25 June 2020
EMA/CHMP/424242/2020
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Methylthioninium chloride Cosmo

International non-proprietary name: methylthioninium chloride

Procedure No. EMEA/H/C/002776/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

ADR	adenoma detection rate
AE	adverse event
ALT	alanine aminotransferase
AMR	adenoma miss rate
ASGE	American Society for Gastrointestinal Endoscopy
ASMF	Active Substance Master File = Drug Master File
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
CHMP	Committee for Medicinal Products for Human use
CI	confidence interval
Cmax	maximum plasma concentration
CRC	colorectal cancer
CSR	Clinical Study Report
CYP	cytochrome P450
DKMA	Danish Medicines Agency
DNA	deoxyribonucleic acid
EC	European Commission
ECG	electrocardiogram
EMA	European Medicines Agency
ESGE	European Society of Gastrointestinal Endoscopy
EU	European Union
FAS	Full Analysis Set
FDA	Food and Drug Administration
FPR	false positive rate
HCP	healthcare professional
HD	high definition
HDWL	high-definition white light
IBD	inflammatory bowel disease
ICH	International Conference on Harmonisation
ICP-MS	Inductively coupled plasma mass spectrometry
IN	intraepithelial neoplasia
IPC	In-process control

IR	Infrared
ISS	Integrated Summary of Safety
IV	intravenous
LDPE	Low density polyethylene
MAA	Marketing Authorisation Application
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare products Regulatory Agency
MMX	Multi-Matrix
NBI	Narrow Band Imaging
NDA	New Drug Application
NMR	Nuclear Magnetic Resonance
NMT	Not more than
NSA	Number of Stained Areas with staining score > 2
OR	odds ratio
PEG	polyethylene glycol
PK	pharmacokinetic(s)
PP	Per Protocol
PVC	Polyvinyl chloride
RH	Relative Humidity
ROI	region of interest
rpm	rotations per minute
SAE	serious adverse event
SAP	Statistical Analysis Plan
SC	staining score
SmPC	Summary of Product Characteristics
SSA	sessile serrated adenoma
t1/2	elimination half life
TEAE	treatment emergent adverse event
TGA	Thermo-Gravimetric Analysis
Tmax	time to reach maximum concentration
TSA	traditional serrated adenoma
UGT	uridine diphosphate glucuronyltransferase
UK	United Kingdom

ULN	upper limit of normal
US	United States
UV	Ultraviolet
XRPD	X-Ray Powder Diffraction
γH2AX	phosphorylated histone H2AX

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Cosmo Technologies Ltd submitted on 7 February 2019 an application for Marketing authorisation to the European Medicines Agency (EMA) for Methylthioninium chloride Cosmo, through the centralised procedure under Article 3 (2) (b) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 31 May 2018. The eligibility to the centralised procedure under Article 3(2)(b) of Regulation (EC) No 726/2004 was based on demonstration of interest of patients at Community level.

The application concerns a hybrid medicinal product as defined in Article 10(3) of Directive 2001/83/EC and refers to a reference product, as defined in Article 10 (2)(a) of Directive 2001/83/EC, for which a marketing authorisation is or has been granted in a Member State on the basis of a complete dossier in accordance with Article 8(3) of Directive 2001/83/EC.

The applicant applied for the following indication.

Methylthioninium chloride Cosmo is indicated as a diagnostic agent enhancing visualisation of colorectal lesions in adult patients undergoing screening or surveillance colonoscopy.

The legal basis for this application refers to:

Hybrid application (Article 10(3) of Directive No 2001/83/EC).

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and bibliographic literature substituting/supporting certain tests or studies.

The chosen reference product is:

Medicinal product which is or has been authorised in accordance with Union provisions in force for not less than 10 years in the EEA:

- Product name, strength, pharmaceutical form: METILÉNKÉK PHARMAMAGIST 1% solution for injection
- Marketing authorisation holder: Pharmamagist Gyógyszeripari, Kereskedelmi és Szolgáltató Kft.
- Date of authorisation: 29-06-2006
- Marketing authorisation granted by:
  - Member State (EEA): Hungary
    - National procedure
- Marketing authorisation number: OGYI-T-20149/01-02

Medicinal product authorised in the Union/Members State where the application is made or European reference medicinal product:

- Product name, strength, pharmaceutical form: METILÉNKÉK PHARMAMAGIST 1% solution for injection
- Marketing authorisation holder: Pharmamagist Gyógyszeripari, Kereskedelmi és Szolgáltató Kft.
- Date of authorisation: 29-06-2006
- Marketing authorisation granted by:
  - Member State (EEA): Hungary
    - National procedure

- Marketing authorisation number: 29-06-2006

Information on paediatric requirements

Not applicable

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Scientific advice

The applicant received Scientific Advice on 24 May 2012 (EMEA/H/SA/2347/1/2012/SME/II), 19 September 2013 (EMEA/H/SA/2347/1/FU/1/2013/II0 and 15 September 2016 (EMEA/H/SA/2347/1/FU/2/2016/II) for the development programme supporting the indication granted by CHMP. The Scientific Advice pertained to the following quality, preclinical and clinical aspects of the dossier:

The main clinical aspects under consideration were:

- The design of the surveillance and screening study in patients undergoing screening colonoscopy. Aspects discussed include the population, the primary and secondary endpoint, the safety database, the sample size and the dose.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Kirstine Moll Harboe Co-Rapporteur: Natalja Karpova

The application was received by the EMA on	7 February 2019
The procedure started on	28 February 2019
The Rapporteur's first Assessment Report was circulated to all CHMP members on	16 May 2019
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	21 May 2019
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	3 June 2019
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	27 June 2019
The applicant submitted the responses to the CHMP consolidated List of	24 January 2020

Questions on	
The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on	2 March 2020
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	12 March 2020
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	26 March 2020
The applicant submitted the responses to the CHMP consolidated List of Outstanding Issues on	26 May 2020
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	08 June 2020
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Methylthioninium chloride Cosmo on	25 June 2020

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Methylthioninium chloride Cosmo is indicated as a diagnostic agent enhancing visualisation of colorectal lesions in adult patients undergoing screening or surveillance colonoscopy.

2.1.2. Epidemiology and risk factors

CRC, or colorectal adenocarcinoma, represents the third most common cancer in men and the second most common cancer in women worldwide. In 2012, there were approximately 746,000 new cases of CRC in men and 614,000 in women, corresponding to 10.0% and 9.2% of all new cancer cases in men and women, respectively. Overall, 1.4 million cases and 693,900 deaths were estimated in 2012 (Torre CA Cancer J Clin 2015). Wide geographical variation is evident between countries in the incidence and mortality of CRC, with almost 55% occurring in developed countries (IARC Cancer Fact sheets). In Europe, CRC was the second most common cancer in 2012, with 447,000 cases, and the second most common cause of death from cancer, with 215,000 deaths (Ferlay Eur J Cancer 2013).

Risk factors for CRC can be divided into lifestyle/behavioural factors and genetic factors, with the former including smoking, obesity and physical inactivity (Labianca Ann Oncol 2013). The major unchangeable risk factor for sporadic CRC is age, with nearly 70% of patients aged over 65 years. There are a number of inherited syndromes which are associated with a higher risk of CRC, including familial adenomatous polyposis; however, these high-risk subjects were excluded from enrolment into the pivotal study (Study CB-17-01/06).

2.1.3. Biologic features, Aetiology and pathogenesis

CRC usually develops over a period of several years, generally starting from pre-cancerous lesions: it has been estimated that at least 95% of CRC cases arise from pre-existing adenomas (Lin Agency for Healthcare Research and Quality 2016), even though the rate of progression of adenoma to cancer is variable. Polyps are lesions located in the mucosa of the large bowel and may be pre-cancerous (neoplastic adenoma) or non-pre-cancerous (non-neoplastic, including hyperplastic polyps) (Brown Cochrane Database Syst Rev 2016). Adenomas are epithelial tumours that can progress to adenocarcinomas and are therefore considered as precursors of CRC. Adenomas can vary greatly in their size and shape and have different degrees of dysplasia and different histologic characteristics.

2.1.4. Clinical presentation, diagnosis and stage/prognosis

In the early stages of the disease, CRC is asymptomatic in most patients. As tumour grows in size and spreads, it may present as focal symptoms (changes in bowel habits, visible blood in stools, abdominal pain etc.) or general symptoms (fatigue, weight loss, jaundice etc.). Diagnosis is made by sigmoidoscopy/colonoscopy (including biopsies). As for most solid tumours, staging is done according to the TNM staging system. Prognosis is heavily dependent on the stage, with close to 90% survival for early stages and less than 15% for advanced stages.

2.1.5. Management

The detection and removal of lesions, and consequently, the removal of adenomas, is a crucial aspect of the prevention of CRC (Kaminski Gastroenterology 2017). It has been shown that for every 1% increase in ADR, there is an associated 3% reduction in the risk of interval CRC and a 5% decrease in risk of fatal interval cancer (Kaminski Gastroenterology 2017; Corley N Engl J Med 2014).

A growing body of scientific evidence now proves that adenomas need to be categorised as either "conventional adenomas" or "serrated adenomas" (part of the serrated polyps group), and that both are recognised as precursors of CRC, so that they both need to be removed during colonoscopy, whereas the "Vienna Classification" classifies conventional adenomas only. Serrated polyps comprise a heterogeneous group of lesions with distinct features and are normally divided into 3 subgroups: traditional serrated adenomas (TSAs), sessile serrated adenomas (SSAs), and hyperplastic polyps. While the last subgroup, hyperplastic polyps, lacks malignant potential, there is now widespread consensus between endoscopists and histologists that TSAs and SSAs must be identified as potential cancer precursors. This is because there is unequivocal evidence that a significant proportion of TSAs and SSAs progress via the so-called "serrated neoplasia pathway" to colorectal adenocarcinoma and they likely account for 10% to 20% of all CRCs. There are also reasons to suspect that this "serrated pathway" may contribute disproportionately to interval or missed cancers. These TSAs and SSAs are usually flat or sessile, occasionally covered by a mucous cap, and consequently may be easily missed during colonoscopy screening. The prevalence and distribution of these lesions are however not well-defined, partly because most of the large population-based screening studies were conducted before the full characterisation and classification of different subtypes of serrated polyps.

If pre-cancerous lesions are detected and removed, CRC can be prevented, and if CRC is diagnosed early, it is a curable disease (CDC 2011). Therefore, in many Western countries, CRC mortality has declined progressively, due to the application of cancer screening programmes, with the removal of the detected mucosal lesions and adenomas, as well as the early detection of pre-cancerous and cancerous lesions, and the introduction of more effective treatments (Labianca Ann Oncol 2013). Prevention strategies have 2 arms; firstly, screening programmes for early detection and removal in

asymptomatic individuals (baseline colonoscopy), and secondly surveillance to follow-up those individuals at high-risk (surveillance colonoscopy) (Zavoral World J Gastroenterol 2014).

There are a number of factors that make CRC an excellent candidate for population screening, including high incidence, long preclinical phase, recognisable and treatable precursor, and correlation of mortality with disease stage (Schreuders Gut 2015). Colorectal cancer screening programmes were introduced in Europe after publication of the EU Council recommendations on cancer screening in 2003. Different screening tests and test algorithms are used in CRC screening programmes across different EU Member States. As of 2015, a total of 23 EU Member States have implemented or have plans to introduce CRC screening programmes (20 population-based and 3 non-population-based) (Ponti European Commission Report 2017). Screening guidelines for CRC generally use endoscopy, either flexible sigmoidoscopy or colonoscopy, as the primary screening tool, or combine them with a less invasive first-line test, such as a screening test for faecal occult blood in stool samples (Brenner BMJ 2014). A positive faecal screening test is then followed up with a flexible sigmoidoscopy or full colonoscopy. In some EU countries, colonoscopy allows the complete and direct examination of the whole colon and combines diagnosis and therapy in a single session, compared with non-invasive 2-stage screening strategies (Hewett Gastrointest Endosc Clin N Am 2015). The examination through the endoscopic probe requires the intestinal mucosa to be cleansed: for this purpose the European Society of Gastrointestinal Endoscopy (ESGE) guidelines for bowel preparation for colonoscopy (Hassan Endoscopy 2013) recommend a day before or split regimen (day before and day of colonoscopy) of 4 litres of polyethylene glycol (PEG) solution for routine bowel preparation, which may be reduced to 2 litres in some situations such as outpatient elective colonoscopy or to same day regimen for afternoon colonoscopies. However, recent data demonstrate that in clinical practice in Europe, actual uptake of the split dose regimen is low, with only 33% of patients opting for this regimen for early morning (8.00 am to 10:00 am) colonoscopies and nearly 40% of patients rejecting this option overall (Radaelli Gut 2017).

Due to the established progression of adenoma to CRC and the difficulty of determining the nature of lesions (e.g., benign lesions, pre-cancerous adenomas or cancerous adenomas) during the colonoscopy procedure, it is currently recommended by multiple international endoscopist societies that all lesions found during colonoscopy are removed (Winawer N Engl J Med 1993, Winawer Bull World Health Organ 1995, Rex Am J Gastroenterol 2002, Ferlitsch Endoscopy 2017, von Renteln Clin Transl Gastroenterol 2017). Subsequently, the nature of the lesion is assessed by histological analysis. Resect and discard' or 'diagnose and leave behind' approaches are also proposed in guidelines (Ferlitsch Endoscopy 2017) where optical diagnosis of diminutive polyps may be made with a high degree of confidence or for diminutive multiple polyps in the rectum where a sampling approach is appropriate. The caveat for these approaches being with regard to the endoscopists expertise in optical biopsy and degree of confidence.

There are several mechanisms by which colonoscopy is able to reduce the incidence and mortality of CRC. Firstly, removal of pre-cancerous lesions during colonoscopy prevents their progression to carcinoma; secondly, colonoscopy enables detection and histological diagnosis of CRC at an earlier, pre-symptomatic stage, which greatly increases survival rates (Lin JAMA 2016). In addition, the number and histological classification of adenomas detected at baseline colonoscopy are used to determine the frequency with which patients will undergo subsequent follow-up surveillance colonoscopy (Lieberman Gastroenterology 2012). According to guidelines, patients in whom no lesions are detected at baseline colonoscopy will not undergo a second colonoscopy for 10 years, while patients considered at higher risk for CRC based on their first colonoscopy will undergo surveillance colonoscopy within 3 to 5 years, depending on the number and nature of the removed lesions. This increased frequency of surveillance colonoscopies reduces the possibility of higher risk patients developing CRC in the interval between colonoscopic examinations (hereafter referred to as interval

cancers) and also helps ensure that any interval cancer that has developed will be detected early (Lieberman Gastroenterology 2012).

Since the early 1990s, several studies have shown the benefit of lower gastrointestinal endoscopy in reducing the risk of CRC, and this has been confirmed by more recent randomised controlled trials (Brenner BMJ 2014). A systematic review of randomised controlled studies demonstrated a 26% reduction in CRC mortality with flexible sigmoidoscopy screening compared to controls (Fitzpatrick-Lewis Clin Colorectal Cancer 2016). Similarly, a recent meta-analysis showed colonoscopy and flexible sigmoidoscopy screening reduced CRC mortality by 61% and 33%, respectively (Zhang Clin Colorectal cancer 2017). In patients with known polyps, a 76% to 90% reduction in the incidence of CRC compared to 3 reference groups as part of the National Polyp Study was demonstrated (Winawer N Engl J Med 1993). More recently, a 53% reduction in mortality with colonoscopic polypectomy compared to the general population has been demonstrated. Mortality from CRC was similar among patients with adenomas and those with non-adenomatous polyps during the first 10 years after polypectomy (relative risk, 1.2; 95% confidence interval [CI], 0.1 to 10.6) (Zauber N Engl J Med 2012). In a study of a large group of endoscopists, it was observed that a 1% increase in the ADR was associated with a reduction in the risk of interval cancer of 3% and a decrease in mortality of 5% (Corley N Engl J Med 2014).

Rationale for the Clinical Indication

Colonoscopy is considered the gold standard for colorectal polyp detection and removal (Pohl Gut 2009), but this procedure is not perfect. However, the effective level of protection offered by colonoscopy against CRC is significantly lower than originally estimated (Hewett J Natl Compr Canc Netw 2010). There is a concern about the sensitivity of colonoscopy, with studies suggesting that as many as a quarter of polyps may be missed (Heresbach Endoscopy 2008; Trivedi QJM 2013). Reasons for this can be technical, anatomical, or lesion-dependent (Matsuda Dig Endosc 2015). Many endoscopists have reported levels of lesion detection significantly lower than recommended targets (Hewett J Natl Compr Canc Netw 2010). There is also evidence that training and experience play a significant role in determining an individual endoscopist's ADR (Peters Clin Gastroenterol Hepatol 2010).

Lesions that are missed, rather than being removed during colonoscopy, may progress to carcinoma. During the colonoscopy procedure, in fact, it is impossible to fully determine which lesions are benign, which are adenomas, and which adenomas are cancerous or pre-cancerous. Therefore, the standard of care in endoscopy is to remove (or sample) all lesions found during colonoscopy, a practice that is recommended by the United States (US) Multi-Society Task Force on CRC (Rex Gastrointest Endosc 2017). The only definitive means of distinguishing the lesion type is through histopathological examination. Failing to detect (and consequently remove) colonic lesions expose patients to the risk of developing interval CRC. Low ADR is one of the primary reasons for post-colonoscopy CRC (Ngu Therap Ad Gastroenterol 2018).

Between 70% and 91% of interval cancers have been suggested to result from missed lesions or incompletely or inappropriately resected lesions (Pohl Clin Gastroenterol Hepatol 2010, Robertson Gut 2014, Benedict World J Gastroenterol 2015), and interval cancer represents a significant portion of CRC (Bressler Gastroenterology 2007). Because the number and nature of adenomas detected at baseline colonoscopy are used to determine the frequency of subsequent surveillance colonoscopies, patients for whom adenomas were completely missed at baseline will have longer surveillance periods, which pose a serious risk for development of interval cancer (Hewett J Natl Compr Canc Netw 2010). Failure to detect lesions therefore ultimately jeopardises the overall effectiveness of colonoscopy. Low ADR is implicated as one of the primary reasons for post-colonoscopy CRC. Acceptable levels of ADR

will depend upon the population receiving colonoscopy, but minimal standards should be defined (Ngu Therap Ad Gastroenterol 2018).

Several factors limit the effectiveness of colonoscopy in detecting lesions: these are primarily related to the patient (i.e., quality of bowel preparation), the equipment (i.e., image resolution and quality) and the endoscopist (i.e., suboptimal procedural technique). In particular, the shape and dimensions of colorectal lesions have a dramatic impact on the rate at which they are missed (Matsuda Dig Endosc 2015). Flat and depressed lesions (non-polypoid) and small lesions are the most challenging lesions to be detected during colonoscopy, due to a lack of contrast with respect to the surrounding tissues. It is estimated that 10% of the colonic surface is poorly surveyed using a standard forward-viewing colonoscope even with good bowel preparation; thus, optimising mucosal visualisation is fundamental to ensuring high-quality colonoscopy (Ngu Therap Ad Gastroenterol 2018). In a multicentre retrospective study, patients diagnosed with colorectal adenomas at a screening colonoscopy underwent a second colonoscopy 3 months later. Among the 2093 patients, 560 patients had adenomas that were missed in the first colonoscopy and were only detected in the second colonoscopy. Of a total of 4632 adenomas, 967 were missed at the first colonoscopy, with an overall 'per-adenoma' adenoma miss rate (AMR) of 20.9%. The 'per-patient' AMR was 43.3% in patients with flat adenomas, compared to 18.6% in patients with only protruding adenomas (odds ratio [OR]: 0.300, 95% CI 0.245 to 0.367) (Xiang World J Gastroenterol 2014). In another retrospective study to investigate the miss rate of lesions in 463 patients, non-polypoid lesions were missed at a rate of 32.7%, compared to a miss rate of 7.5% for pedunculated or sub-pedunculated polyps (adjusted OR: 3.62, 95% CI 2.40 to 5.46) (Kim Intest Res 2017). Non-polypoid lesions are relatively common worldwide, and failure to detect these lesions is a particular concern. In fact, patients with non-polypoid lesions have an increased risk of developing advanced neoplasia at surveillance colonoscopy compared to patients with polypoid lesions only, and patients with non-polypoid lesions are at high risk of developing CRC (McGill Clin Gastroenterol Hepatol 2017). Tandem colonoscopy has also shown smaller lesions are understandably more frequently missed by all endoscopists (Rex Gastroenterology 1997). The ADR, defined as the proportion of colonoscopies during which at least one adenoma is found, is used as an important marker of quality in colonoscopy by the most eminent and important endoscopy societies in the world, including the ESGE and the American Society for Gastrointestinal Endoscopy (ASGE) (Matsuda Digestive Endoscopy 2015; Kaminski Endoscopy 2017; Rex Gastrointest Endosc 2015). ADR represents the primary measure of the quality of mucosal inspection, and is the single most important quality measure in colonoscopy, recognised in the medical literature as the 'gold standard' in colonoscopy quality (Liem Transl Gastroenterol Hepatol 2018). Endoscopists with a low ADR might fail to identify patients with pre-cancerous lesions, putting patients at risk of cancer twice, once by failure to clear the colon and secondly by recommending inappropriately long intervals between examinations (Rex Gastrointest Endosc 2015). Furthermore, ADR is inversely correlated with the rate of interval cancers (Kaminski N Engl J Med 2010), and ADR has been validated as an independent predictor of the risk of interval cancer. An increase in ADR represents a clear and quantitative improvement in CRC prevention, through both a more complete clearance of adenomas at baseline colonoscopy and shorter surveillance intervals. Even incremental improvements in ADR are clinically significant: a 1% increase in ADR is associated with a 3% reduction in the risk of interval cancer, and a 5% decrease in CRC mortality (Kaminski Gastroenterology 2017, Corley N Engl J Med 2014). Assessing and improving ADR is therefore at the core of a successful CRC prevention programme (Fayad Gastroinest Endosc Clin N Am 2015).

