N=1
Prior to rectosigmoidoscopy	N		1
	Mean		60.0
	SD		NC
	CV%		NC
	Min		60
	Median		60.0
	Max		60
After the end of rectosigmoidoscopy	N	1	1
	Mean	50.0	60.0
	SD	NC	NC
	CV%	NC	NC
	Min	50	60
	Median	50.0	60.0
	Max	50	60

Note: Subjects are summarised according to the IMP they actually received

No assessment was performed during rectosigmoidoscopy

Source: Listing 16.2.9.1 - Vital signs

Program: Tables\c111-vs-tbl.sas

Table 14.3.5.1 Vital signs (Safety set)

Heart rate (beats/min)

	Statistics	Methylene Blue enema 0.002% N=4	Methylene Blue enema 0.02% N=1
Prior to rectosigmoidoscopy	N	4	1
	Mean	72.5	90.0
	SD	13.2	NC
	CV%	18.2	NC
	Min	55	90
	Median	75.0	90.0
	Max	85	90
After the end of rectosigmoidoscopy	N	4	1
	Mean	70.8	70.0
	SD	12.7	NC
	CV%	18.0	NC
	Min	60	70
	Median	69.0	70.0
	Max	85	70

Note: Subjects are summarised according to the IMP they actually received
No assessment was performed during rectosigmoidoscopy
Source: Listing 16.2.9.1 - Vital signs
Program: Tables\c111-vs-tbl.sas

Table 14.3.5.1 Vital signs (Safety set)

Oxygen saturation (%)

	Statistics	Methylene Blue enema 0.002% N=4	Methylene Blue enema 0.02% N=1
Prior to rectosigmoidoscopy	N	4	1
	Mean	98.0	98.0
	SD	1.4	NC
	CV%	1.4	NC
	Min	97	98
	Median	97.5	98.0
	Max	100	98
After the end of rectosigmoidoscopy	N	4	1
	Mean	98.3	99.0
	SD	1.7	NC
	CV%	1.7	NC
	Min	96	99
	Median	98.5	99.0
	Max	100	99

Note: Subjects are summarised according to the IMP they actually received
No assessment was performed during rectosigmoidoscopy
Source: Listing 16.2.9.1 - Vital signs
Program: Tables\c111-vs-tbl.sas

Table 14.3.5.2 IMP dispensation (Safety set)

	IMP dispensation	Methylene Blue enema 0.002% N=4 n (%)	Methylene Blue enema 0.02% N=1 n (%)	Overall N=5 n (%)
IMP dispensation	Y	4 (100.0)	1 (100.0)	5 (100.0)
	N	0 (0.0)	0 (0.0)	0 (0.0)

Note: The denominator for calculating the percentages is the total number of subjects of each IMP group

Source: Listing 16.2.5.1 - IMP administrations

Program: Tables\c111-ex-tbl.sas

15 REFERENCE LIST

1. Canto MI, Setrakian S, Willis JE, Chak A, Petras RE, Sivak MV. Methylene blue staining of dysplastic and nondysplastic Barrett's esophagus: an in vivo and ex vivo study. *Endoscopy*. 2001 May;33(5):391-400
2. Bruno MJ. Magnification endoscopy, high resolution endoscopy, and chromoscopy; towards a better optical diagnosis. *Gut* 2003;52(Suppl IV):iv7–iv11
3. Becker C, Fantini MC, Wirtz S, Nikolaev A, Kiessslich R, Lehr HA, Galle PR, Neurath MF. *In vivo* imaging of colitis and colon cancer development in mice using high resolution chromoendoscopy. *Gut* 2005;54:950–954
4. Roncucci L, Stamp D, Medline A, Cullen JB, Bruce WR. Identification and quantification of aberrant crypt foci and microadenomas in the human colon. *Hum Pathol*. 1991 Mar;22(3):287-94
5. Rollins AM, Sivak MVJ. Potential new endoscopic techniques for the earlier diagnosis of pre-malignancy. *Best Pract Res Clin Gastroenterol*.2001;15:227–247
6. Quirke P., Risio M., Lambert R., von Karsa L. and M. Vieth. Quality assurance in pathology in colorectal cancer screening and diagnosis – European recommendations. *Virchows Arch* 2011;458:1-19
7. Carroll RE. Colon preparation for magnification endoscopy: a rapid novel approach. *Endoscopy* 2004;36(7):609-611
8. Lai EJ, Calderwood AH, Doros G, Fix OK and BC Jacobson. The Boston Bowel Preparation Scale: A valid and reliable instrument for colonoscopy-oriented research. *Gastrointestinal Endoscopy*. 2009 March; 69(3 Pt 2): 620–625
9. http://www.fda.gov/Drugs/DrugSafety/ucm263190.htm accessed on 22JAN13 through http://www.spectrummedicalgroup.com/ps/ar/Docs/Methylene%20Blue.pdf
10. SAS/STAT® User's Guide