Considering the relevance of ADR as the most important quality indicator of colonoscopy for CRC screening or surveillance, and the growing evidence about its impact on the decrease of interval cancer risk and death, there has been much research and effort made in developing tools or techniques, such as chromoendoscopy or virtual (or electronic) chromoendoscopy, with the aim of increasing the ADR and decreasing the miss rate of pre-cancerous lesions.

Chromoendoscopy

One technique that has been found to significantly improve ADR for both average- and high-risk CRC screening populations is chromoendoscopy (Pohl Gut 2011). Chromoendoscopy involves using a dye to highlight the mucosal architecture, and improve detection of mucosal irregularities and contours of lesions. These contrast dyes are either absorbed by the mucosa (in the case of vital dyes such as methylthioninium chloride) or remain on the mucosal surface (in the case of non-vital dyes such as indigo carmine) (Bartel Gastrointest Endoscopy Clin N Am 2015). Currently in chromoendoscopy, the dye is 'sprayed' onto the mucosal surface using a catheter via the biopsy channel during colonoscopy. The application of the dye can be 'targeted' to areas or lesions of interest, or 'pan-colonic' involving spraying the whole of the colonic mucosa (Brown Cochrane Database Syst Rev 2016). Contrast dyes are safe and cheap but their use requires time and is cumbersome, which has limited their general implementation. Spray chromoendoscopy, independently of the application modality (pan-colonic or targeted spraying), has been proven to be more effective than standard colonoscopy in the detection of non-polypoid lesions in published studies, both in subjects at average risk for CRC (Kahi Am J Gastroenterol 2010; Pohl Gut 2011) and in patients at high-risk for CRC (e.g., inflammatory bowel disease [IBD] patients) (Kiesslich Gastroenterology 2003; Kiesslich Gastroenterology 2007).

In clinical studies, chromoendoscopy has shown a generally higher ADR than normal colonoscopy. For example, the ADR with standard colonoscopy ranged between 25% and 31%, whereas ADR with chromoendoscopy ranged between 33% and 39% (Brooker Gastrointest Endosc 2002, Le Rhun Clin Gastroenterol Hepatol 2006). A Cochrane review in 2016 looked at chromoendoscopy versus conventional endoscopy for the detection of polyps in the colon and rectum (Brown Cochrane Database Syst Rev 2016). Seven trials were included involving 2727 participants, with 5 of the trials being similar enough to allow pooled results, and the other 2 trials included in a subgroup analysis. Overall, chromoendoscopy was significantly more likely to detect people with at least one neoplastic lesion (OR 1.53, 95% CI 1.31 to 1.79), and at least one diminutive neoplastic lesion (< 5 mm) (OR 1.51, 95% CI 1.19 to 1.92). None of the studies reported adverse events related to the use of the contrast dye (usually indigo carmine). Analysis of the 7 studies also suggested that chromoendoscopy consistently enhances the detection of premalignant polyps in the colon and rectum (Figure 2). There was no apparent increase in the detection of larger lesions or advanced pathology, which is consistent with the expectation that chromoendoscopy should particularly enhance the detection of small and flat lesions, which otherwise may be hard to detect.

Despite the fact that spray chromoendoscopy increases ADR and is endorsed by several guidelines for patients at high-risk of CRC, such as patients with IBD (Cairns Gut 2010; Cancer Council Australia Colonoscopy Surveillance Working Party 2011; Mowat Gut 2011; NICE 2011; Dignass J Crohns Colitis 2012; Annese J Crohns Colitis 2013; Kaminski Endoscopy 2014), its use in routine clinical practice is limited. A particular disadvantage of chromoendoscopy is that it takes significantly longer than typical colonoscopy, with an average length of 36.9±14.5 minutes versus 27.3±6.2 minutes in a typical colonoscopy (Stoffel Cancer Prev Res (Phila) 2008). Chromoendoscopy is also more labour-intensive than conventional colonoscopy, and can be quite messy (Brown Cochrane Database Syst Rev 2016). Non-uniform staining of the colon and possibility of missing lesions is also an issue, as the technique is operator-dependent in terms of extent of area sprayed, and the washing of the excess dye within a few minutes after spraying prevents the vital dye from penetrating deeply into the mucosal cell architecture to maximise the contrast between the lesion and the surrounding tissue (Trivedi QJM 2013).

Several alternative tools have been developed with the aim of avoiding the drawbacks related to the practical application of chromoendoscopy, while retaining its benefits. These tools include the use of optical filters or software-based technologies to improve detection of lesions by virtual

chromoendoscopy (Bartel Gastrointest Endosc Clin N Am 2015), such as Narrow-Band Imaging (NBI) (Emura World J Gastroenterol 2008), Flexible Spectral Imaging Colour Enhancement (Fujifilm, Japan), and iScan (Pentax, Japan). Virtual chromoendoscopy attempts to replicate the results of chromoendoscopy in terms of increased detection of lesions with digital techniques.

However, notwithstanding the massive investments made by the equipment makers, no new tool has been able to generate an increase in ADR at least resembling that achievable with chromoendoscopy (Nagorni Cochrane Database Syst Rev 2012; Hong Gastrointest Endosc 2012). Furthermore, the use of such techniques has not unequivocally been demonstrated to improve the ADR with respect to conventional colonoscopy in randomised, controlled clinical trials. The most significant advancement in the equipment of colonoscopy in recent years has been the introduction of high-definition white light (HDWL) colonoscopy. Nonetheless, HDWL colonoscopies have generated, on average, an increase of ADR equal to 3.5% compared with standard definition colonoscopy (Subramanian Endoscopy 2011), incomparable with the reported ADR gain with chromoendoscopy versus standard colonoscopy. Given the significant improvement of HDWL colonoscopy over standard colonoscopy and the broad adoption of this technological advancement over and above others, as described above, it is pertinent to note that all the endoscopies conducted in the pivotal Phase 3 study with Methylthioninium chloride Cosmo tablets (both the active and placebo groups) were conducted by experienced colonoscopists (> 500 colonoscopies/year) using the most current standard HDWL equipment.

2.2. About the product

The initially applied indication was "Methylthioninium chloride-MMX is indicated as an aid for the enhanced visualization and detection of colorectal lesions in adult patients undergoing screening / surveillance colonoscopy for colorectal cancer. An increased detection of colorectal lesions translates into an increase in the adenoma detection rate (ADR)."

The final agreed indication following the CHMP review is: "Methylthioninium chloride Cosmo is indicated as a diagnostic agent enhancing visualisation of colorectal lesions in adult patients undergoing screening or surveillance colonoscopy."

Cosmo have developed Methylthioninium chloride Cosmo as a self-administered tablet formulation to be taken orally during or after the intake of the bowel cleansing preparation. Tablet administration should be completed in the evening prior to the colonoscopy to ensure there is enough time for the tablets to reach the colon and locally release the methylthioninium chloride prior to the colonoscopy. Each tablet contains methylthioninium chloride European Pharmacopoeia equivalent to 25 mg of anhydrous methylthioninium chloride. The recommended total dose is 200 mg (8 × 25 mg tablets), taken with the bowel cleansing regimen chosen by the healthcare professional (HCP) or with water.

Methylthioninium chloride is widely used in humans for a variety of medical purposes, as both a medication and a dye, and is a registered drug in many countries for indications such as the treatment of acquired and hereditary methaemoglobinaemia (including drug-induced and idiopathic methaemoglobinaemia), the prevention of ifosfamide-induced encephalopathy in human cancer management, the prevention of urinary tract infections, the intraoperative visualisation of nerves and endocrine glands as well as of pathologic fistulae, and the sterilisation of transfusion plasma.

It is currently approved centrally in Europe by the European Medicines Agency (EMA) as Methylthioninium chloride Proveblue® 5 mg/mL solution for injection for use in adults, children and adolescents for the acute symptomatic treatment of medicinal and chemical products-induced methaemoglobinaemia; as well as a Class IIa medical device ProveDye, indicated as a visualisation aid for surgical procedures such as the delineation of tissues and operative pieces, seal tests for sutures, detection of leaks, and fistula detection.

2.3. The development programme/compliance with CHMP guidance/scientific advice

A total of 7 clinical studies of Methylthioninium chloride Cosmo have been conducted, including:

• 2 uncontrolled, Phase 1 studies (Studies CB-17-01/01 and CB-17-01/02)

• 4 uncontrolled, Phase 2 studies (Studies CB-17-01/03, CB-17-01/04, CB-17-01/05 and CB-17-01/08)

• 1 large, multicentre, randomised, double-blind, placebo-controlled, pivotal, Phase 3 study (Study CB-17-01/06).

A total of 1087 subjects received Methylthioninium chloride Cosmo (any dose) during clinical development, with 798 subjects receiving the full 200 mg dose (8 × 25 mg tablets) in the dose and dosage form intended for commercial use. Prior to this MAA, Methylthioninium chloride Cosmo has been filed for regulatory approval in the United States and Canada for similar indications:

The applicant sought Scientific Advice from the EMA, as well as the US FDA and UK MHRA. Specifically, from the EMA, Scientific Advice was sought in 2012 (EMA/CHMP/SAWP/288902/2012; Procedure No.: EMEA/H/SA/2347/1/2012/SME/II), 2013 (EMA/CHMP/SAWP/550700/2013; EMEA/H/SA/2347/1/FU/l/2013/II) and 2016 (EMA/CHMP/SAWP/590139/2016; EMEA/H/SA/2347/1/FU/2/2016/II).

Topics discussed at scientific advice meetings included:

- The acceptability of a single pivotal confirmatory efficacy study.
- The regulatory strategy for MA application.
- The acceptability of the nonclinical package.
- The mechanisms for control of bias.
- Emergent clinical management within the intended indication and its impact on the clinical development program.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as prolonged-release tablets containing 25 mg of methylthioninium chloride as active substance.

Other ingredients of the tablet core are: stearic acid 50 (E570), soya lecithin (E322), microcrystalline cellulose (E460), hypromellose 2208 (E464), mannitol (E421), talc (E553b), silica colloidal anhydrous (E551) and magnesium stearate (E470b). Other ingredients of the tablet coating are: methacrylic acid

- methyl methacrylate copolymer (1:1), methacrylic acid - methyl methacrylate copolymer (1:2), talc (E553b), titanium dioxide (E171), triethyl citrate (E1505).

The product is available in polyamide/aluminium/PVC foil blister with aluminium push-through foil as described in section 6.5 of the SmPC.

2.4.2. Active substance

General information

The chemical name of methylthioninium chloride is 3,7-bis(dimethylamino)phenothiazin-5-ylium chloride (methylene blue), corresponding to the molecular formula $C_{16}H_{18}ClN_3S$, xH_2O (the active substance is a mixture of mainly pentahydrate and other hydrates forms). It has a relative molecular mass of 319.9 + x*18 and the following structure:

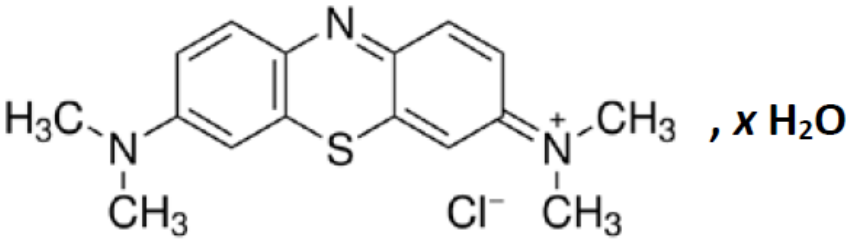


Figure 1: active substance structure

The chemical structure of the active substance was elucidated by a combination of infrared (IR) absorption spectrophotometry, ultraviolet (UV) absorption spectrophotometry, nuclear magnetic resonance (NMR) spectroscopy (^1H, ^{13}C) and mass spectrometry.

The solid state properties of the active substance were measured by X-ray powder diffraction (XRPD) and thermogravimetric analysis (TGA).

The active substance is a dark blue or dark green crystalline hygroscopic powder with a metallic sheen. It is slightly soluble in water and ethanol (96%).

Methylthioninium chloride has a non - chiral molecular structure.

There is a monograph of methylthioninium chloride in the European Pharmacopoeia. The Active Substance Master File (ASMF) procedure is used within the current Marketing Authorisation Application (two ASMFs are submitted).

Polymorphism has been observed for the active substance. Based on literature, methylthioninium chloride exists in different hydrate structures: Form A is a pentahydrate, Form B and C are dihydrates, Form D contains more than 2 equivalents of water and Form E is a monohydrate. Form A is considered to be the most stable thermodynamically at room temperature and up to 70% RH. Furthermore, as described in literature, crystallisation of methylthioninium chloride from water generally gives a mixture of mainly pentahydrate and additional dihydrated forms. It has been demonstrated that the active substance obtained from two sources is a mixture of mainly pentahydrate and other hydrates forms, which is also in line with the monograph of methylthioninium chloride in the Ph. Eur., which states that the active substance contains a variable quantity of water (with prescribed limits). No issues related to the solubility of the active substance are expected and its bioavailability is not linked to a specific crystalline status, since methylthioninium chloride can be defined as highly soluble at physiological pH. In line with ICH Q6A, no acceptance criteria are set for polymorphism in active

substances since there is no finished product safety, performance, or efficacy issue linked to a specific polymorph of the active substance.

The active substance is manufactured according to two synthetic routes. It was demonstrated that the active substance manufacturing process of both suppliers consistently produces the active substance in which pentahydrate crystals (Form A) are mainly evident. It was further demonstrated that the polymorphic form does not change during storage.

Manufacture, characterisation and process controls

Detailed information on the manufacturing of the active substance has been provided in the ASMF restricted parts of two ASMF holders and was considered satisfactory.

During initial development, the active substance was sourced from the first manufacturer, Manufacturer B only. All clinical trial batches of the finished product used the active substance sourced from Manufacturer B in their manufacture. An additional active substance supplier, Manufacturer A was selected to support commercialisation. A comparability study was conducted for the active substance made by Manufacturer A and B, as well as a comparison of finished product batches made using active substance batches from both manufacturers. The study demonstrated that the active substance sourced from the two manufacturers is comparable.

The active substance obtained from Manufacturer A is synthesised in three chemical reaction steps, purification and physical processing step using well-defined starting materials with acceptable specifications. Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

The active substance obtained from Manufacturer B is synthesised in five chemical reaction steps, purification and physical processing step, using well-defined starting materials with acceptable specifications. Of note, the active substance manufacturing process was updated during the procedure at the request of CHMP, to include additional synthesis steps. Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

The characterisation of the active substance and its impurities, sourced from both manufacturers is in accordance with the EU guideline on chemistry of new active substances. Potential and actual impurities were well discussed with regards to their origin and characterised. The risk of genotoxic impurity formation has been assessed in line with ICH M7 and relevant control strategies are in place. Several synthesis impurities have been identified as potentially genotoxic. Genotoxic impurities are further discussed under the active substance specification section. The potential risk of nitrosamine impurity formation and the presence of elemental impurities are discussed under the finished product.

The active substance provided by the additional active substance manufacturer is packaged in packaging which complies with the EC directive 2002/72/EC and EC 10/2011 as amended.

The active substance provided by the initial supplier is packaged in packaging which complies with the EC directive 2002/72/EC and EC 10/2011 as amended.

Specification

The active substance specification includes tests for: appearance, identification (IR), identification of chlorides (Ph. Eur.), related substances (HPLC), metals (Ph. Eur.), loss on drying (Ph. Eur.), sulfated ash (Ph. Eur.) and assay (HPLC), according to official European Pharmacopoeia monograph of

Methylthionium chloride and additional in house tests for residual solvents (GC) and synthesis impurities (HPLC).

The proposed active substance specification complies with the Ph. Eur. monograph 1132 Methylthioninium chloride, including supplements. Specifications of the active substance sourced by different manufacturers contains appropriate limits for process-related impurities. The limits for potentially genotoxic impurities are compliant with acceptable daily intake in line with the ICH M7 guideline.

Microbiological purity of the active substance is controlled by the active substance Manufacturer A and the finished product manufacturer routinely controls microbiological purity of prolonged-release tablets with appropriate limits according to Ph.Eur. The justification of non-control of microbiological purity of the active substance by finished product manufacturer is acceptable.

All the analytical methods applied by the finished product manufacturer for active substance testing are Ph. Eur. methods except for specific tests for residual solvents and for synthesis impurities. The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data of three active substance batches from each manufacturer are provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data from three commercial scale batches of active substance from Manufacturer A stored in the package representative of proposed commercial packaging for up to 36 months under long term conditions (5 ºC ± 3°C), for up to 36 months under accelerated conditions (25 ºC / 60% RH), for up to 12 months at 30 ºC / 65% RH and for up to 6 months at 40 ºC / 75% RH according to the ICH guidelines were provided. Photostability testing following the ICH guideline Q1B was performed on one batch. Results on stress conditions (acidity, alkalinity, oxidation, exposed to light, temperature 80 ºC) were also provide on one batch.

The tested parameters were the same as those at active substance release. The analytical methods used were the same as for release and were stability indicating.

Long term stability shows all results within specification. Photostability study showed that the active substance manufactured by Manufacturer A is photostable.

Results of forced degradation test show that the active substance is sensitive to alkaline and acidic conditions and more stable under oxidation and high temperature conditions.

Any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA.

The stability results indicate that the active substance manufactured by Manufacturer A is sufficiently stable. The stability results justify the proposed retest period.

Stability data from three commercial scale batches of active substance from Manufacturer B, the initial active substance manufacturer, stored in the intended commercial package for up to 36 months under long term conditions (5 ºC ± 3°C) and for up to 6 months under accelerated conditions (25 ºC / 60% RH) according to the ICH guidelines were provided. Photostability testing following the ICH guideline Q1B was performed on one batch. Results on stress conditions (acidity, alkalinity, oxidation, exposed to light, temperature 80 ºC) were also provide on one batch.

The analytical methods used were the same as for release and were stability indicating.

Long term stability shows all results within specification. Study under accelerated conditions shows all results within specifications.

Results of forced degradation test show that the active substance is sensitive to alkaline and acidic conditions and relatively stable under oxidation and high temperature conditions.

Any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA. The stability results indicate that the active substance manufactured by Manufacturer B the initial active substance supplier is sufficiently stable. The stability results justify the proposed retest period.

2.4.3. Finished medicinal product

Description of the product and Pharmaceutical development

The finished product is presented as prolonged-release tablets presented in one strength. Each tablet contains 25 mg of the active substance methylthioninium chloride. The tablets are off-white to light blue, round, biconvex with a diameter of 9.5 mm and a thickness of 5.3 mm.

The aim of pharmaceutical development was to develop a locally released vital dye formulation indicated for enhanced visualisation and detection of colorectal lesions in patients undergoing screening or surveillance colonoscopy. The tablets are formulated using a patented Multi-Matrix (MMX) structure that allows the delivery of the active substance directly inside the colon. With this technology, the maximum local bioavailability of the active substance is achieved and, consequently, the contrast enhancing effect is optimised. The MMX technology allows the delivery of active pharmaceutical ingredients into the lumen of the colon through tablets in a delayed and controlled extent with the effect that the active pharmaceutical ingredients can be applied to the full length of the colon. The controlled release over the length of the colon simplifies the application for the patients and allows for the topical application of the active pharmaceutical ingredients to the bowel surface.

The active substance methylthioninium chloride is described in a monograph in Ph. Eur. Methylthioninium chloride is highly bioavailable, with virtually 100% of the oral dose absorbed. A comparison of the active substance sourced from the proposed suppliers, Manufacturer A and Manufacturer B (used during clinical development work) was performed, considering the physical properties that could affect the performance of the finished product formulation. The characteristics was found similar for the two sources.

The potential influence of active substance particle size on the manufacture, quality and stability of the finished product has been adequately discussed. At pH ranging from 2 to 7.2, covering the intestinal tract, the solubility of the active substance is ranging between approximately 10 to 40 g/l and therefore the active substance can be considered as "highly soluble" in physiological pH. The active substance is considered a highly soluble drug since it shows dose/solubility volume $\leq$ 250 mL throughout the physiological pH range. In addition, the finished product is formulated to deliver the active substance in a controlled release manner, therefore the active substance particle size is not considered a critical parameter.

The two active substance manufacturers have characterised the physical state of the manufactured active substance. Both sources have demonstrated to consistently produce active substance containing mainly the pentahydrate form although no direct, formal quantification is provided. The performance of the active substance is mainly controlled by the formulation. No issues related to the solubility of the active substance are expected and its bioavailability is not linked to a specific crystalline status, since

methylthioninium chloride can be defined as highly soluble at physiological pH. Methylthioninium chloride also does not have a narrow therapeutic index. Performance of the finished product, linked with solubility of the active substance, is sufficiently controlled at release with dissolution testing and appropriate acceptance criteria. In line with ICH Q6A, no acceptance criteria are set for polymorphism in active substances since there is no finished product safety, performance, or efficacy issue linked to a specific polymorph of the active substance.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards, except lecithin, which complies with a USP/NF monograph, since there is no Ph. Eur. Monograph for this substance. Reference to USP/NF for the excipient lecithin has been justified and a tabulated specification of excipient is included in the dossier. Methylthioninium chloride Cosmo contains 3 mg soya lecithin per prolonged-release tablet, which is an excipient with a known effect. Appropriate warnings have been included in the product information. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC and in paragraph 2.1.1 of this report.

No formal compatibility studies between the active substance and each of the excipients were performed during the finished product development. The excipients were chosen based on the manufacturers experience (prior knowledge) with other products using the MMX technology. The compatibility has been adequately confirmed by the stability studies of the finished product.

The chosen formulation is considered adequate for the intended indication. The selection and concentration of excipients in the final formulation was based on the physicochemical characteristics of each excipient. The choice of excipients in order to manufacture the multi matrix structure has been explained and justified. The applicant has discussed the functionality-related characteristics of the excipients. The tests that are included in the excipient specifications are suitable for release controlling excipients and their functionality in the multi matrix system.

The multi matrix structure uses a combination of different controlled release mechanisms, such as swelling and diffusion. The tablet core multi-matrix structure is composed primarily of hydrophilic and inert (amphiphilic/lipophilic) matrices. The methylthioninium chloride is partially embedded in the inert matrix (lecithin / stearic acid), which is dispersed in the hydrophilic matrix (hypromellose).

After the pH dependent coating is dissolved, the tablet core is exposed to solvents in the local environment, which cause the hydrophilic matrix to swell. The solvent then penetrates the inert matrix, which is dispersed in the hydrophilic matrix and causes the release of the drug through diffusion from the matrix into the colon.

Multiple administration of 8 tablets each containing 25 mg methylthioninium chloride, during the bowel preparation procedure was considered as optimal.

The same formulation was used in the clinical trials as in the development studies.

The manufacturing process, is a standard dry-granulation direct compression method, including the following stages: pre-mixing, final blending preparation, tableting and film-coating. The development of the formulation and manufacturing process is considered adequately described.

The primary packaging is polyamide/aluminium/PVC foil blister with aluminium push-through foil. The material complies with Ph.Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Manufacture of the product and process controls

The manufacturing process consists of four main steps: pre-mixing, final blending preparation, tableting and film-coating. The process is considered to be a non-standard manufacturing process (prolonged release dosage form) however, standard processing steps are used.

Major steps of the manufacturing process have been validated on three consecutive commercial scale batches. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form.

Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: appearance, size, methylthioninium chloride identification (HPLC, UV/VIS), uniformity of dosage units (Ph. Eur.), methylthioninium chloride assay (HPLC), related substances (HPLC), dissolution (Ph. Eur.), ethanol (GC), dye identification (colorimetric) and microbial analysis (Ph. Eur.).

A risk assessment of the potential presence of nitrosamine impurities in the methylthioninium chloride tablets was provided at the request of CHMP. All potential sources for the formation of nitrosamines have been evaluated. These includes the active substance from Manufacurer A and Manufacturer B, excipients, manufacturing process conditions, manufacturing equipment, container closure system and storage conditions. No risk for the presence of any nitrosamine impurities in the methylthioninium tablets was identified. The nitrosamine risk evaluation was considered to be sufficient.

The potential presence of elemental impurities in the finished product has been assessed on a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Batch analysis data on 3 batches using a validated ICP-MS method was provided, demonstrating that each relevant elemental impurity was not detected above 30% of the respective PDE. The information on the control of elemental impurities is satisfactory.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are provided for 6 commercial scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

The finished product is released on the market based on the above release specifications, through traditional final product release testing.

Stability of the product

Stability data from 6 commercial scale (3 manufactured using active substance manufactured by the initial active substance supplier, Manufacturer B and 3 manufactured using active substance manufactured by the additional supplier, Manufacturer A,) batches of finished product stored for up to 36 months under long term conditions (25 ºC / 60% RH), for up to 12 months under intermediate conditions (30 ºC / 65% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. The batches of medicinal product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing. Additional supportive stability data was provided on 3 supportive batches for clinical use. The supportive batches were produced with the same drug product composition and container closure system as the registration batches. Furthermore, stability data at 25 °C / 60% RH and 5°C (18 months) on bulk product packaged in double LDPE bags placed in HDPE drums are available for two batches.

A comparable stability profile between registration and the supportive development batches has been demonstrated, at both long term and accelerated conditions. No significant differences were observed between batches manufactured using active substance obtained from different manufacturers. No out of specification result was observed in all the tested conditions and no significant change was evidenced. All the batches met the proposed specifications for all storage conditions. Stress stability studies conducted demonstrated that the assay and related substances methods were stability indicating.

Samples were tested (based on the timepoint) for appearance, identification by HPLC, uniformity of dosage unit, assay, related substances, dissolution test, dye identification, microbiological analysis. The analytical procedures used are stability indicating. Analytical methods have been optimized during the development, therefore the methods used for testing assay, related substances and dissolution for release and stability testing of the supportive stability batches are not fully identical. The details of the methods development were summarized by the applicant, a comparison between the methods used for release and stability of registration batches with the methods previously used on the supportive stability batches was provided and the study results show substantial equivalence of the methods.

In accordance with EU GMP guidelines, any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA.

Photostability studies were conducted according to ICH Q1B and it was shown that the finished product is photo-stable and no special packaging protection from light is needed.

Based on available stability data, the proposed shelf-life of 3 years and no special storage conditions as stated in the SmPC (section 6.3) are acceptable.

Adventitious agents

No excipients derived from animal or human origin have been used.

2.4.4. Discussion on chemical, and pharmaceutical aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

The synthetic route used by the two active substance manufacturers Manufacturer B and Manufacturer A are different, however the quality of the active substance obtained by both manufacturers is the same and complies with the monograph in Ph. Eur. for methylthioninium chloride. In addition, both have specification for residual solvents, which is specific to each manufacturer.

Regarding the synthesis used by the initial active substance manufacturer Manufacturer B, the starting materials have been redefined to an earlier step in the synthesis and all relevant sections of the ASMF have been updated. Discussion on potentially genotoxic impurities has been amended.

The finished product manufacturer controls the active substance in accordance with the monograph in Ph. Eur. and additional supplier specific tests for residual solvents.

Sufficient information regarding the potential presence of nitrosamine impurities has been provided and no risk for the presence of any nitrosamine impurities in the methylthioninium tablets was identified.

The finished product Methylthioninium chloride Cosmo 25 mg is modified release film-coated tablets manufactured using a Multi-Matrix System (MMX) Technology. The type of formulation is considered justified for the intended indication and the formulation development is adequately described.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.4.6. Recommendations for future quality development

Not applicable.

2.5. Non-clinical aspects

2.5.1. Introduction

The nonclinical package in support of this application is comprised of published studies on methylthioninium chloride in peer-reviewed literature complemented by studies conducted with methylthioninium chloride and Methylthioninium chloride Cosmo tablets. Methylthioninium chloride has been known and used for more than a century, therefore this application is based on comprehensive data related to different applications for human use, particularly, both systemic and local applications.

The proprietary Methylthioninium chloride Cosmo prolonged release formulation significantly changes the systemic absorption of methylthioninium chloride compared to when it is administered "as is" as drug substance, therefore the assessment of the actual exposure, and its effects, in nonclinical studies with MMX gastro-resistant, prolonged release tablets have also been determined. The species selected for oral administration was the dog, as the administration of MMX tablets impeded treatment of smaller animals, e.g. rats and rabbits. GLP-compliant studies in the dog included a 28-day toxicology study (including toxicokinetic evaluation) and the assessment of safety pharmacology parameters (blood pressure, heart rate, ECG and body temperature). In addition, in view of the complexity of the pharmacokinetics of methylthioninium chloride, which involves interconversion to leucomethylene blue, and a high volume of distribution, the assessment of plasma protein binding, transporter effects and inhibition/induction of hepatic enzymes of methylthioninium chloride, using drug substance, was also investigated.

Studies Conducted by Cosmo Technologies:

Study No.	Study Title	GLP-Compliant
CPS-04	Methylene Blue: Investigation of in vitro plasma protein binding in rat, dog and human	Yes
CPS-05	Methylene Blue: P-glycoprotein (P-gp), OAT1 and OAT3 interactions	Yes
CPS-06	Methylene Blue: Assessment of the potential to inhibit human hepatic cytochrome P450 (CYP) enzymes in vitro	Yes

CPS-07	Methylene Blue: Assessment of the potential to induce human hepatic cytochrome P450 (CYP) 1A2, 2B6 and 3A4 using human hepatocytes in culture	Yes
20160067PCCPB	Methylene blue MMX® 25 mg tablets. Evaluation of effects on blood pressure, heart rate, electrocardiogram and body temperature after single oral administration to conscious dogs	Yes
20160066TCPB	Methylene blue MMX 25 mg tablets. 28-day oral toxicity study in the dog including recovery and toxicokinetics	Yes

2.5.2. Pharmacology

Primary pharmacodynamic studies

Specific studies on the primary pharmacology of methylthioninium chloride drug substance have not been conducted. Methylthioninium chloride has been used for many years and is currently used in the treatment of methemoglobinemia [Beutler 2005].

Secondary pharmacodynamic studies

Specific studies on the secondary pharmacology of methylthioninium chloride have not been conducted, as methylthioninium chloride has a well-established efficacy and safety profile and many years of medicinal use.

Safety pharmacology programme

A limited safety pharmacology programme was conducted with Methylthioninium chloride Cosmo 25 mg tablets, focusing on cardiovascular safely in a 28-day repeat oral toxicity study in beagle dogs (Study No. CERB 20160066TCPB). Methylthioninium chloride Cosmo at 200 mg, 400 mg and 600 mg did not induce any statistically significant changes in mean, systolic and diastolic arterial blood pressure, heart rate, body temperature, cardiac conduction times (i.e., PR, PQ intervals and QRS complex durations), ventricular repolarisation duration (QTc and QT shift), QT interval short term variability or ST segment when compared to the placebo control group. No test article-related changes in the sympatho-vagal balance were observed. No test article-related disturbances in the Lead II electrocardiogram were noted. All animals that received Methylthioninium chloride Cosmo tablets had blue/green faeces during the experiment.

Respiratory System

No respiratory studies have been conducted. In a published one-month oral gavage study in rats, doses of 1000 or 2000 mg/kg caused methemoglobinemia and respiratory changes [NTP 1990]; the respiratory changes were consistent with reduced levels of hemoglobin which would be expected to result in a compensatory increase in respiratory parameters such as rate and volume. No evidence of methemoglobinemia was noted in the 28-day repeat oral toxicity study in beagle dogs (Study No. CERB 20160066TCPB), indicating that respiratory changes are unlikely following oral dosing with Methylthioninium chloride Cosmo tablets.

Central Nervous System (CNS)

No studies have been conducted. In a study reported in the literature, the incubation of slices of young rat cerebellum incubated for one hour with methylene blue (10 to 100 µM), caused a progressive destruction of the differentiating cells [Garthwaite 1988]. However, these effects were noted at free concentrations of methylene blue, orders of magnitude higher than observed in the clinic. In addition, a suppression of evoked excitatory field potentials in hippocampal slices at 1h following incubation with 10 µM methylene blue has been reported; in dissociated neuronal cultures from the subventricular zone, this was associated with an increase in dying cells at doses of ≥10 µM methylene blue [Vutskits 2008, Garthwaite 1988].

No specific studies to investigate central nervous system effects have been conducted by the Sponsor. In a one-month oral gavage toxicity study (125 to 2000 mg/kg/day) in rats, methylene blue induced no significant neurological deficits at 2 weeks or at study termination [NTP 1990]. No central nervous system effects were noted in the cardiovascular safety pharmacology study in dogs or in the 28-day oral repeat-dose toxicity study in Beagle dogs, administering Methylthioninium chloride Cosmo 25 mg tablets (Study No. 20160066TCPB).

Cardiovascular system

A GLP study was conducted (Study no. 20160067PCCPB) to examine the effects of administration of Methylthioninium chloride Cosmo 25 mg tablets on blood pressure, heart rate, body temperature and electrocardiograms in Beagle dogs. Four male Beagle dogs (21-39 months of age, 12.3-15.0 kg body weight) equipped with implanted telemeters received placebo or 200, 400 or 600 mg Methylthioninium chloride Cosmo 25 mg tablets via oral gavage in a cross-over design with 72 hours of washout between doses. There were two phases in the study: the first involved dosing with telemetry measurements, with the second part involving additional dosing for blood collection and observations. In phase I, the animals received 3 capsules at 1 hour intervals containing 9, 9 and 6 placebo tablets for the placebo group, 3, 3 and 2 tablets for the 200 mg group, 6, 6 and 4 tablets for the 400 mg dose group, and 9, 9 and 6 tablets for the 600 mg dose group.

Measurements included arterial blood pressure, heart rate, body temperature, and epicardial Lead II electrocardiograms started at least 2 hours prior to dosing and continued for at least 24 hours after dosing with the third capsule.

In phase II, the animals were dosed again with Methylthioninium chloride Cosmo 25 mg tablets at 600 mg for complementary investigations such as blood sampling and observation of animals.

Whole blood concentration results confirmed that all treated animals (males and females) were exposed to Methylthioninium chloride Cosmo 25 mg tablets throughout the study on D1 and D25. 72 hours after the last dosing, methylene blue was not quantifiable whatever the dose. Variability in whole blood concentration between animals was high, one or two blood exposure peaks were seen depending on animals and independently from dose levels, gender and dosing days.

Methylthioninium chloride Cosmo 25 mg tablets at 200 mg, 400 mg and 600 mg did not induce any statistically significant changes in mean, systolic and diastolic arterial blood pressure, heart rate, body temperature, cardiac conduction times (i.e., PR, PQ intervals and QRS complex durations), ventricular repolarisation duration (QTc and QTshift), QT interval short term variability or ST segment when compared to the placebo control group.

No test article-related changes in the sympatho-vagal balance were observed. No test article-related disturbances in the Lead II electrocardiogram were noted. All animals that received Methylthioninium chloride Cosmo 25 mg tablets had blue/green faeces during the experiment.

Pharmacodynamic drug interactions

Studies on pharmacodynamic drug interactions have not been conducted.

2.5.3. Pharmacokinetics

The nonclinical pharmacokinetics (PK) of methylthioninium chloride MMX is presented as a compilation of the information available in the public domain on methylthioninium chloride complemented by studies conducted on methylthioninium chloride by the applicant. No dedicated PK studies were conducted for Methylthioninium chloride Cosmo.

Methods of analysis

For the support of non-clinical studies, a Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) bioanalytical method for the quantification of methylene blue in dog K2EDTA whole blood by LC-MS/MS was validated according to generally recommended acceptance criteria for samples stored at ≤ -18ºC. Method validation parameters including response function, selectivity, carryover, precision, accuracy, recovery, matrix effect, bench-top stability at processing temperature, re-injection stability at 10°C, F/T stability, stock solution stability, spike solution stability, long-term stability at ≤ -18ºC and at ≤ -70ºC, dilution integrity and the determination of the maximum batch size were evaluated.

Absorption

No dedicated absorption studies were performed with Methylthioninium chloride Cosmo. A summary of published studies was included in support of the absorption of methylene blue. Methylene blue was absorbed from the small intestinal tract in rats, guinea pigs and rabbits to a similar extent [Watanabe and Fujita 1977, Watanabe and Mori 1977]. Following IV and oral administration to the dog, it was determined that approximately 60 to 70% of the oral dose was absorbed [Disanto 1972, Disanto 1972, Watanabe and Fujita 1977].

Pharmacokinetic profiles are relatively complex due to the binding of methylthioninium chloride and leuko-methylene blue with tissues and the difficulty of analysis [Disanto 1972, Disanto 1972, Watanabe and Fujita 1977, Watanabe and Mori 1977, Yang 2011]. In animals, such as the sheep, half-life is relatively short but the apparent volume of distribution is very high, likely due to the extensive tissue distribution and binding [Burrows 1984].

Sponsor-led studies were performed to assess the involvement of P-glycoprotein in absorption. Toxicokinetic evaluation was also performed in a 28-day repeat-dose study with Methylthioninium chloride Cosmo 25 mg tablets in beagle dogs (Study No. 20160066TCPB). In brief, male and female beagle dogs were administered a total daily dose of placebo, 200 mg, 400 mg and 600 mg Methylthioninium chloride Cosmo orally every 4 days over a 28-day period. Whole blood was collected from the cephalic vein pre-dose and 2, 6, 8, 12, 16, 24, 48 and 72 hours post-dosing on Days 1 and 25. A pre-dose blood sample was also collected on Day 13 of dosing. Whole blood concentration results confirmed that all treated animals (males and females) were exposed to methylthioninium chloride throughout the study on D1 and D25. 72 hours after the last dosing, methylthioninium chloride was not quantifiable at all dose levels. Variability in whole blood concentration between animals was high, and one or two blood exposure peaks were seen depending on animals independently from dose levels, gender and dosing days. Because of delayed blood exposure peaks, half-life (t1/2) could not be calculated in most animals independently from dose levels and dosing days. Despite heterogeneity of blood exposure peaks, Cmax and AUClast were dose dependent. Due to heterogeneity of blood

exposure, linearity of Cmax and AUClast values according to dose levels was not assessed. Observational absorption date is presented in Table 2.

Table 2. Minimal and maximal values of exposure following the day of treatment with Methylthioninium chloride Cosmo 25 mg tablets

Treatment	Day	Cmax(obs) (ng/mL)	AUClast (ng*hr/ml)
Methylene blue 200 mg	D1	43.8-231	151.2-1972.8
	D25	16.6-69.7	33.2-366.4
Methylene blue 400mg	D1	114-432	456-4697.2
	D25	23.4-120	139.6-1355.7
Methylene blue 600 mg	D1	135-948	930.9-8045.6
	D25	44-493	364.1-8668

Distribution

No dedicated distribution studies were performed with Methylthioninium chloride Cosmo. A summary of published studies was included in support of the absorption of methylthioninium chloride. In animals, such as the sheep, the half-life of methylthioninium chloride is relatively short but the apparent volume of distribution is very high, likely due to the extensive tissue distribution and binding [Burrows 1984].

There have been high concentrations of methylthioninium chloride measured in liver, kidney, lung, and heart after IV administration in rats but the full distribution of methylthioninium chloride and the rate of elimination from all tissues are not known [Disanto 1972]. A GLP-compliant plasma protein binding study with methylthioninium chloride was conducted in rats, dogs and humans (Study No. CPS/04). The test article was soluble in the buffer used at nominal concentrations of up to 12 µg/mL. Preliminary system suitability tests were performed with human plasma and protein-free plasma spiked with methylthioninium chloride at a nominal concentration of ca. 12 µg/mL. The techniques of equilibrium dialysis, ultrafiltration and ultracentrifugation were evaluated for their suitability to determine the plasma protein binding of methylthioninium chloride. The stability and non-specific binding of methylthioninium chloride during these experiments were determined. Following review of the suitability experiments and in conjunction with the Sponsor the selected technique for the determination of the plasma protein binding of methylthioninium chloride was ultrafiltration.

The plasma protein binding of methylthioninium chloride in rat, dog and human plasma was then determined through ultrafiltration, at the following nominal concentrations: 12, 2.4, 1.2, 0.24, 0.12 µg/mL. Spiked plasma was allowed to equilibrate for approximately 10 minutes in a heated incubator at 37°C, then was loaded into duplicate ultrafiltration devices and centrifuged at 1,525 x g for 20 minutes at 37°C. The concentration of test compound in the spiked plasma and ultrafiltrate was then determined by LC-MS/MS analysis. All residual samples were stored at ca. -20°C.

Following ultrafiltration, the plasma protein binding of methylthioninium chloride in rat plasma ranged from 79.8% - 88.5%, in dog plasma it ranged from 77.3% - 88.1%, and in human plasma it ranged from 62.9% - 86.3% over the concentration range studied. In each species, the plasma protein binding was approximately concentration-dependent.

In a more recent study (Pohler 2004), the adsorption, distribution and excretion of 14C-labeled methylthioninium chloride following oral and 24h infusion, respectively, were investigated in rats. A nominal dose level of 20 mg/kg body weight of methylthioninium chloride was administered by gavage or by 24h infusion. In addition, the profile of the metabolite Azure B was also investigated after IV

infusion in blood and tissues. The observation time was 96 hours. The study demonstrated that the radioactivity is completed excreted within 96 hours from dosing, less than 1% is remaining after 96 hours, bioavailability was approximately 50% and no accumulation of methylthioninium chloride was seen. Although during the study the radioactivity content in the intestinal mucosa was not assessed, it is assumed that the concentrations in the intestinal mucosa would have been larger after oral than after iv administrations. As the tissue content after 96 hours from dosing is very limited, it is reasonable to assume that no accumulation of methylthioninium chloride and its metabolites occurred after 96 hours from administration of methylthioninium chloride at 20 mg/kg in the rat. 14C-methylthioninium chloride has a very short initial half-life of few minutes, but a longer terminal half-life of approximately from 13 to 18 hours. The PK profile was comparable after os and IV administrations. Similar PK profiles of Azure B and methylthioninium chloride were observed. Slightly longer terminal phase was estimated for Azure B. Kidney and liver are the organs with the major concentration of radioactivity.

Metabolism

Once absorbed there is conversion of methylthioninium chloride to leucomethylene blue (LMB) such that the majority of material available systemically is LMB: a NADPH-dependent enzyme reaction is involved in this biotransformation [Beutler 2005]. The interconversion of methylthioninium chloride to leuko-methylene blue occurs consistently in a range of species studied, including rat, rabbit, guinea pig, dog and man [Disanto 1972, Disanto 1972, Watanabe and Fujita 1977, Watanabe and Mori 1977, Yang 2011]. In rats and mice, there is also an indication that other metabolites are formed by N-demethylation of methylthioninium chloride (Azure A and B) [Yang 2011]. These metabolites are also seen in urine of humans treated with methylthioninium chloride. The information available therefore indicates that metabolism in the typical pre-clinical species employed, including the dog, are reflective of metabolism in man.

The applicant has conducted studies to examine the ability of methylthioninium chloride to inhibit and induce human cytochromes P450. No interspecies comparison studies were conducted.

Enzyme inhibition

CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4/5 Inhibition Experiments

A GLP-compliant study (Study No. CPS/06) was conducted in human liver microsomes to determine the inhibition profile of methylthioninium chloride towards human cytochrome P450 isozymes 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4/5. Methylene blue (at nominal concentrations of 0, 0.05, 0.5, 5, 50 and 500 µM) was incubated with pooled human liver microsomes, β-NADPH, phosphate buffer and CYP-selective chemical substrates for 10 minutes. The experiments were conducted with and without 30 minutes pre-incubation (with and without cofactor) prior to the addition of selective substrate, to assess potential time-dependent inhibition and potential contribution of mechanism-based inhibition.

Methylthioninium chloride was found to inhibit all the CYP enzymes investigated (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4/5) at the concentrations investigated in this study (nominal concentrations of 0.05 – 500 µM). CYP3A4/5 with midazolam as substrate was affected to the greatest degree, with >99.0% inhibition. CYP1A2 was inhibited the least (73.1% to >92.6% inhibition). TDI potential is indicated if there is a >2-fold shift in IC50 following a 30-minute pre-incubation period. Therefore, with an IC50 shift of 2.63-fold and 2.18-fold for CYP2B6 and CYP2C9, respectively, it is likely that the inhibition by methylthioninium chloride of these enzymes was time-dependent.

The IC50 shifts of 1.16-fold (CYP2C8), 1.38-fold (CYP2D6), 1.35-fold (CYP3A4/5 with testosterone as substrate) and 1.18-fold (CYP3A4/5 with midazolam as substrate) indicate that it is possible that the

inhibition by methylthioninium chloride of these enzymes was time-dependent, in part at least. Due to the shift of <2-fold in the IC50 following a 30-minute pre-incubation period, it cannot be concluded if time-dependent inhibition occurred in these assays, due to the potent reversible inhibition being observed. Time-dependent inhibition was unlikely to have contributed to the inhibition of CYP1A2 and CYP2C19, with IC50 shifts of 0.700-fold and 0.349-fold respectively.

CYP1A2, 2B6 and 3A4 Induction Experiment

A GLP-compliant study (Study No. CPS-07) was conducted in cultured human hepatocytes to assess the potential of methylthioninium chloride to induce CYP1A2, 2B6 and 3A4, the major inducible CYP450 enzymes in drug metabolism. Prior to conducting the induction experiments, the cytotoxic (hepatotoxic) effect of methylthioninium chloride on cultured cryopreserved human hepatocytes from one donor was assessed by visual microscopic inspection and a cell proliferation assay to define the maximum concentration of methylthioninium chloride that could be evaluated for CYP induction in this study. No cytotoxic effects on the human hepatocytes were observed with up to nominal concentrations of 40 µM methylthioninium chloride compared to the relevant solvent control after 72 hours incubation. At a nominal concentration of 80 µM cell viability was reduced to 72.5%, indicating possible cytotoxicity at this concentration. Based upon these data it was decided to perform the assessment of methylthioninium chloride -mediated CYP induction with nominal concentrations of methylthioninium chloride

Following this, cryopreserved human hepatocytes from three individual donors were cultured and exposed to multiple doses of methylthioninium chloride for 72 hours at nominal concentrations to assess the effects on CYP1A2, 2B6 and 3A4 mRNA expression. Following exposure, RNA was extracted from the cells and analyzed for levels of mRNA of the target genes using validated RT-qPCR methods. In addition to methylthioninium chloride, cultured hepatocytes from the same three donors were exposed to the prototypical inducing compounds omeprazole (CYP1A2), phenobarbital (CYP2B6) and rifampicin (CYP3A4), as well as to the non-inducing agent flumazenil.

The maximum methylene blue-mediated inductive responses (fold change in mRNA) for CYP1A2, 2B6 and 3A4, compared to solvent only control values, are summarized below:

CYP	**Maximum Mean Fold Change in mRNA (methylene blue concentration)**		
	Donor 1	**Donor 2**	**Donor 3**
CYP1A2	6.51 (40 µM)	7.60 (40 µM)	24.1 (24 µM)
CYP2B6	3.43 (0.4 µM)	3.50 (0.4 µM)	26.9 (24 µM)
CYP3A4	5.27 (0.4 µM)	2.31 (0.4 µM)	3.17 (24 µM)

For donors 1, 2 and 3, the values for omeprazole (AhR)-mediated CYP1A2 induction, phenobarbital (CAR)-mediated CYP2B6 induction and rifampicin (PXR)-mediated CYP3A4 induction were above the minimum values accepted by current industry best practice, at the concentrations dosed. The expected induction results were obtained with these compounds and therefore the data obtained in this study (and conclusions drawn from it) are considered valid.

The results from this study demonstrate that the test material methylthioninium chloride at nominal concentrations of 0.8 µM and above is a potential inducer of CYP1A2 and CYP2B6 in human hepatocytes following 3 days (72 hours) exposure. Methylthioninium chloride is not an inducer of CYP3A4 at nominal concentrations up to 40 µM.

Excretion

Excretion is via urine and faeces; the rates and routes appear to vary between species [Disanto 1972, Watanabe and Fujita 1977, Watanabe and Mori 1977].

No specific studies on excretion of methylthioninium chloride following oral administration of Methylthioninium chloride Cosmo 25 mg tablets have been performed.

Pharmacokinetic drug interactions

P-glycoprotein (P-gp), OAT1 and OAT3 Interaction experiments

A GLP-compliant study (Study No. CPS-05) was conducted in Caco-2 cells and MDCKII cells to investigate the interactions of methylthioninium chloride with the ABC transporter P-glycoprotein (P-gp; MDR1) and the renal SLC transporters OAT1 and OAT3.

The methylene blue concentrations for the interactions experiment were selected on the basis of the solubility of the compound in the buffer used and was determined before initiation of the experiments. Following the solubility assessment, the following methylene blue concentrations were chosen for P-gp and OAT1 and OAT3 interaction assessments: 0, 0.565, 5.65, 16.8 and 56.5 µM. Methylene blue was permeable across the Caco-2 cell monolayer in both the A – B and B – A, directions. However, P_{app} values were higher in the B – A direction, suggesting an efflux transporter-mediated movement of methylene blue. Methylene blue may be a low absorption compound in humans via P-gp transport, as the P_{app} values in the A – B direction (simulating movement from the lumen of the GI tract to the systemic circulation) after 120 minutes were generally less than 2 x 10-6cm.sec^{-1}.

P-gp inhibition

Following P-gp inhibition experiment, a reduction in the efflux ratios for digoxin was seen throughout the incubation period, when compared to wells incubated with digoxin alone. This trend indicated that methylthioninium chloride has the potential to be a weak inhibitor of P-gp, with an estimated IC50 of 49.7 µM.

The efflux ratio of methylthioninium chloride when incubated alone with MDCKII cells was ≥2 at concentrations of 16.8 µM and 56.5 µM at each timepoint, indicating methylthioninium chloride was a substrate of P-gp. Based on the A – B permeability parameter (Papp), and the involvement of P-gp, absorption of methylthioninium chloride via P-gp may be limited in humans.

OAT1 inhibition and substrate experiments

Methylthioninium chloride was found to unlikely be either an inhibitor or a substrate of OAT1; there was a <5% inhibition of the uptake of the OAT1 substrate p-aminohippuric acid, and a <20% inhibition of methylthioninium chloride uptake into transfected cells by the OAT1 inhibitor probenecid.

OAT3 inhibition and substrate experiments

Methylthioninium chloride was found to unlikely be an inhibitor of OAT3, since there was approximately 30% inhibition of the uptake of the OAT3 substrate estrone-3-sulphate (IC50 was >56.5 µM). Methylthioninium chloride was found to be a possible substrate of the OAT3, since the uptake into transfected cells was inhibited 30-40% by the OAT3 inhibitor probenecid.

2.5.4. Toxicology

Single dose toxicity

Single-dose studies were not conducted in any nonclinical species. The LD50 and acute toxicity for various routes of administration in various nonclinical species has been provided based on the literature. The LD50 for oral administration was 3500 mg/kg (mice) 1180 mg/kg (rat), 1000 mg/kg (rabbit) and 500 mg/kg (dog). These doses are considerably higher than the maximum clinical dose (200 mg per patient or 3.3 mg/kg based on a 60kg human). Acute toxicity findings included hemoconcentration, hypothermia, acidosis, hypercapnia, hypoxia, increases in blood pressure, changes in respiratory frequency and amplitude, corneal injury, conjunctival damage, and Heinz body formation. These effects are unlikely to be observed at the anticipated clinical exposure with Methylthioninium chloride Cosmo.

Repeat dose toxicity

The National Toxicology Program in the U.S.A has conducted six in vivo studies on methylene blue. Three (3) studies were conducted in F344/N rats and the other 3 in B6C3F1 mice. In each species, a one-month toxicity, a three-month toxicity [NTP 1990, National Toxicology Program 2008, Auerbach 2010] and a two-year carcinogenicity study [National Toxicology Program 2008] were performed subsequently. In the NTP, doses ≥ 500 mg/kg/day were associated with lethality in both species over 1 month of dosing. Methemoglobinemia, regenerative Heinz body anaemia, reduced body weight and organ weight, bone marrow hyperplasia and liver lesions were some of the effects noted in methylene blue-treated animals. In a 3-month study at doses up to 200 mg/kg/day were administered to both mice and rats. Similar effects were observed including methemoglobinemia, regenerative Heinz body anaemia, reduced body weight and organ weight at all dose levels. NOAELs were not identified in either study.

The applicant also conducted a GLP-compliant 28-day repeat dose oral dose toxicity study on with Methylthioninium chloride Cosmo tablets in Beagle dogs, the findings of which are detailed in the following table.

Table: Sponsor-led repeat dose toxicity study with Methylthioninium Chloride Cosmo 25 mg tablets.

Study	Species/ Sex/ Number / Group	Duration	Dose/Route (mg/day)	Major findings
20160066TCPB	Beagle dog Main study: n=3/sex/ dose Recovery study: n=2/sex/ 0 and HD groups	28 days and 14 day recovery	Oral Every 4 days 0, 200 (LD), 400 (MD), 600 (HD)	*Survival:* No mortality occurred in this study. *Clinical observations/pathology:* No clinical signs were noted, except for coloured faeces in animals treated with Methylene Blue MMX (predicted response due to biliary excretion of methylene blue). Liquid faeces observed in placebo and test article groups, persisted in recovery phase. No changes in body temperatures, body weight gain, food consumption during the study. No ophthalmological abnormalities were noted at any stage. No test article-related changes in 6-lead electrocardiograms or in systolic blood pressure on the last week of dosing or at the end of the recovery period. No test article-related changes were noted in hematology or coagulation

				parameters. No change in urinary parameters was noted. Higher total and direct bilirubin concentration in MD and HD animals on D8 and a higher urea concentration in animals treated with Methylene blue MMX 25-mg tablets at HD on D8 when compared to the placebo group and pre-dose values. No test-article changes in organ weights. HD animals (4/6) displayed points and dark areas on spleens. Points in the caecum, rectum, foam in stomach or red duodenum observed in control and treated groups. No histopathological changes observed. **NOAEL: 600 mg**

Toxicokinetics

Toxicokinetic evaluation was performed at D1 and D25. Whole blood concentration results confirmed that all treated animals (males and females) were exposed to methylthioninium chloride throughout the study on D1 and D25. 72 hours after the last dosing, methylthioninium chloride was not quantifiable whatever the dose. Variability in whole blood concentration between animals was high, one or two blood exposure peaks were seen depending on animals independently from dose levels, gender and dosing days. Because of delayed blood exposure peaks, half-life (t1/2) could not be calculated in most animals independently from dose levels and dosing days. Despite heterogeneity of blood exposure peaks, Cmax and AUC_{last} appeared dose dependent. Due to heterogeneity of blood exposure, linearity of Cmax and AUC_{last} values according to dose levels was not assessed. At the NOAEL of 600 mg (every 4 days), AUC_{last} ranged between 364-8668 ng/ml.hr in males, and 1977-3910 ng/ml.hr in females at D25. Exposure margins at the NOAEL were calculated based on clinical exposure in PK study CB-17-01/02 (AUC_{0-t} 25.16 ± 7.42 µg/ml.hr) in the below table.

Table: Exposure margins with Methylthioninium chloride Cosmo 25 mg tablets.

Methylthioninium Chloride MMX (mg)	**Day**	**Cmax (obs) (ng/mL)**	**AUClast (ng*hr/mL)**	**Exposure margins**
200 mg	D1	43.8-231	151.2-1972.8	5.9×10^{-3} – 0.077
	D25	16.6-69.7	33.2-366.4	5.5×10^{-6} – 0.015
400mg	D1	114-432	456-4697.2	0.018 – 0.18
	D25	23.4-120	139.6-1355.7	5.5×10^{-3} – 0.05
600 mg (NOAEL)	D1	135-948	930.9-8045.6	**0.03 – 0.34**
	D25	44-493	364.1-8668	**0.014 – 0.33**

In the 28-day toxicity study in dogs (20160066TCPB), as part of the examinations at necropsy, the whole gastrointestinal (GI) tract of treated dogs was examined. As Methylthioninium chloride Cosmo 25mg tablets are specifically designed to release the active substance throughout the whole length of the colon, where the highest local concentration is achieved in vivo, the macroscopic and microscopic findings in the GI tract, and particularly of the colon, might be deemed as indicative of any possible local effects due to the product. On Day 29, there were macroscopic points in colon of animals treated with Methylthioninium chloride Cosmo tablets across all the dose levels. On Day 43, these changes

were no longer present. There were other macroscopic changes, mainly in the gastrointestinal tract, such as points in cecum or rectum, foam in the stomach or red duodenum on Days 29 and 43. The incidence was similar in animals treated with Methylthioninium chloride Cosmo tablets or placebo. No significant histologic changes were noted. These results indicated a good local tolerability of the Methylthioninium chloride Cosmo tablets in the GI tract, since no significant signs of toxicity were found after administration of Methylthioninium chloride Cosmo tablets at multiples of the human dose.

Genotoxicity

Genotoxicity studies were not conducted by the applicant. The applicant has included results from a number of published studies evaluating the mutagenic and clastogenic potential of methylene blue. The findings are summarised below.

Table 8a. Overview of bacterial mutagenicity studies with methylene blue and metabolites:

Study reference	**Test system**	**Concentrations/ Concentration range/ Metabolising system**	**Results Positive/negative/equivocal**
NTP (2008)	S. typhimurium **TA100, TA98**	1 to 200 µg/plate +/- S9 (rat or hamster liver)	Positive signal in TA98 for methylene blue
NTP (1990, 2008)	S. typhimurium **TA100, TA98** E.coli **WP2**	0.25 to 150 µg/plate +/- S9 (rat liver) 0.25 to 1,500 µg/plate	Positive signal in TA100 and TA98, and E.coli strain WP2 for methylene blue, Azure A, B and C
Chung (1981)	S. typhimurium TA98	5- 1000 ug +/- S9 (rat liver)	Positive signal with methylene blue
Yamaguchi (1981)	S. typhimurium 3 strains, including TA1530 and 4 derivative mutant strains	not available	Methylene blue is mutagenic in a variety of Salmonella typhimurium tester strains, ± EDTA-Fe or S9 activation.
Kubinsky (1981)	E.coli Strain not specified	70-700µM +/- S9 (rat liver)	Positive signal for methylene blue

Table 8b. Overview of chromosomal aberration studies with methylene blue.

Study reference	**Test system**	**Concentrations/ Concentration range/ Metabolising system**	**Results Positive/negative/equivocal**
Gene mutations in mammalian cells NTP (2008) *in vitro*	CHO-cells	0.17- 5 µg/ml +/- S9	Positive signal. Sister chromatid exchanges (SCE) induced by methylene blue
Gene mutations in mammalian cells NTP (1991) *in vitro*	CHO-cells	Trial 1: 0.5 -5 µg/ml Trial 2: 0.63 to 25 µg/ml (+ S9) Trial 1: 0.17-1.7 µg/ml Trial 2: 0.63 to 25 (- S9)	Without S9 activation methylene blue (trihydrate) was weakly positive 0.17 to 1.7 µg/mL) or positive (0.63 to 25 µg/mL) for SCE. With S9 activation, methylene blue was weakly positive (0.5 to 5 µg/mL) or positive (0.63 to 2.5 µg/mL)
Wagner (1995) Mouse lymphoma assay *in vitro*	Mouse lymphoma cells	10-50 µg/ml (-S9) 2.5 -15 µg/ml (+S9)	Positive signal at 10 µg/mL with S9 activation and 30 µg /mL without metabolic activation increased thymidine kinase mutation frequency by 3.9-fold in mouse lymphoma cells.
Wagner (1995) Mouse micronucleus assay *in vivo*	Mouse micronucleus assay	15.5- 62 mg/kg +/- S9	No increase in the frequency of micronucleated erythrocytes was observed in bone marrow of mice given 62 mg/kg of methylene blue via intravenous administration.
Speit (1982) *in vivo*	Chinese hamsters	1- 12 mg/kg (ip)	No increase in SCE in bone marrow cells of adult hamsters following methylene blue doses (1-12 mg/kg).
NTP (2008) Mouse micronucleus assay *in vivo*	Mouse bone marrow and peripheral blood erythrocytes	25-150 mg/kg (single, i.p) 25-200 mg/kg (3 mo, gavage)	No increase in the frequency of micronucleated erythrocytes was observed in bone marrow or blood of male mice sampled 48 hours after a single i.p. injection of 25- 150 mg/kg methylene blue. No increases in micronucleated erythrocytes were observed in peripheral blood samples taken from male and female mice at the end of the 3-month toxicity study
CB-17-01/08 Clinical study to assess dsDNA damage after exposure of stained mucosa to white light during colonoscopy	γH2AX analysis of colon biopsy	Methylene blue MMX tablets	Exposure of the colonic mucosa to methylene blue and white light during standard colonoscopy did not show any evidence of DNA damage as evaluated by detection of γH2AX.

Table 8c. Other genotoxicity studies.

Study reference	Test system	Results Positive/negative/equivocal
Li (2000) Binding to DNA	Spectroscopic analysis of methylene binding to DNA	Methylene blue binds to dsDNA.
Hagmar (1992) Binding to chromatin	Spectroscopic analysis of methylene binding to chromatin	With increasing salt concentration methylene blue is displaced from chromatin to a higher extent than from DNA.
Villanueva (1993) DNA-protein cross-linking	Spectroscopic analysis of DNA-protein cross-linking by methylene blue	Methylene blue induces light dose-dependent increases in DNA-protein crosslinks (calf thymus DNA, calf thymus histone Type II) that is attributable to production of singlet oxygen

Carcinogenicity

No dedicated carcinogenicity studies were conducted by the applicant. A 2-year study from the NTP (2008) was included in support of the application and summarised below.

Table: Overview of the NTP 2-year carcinogenicity study

Study ID /GLP	Species/No. of animals	Dose/Route mg/kg/day	Major findings
NTP 2008 2 year study GLP-compliant	F344/N rats B6C3F1 mice Main study: n=50/species/sex/gr/ n=10/sex/gr rats for hematology at 2wk, 3, 12 and 18 mo n=30/sex/gr mice for hematology at 2wk, 3, 12 and 18 mo	0, 5, 25, 50 (rats) 0, 2.5, 12.5, 25 (mice) daily admin 5 days per week for 14 weeks oral gavage	<u>Rat</u> *Survival*: Survival of all dosed groups of rats was similar to that of the vehicle. controls. *Pathology:* Mean BW of 25 and 50 mg/kg male rats were less than those of the vehicle controls after weeks 29 and 21, respectively. In the 25 and 50 mg/kg females, mean body weights were less after weeks 73 and 53, respectively. Dosed male and female rats developed methemoglobinemia, and females developed a regenerative Heinz body anemia. The incidences of pancreatic islet cell adenoma and adenoma or carcinoma (combined) were increased in all dosed groups of males, were significantly increased in 25 mg/kg males, and exceeded the historical range in controls (all routes). The incidence of pancreatic islet cell hyperplasia was significantly increased in the 50 mg/kg males. In the spleen, the incidence of hematopoietic cell proliferation in 50 mg/kg males was significantly increased; the incidences of capsular fibrosis were significantly increased in all dosed groups of males and in 5 and 50 mg/kg females. <u>Mice</u> *Survival*: Survival of dosed male and female groups exceeded that of the vehicle controls in a generally dose-related manner. *Pathology:* Mean body weights of dosed female mice began to increase after weeks 29, 61, and 85, reaching final values that were 113%, 111%, and 106% of vehicle controls for the 2.5, 12.5, and 25 mg/kg

			groups, respectively. Dosed mice developed methemoglobinaemia and a regenerative Heinz body anaemia. The incidences of carcinoma and of adenoma or carcinoma (combined) of the small intestine occurred with a positive trend in males. The incidences of malignant lymphoma occurred with a positive trend in females, and the incidence in 25 mg/kg males exceeded the historical control range. The incidences of hematopoietic cell proliferation of the spleen were significantly increased in 12.5 and 25 mg/kg males and in 25 mg/kg females. The incidences of inflammation of the nose were significantly increased in 12.5 and 25 mg/kg females.

Reproduction toxicity

No dedicated reproductive and developmental toxicity studies have been conducted by the applicant. The findings from published studies were included in support of this application.

In vitro, methylthioninium chloride has been shown to reduce motility of human sperm in a dose dependent manner [Coddington 1989]. It has also been shown to inhibit the growth of cultured two-cell mouse embryos [Coddington 1989]. In vivo, there were no consistent effects of methylthioninium chloride trihydrate administration on reproductive system measures in male or female rats after 3 months of oral administration of methylthioninium chloride up to 200 mg/kg, however, male mice had decreased sperm motility and increased epididymal sperm counts at 200 mg/ kg. No significant difference in oestrus cycle in rats or mice was identified in any group [National Toxicology Program 2008].

In rats and rabbits, teratogenic effects have been reported, with fetal and maternal toxicity [National Toxicology Program 1993, National Toxicology Program 1994]. In rats, methylthioninium chloride has been shown to increase resorption rates [Telford 1962]. Methylthioninium chloride also causes preterm delivery and fetal growth restriction in 45%, 50% and 83% of animals after subcutaneous injection at doses of 50, 60 or 85 mg/kg [Tiboni, Giampietro, 2001]. Transplacental exposure of mice to up to 70 mg/kg of methylthioninium chloride also caused a dose-dependent increase in embryolethality and in axial skeleton and neural tube defects [Tiboni and Lamonaca 2001]. In studies conducted by the National Toxicology Program, in rats the NOAEL for developmental toxicity was 125 mg/kg/d; in rabbits, the maternal NOAEL was 50 mg/kg/d [National Toxicology Program 1993, National Toxicology Program 1994]. Based upon these data, Methylthioninium chloride Cosmo tablets should not be used during pregnancy.

2.5.5. Ecotoxicity/environmental risk assessment

Screening for Persistence, Bioaccumulation and Toxicity

A review of available information has revealed that logPow (log partition coefficient (water and 1-octanol)) for methylthioninium chloride has been reported as -0.9 [Proveblue EPAR] and -0.1 [Wainwright 1997]. These values are well below the action limit of 4.5, and therefore no Persistence, Bioaccumulation and Toxicity screening is necessary.

Concentration in surface water

The following assumptions were made in the calculation of $PEC_{surface\ water}$:

- DOSEai

- The maximum daily dose consumed per inhabitant was 200 mg, in line with the MRHD in the proposed SmPC.

- Fpen
    - In the case of Methylthioninium chloride Cosmo, the expected population is the number of patients who undergo colorectal screening in the EU. Data published by Eurostat estimates that there are over 4.8 million colonoscopies conducted in the EU annually [Eurostat 2014]. This revised estimate of the number of colorectal endoscopy procedures conducted annually in Europe is a more accurate estimate of market penetration for Methylthioninium chloride Cosmo and has been utilised for the calculations in this ERA. It is assumed that every patient is subjected to only one colonoscopy, so that the total number of colonoscopies is the total number of patients exposed in a given year. In line with the EMA guidance, Q&A, a market share of 100% (i.e. every patient undergoing colonoscopy receives Methylthioninium chloride Cosmo) has also been assumed.

- WasteWinhab
    - CHMP default value of 200 litres/day ($0.2m^3$)

- Dilution
    - CHMP default value of 10

The total dose per inhabitant per day [DOSEai x Fpen] can therefore be calculated based on the number of colorectal endoscopy procedures conducted annually in Europe and the total EU population, also published in the 2014 EuroStat report.

Dose per patient per procedure	200 mg
Number of procedures in Europe	4866574 / annum [Eurostat 2014]
Percentage market penetration/use (Fpen = 1)	100% of all colorectal endoscopy procedures
Total population of EU	512 500 607 [Eurostat 2014]
Total dose per annum (Total dose x no of colonoscopies in EU)	973 314 800 mg
Total dose per inhabitant per annum (Total dose per annum/ total EU population)	1.899 mg
Total dose (mg) per inhabitant (inh) per day (d) (DOSEai x Fpen)	$0.005 mg.inh^{-1}.d^{-1}$

Calculation
The Predicted Environmental Concentration is estimated by:

$$PEC_{SURFACE\ WATER} = \frac{DOSEai \times Fpen}{WASTEWinhab \times DILUTION}$$

$$PEC_{SURFACE\ WATER} = \frac{0.005 mg.inh^{-1}.d^{-1}}{200\ (L.\ d^{-1}) \times 10}$$

$PEC_{SURFACE\ WATER}$ = 0.0000025 mg/L

$PEC_{SURFACE\ WATER}$ = 0.0025 µg/L

The calculated $PEC_{SURFACE\ WATER}$ is less than the threshold of concern, 0.01 µg/L day specified in the CHMP guideline (EMEA/CHMP/SWP/4447/00 corr 2).

Summary of main study results

Substance (INN/Invented Name): Methylthioninium chloride Cosmo			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107	-0.9 [Proveblue EPAR] -0.1 [Wainwright 1997]	Potential PBT (Y/N): N
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
$PEC_{surfacewater}$, default or refined (e.g. prevalence, literature)	0.0025	µg/L	> 0.01 threshold (Y/N): N

2.5.6. Discussion on non-clinical aspects

The data presented in the nonclinical pharmacology section is largely in line with relevant guidelines including ICH M3 R2, ICH S7A and "Guideline on the non-clinical documentation for mixed marketing authorisation applications", (CPMP/SWP/799/95). The pharmacology package consists of bibliographic reference to the primary pharmacodynamic properties of the active substance methylthioninium chloride and a mixed safety pharmacology package of reference to published studies and sponsored studies. The primary and secondary pharmacodynamic (PD) properties of methylthioninium chloride are well-characterised clinically and it is agreed that nonclinical studies would not be of added value given that the specificity of staining of specialised columnar epithelium in the colon has also been established clinically. The multi-matrix (MMX) formulation of Methylthioninium chloride Cosmo acts as a passive carrier of methylthioninium chloride to the colon lumen and mucosa and does not alter the known pharmacodynamics of methylthioninium chloride.

Respiratory changes due to reduced haemoglobin (a known effect of methylthioninium chloride) has been identified at high doses (⩾ 1000 mg/kg) in a National Toxicology Program (NTP) study, however respiratory changes or methemoglobinemia was not observed in a 28-day oral toxicity study in dogs and are unlikely to be observed at the anticipated clinical dose of 200 mg with Methylthioninium chloride Cosmo. Published studies identified neuronal death in cerebellar slices and neuronal cultures from the subventricular zone. In vivo, no neurological deficits were observed at doses ranging 125-2000 mg/kg/day in rats (NTP study) or in the sponsored 28-day repeat dose toxicity study in dogs. Cardiovascular safety was investigated in a GLP-compliant study with Methylthioninium chloride Cosmo in dogs. There were no test article-related changes in cardiovascular parameters including blood pressure, heart rate, cardiac conduction and ventricular repolarisation duration. No additional safety

pharmacology studies were performed. Given the exposures expected at the clinical dose, the observations from the aforementioned studies are unlikely to be observed in humans and clinical experience supersedes nonclinical data in this regard.

The pharmacokinetic (PK) package presented in support of Methylthioninium chloride Cosmo is largely based on bibliographical data, and supplemented with observational toxicokinetic (TK) data from a 28-day repeat dose study in beagle dogs and a suite of in vitro drug interaction experimentsThe PK of methylthioninium chloride in nonclinical species is dissimilar to the known human PK and thus nonclinical evidence is inherently limited. PK data was obtained from toxicokinetic (TK) data from the 28-day repeat dose dog study with Methylthioninium chloride Cosmo 25 mg tablets. Dose dependent exposure was observed as demonstrated by observational Cmax and AUC values with significant inter-animal variability. T1/2 could not be determined in this study due to delayed exposure peaks, likely due to the MMX formulation. The PK/TK data in the beagle dog study indicates poor absorption following oral administration of Methylthioninium chloride Cosmo and is supported by bibliographic evidence of poor absorption of methythioninium chloride in nonclinical species. The clinical PK profile of Methylthioninium chloride Cosmo (25 mg tablets) is also characterised by poor systemic absorption, however variability is considerably lower and dose proportionality is observed. The potential impact of variability in absorption in nonclinical species in relation to the MMX formulation and appropriate dose levels for clinical use in the proposed indication was raised during the procedure. Although variability in methylene blue absorption could be species dependent, it could also be impacted by other factors including gastric emptying, intestinal motility and gastrointestinal pH. Dose selection for clinical use was determined using standard spray chromoendoscopy. No animal model was used to confirm the release characteristics of Methylthioninium chloride Cosmo formulation, however the impact of systemic exposure with this formulation is low and no further nonclinical studies are warranted.

In rats, IV administration of methylene blue leads to high concentrations in the kidney, lung, liver, heart and brain. No tissue distribution data was provided for other nonclinical species, including dogs. This would usually be insufficient to support the application; however given the limited systemic bioavailability with Methylthioninium chloride Cosmo it is unlikely that there will be significant distribution to sites other than the colon. Distribution studies of methylene blue in mucosal tissues of the intestine after oral use were not included in the original submission. A literature review identified a study that provided indirect (faecal sample measurements) evidence suggesting methylene blue accumulation in the intestinal mucosa is more profound after oral administration in comparison to the intravenous administration. None of the studies in the literature review examined the distribution of methylene blue (or MMX formulation) specifically on the intestinal surface. However, the evidence provided together with the existing data from the long-term medical use of methylthioninium chloride (chromoendoscopy using spray probe) is supportive for the clinical use of methylthioninium chloride formulation administered via the gastrointestinal tract.

Metabolites of methythioninium chloride are anticipated to have greater lipophilicity than the parent compound and may accumulate in tissues (IARC monograph Methylene Blue, 2018). A comparative accumulation of leucomethylene blue (LMB), Azure A and B – the major metabolites of MB, in tissues, possibly related to their increased lipophilicity, was discussed by the applicant. The PK profile of Azure B was similar to that of methylthioninium chloride, when given as IV infusion (14C-radiolabelled), with a slightly longer elimination phase from tissues. Kidney and liver are the organs with the major concentration of radioactivity. Although longer persistence of Azure B in tissues was shown, it however not necessarily resulted in tissue accumulation. Plasma binding activity studies were performed to supplement the distribution package. High protein binding activity was identified in rats, dogs and humans and was approximately concentration-dependent.

Based on the bibliographic studies provided, the metabolism of methylthioninium chloride is not fully understood nonclinically/clinically. The first step involves rapid conversion of methylthioninium chloride

to leucomethylene blue, which is observed in a range of non-clinical species including mice, rats, rabbits and dogs, in addition to humans. Two additional metabolites have been characterised, Azure A and B, and are also present in nonclinical species and humans. Sponsor-led studies were performed to investigate methylthioninium chloride drug interaction and methylthioninium chloride was found to be a strong inhibitor of several CYP450 enzymes (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4/5). The excretion of methylthioninium chloride is primarily via the urine and feces in nonclinical species, comparing to high urinary excretion in humans. Methylthioninium chloride was also identified as an inhibitor of P-gp and limited P-gp-mediated absorption was observed in the intestine. Methylthioninium chloride was also a potential substrate for OAT3. These drug interactions have been appropriately listed in SmPC section 4.5. Methylthioninium chloride has also been identified as a potent inhibitor of renal transporters OCT2, MATE1 and MATE2-K (Methylthioninium chloride Proveblue SmPC Section 4.5 (EMA/CHMP/280202/2018)). This has been included in Section 4.5 of the SmPC.

The use of dogs as the nonclinical species for toxicology studies, and thus the derived TK data, is of limited relevance due to low systemic absorption in dogs. However, the selection is justified on the basis of the technical difficulties with oral dosing of the MMX tablets in smaller animals e.g. rats or rabbits. Additionally, bioavailability is predicted to be low regardless of the nonclinical species used due to the prolonged release formulation. Overall, the PK package, is considered sufficient to support the application for Methylthioninium chloride Cosmo.

Single-dose studies were not conducted in any nonclinical species.The LD50 for various routes of administration in various nonclinical species has been provided based on the literature. The LD50 for oral administration was 3500 mg/kg (mice) 1180 mg/kg (rat), 1000 mg/kg (rabbit) and 500 mg/kg (dog). These doses are considerably higher than the maximum clinical dose (200 mg per patient or 3.3 mg/kg based on a 60kg human). Acute toxicity findings included hemoconcentration, hypothermia, acidosis, hypercapnia, hypoxia, increases in blood pressure, changes in respiratory frequency and amplitude, corneal injury, conjunctival damage, and Heinz body formation. These effects are unlikely to be observed at the anticipated clinical exposure with Methylthioninium chloride Cosmo.

Repeat-dose toxicity was investigated in a 28-day oral study in beagle dogs with Methylthioninium chloride Cosmo 25 mg tablets. Findings from the GLP-compliant studies conducted as part of the National Toxicology Program (NTP) were also included in support of the application and were conducted with oral administration of methylthioninium chloride in mice and rats. In the NTP, doses $\geqslant$ 500 mg/kg/day were associated with lethality in both species over 1 month of dosing. Methemoglobinemia, regenerative Heinz body anaemia, reduced body weight and organ weight, bone marrow hyperplasia and liver lesions were some of the effects noted in methylene blue-treated animals. In a 3-month study at doses up to 200 mg/kg/day were administered to both mice and rats. Similar effects were observed including methemoglobinemia, regenerative Heinz body anaemia, reduced body weight and organ weight at all dose levels. NOAELs were not identified in either study. In the GLP-compliant 28-day oral study in beagle dogs, no toxicities were observed with 200, 400 or 600 mg doses of Methylthioninium chloride Cosmo when administered every 4 days (a total of 7 doses). A NOAEL of 600 mg was identified in this study. Toxicokinetic (TK) evaluation was performed at Day 1 and 25. Absorption of the test article was highly variable with delayed exposure peaks and linearity could not be confirmed. The variability of intra-animal exposure data on Day 1 and 25 confounds an assessment on the potential for accumulation, however this is not a significant concern given the likely frequency of use with the proposed indication.

Due to the relatively limited absorption of Methylthioninium chloride Cosmo in dogs, no safety margins were achieved against the maximum clinical dose (200 mg,) at the NOAEL (600 mg). From the pivotal clinical PK study with Methylthioninium chloride Cosmo 25 mg tablets, a single dose (8 x 25 mg tablets) gave mean exposures Cmax and AUC0-t of 1.15 ± 0.26 µg/ml and 25.16 ± 7.42 µg/ml.hr, respectively. Comparison of exposures in the repeat-dose dog study and clinically demonstrated

negative margins at all dose levels. Despite the absence of safety margins, there is significant clinical experience with methylthioninium chloride and toxicities are well-known. In consideration of the proposed indication for patients undergoing surveillance/screening for colorectal cancer, patient exposure will be infrequent (maximum once-yearly according to the RMP) and the potential for systemic toxicity is likely to be low. In relation to local tolerance, the observation of the liquid faeces in the animals in the control and test groups were of notable incidence and may demonstrate local intolerance of the final drug product itself, unrelated to the test article, implying a possible role of excipient in this side effect (Study 20160066TCPB). It is stated that medicinal product does not include a novel excipient, and all of the excipients used for the medicinal product are commonly used in pharmaceuticals for oral administration, therefore an assessment of the information regarding its safety is not provided. Since the observed side effect persisted also in the recovery period of 14 days after drug withdrawal, it could represent a significant issue. The applicant provided additional information on the study to address this. Liquid faeces were observed in both control and treated animals, with high variability in frequency during the time, including the off-dose period. A confounding factor is represented by collective allocation of animals in boxes, that impeded the individual observation. In addition, collective caging may contribute to spread parasitic infection which are often undetected (e.g. giardiasis) and are often asymptomatic, with the exception of gastrointestinal disturbances. This could explain the persistence of symptoms beyond the treatment period. Based on these considerations, the relevance of this effects to humans is unclear, both because the components of the MMX tablets are well known as GRAS substances, and because the occurrence of liquid faeces during clinical trials was never recorded. The applicant's justification was considered acceptable.

No nonclinical genotoxicity studies have been conducted with Methylthioninium chloride Cosmo. The applicant has included results from a number of published studies with methylthioninium chloride evaluating the mutagenic and clastogenic potential of methylthioninium chloride. Additionally, the applicant conducted a clinical study to assess the potential for DNA damage in colonic mucosa when white light is applied to the stained area and no evidence was observed in biopsy samples taken after the first and second procedure. Based on the information provided from studies in salmonella typhimurim and E.coli strains, methylene blue is considered to be both mutagenic and clastogenic. Evidence of induced sister chromatid exchanges and chromosomal aberrations in CHO cells indicate clastogenic potential. Positive findings in vitro were not observed in vivo at doses of 62 mg/kg (intravenous), 12 or 150 mg/kg (intraperitoneal) and 200 mg/kg (oral gavage). However, methylene blue is considered genotoxic and this has been noted in Section 5.3 of the SmPC.

The GLP-compliant 2-year oral study in mice and rats conducted as part of the National Toxicology Program suggests methylene blue may demonstrate carcinogenic potential, however methylthioninium chloride it is not of significant concern for this application due to the low systemic exposure, short duration of treatment and infrequency of administration.

A phase I environmental risk assessment (ERA) for Methylthioninium chloride Cosmo was performed. PBT screening was assessed using a referenced log Kow. As methylene blue is an ionisable substance the applicant was requested to provide a log Dow in line with "Guideline on the environmental risk assessment of medicinal products for human use", (EMEA/CHMP/SWP/4447/00 corr 2) and 'Questions and answers on 'Guideline on the environmental risk assessment of medicinal products for human use', (EMA/CHMP/SWP/44609/2010 Rev. 1). The justification for refinement of the FPen to reflect a more accurate estimate of market penetration is appropriate, based on an estimate of the number of colorectal endoscopy procedures conducted annually in the EU. An appropriate statement on disposal has been included in section 6.6 of the SmPC. An experimentally derived n-octanol/water partition coefficient will be provided by the MAH post authorisation.

2.5.7. Conclusion on the non-clinical aspects

Due to the nature of this type of application the nonclinical package is heavily reliant on evidence from published studies with methylthioninium chloride, the active substance of Methylthioninium chloride Cosmo 25mg tablets. The pharmacology of methylthioninium chloride is considered to be well established. The pharmacokinetics of orally-administered methylthioninium chloride is complex and varies across nonclinical species, thus limiting the value of PK studies with Methylthioninium chloride Cosmo as it is a prolonged-release formula with low bioavailability.

Overall, the nonclinical package supports the application for Methylthioninium chloride Cosmo in the proposed indication. It is considered that the toxicology of methylthioninium chloride is well-known and the potential risk with this prolonged-release tablet is low. The toxicology of methylthioninium chloride is well-documented in terms of genotoxicity and carcinogenicity although these are not of significant concern for the proposed indication in this application. Methylthioninium chloride is a known reproductive toxicant and appropriate warnings regarding its use during pregnancy/lactation and in WOCBP have been included in the SmPC.

2.6. Clinical aspects

2.6.1. Introduction

The Methylthioninium chloride Cosmo 25 mg tablet is a gastro-resistant, prolonged release tablet, containing methylthioninium chloride equivalent to 25 milligrams of anhydrous methylthioninium chloride. The indication is as a diagnostic agent enhancing visualisation of colorectal lesions in adult patients undergoing screening or surveillance colonoscopy.

The active substance, methylthioninium chloride (methylene blue) is the same active substance as in the EU reference medicinal product METILÉNKÉK PHARMAMAGIST 1% oldatos injekció approved in Hungary. Medicinal products containing methylthioninium chloride have been approved for many years. Methylthioninium chloride is already approved by the European Medicines Agency (EMA) as methylthioninium chloride Proveblue for use in adults, children and adolescents for the acute symptomatic treatment of medicinal and chemical product induced methaemoglobinaemia (IV formulation).

For Methylthioninium chloride Cosmo, the active substance is the same as in the proposed reference product, as well as other EU approved medicinal products, however the indication, strength, pharmaceutical form and route of administration for Methylthioninium chloride Cosmo are different.

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

- ***Tabular overview of clinical studies***

Type of Study	Study Identifier	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects Treated	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Safety, PK	CB-17-01/01/ CRO-PK-10-236	5.3.3.1	Safety, PK, and bioavailability	Open label, serial cohort, cross-over design	Single oral doses of 200 and 400 mg Methylene Blue MMX. Cross-over following 7 days to 100 mg methylene blue as 10 ml of a 1% solution	22	Healthy volunteers	Single treatment	Complete; Full
Safety, PK	CB-17-01/02/ CRO-PK-11-249	5.3.3.1	PK and colon staining	Open-label, uncontrolled, serial cohort	Methylene Blue MMX 25 mg tablets, single ascending oral doses of 100 and 200 mg	23	Healthy volunteers	Single treatment	Complete; Full
PD	CB-17-01/03/ CRO-11-108	5.3.4.2	Colon staining	Open-label, uncontrolled	Methylene Blue MMX 25 mg tablets, oral doses of 150 or 200 mg	114	Subjects scheduled for colonoscopy	Single treatment	Complete; Full
Efficacy	CB-17-01/06	5.3.5.1	Adenoma detection rate	Randomized, double-blind, placebo-controlled	200 mg Methylene Blue MMX (8 × 25 mg tablets) 100 mg Methylene Blue MMX (4 × 25 mg Methylene Blue MMX tablets and 4 × placebo tablets) Placebo (8 × placebo tablets)	200 mg Methylene Blue MMX (N=488) 100 mg Methylene Blue MMX (N=241) Placebo (N=479)	Subjects scheduled for screening or surveillance colonoscopy	Single treatment	Complete; Full
Efficacy	CB-17-01/05/ CRO-11-110	5.3.5.2	Detection of Adenomas and polyps	Open label, uncontrolled	200 mg Methylene Blue MMX (8 × 25 mg tablets)	96 subjects Full Dose group; 62 subjects Split Dose group	Subjects scheduled for colonoscopy	Single treatment	Complete; Full (Separate reports for Full Dose group and Split Dose group)
Efficacy	CB-17-01/04/ CRO-11-109	5.3.5.4	Intraepithelial neoplasia detection and colon staining	Open label, uncontrolled	200 mg Methylene Blue MMX (8 × 25 mg tablets)	53	Subjects with ulcerative colitis undergoing colonoscopy	Single treatment	Complete; Full
Safety	CB-17-01/08/ CRO-13-113	5.3.5.4	Effects on double stranded DNA	Open label, uncontrolled	200 mg Methylene Blue MMX (8 × 25 mg tablets)	10	Subjects undergoing colonoscopy who require a second colonoscopy for medical reasons	Single treatment	Complete; Full

PD = pharmacodynamic; PK pharmacokinetic

2.6.2. Pharmacokinetics

The active substance, methylthioninium chloride, is well-known from other EU approved products, however the indication, strength, pharmaceutical form and route of administration for Methylthioninium chloride Cosmo 25 mg tablet are different. Single doses of 500 mg have been administered safely to healthy subjects, and for an indication like treatment of methaemoglobinaemia daily IV doses of 2 mg/kg is administered.

Methylthioninium chloride Cosmo is designed to act locally in staining the colonic mucosa as an aid for the enhanced visualization and detection of colorectal lesions, they exert their effect at the site of application. The Methylthioninium chloride Cosmo tablet contains methylthioninium chloride at a concentration of 25 mg per tablet. Methylthioninium chloride Cosmo is self-administered by the patient at a total oral dose of 200 mg (8 × 25 mg tablets), administered in divided doses according to a simple administration schedule coordinated with the bowel cleansing preparation regimen prior to colonoscopy.

Methylthioninium chloride is absorbed and becomes systemically available, and the bioavailability and pharmacokinetics of methylthioninium chloride following administration in MMX tablets, were investigated and analysed descriptively in the two Phase I studies (table 1).

Table 1: Summary of completed clinical pharmacokinetic studies with Methylthioninium chloride Cosmo

Study and Phase	Study Design	Test Formulation/Dose	Reference Comparator	Subjects Receiving Methylthioninium chloride Cosmo
CB-17-01/01 Phase 1	Single ascending doses, open-label, randomised, cross-over safety, bioavailability study in healthy volunteers, to assess the safety and bioavailability of Methylthioninium chloride Cosmo 200 mg tablets, administered as single ascending oral doses, compared to methylthioninium chloride 1% administered as single IV dose of 100 mg	Methylthioninium chloride Cosmo 200 mg prolonged release tablets/ Single oral doses of 200 mg (cohort 1) or 400 mg (cohort 2). After a cross-over wash-out period of 7 days, 100 mg of methylthioninium chloride IV (slow IV injection) as 10 ml of a 1% solution in cohort 1.	Methylthioninium chloride 1% vials 10 mL (each containing 100 mg of methylthioninium chloride)	22
CB-17-01/02 Phase 1	Single ascending dose, open-label, uncontrolled, safety, bioavailability, and colon staining study in healthy male volunteers	Methylthioninium chloride Cosmo 25 mg prolonged release tablets/ Single ascending oral doses of 100 (4 × 25 mg) and 200 mg (8 × 25 mg).	None	23

IV = intravenous; MMX = Multi-Matrix.

In vitro studies were conducted with methylthioninium chloride to determine the plasma protein binding (Study CPS/04). In addition, potential interactions of methylthioninium chloride with cytochrome P450 (CYP) enzymes (Studies CPS/06 and CPS/07) and efflux/uptake transporters were assessed (Study CPS/05), presented in Table 2.

Table 2: *In Vitro* Human Biomaterial Studies

Description	Assay System	Report Number
Distribution Study		
Plasma Protein Binding	Plasma (rat, dog, human)	Study No. CPS/04

Metabolism and Drug Interaction Study		
Inhibition of CYP P450 enzymes	Liver, Microsomes (human)	Study No. CPS/06
Induction of CYP P450 enzymes	Hepatocytes (human)	Study No. CPS/07
P-gp, OAT1 and OAT3 Interactions	P-gp: CacoReady™ and PreadyPort™ OAT1 and OAT3: TransportoCell™	Study No. CPS/05

CYP = cytochrome P450; OAT = organic anion transporter; P-gp = P-glycoprotein.

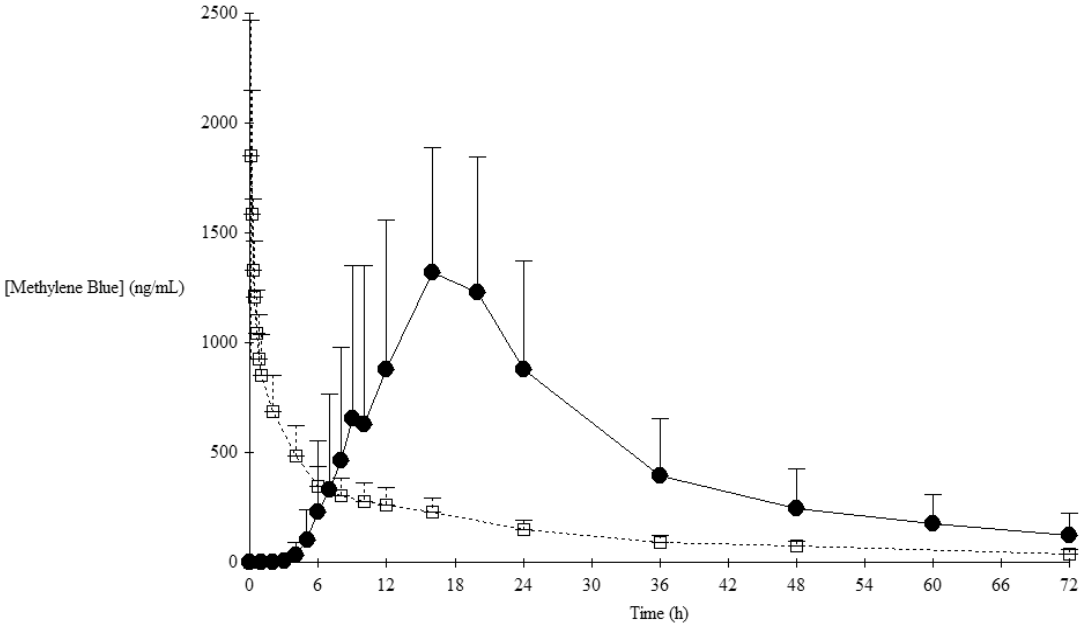
Both PK studies were conducted in healthy subjects, 36 males and 10 postmenopausal females, from 18-65 years of age.

Absorption

The MMX tablet formulation has been developed to allow drug delivery in the colon. Absorption is delayed and the drug is not detectable in blood until after 3 hours for the 200 mg dose and 5 hours for the 400 mg dose.

The mean absolute bioavailability (Fabs) of methylthioninium chloride when administered as a single Methylthioninium chloride Cosmo 200 mg tablet was calculated to be approximately 140%. One mechanism for this unexpected high value could be saturation of efflux transporter mechanisms, causing a bottleneck in the elimination of methylthioninium chloride from the body. High inter-subject variability could also contribute.

Figure 2: Blood methylthioninium chloride concentration versus time profiles after single dose administration of either 200 mg of Methylthioninium chloride Cosmo or 100 mg of Methylene Blue 1% Injectable Solution



To avoid potential unwanted expulsion of the 200 mg monolithic tablet before adequate delivery of dye, a 25 mg tablet was developed to allow dived dosing and decrease the risk that all tablets are expelled before dye is delivered. This formulation was used in study CB-17-01/02.

PK parameters of methylthioninium chloride administered as single oral doses of Methylthioninium chloride Cosmo 100 mg (4 × 25 mg tablets) and 200 mg (8 × 25 mg tablets) concomitantly with the administration of the bowel cleansing preparation are detailed in Table 3. Inter-individual variability in methylthioninium chloride PK is high.

Table 3: Study CB-17-01/02: Plasma methylthioninium chloride Pharmacokinetic Parameters

Treatment	C_{max} (µg/mL)	T_{max} (h)	AUC_{0-t} (µg/mL × h)	$AUC_{0-\infty}$ (µg/mL × h)	$t_{1/2}$ (h)	λz (1/h)
100 mg Methylthioninium chloride Cosmo	0.57 ± 0.18	12.00 (9.00 to 16.00)	10.72 ± 6.68	11.53 ± 6.60	13.87 ± 5.09	0.05 ± 0.02
200 mg Methylthioninium chloride Cosmo	1.15 ± 0.26	16.00 (10.00 to 24.00)	25.16 ± 7.42	28.56 ± 9.76	15.08 ± 5.85	0.05 ± 0.02

Bioequivalence

The commercial formulation contains 25 mg of methylthioninium chloride per tablet, and is the same formulation that was used in Phase 1 Study CB-17-01/02, Phase 2 and Phase 3 studies, and which will be used for commercial manufacturing. The only difference between the clinical batches and the commercial batches is that an additional drug substance supplier has been selected for the commercialisation phase. Thus, no bioequivalence studies of the 25 mg formulation were necessary between Phase 1, 2, or 3 studies.

Distribution

The apparent distribution volume of methylthioninium chloride after single iv injection of 100 mg was between 160 and 580 L. Methylthioninium chloride has a high volume of distribution indicating both extensive distribution to extravascular compartments and high protein binding. With the single administration of 200 mg Methylthioninium chloride Cosmo in conjunction of endoscopy, there is no concern of clinically relevant accumulation.

Protein binding of methylthioninium chloride is low/moderate with a range of 62.9% to 86.3% in human plasma.

Elimination

No ADME study has been conducted.

Elimination $t_{1/2}$ ranged between 14 and 27 hours after the lower dose and between 6 and 26 hours after the higher dose. After single dose of 200 mg of Methylthioninium chloride Cosmo the dye was quantifiable in blood after 3-12 h of the dosing due to the modified release profile of MMX tablets. After single dose of 400 mg of Methylthioninium chloride Cosmo the dye became quantifiable in blood after 5-12 h of the dosing. Systemic exposure to the active principle reached a peak in a median time of 16 h.

Following oral administration in man, methylthioninium chloride is largely (78%) metabolised in the body to leukomethylene blue, which is excreted primarily in the urine along with the unchanged methylthioninium chloride. Urinary excretion is around 40 % of the oral dose. The remaining methylthioninium chloride likely is removed by 1) distribution and binding to tissues, 2) metabolism or 3) biliary excretion, expectedly a combination of all.

Metabolism

Methylthioninium chloride is metabolised by CYPs 1A2, 2C19 and 2D6 in vitro; however, the predominant in vitro pathway appears to be uridine diphosphate-glucuronyltransferase (UGT)-mediated conjugation by multiple UGT enzymes, including UGT1A4 and UGT1A9.

Azure B, which is a minor impurity in methylthioninium chloride, is also formed in humans as a metabolite of methylthioninium chloride. The PK of metabolites has not been investigated, but the clinical impact of methylthioninium chloride metabolites is expected to be minimal given the single application of methylthioninium chloride in relation to an endoscopic procedure.

Dose proportionality and time dependency

In Study CB-17-01/01, peak blood concentration did not increase proportionally with the dose escalation. On average, C_{max} was very similar after both the 200 mg dose (1.66 ± 0.50 µg/mL) and the 400 mg dose (1.64 ± 0.73 µg/mL) of Methylthioninium chloride Cosmo. AUC was, on average, 32.94 µg/mL × h after a 200 mg dose and 38.08 µg/mL × h after a 400 mg dose. This could be caused by partial saturation of the transporter mechanism, which is partly responsible for drug absorption, so that at the dose of 400 mg, part of the dye probably remains in the gastrointestinal tract without being absorbed.

In Study CB-17-01/02, single oral doses of Methylthioninium chloride Cosmo 100 mg (4 × 25 mg tablets) and 200 mg (8 × 25 mg tablets) were administered concomitantly with the administration of the bowel cleansing preparation.

The rate and extent of exposure (Cmax and AUC, respectively) increased proportionally with the dose: Cmax was, on average, 2 fold higher after the 200 mg dose (1.15 ± 0.26 µg/mL) than after the 100 mg dose (0.57 ± 0.18 µg/mL). Also, AUC0-t and AUC0-∞ were on average 2.5 fold higher after 200 mg than after 100 mg.

Table 4: Methylthioninium chloride Cosmo pharmacokinetic parameters in blood (Phase 1 Studies CB-17-01/01 and CB-17-01/02)

Study	Dose (Regimen)	C_{max} (µg/mL)	T_{max} (h)	AUC_{0-t} (h × µg/mL)	$AUC_{0-\infty}$ (h × µg/mL)	$t_{1/2}$ (h)
CB-17-01/01	200 mg Methylene Blue MMX (Single 200 mg tablet)	1.66 ± 0.50	16.00 (9.00 to 20.00)	32.94 ± 14.30	36.82 ± 17.57	20.19 ± 4.68
	400 mg Methylene Blue MMX (2 × 200 mg tablets)	1.64 ± 0.73	16.00 (12.00 to 24.00)	38.08 ± 23.07	42.11 ± 26.22	17.25 ± 7.43
	100 mg Methylene Blue (IV; 10 mL of 1% injectable solution)	2.07 ± 0.57	0.10 (0.10 to 0.20)	11.86 ± 2.80	13.47 ± 3.29	26.71 ± 8.12
CB-17-01/02	100 mg Methylthioninium chloride Cosmo (4 × 25 mg tablets)	0.57 ± 0.18	12.00 (9.00 to 16.00)	10.72 ± 6.68	11.53 ± 6.60	13.87 ± 5.09
	200 mg Methylene Blue MMX (8 × 25 mg tablets)	1.15 ± 0.26	16.00 (10.00 to 24.00)	25.16 ± 7.42	28.56 ± 9.76	15.08 ± 5.85

$AUC_{0-\infty}$ = area under the concentration versus time curve up to infinity; AUC_{0-t} = area under the concentration versus time curve up to the last sampling time; C_{max} = maximum plasma concentration; CSR = clinical study report; h = hours; max = maximum; min = minimum; MMX = Multi-Matrix; SD = standard deviation; $t_{1/2}$ = elimination half-life; T_{max} = time to maximum plasma concentration.
Values are arithmetic means ± SD, except for T_{max} = median (min to max).

In general, dose proportionality was seen between the 100 mg and 200 mg dose. For the 400 mg dose, peak blood concentration did not increase proportionally. The explanation has not been investigated, but saturation of transporter mechanisms is hypothesized.

No studies have been conducted to investigate methylthioninium chloride time dependency. Time dependency is not likely to be clinically relevant for single dose administration.

Pharmacokinetics in target population

The applicant has not conducted studies to assess PK characteristics in the 'patient' population as Methylthioninium chloride Cosmo's effectiveness is not considered to be related to systemic ADME characteristics, given that the product is a locally applied vital dye of the colonic mucosa. Initial tolerability studies were not conducted in the 'patient' population as the active substance is well known.

No PK studies in special population have been conducted. The 46 subjects included in the two PK studies were overall highly homogenous and no Pop PK analyses have been conducted to assess the impact of covariates in PK.

Demographic baseline data of study CB-01/01 and CB-01/02 are presented below:

CB-01/01

Sex		Age	Height	BW
Males	Females	(y)	(cm)	(kg)
12 (54.5%)	10 (45.5%)	46.0±12.4	168.7±11.1	72.1±7.2

CB-01/02

Demographic data	Safety population		PK population		PP population	
	Period 1 N=5	Period 2 N=18	Period 1 N=5	Period 2 N=17	Period 1 N=3	Period 2 N=16
Age (years)						
Mean ± SD	40.6±2.3	33.7±8.4	40.6±2.3	33.2±8.4	40.3±3.1	32.6±8.2
Median (range)	41.0 (37-43)	34.5 (21-47)	41.0 (37-43)	34.0 (21-47)	41.0 (37-43)	34.0 (21-47)
Body weight (kg)						
Mean ± SD	80.5±6.4	79.7±9.4	80.5±6.4	80.5±9.0	79.7±5.5	81.2±8.8
Median (range)	76.8 (74.6-88.7)	79.0 (65.8-97.2)	76.8 (74.6-88.7)	79.7 (68.0-97.2)	76.8 (76.3-86.0)	80.0 (68.0-97.2)
Height (cm)						
Mean ± SD	178.0±5.1	178.4±5.6	178.0±5.1	179.2±4.6	177.0±4.4	179.7±4.3
Median (range)	179 (172-185)	180 (165-188)	179 (172-185)	180 (170-188)	179 (172-180)	180 (170-188)
Race						
White – n (%)	5 (100.0)	18 (100.0)	5 (100.0)	17 (100.0)	3 (100.0)	16 (100.0)

Period 1: 100 mg dose group; period 2: 20 mg dose group

Methylthioninium chloride Cosmo should be used with caution in patients with moderate to severe renal impairment as there are no data in this patient group and methylthioninium chloride is predominantly renally eliminated. Based on clinical trial analysis (see Clinical Safety), no dose adjustment is required in patients with mild or moderate hepatic impairment. There is no experience in patients with severe hepatic impairment. PK data in patients ≥ 75 years of age are limited, but the safety profile is overall similar across age groups. No dose adjustment is required for elderly patients. No studies in children have been conducted. Methylthioninium chloride Cosmo should not be used in children (as reflected in sections 4.2 and 5.2 of the SmPC).

	Age 65-74 (Older subjects number /total number)	Age 75-84 (Older subjects number /total number)	Age 85+ (Older subjects number /total number)
PK Trials (all)	0	0	0

Pharmacokinetic interactions

Serotonin syndrome has been reported with the use of methylthioninium chloride class productsProvepharm SAS 2016). Safety announcements released by the US FDA and the UK Medicines and Healthcare products Regulatory Agency have indicated that most cases of CNS toxicity/serotonin syndrome occurred in the context of IV administration of methylthioninium chloride as a visualising agent in preparation for parathyroid or thyroid surgery (US FDA 2011; UK MHRA 2009). Most reports of serotonin syndrome with IV methylthioninium chloride have been associated with concomitant use of serotonergic drugs, some tricyclic antidepressants and other psychiatric medications, and monoamine oxidase inhibitors Provepharm SAS 2016). Not all serotonergic drugs have equal capacity to cause serotonin syndrome with IV methylthioninium chloride. The cases of serotonin syndrome with IV methylthioninium chloride occurred in patients taking specific serotonergic psychiatric drugs, namely a selective serotonin reuptake inhibitor (SSRI), a serotonin-norepinephrine reuptake inhibitor. For Methylthioninium chloride Cosmo, the clinical impact is expected to be minimal due to the short treatment duration also when co-administered with sedatives.

No clinical DDI studies have been conducted.

Pharmacokinetics using human biomaterials

Potential interactions of methylthioninium chloride cytochrome P450 (CYP) enzymes and efflux/uptake transporters were assessed in vitro.

The inhibitory potential of methylthioninium chloride to inhibit CYP isozymes is substantial. In vitro, methylthioninium chloride directly inhibited human CYP isozymes 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4/5 at therapeutic concentrations. Potential time-dependent inhibition was found for CYP isozymes 2B6 and 2C9, and irreversible mechanism-based inhibition > 70% was found for all CYP isozymes tested. In more details, the inhibition profile of methylthioninium chloride in the concentration range of 0 µM to 500 µM towards human CYP isozymes 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4/5 was assessed in vitro in pooled human liver microsomes in the presence of reduced β-nicotinamide adenine dinucleotide phosphate (β-NADPH), phosphate buffer, and a substrate.

Total time-dependent inhibition (TDI) was determined by pre-incubation of methylthioninium chloride and the CYP isozyme for 30 minutes with cofactor before addition of the substrate. Total TDI by 500 µM methylthioninium chloride ranged from > 92.6% for CYP1A2 (phenacetin used as the substrate) to > 99.3% for CYP3A4/5 (midazolam used as the substrate). The IC50 was in the range of 0.0659 µM (CYP1A2) to 0.835 µM (CYP2B6). A shift in the IC50 of > 2-fold after the 30-minute pre-incubation period indicates TDI potential. This threshold was passed by CYP2B6 (2.63-fold) and CYP2C9 (2.18-fold), showing likely TDI by methylthioninium chloride. In addition, possible TDI by methylthioninium chloride was indicated for CYP2D6 (1.38-fold), CYP3A4/5 (1.35-fold with testosterone as the substrate; 1.18-fold with midazolam as the substrate) and CYP2C8 (1.16-fold), although this cannot be definitively concluded due to the potent reversible inhibition observed. For CYP1A2 (0.700-fold) and CYP2C19 (0.349-fold), TDI by methylthioninium chloride was unlikely.

Irreversible mechanism-based inhibition (MBI) was determined with pre-incubation for 30 minutes without cofactor (Table 3). Irreversible MBI by 500 µM methylthioninium chloride ranged from 73.1% for CYP1A2 (phenacetin used as the substrate) to > 99.0% for CYP3A4/5 (midazolam used as the substrate). The IC50 was in the range of 0.0445 µM (CYP1A2) to 1.08 µM (CYP2D6).

Methylthioninium chloride induces CYP1A2 and CYP2B6 in human hepatocytes at concentrations ≥ 0.8 µM, which are below therapeutic concentrations, as Cmax for methylthioninium chloride is 1.66 µg/mL equivalent to 5.2 µM (methylthioninium chloride relative molecular mass 319.85).

Methylthioninium chloride was in vitro identified to be a substrate both for the renal solute carrier transporters P-gp and OAT3 and potentially acts as a mild inhibitor of P-gp (concentration of drug causing 50% inhibition of enzyme activity = 49.7 µM). Methylthioninium chloride is not a substrate or an inhibitor of OAT1.

The clinical consequences of changes in plasma concentrations of co-administered drugs which are substrates of these metabolic enzymes and transporters are not known but cannot be excluded. However they are considered likely to be minimal due to the single administration.

Methylthioninium chloride has also been identified as a potent inhibitor of renal transporters OCT2, MATE1 and MATE2-K (Methylthioninium chloride Proveblue SmPC Section 4.5 (EMA/CHMP/280202/2018)).

Exposure relevant for safety evaluation

The daily IV dose indicated for the treatment of methaemoglobinaemia with the products available on the market at the time this programme began was up to 2 mg/kg of body weight. Thus, in a man of 60 kg, the maximal daily dose would be 120 mg. In the US, the maximal daily oral dose of methylthioninium chloride indicated for the treatment of bladder infections with Urolene blue® is 195 mg, while for the treatment of infections of the urinary tract with Mictasol bleu®, a maximal daily dose of 180 mg is recommended. In the treatment of ifosfamide induced encephalopathy, daily IV injections of up to 300 mg of methylthioninium chloride are recommended. ProveDye®, indicated as a visualisation aid for surgical procedures, is used at a range of doses (1 mg to 30 mg) depending on the procedure being performed.

2.6.3. Pharmacodynamics

Mechanism of action

Methylthioninium chloride is known to be a "vital dye", meaning "a dye or stain agent capable of penetrating living cells or tissues and not inducing immediate evident degenerative changes". Methylthioninium chloride is taken up across the cell membrane into the cytoplasm of actively absorbing cells such as those found in the small intestine and colon.

The Multi-Matrix (MMX) formulation of methylthioninium chloride was developed as an oral drug product to provide a locally released dye for use during colonoscopy that would maximise the staining contrast in mucosal lesions while minimising variability inherent in operator delivered sprays. The formulation is designed with extended release characteristics using a film coating that dissolves at pH 7 or above, a condition normally achieved in the terminal ileum, so that methylthioninium chloride is progressively released directly into the colon. Once the film coating has dissolved, the MMX formulation determines a slow release of the dye, resulting in its homogeneous and prolonged dispersion on the surface of the colonic mucosa during the tablets' transit through the colon. The maximum local bioavailability of the methylthioninium chloride in the colon is thus achieved and, consequently, the contrast enhancing effect is optimised.

There is no molecular or other interaction between methylene blue and MMX tablets, which may alter the pharmacodynamics (PD) of methylene blue. The MMX acts as a passive carrier of methylene blue to the colon lumen and mucosa, preventing its release in the small bowel. There is no evidence of any structural change in methylene blue due to the gastric transit, and pH and time are responsible for the extended delivery of methylene blue from the tablet to the targeted colorectal mucosa. Thus, the methylene blue that is passively delivered by MMX at the colorectal level is exactly the same molecule with the same PD properties that is used in routine chromoendoscopy.

The pharmacodynamics of methylene blue are well known and it is still widely used for long time in humans for a variety of medical purposes, as both a medication and a dye. No new relevant studies on the mechanism of action of the compound have been conducted by the applicant, the efficacy of staining is evaluated during the clinical studies.

Primary pharmacology

- Colon staining assessments were performed in five clinical studies, CB-17-01/02, CB-17-01/03, CB-17-01/04, CB-17-01/05, and CB-17-01/08. Four regions of interest (ROIs) in the colon were defined (ascending colon, transverse colon, descending colon and rectosigmoid colon)

and each given a staining score based upon the proportion of colon mucosa stained as assessed by the investigator against predefined criteria, using a 0-5 scoring system.

Results were expressed as staining score (SC) for each ROI, Total Staining Score (TSC) i.e., the sum of all SCs and NSA= Number of Stained Areas with SC > 2.

Colonic mucosa staining quality was satisfactory (i.e., TSC ≥ 8 and no over-staining in any ROI) in all subjects evaluated in both dose groups (100 mg and 200 mg), but a better result was achieved in the 200 mg dose group.

Ten different tablet administration schedules for the total amount of 150 mg (schedules A, B, C) or 200 mg (schedules D, E, F, G, H, I, and J) doses were investigated in small groups of subjects during the study, as shown in Table 11.

Table 5: Study CB-17-01/03: Methylthioninium chloride Cosmo Tablet Administration Schedule

Dose	Mode of Administration	Number of tablets taken in relation to the intake of the bowel cleansing preparation				
		At the beginning of the bowel cleansing preparation	After the 1st L	After the 2nd L	After the 3rd L	At the end
150 mg	A	2	2	2	0	0
	B	6	0	0	0	0
	C	0	0	0	0	6
200 mg	D	4	2	2	0	0
	E	0	0	0	0	8
	F	2	2	2	0	2
	G	4	2	2	0	0
	H	0	0	0	4	4
	I	0	0	4	4	0
	J	0	0	2	3	3

CSR = clinical study report; MMX = Multi-Matrix

NSA and TSC for each dose and mode of administration are summarised below. There was a trend towards better staining when tablets were administered late and in the end of the cleansing.

Table 12: Study CB-17-01/03: Most frequent NSA in the PP Population (N = 109) and the FAS (N = 112)

Population	Methylene Blue MMX Tablets																					
PP N = 109	150 mg (N = 23)						200 mg (N = 86)															
	A N = 7		B N = 7		C N = 9		D N = 19		E N = 9		F N = 8		G N = 2		H N = 20		I N = 15		J N = 13	
	Regions	Subjects n (%)	Regions	Subjects n (%)	Regions	Subjects n (%)	Regions	Subjects n (%)	Regions	Subjects n (%)	Regions	Subjects n (%)	Regions	Subjects n (%)	Regions	Subjects n (%)	Regions	Subjects n (%)	Regions	Subjects n (%)
	2	3 (42.9)	0	6 (85.7)	1	5 (55.6)	1	7 (36.8)	1	3 (33.3)	4	3 (37.5)	1 and 4	1 (50.0)	2	9 (45.0)	4	6 (40.0)	3	5 (38.5)
FAS N = 112	(N = 24)						(N = 88)													
	A N = 8		B N = 7		C N = 9		D N = 20		E N = 9		F N = 8		G N = 2		H N = 20		I N = 16		J N = 13	
	2	3 (37.5)	0	6 (85.7)	1	5 (55.6)	1	7 (35.0)	1	3 (33.3)	4	3 (37.5)	1 and 4	1 (50.0)	2	9 (45.0)	4	6 (37.5)	3	5 (38.5)

FAS = Full Analysis Set; MMX = Multi-Matrix; NSA = Number of Stained Areas with staining score > 2; PP = Per Protocol.

Table 13: Study CB-17-01/03: Most frequent TSC and Mean ± SD TSC by Mode of Administration in the PP Population (N = 109) and the FAS (N = 112)

Population	Methylene Blue MMX Tablets																			
PP N = 109	150 mg (N = 23)						200 mg (N = 86)													
	A N = 7		B N = 7		C N = 9		D N = 19		E N = 9		F N = 8		G N = 2		H N = 20		I N = 15		J N = 13	
	TSC	Subjects n (%)	TSC	Subjects n (%)	TSC	Subjects n (%)	TSC	Subjects n (%)	TSC	Subjects n (%)	TSC	Subjects n (%)	TSC	Subjects n (%)	TSC	Subjects n (%)	TSC	Subjects n (%)	TSC	Subjects n (%)
	8	3 (42.9)	1	3 (42.9)	5	3 (33.3)	4	3 (15.8)	13	3 (33.3)	6	2 (25.0)	5 and 16	1 (50.0)	7, 9, 11	3 (15.0)	7 and 16	3 (20.0)	12	6 (46.2)
Mean TSC	7.6 ± 3.6		2.3 ± 2.4		8.1 ± 3.6		6.7 ± 4.9		9.8 ± 4.4		9.3 ± 4.1		10.5 ± 7.8		10.0 ± 3.2		11.9 ± 3.4		11.6 ± 3.5	
FAS N = 112	(N = 24)						(N = 88)													
	A N = 8		B N = 7		C N = 9		D N = 20		E N = 9		F N = 8		G N = 2		H N = 20		I N = 16		J N = 13	
	8	3 (37.5)	1	3 (42.9)	5	3 (33.3)	4	3 (15.0)	13	3 (33.3)	6	2 (25.0)	5 and 16	1 (50.0)	7, 9, 11	3 (15.0)	7 and 16	3 (18.8)	12	6 (46.2)
Mean TSC	6.8 ± 4.0		2.3 ± 2.4		8.1 ± 3.6		7.0 ± 5.0		9.8 ± 4.4		9.3 ± 4.1		10.5 ± 7.8		10.0 ± 3.2		11.4 ± 3.8		11.6 ± 3.5	

CSR = clinical study report; FAS = Full Analysis Set; MMX = Multi-Matrix; PP = Per Protocol; SD = standard deviation; TSC = total staining score

Secondary pharmacology

Potential effect of the oral administration of 200 mg Methylthioninium chloride Cosmo tablets on the integrity of colonic epithelial double stranded DNA during a full chromoendoscopy in comparison with a standard white light colonoscopy without the use of Methylthioninium chloride Cosmo was investigated in a small exploratory study (Study CB-17-01/08). There was no evidence of damage to colonic epithelial double stranded DNA in the study (data not shown).

2.6.4. Discussion on clinical pharmacology

The Methylthioninium chloride Cosmo 25 mg tablet is a gastro-resistant, prolonged release tablet. The active substance, also known as methylthioninium chloride, has been approved for many years in different formulations and for different indications.

The MMX formulation improves delivery of methylthioninium chloride in the colon, where it is locally released and acts as a visual dye to enhance the staining contrast in mucosal lesions. For the proposed indication, the proposed posology of Methylthioninium chloride Cosmo is as single oral administration of a split 200 mg dose during and after oral bowel cleansing preparation.

Systemic absorption after oral administration is substantial. Single doses of 500 mg have been administered safely to healthy subjects, and for other indications daily IV doses of 2 mg/kg are administered. The absolute bioavailability was 140 %. This is unusual, but besides high inter-subject variability, an underlying mechanism could be saturation of efflux transporter mechanisms causing a bottleneck in the elimination of methylthioninium chloride from the body. The absolute bioavailability was roughly calculated in two different populations on mean AUC0-∞ values from the ratios of AUC0-∞ values after single oral dose of 400 mg and single i.v. injection of 100 mg to be 78%.

A 25 mg tablet was developed to allow dived dosing and decrease the risk that all tablets are expelled before dye is delivered. The intended delay in absorption was demonstrated, as the drug was not detectable in blood until after 3 hours for the 200 mg dose and 5 hours for the 400 mg dose. The divided dose (8 x 25 mg) resulted in lower Cmax values compared to a single dose (1x 200 mg), which is preferred in relation to safety.

Dose proportionality was seen between the 100 mg and 200 mg dose. For the 400 mg dose, peak blood concentration did not increase proportionally, possibly due to saturation of transporter mechanisms. Lack of dose proportionality between the 200 mg and 400 mg dose support the 200 mg dose.

Methylene blue has a high volume of distribution indicating both extensive distribution to extravascular compartments and high protein binding. With the single administration of Methylthioninium chloride Cosmo in conjunction of endoscopy, there is no concern of clinically relevant accumulation.

Urinary excretion in the two PK studies was around 40 % of the oral dose. This is in line with what has previously been found for methylthioninium chloride.

The summary of methylthioninium chloride metabolism is mainly based on information available from EU-approved methylthioninium chloride products. Published literature describing the metabolism of methylthioninium chloride is scarce. Methylthioninium chloride is well-known as active substance from other EU approved products and even though the data presented to support the metabolism of methylene blue are suboptimal, metabolism has been adequately described. Methylthioninium chloride is metabolised by CYPs 1A2, 2C19 and 2D6 in vitro; however, the predominant in vitro pathway is – according to product information from other methylthioninium chloride products - uridine diphosphate glucuronyltransferase (UGT) mediated conjugation by multiple UGT enzymes, including UGT1A4 and UGT1A9.

Potential interactions of methylthioninium chloride with cytochrome P450 (CYP) enzymes and efflux/uptake transporters were assessed in vitro. No clinical DDI studies have been conducted, which is acceptable for this single dose administration treatment. The inhibitory potential of methylthioninium chloride to inhibit CYP isozymes is substantial. In vitro, methylthioninium chloride directly inhibited human CYP isozymes 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4/5 at therapeutic concentrations. Potential time-dependent inhibition was found for CYP isozymes 2B6 and 2C9, and irreversible mechanism-based inhibition > 70% was found for all CYP isozymes tested. The clinical impact of the inhibitory potential of methylthioninium chloride is expected to be limited due to the short treatment duration.

Methylthioninium chloride induces CYP1A2 and CYP2B6 in human. However, also here the risk of clinically relevant DDIs is considered to be minor due to the relative short treatment durations and almost complete elimination of methylthioninium chloride after 72 hours.

Methylthioninium chloride was in vitro identified to be a substrate both for the renal solute carrier transporters P-gp and OAT3 and potentially acts as a mild inhibitor of P-gp. Decrease in excretion efficiency would not be expected to be clinically relevant for a split, single dose administration.

Both PK studies were conducted in healthy subjects, 36 males and 10 postmenopausal females, from 18-65 years of age, and therefore PK has been investigated in a very homogenous population. No studies in special populations, i.e. subjects with impaired renal or hepatic function, elderly or children have been conducted. This is acceptable. Based on clinical trial analysis (see Clinical Safety) there is no evidence that hepatic impairment impacts on the safety profile of Methylthioninium chloride Cosmo. No warnings or dose adjustments are required in respect of hepatic impairment. There are no data in patients with severe hepatic impairment. No dose adjustment is required in patients with mild renal impairment (see Clinical Safety), but should be used with caution in patients with moderate to severe renal impairment as there are no data in this patient group and methylthioninium chloride is predominantly renally eliminated (relevant information is reflected in SmPC sections 4.2 and 5.2). No Pop PK studies have been conducted, which is acceptable.

The mechanism of action of methylthioninium chloride is well-known. The MMX formulation with extended release and protection against dissolving at pH < 7 enhance release of methylthioninium chloride into the colon and the split dose administration protects against the risk of potential expulsion as a result of the concomitant administered full bowel preparation.

Colonic mucosa staining quality was satisfactory (i.e., TSC ≥ 8 and no over-staining in any ROI) in all subjects evaluated in both dose groups (100 mg and 200 mg), but a better result was achieved in the 200 mg dose group. This is supportive of the 200 mg dose.

The methylthioninium chloride that is passively delivered by MMX at the colorectal level is exactly the same molecule with the same PD properties that is used in routine chromoendoscopy. The literature appears to be comprehensive with regard to the specific action of methylthioninium chloride and inactive content of MMX tablets in the application in chromoendoscopy under consideration. The material presented is considered sufficient regarding pharmacodynamic.

The methylthioninium chloride is not selective in identifying a specific type of lesion from other types of lesions, it does not provide any insight for differentiation of certain type of lesion. Available evidence suggests that methylthioninium chloride has a different uptake in normal mucosal cells compared to dysplastic or pre-neoplastic mucosal cells which allows for an enhancement of contrast and facilitates lesion detection. As the product is aimed to improve current practice, provided information regarding efficiency and PD correlation could be considered acceptable.

Different dosing regimens of the 8x 25 mg tablets before, during and after the bowel preparation have been included in the clinical trials. Firm conclusions are challenged by small numbers and different doses. The dosing instructions have been adapted to be independent of the bowel cleansing regimen with the two most important aspects being sufficient time between first Methylthioninium chloride Cosmo administration and colonoscopy and that first dose should be administered after the first portion of the bowel preparation (this is reflected in SmPC 4.2).

2.6.5. Conclusions on clinical pharmacology

The clinical pharmacology program to support the application is limited, but adequate to supplement what is well-known about the PK of methylthioninium chloride. The split 200 mg dose split is supported from a PK point of view.

2.7. Clinical efficacy

The demonstration of efficacy of Methylthioninium chloride Cosmo as a diagnostic agent enhancing visualisation of colorectal lesions in adult patients undergoing screening or surveillance colonoscopy is

based on three studies; a single pivotal randomised, double blind, placebo controlled phase 3 study and two supportive, uncontrolled, un-blinded studies.

Study Number	Phase	Study Design	Subject Population	Number of Subjects Treated/Treatment Groups	Primary Endpoint(s)
CB-17-01/04	2	Open-label, uncontrolled	Subjects with long lasting ulcerative colitis undergoing colonoscopy	53 200 mg Methylthioninium chloride Cosmo (8 × 25 mg tablets)	Intraepithelial neoplasia detection and colon staining
CB-17-01/05	2	Open-label, uncontrolled	Subjects scheduled for colonoscopy	158 200 mg Methylthioninium chloride Cosmo (8 × 25 mg tablets) 96 subjects Full Dose group; 62 subjects Split Dose group	Detection of adenomas and polyps
CB-17-01/06	3	Randomised, double-blind, placebo controlled	Subjects scheduled for screening or surveillance colonoscopy	1208 randomised 2:1:2 as 200 mg Methylthioninium chloride Cosmo (8 × 25 mg tablets) (N=488) 100 mg Methylthioninium chloride Cosmo (4 × 25 mg Methylthioninium chloride Cosmo tablets and 4 × placebo tablets) (N=241) Placebo (8 × placebo tablets) (N=479)	Adenoma detection rate

2.7.1. Dose-response study

No designated dose finding studies were performed. In addition to the studies above, dose-response information was retrieved from Study CB-17-01/03.

Study Number	Phase	Study Design	Subject Population	Number of Subjects Treated/Treatment Groups	Primary Endpoint(s)
CB-17-01/03	2	Open-label uncontrolled	Subjects scheduled for colonoscopy	114 Methylthioninium chloride Cosmo 25 mg tablets, oral doses of 150 or 200 mg	Colon staining

For details about CB-17 01/03 see section Clinical Pharmacology.

For details about CB-17 01/05 see section on Supportive Studies.

The recommended dose of Methylthioninium chloride Cosmo is a single dose of 200 mg, to be administered in conjunction with the bowel cleansing preparation prior to colonoscopy.

Phase 2 Study CB-17-01/03 suggested that a dose of Methylthioninium chloride Cosmo 200 mg provided better colon staining than a dose of 150 mg. Phase 3 Study CB-17-01/06 also supports a conclusion that the Methylthioninium chloride Cosmo 200 mg dose is better than a lower dose, such as 100 mg, for the detection of adenomas and carcinomas during colonoscopy.

To control for the potential for acquisition bias, by masking the active arm of the pivotal study from the investigator (endoscopist) and provide subject blinding between the placebo and Methylthioninium chloride Cosmo 200mg groups after dosing, a 100 mg dose group was included in the pivotal CB-17-01/06 study. Fewer subjects were enrolled in the 100 mg dose group, and the study was not powered to show statistically significant differences between the Methylthioninium chloride Cosmo 100 mg group and the placebo group, or between the Methylthioninium chloride Cosmo 100 mg group and the

200 mg group. However, a clear dose-response trend was observed for most of the relevant endpoints, including the primary efficacy endpoint and key secondary and exploratory endpoints. The low dose group (Methylthioninium chloride Cosmo 100 mg) consistently obtained values numerically intermediate between full dose and placebo for all the main efficacy endpoints.

To further investigate the dose-response relationship, several additional analyses were conducted on selected endpoints for dose-response using the Cochran-Armitage trend test (one-sided). With respect to the primary endpoint, in the FAS, the proportion of subjects with at least one histologically proven adenoma or carcinoma in the Methylthioninium chloride Cosmo 100 mg group was intermediate between placebo and Methylthioninium chloride Cosmo 200 mg, with a statistically significant test for dose response (Cochran-Armitage trend test p = 0.0042). Statistically significant dose response was also demonstrated for other endpoints, including the proportion of subjects with at least one excised non-polypoid lesion (p = 0.0025) and proportion of subjects with at least one non-polypoid histologically proven adenoma or carcinoma < 10 mm (p = 0.0027). Thus, Methylthioninium chloride Cosmo shows a very clear dose-response relationship throughout the 100 mg and 200 mg dosing range, with 200 mg being the most effective dose. Overall, the results throughout the clinical development programme and the dose-response relationship evident in Study CB-17-01/06 provides support for the selection of 200 mg as the recommended total dose of Methylthioninium chloride Cosmo, to be administered in a divided dose of 8 × 25 mg tablets although higher doses were not investigated.

2.7.2. Main study

Study CB-17-01/06: Multi-centre randomised, placebo-controlled, double blind study in patients undergoing schedule screening or surveillance colposcopy for colorectal cancer (CRC)

Methods

Study Participants

The study included outpatients attending selected endoscopy centres for screening or surveillance colonoscopy for polyps or colorectal cancer. The main inclusion and exclusion criteria are listed below.

INCLUSION CRITERIA

- Males or females, aged between 50 and 75.
- A female is eligible to enter and participate in this study if she is:

  of non-childbearing potential including pre-menopausal females with documented (medical report verification) hysterectomy or double oophorectomy or postmenopausal or of childbearing potential, has a negative serum pregnancy test at screening and urine pregnancy test prior to start the study drug, and abstain completely from sexual intercourse or agrees to using highly effective contraceptive methods (e.g. intrauterine device, hormonal contraceptive drug, tubal ligation) during the study until completion of the follow-up procedures.
- Outpatients scheduled for screening or surveillance colonoscopy for colorectal cancer.

EXCLUSION CRITERIA

- Patients at high risk of colorectal cancer e.g. ulcerative colitis

- Pregnancy or lactation.
- Previous medical history of, or suspected hypersensitivity to, the methylthioninium chloride and/or formulations' ingredients.
- Previous medical history of, or suspected hypersensitivity to, the PEG based bowel cleansing preparation and/or bowel cleansing formulations' ingredients.
- Previous medical history of gastrointestinal obstruction or perforation, toxic megacolon, major colonic resection, severe diverticulitis, heart failure (Class III or IV), serious cardiovascular disease, ulcerative colitis or Crohn's disease.
- ALT, AST, GGT, Bilirubin, Creatinine or Urea greater than 2.5 x the upper limit for normal range, based on local laboratory testing.
- The presence of serious cardiovascular disease, including very large abdominal aortic aneurysms (particularly if they are symptomatic), patients who are immediately postoperative, and patients who have suffered recent myocardial infarction (within 3 weeks), pulmonary embolism, or are currently hemodynamically unstable.
- The presence of liver disease with coagulopathy.
- A history of anaemia (previously recorded haemoglobin of less than 10mg/dL) within the last 30 days prior to enrolment.
- Known or suspected deficiency of glucose-6-phosphate dehydrogenase
- Known or suspected deficiency of NADPH reductase
- Treatment within 5 weeks prior to randomisation with Fluoxetine (Prozac).
- Concurrent treatment, or previous treatment within 2 weeks with any of the prohibited psychiatric medications that may interact with methylthioninium chloride as listed under Prohibited medications; Selective Serotonin Reuptake Inhibitors (SSRI), Serotonin-Norepinepherine Reuptake Inhibitors (SNRI's), listed Tricyclic anti-depressants or Monoamine oxidase A inhibitors.
- Current enrollment in any other clinical trial, or previous enrollment in a clinical trial within the last 30 days.
- Other medical condition that in the investigators opinion would make the administration of the study drug or procedures hazardous to the subject.
- Current enrolment in any other clinical trial, or previous enrolment in a clinical trial within the last 30 days.
- Other medical condition that in the investigator's opinion would make the administration of the study drug or procedures hazardous to the subject.

Treatments

Bowel cleansing preparation

All subjects received a full dose regimen of 4 litres PEG-based bowel cleansing preparation starting in the late afternoon (after 6 pm) before the colonoscopy day. The subjects drank at least 250 mL of solution every 15 min, so that the intake of the cleansing preparation, and study drug was completed in 4 hours.

Subjects had to adopt a low residue diet for three days prior to the colonoscopy. On the third day of the low residue diet patients they had to fast for at least 3 h before starting intake of the bowel prep and study drug. During the intake of bowel preparation, the subjects were recommended to drink additional non-gaseous water according to the product instructions. Subjects were to be fasting from 3-4 h prior to beginning of the intake of the bowel preparation solution until the completion of the colonoscopy the following day. Non-gaseous water and clear liquids intake were freely allowed.

Dose regimen

The subjects were randomized 2:2:1 into three groups:

Group One received an oral dose of 200 mg of Methylthioninium chloride Cosmo 25 mg tablets: 3 Methylthioninium chloride Cosmo 25 mg tablets (75 mg) after the first 2 litres of bowel preparation, 3 Methylthioninium chloride Cosmo 25 mg tablets (75 mg) after a total of 3 litres of bowel preparation and, finally, 2 Methylthioninium chloride Cosmo 25 mg tablets (50mg) after a total of 4 litres of bowel preparation had been consumed.

Group Two received an oral dose of matching placebo tablets: 3 tablets after the first 2 litres of bowel preparation, 3 tablets after a total of 3 litres of bowel preparation and, finally, 2 tablets after a total of 4 litres of bowel preparation had been consumed.

Group Three was included only for masking purposes in order to reduce the acquisition bias due to the lack of investigator and subject blinding between placebo and Methylthioninium chloride Cosmo 200 mg groups. This unpowered masking group was treated with 100 mg Methylthioninium chloride Cosmo 25 mg tablets: 1 tablet (25mg) of Methylthioninium chloride Cosmo 25mg tablets and two placebo tablets after the first 2 litres of bowel preparation, additional 2 tablets (50 mg) of Methylthioninium chloride Cosmo 25mg tablets and one placebo tablet after a total of 3 litres of bowel preparation, and, finally, 1 tablet (25 mg) of Methylthioninium chloride Cosmo 25 mg tablet, and one placebo tablet after a total of 4 litres of bowel preparation solution had been consumed.

Objectives

The objective of the study was the evaluation of the Adenoma or Carcinoma detection rate in patients undergoing a full colonoscopy after colonic mucosal staining and contrast enhancing with Methylthioninium chloride Cosmo tablets compared to placebo tablets. The adenoma detection rate (ADR) was defined as the proportion of patients with at least one histologically proven Adenoma or Carcinoma.

Outcomes/endpoints

Primary endpoint (adenoma detection rate (ADR)):

The primary endpoint of this study was to assess the detection efficacy of chromoendoscopy performed with 200 mg Methylthioninium chloride Cosmo 25 mg tablets versus placebo tablets (white light endoscopy) in terms of the proportion of patients with at least one histologically proven Adenoma or Carcinoma. Adenoma was defined as a histologically proven Vienna Grade 3 to 4.2 or a histologically proven Traditional Serrated Adenoma (TSA), or a histologically proven Sessile Serrated Adenoma (SSA). Histologically proven Carcinoma was defined as Vienna Grade 4.3 to 5b.

The Vienna Classification and TSA and SSA grading was made by blinded central laboratory pathologists who reviewed slides prepared from an additional tissue section of each paraffin embedded specimen prepared at the local centre laboratory in accordance with the specific histology charter.

Secondary endpoints (false positive rate (FPR)):

False positive rate between treatment and placebo control arms; the rate was defined as the proportion of patients with no histologically confirmed Adenoma or Carcinoma within any of the patient's excised lesions and the patient having undergone at least one excision;

Proportion of patients with at least one histologically proven Adenoma;

Proportion of patients with at least one histologically proven Carcinoma;

Number of histologically proven Adenomas Vienna categories 3, 4.1 and 4.2 or a histologically proven TSA, or a histologically proven SSA and Carcinomas (Vienna categories 4.3, 4.4, 5.a and 5.b) detected per patient;

Number of histologically proven hyperplastic polyps, fibroblastic polyps and mixed polyps detected per patient;

Exploratory endpoints:

Proportion of patients with at least one polypoid lesion;

Number of polypoid lesions detected per patient;

Proportion of patients with at least one non polypoid lesion;

Number of non-polypoid lesions detected per patient;

Number of histologically proven Carcinomas (Vienna categories 4.3, 4.4, 5.a and 5.b) detected per patient;

Proportion of patients with at least one histologically proven hyperplastic, fibroblastic or mixed polyp;

Number of histologically proven Adenomas and Carcinomas detected in the right colon (i.e. caecum and ascending colon) per patient;

Number of polypoid lesions detected in the right colon (i.e. caecum and ascending colon) per patient;

Number of non-polypoid lesions detected in the right colon (i.e. caecum and ascending colon) per patient;

Number of histologically proven hyperplastic polyps, fibroblastic polyps and mixed polyps detected in the right colon (i.e. caecum and ascending colon) per patient;

Lesion size (total number of lesions <5 mm, lesions ≥5 mm and <10 mm and lesions ≥10 mm);

Boston Bowel Preparation Score (BBPS) for bowel cleansing preparation quality;

Measures of colonoscopy performance:

Time to reach the caecum;

Withdrawal time from the caecum to exit, excluding interventional time if any.

Consensus between local and central reading of the endoscopy video with regards to the need for excision of the identified lesions.

Changes in the definition of the primary endpoint while the study was ongoing

The Study Protocol originally provided for adenomas to be defined according to the "Vienna Classification" as the histological Grade 3 to 4.2. But the "Vienna Classification" does not take into account certain lesions named Traditional Serrated Adenomas (TSAs) and Sessile Serrated Adenomas

(SSAs), although the Study Protocol provided for their separate identification and listing as mucosal lesions.

At the time the Study Protocol was filed, certain major scientific findings were not yet globally acknowledged. Now there is widespread consensus between endoscopists and histologists that TSAs and SSAs are precursors of colorectal carcinomas and need to be removed because of their nature, independently from their score under the "Vienna Classification". Exclusion of TSAs and SSAs from the primary end-point would lead to an incomplete assessment of lesions with malignant potential. The objective of the study was to identify all lesions that need to be removed which have the potential to degenerate and turn into cancers. Therefore, TSAs and SSAs were included in the primary endpoint in addition to the "Vienna Classification" grade 3 to 4.2 lesions. The calculation of the adenomas detection rate and into all the other efficacy evaluations based on the histological classification.

Sample size

A retrospective study on 5175 subjects found out an adenoma and carcinoma detection rate around 30% (95% CI [26.7%-31.8%]) for white light colonoscopy. Considering an improvement of 10%, deemed to be clinically significant, due to the use of Methylthioninium chloride Cosmo® (this hypothesis is strongly supported by the results on the first 100 subjects of phase II trial) the expected adenoma a carcinoma detection rate for chromoendoscopy is 40%.

The superiority of Methylthioninium chloride Cosmo® full dose versus Placebo was tested in terms of the adjusted odds ratio derived from a logistic regression model.

Under the hypothesis of a 2:2:1 allocation among full dose test group, placebo control group and low dose masking group, the sample size required in order to show superiority of full dose chromoendoscopy with respect of white light colonoscopy in terms of adenoma and carcinoma detection rate increase is listed below.

α two-sided	β	Power $(1-\beta)$	$\pi_{\text{Full Dose}}$	π_{Placebo}	n Full Dose/ Placebo	n Low Dose	N total
0.05	0.1	0.9	0.4	0.3	481	241	1203

Considering an exclusion rate from the Full Analysis Set around 5%, at least 1270 subjects have to be enrolled.

Randomisation

The subjects are randomised using a central system. Randomisation is stratified by centre and reason for colonoscopy (screening, surveillance within 2 years from previous colonoscopy and surveillance after more than 2 years from previous colonoscopy). Subjects are allocated to the full dose of Methylthioninium chloride Cosmo arm, placebo control arm or low dose of Methylthioninium chloride Cosmo arm according to a 2:2:1 ratio.

Blinding (masking)

The trial was double blind at randomisation. The investigator and the subject were not aware of the treatment the subject was assigned, although during colonoscopy and afterwards due to the blue dye characteristics the endoscopist and subject were likely to have become aware of the assigned treatment.

Due to the lack of blindness, a low dose arm of Methylthioninium chloride Cosmo was included for masking purposes only. Only the full dose arm of Methylthioninium chloride Cosmo and the placebo arm were included in the primary efficacy analysis, while the low dose arm of Methylthioninium chloride Cosmo was included in the evaluation of demographic, baseline and background characteristics, in the secondary and exploratory efficacy analysis and in the evaluation of safety and tolerability.

Statistical methods

Analysis sets

Intention-to-Treat Set (ITT): all randomised subjects regardless of investigational medicinal product intake, colonoscopy execution and colon cleansing. This analysis set was used for sensitivity analyses.

Full Analysis Set (FAS): all randomised subjects who received at least one dose of the investigational medicinal product and underwent colonoscopy (regardless of the completion status). This analysis set was be used for the primary efficacy analysis.

Per Protocol Set (PP): all randomised subjects who will fulfilled the study protocol requirements in terms of investigational medicinal product intake and collection of primary efficacy data (full colonoscopy successfully executed), who had an acceptable colon cleansing (defined as a Boston Bowel Preparation Score ≥ 1 in the right colon, transverse colon and left colon; missing scores will not lead to exclusion), who did not have any inclusion/exclusion criteria violation and with no major deviations that may have affected study results. This analysis set was to be used for sensitivity analyses.

Safety Set: all subjects who received at least one dose of the investigational medicinal product. This analysis was used for safety analyses.

<u>Subjects assigned to the wrong randomization category</u>

The randomisation list is stratified by centre and reason for colonoscopy (screening, surveillance within 2 years from previous colonoscopy and surveillance after more than 2 years from previous colonoscopy). Some subjects were by mistake assigned to a stratum of the randomisation list not consistent with their actual reason for colonoscopy. Subjects will be analysed according to the treatment they actually received and to their actual reason for colonoscopy, despite disagreement with the randomisation list.

Primary efficacy analysis

The proportion of subjects included in the Full Analysis Set with at least one histologically proven Adenoma or Carcinoma found during colonoscopy was summarised by treatment (Methylthioninium chloride Cosmo Full Dose, Methylthioninium chloride Cosmo Low Dose and Placebo). Odds ratio, difference in proportions and their 95% confidence intervals was presented.

The proportion of subjects included in the Full Analysis Set (Methylthioninium chloride Cosmo Full Dose and Placebo only) with at least one histologically proven Adenoma or Carcinoma found during colonoscopy was analysed through a logistic regression and treatment, centre, age, sex, reason for colonoscopy (screening, surveillance within 2 years from previous colonoscopy and surveillance after more than 2 years from previous colonoscopy) and number of excisions (categorised as "≤ 3", "4 - 6" and "> 6") were included in the regression model as fixed effects.

Sensitivity analysis for the primary endpoint

Per protocol analysis: The proportion of subjects included in the Per Protocol Set (Methylthioninium chloride Cosmo Full Dose arm and Placebo arm only) with at least one histologically proven Adenoma or Carcinoma found during colonoscopy was analysed through the same logistic regression model as used for the primary endpoint.

Centre by treatment interaction: In order to evaluate the presence of a centre by treatment interaction, the proportion of subjects included in the Full Analysis Set (Methylthioninium chloride Cosmo Full Dose arm and Placebo arm only) with at least one histologically proven Adenoma or Carcinoma found during colonoscopy was analysed by the same logistic regression model as used for the primary endpoint.

Multiple imputation: The proportion of subjects included in the FAS (Methylthioninium chloride Cosmo Full Dose arm and Placebo arm only) with at least one histologically proven Adenoma or Carcinoma found during colonoscopy was analysed through the same logistic regression model as used for the primary endpoint. Missing values of the primary endpoint were replaced according to a multiple imputation under MAR assumption method. The multiple imputation model will use treatment, centre, age, sex, reason for colonoscopy, and number of excisions as covariates. A monotone missing pattern was used. The number of imputation data sets was set to 100.

Worst case analysis: The proportion of subjects included in the FAS (Methylthioninium chloride Cosmo Full Dose arm and Placebo arm only) with at least one histologically proven Adenoma or Carcinoma found during colonoscopy were analysed through the same logistic regression model as used for the primary endpoint. Missing values of the primary endpoint were replaced according to a worst-case method. For each subject, missing values of the primary endpoint were replaced with the worst value of that variable (i.e. 0 - Failure) if the subject was assigned to the Methylthioninium chloride Cosmo Full Dose arm and with the best value of that variable (i.e. 1 - Success) if the subject was assigned to the Placebo arm.

Handling of missing data

Intention-to-Treat Set (ITT): all missing values of the primary endpoint were replaced according to the MI under MAR assumption method and according to the WC method in order to perform two distinct sensitivity analyses. Missing values of the secondary endpoints were not be replaced.

Full Analysis Set (FAS): all subjects of the FAS underwent colonoscopy and the presence/absence of at least one histologically proven Adenoma or Carcinoma detected during colonoscopy could be evaluated for each subject. No replacement of missing data was required for the primary endpoint. Missing values of the secondary endpoints were not be replaced.

Per Protocol Set (PP): all subjects of the PP underwent colonoscopy and the presence/absence of at least one histologically proven Adenoma or Carcinoma detected during colonoscopy could be evaluated for each subject. No replacement of missing data was required for the primary endpoint. Missing values of the secondary endpoints were not be replaced.

Safety Set: all missing safety and tolerability data (vital signs, clinical laboratory test results) were not be replaced.

Secondary endpoints

The secondary and exploratory endpoints were summarised by descriptive statistics only (mean, standard deviation [SD], coefficient of variation [CV%], minimum, median and maximum values for quantitative variables and by frequencies for qualitative variables) with the exception of the FPR.

The FPR was evaluated as the proportion of patients who did not have histologically confirmed Adenoma or Carcinoma but did have at least one excision. The False Positive Rate (FPR) was compared between Methylthioninium chloride Cosmo Full Dose and Placebo according to the following hypothesis test:

H0 = FPR Full Dose - FPR Placebo ≥ PThreshold

Ha = FPR Full Dose - FPR Placebo < PThreshold

The null hypothesis was rejected if the upper bound of the 95% confidence interval of the difference FPR Full Dose - FPR Placebo was less than the proportion PThreshold.

The false positive rate retrieved from the first 100 subjects of phase II trial was around 26% and the ones retrieved from literature ranged from 24% to 30%. The proportion of subjects with excisions with respect to the enrolled ones ranged from 41% to 61%.

Considering the worst case (i.e. a FPR of 30% for Methylthioninium chloride Cosmo Full Dose and a FPR of 24% for Placebo) and supposing that around 50% of enrolled subjects per arm (i.e. 250 subjects per arm) would undergo at least one excision, the 95% confidence interval of the difference of the FPRs could be determined to be [-1.8% - 13.8%]. According to this result, a PThreshold of 15% for the FPR difference between Methylthioninium chloride Cosmo Full Dose Placebo was established. The same PThreshold of 15% and the same hypothesis test were to be used for the comparisons between Methylthioninium chloride Cosmo Low Dose and Placebo and between Methylthioninium chloride Cosmo Full Dose and Methylthioninium chloride Cosmo Low Dose.

Changes from the Statistical Analyses Plan

There were the following additions to the planned analyses as described in the SAP Final Version 1.0 dated 08 April 2016:

Proportion of patients with at least one histologically proven Adenoma or Carcinoma (FAS), Fisher's exact tests were performed for the three pairwise comparisons Methylthioninium chloride Cosmo Full Dose versus Placebo, Methylthioninium chloride Cosmo Low Dose versus Placebo and Methylthioninium chloride Cosmo Full Dose versus Methylthioninium chloride Cosmo Low Dose. This test was not foreseen in the SAP and was added during the analysis, but does not replace the primary analysis based on the logistic regression model.

Additional tables were produced, as additional informative and exploratory analyses, to summarize the FPR calculation at excision level for the FAS and PP Set, respectively. The FPR at excision level was to be calculated as the proportion of histologically negative lesions among all the excisions.

Other changes included addition of tables and clarification of data sets.

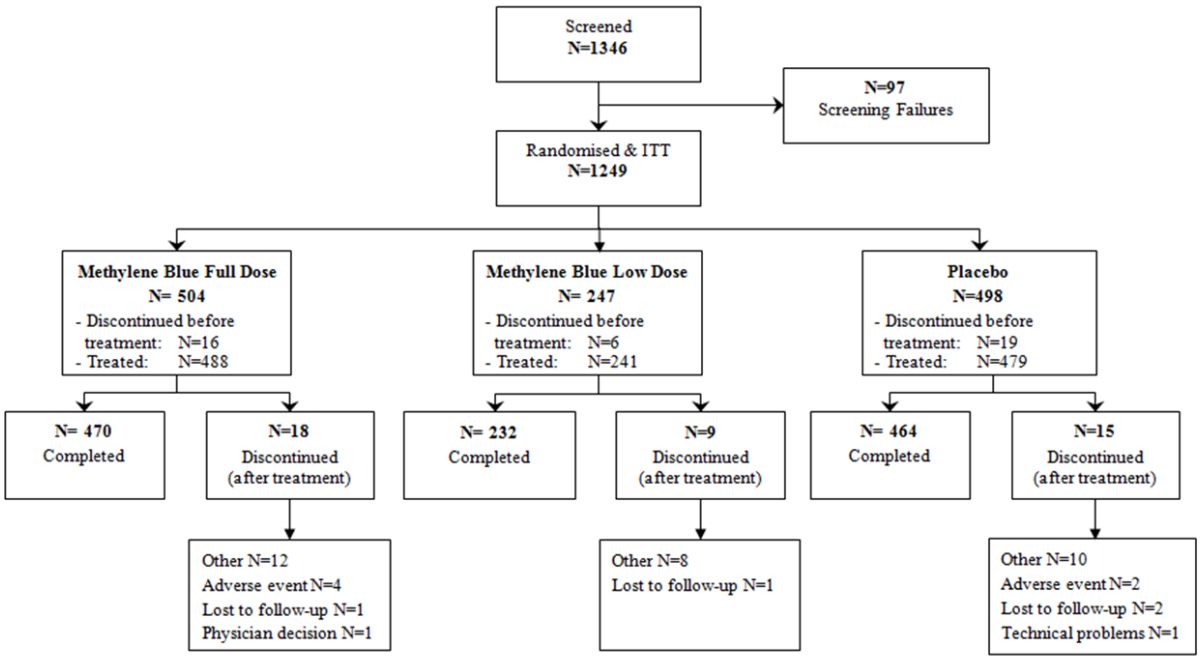
Additional analysis (not listed in the SAP) were presented after interaction with the EMA.

The primary analysis was redone excluding the post-randomization variable "number of excisions".

The primary analysis was re calculated using the definition presented in the older version of the protocol (version 1.2 Amendment 1, date 16 September 2013) and thus excluding SSAs and TSAs.

Results

Participant flow



Recruitment

Patients were recruited from 20 centres in Europe and North America from 15 December 2013 (First Patient Enrolled) to 20 October 2016 (Last Patient Completed).

Conduct of the study

The protocol was amended twice. First amendment intended to make the protocol applicable to both EMA and FDA. The second amendment involved a change of the primary efficacy endpoint to include SSA and TSA in the ADR (see statistical analysis).

Protocol deviations were defined as major or minor during the blind review meeting/conference.

For the ITT Set, a total of 593/1249 patients (47.5%) had deviations, with similar proportions of patients with deviations across the treatment groups.

Major deviations were reported for a total of 112/1249 patients (9.0%) in the ITT Set, with a slightly higher proportion of patients in the Methylthioninium chloride Cosmo Full Dose group (49/504 patients; 9.7%), when compared to the Methylthioninium chloride Cosmo Low Dose group (22/247 patients; 8.9%) and the placebo group (41/498 patients; 8.2%).

Overall, reasons for major deviations included:

- Lack of any data post randomisation (43 patients; 3.4%);
- Failure to take at least one dose of IMP (41 patients; 3.3%);
- Full colonoscopy not successfully executed (25 patients; 2.0%);
- 3rd or 4th endoscopist performed procedure (24 patients; 1.9%);

- Lack of cleansing (16 patients; 1.3%);
- Lack of compliance to the IMP (8 patients; 0.6%);
- Failure to satisfy any inclusion/exclusion criteria (4 patients; 0.3%);
- Non study endoscopist performed procedure (2 patients; 0.2%);
- Missing information about IMP intake (1 patient; 0.1%).

Minor deviations were reported for a total of 564/1249 patients (45.2%) in the ITT Set, with similar proportions of patients with minor deviations across the treatment groups.

Overall, minor deviations reported by >5% of patients in the ITT Set, included:

- Incorrect bowel preparation procedure (91 patients; 7.3%);
- Follow up visit out of window (88 patients; 7.0%);
- No recording from colonoscopy (77 patients; 6.2%).

Baseline data

Demographic characteristics varied slightly across the treatment groups. No clinically relevant differences regarding these characteristics were observed when comparing the different treatment groups.

Table 11-2: Demography and Reason for Colonoscopy (ITT Set)

		Methylene Blue Full Dose N=504 N (%)	Methylene Blue Low Dose N=247 N (%)	Placebo N=498 N (%)	Overall N=1249 N (%)
Sex					
Female	N (%)	202 (40.1)	105 (42.5)	191 (38.4)	498 (39.9)
Male	N (%)	302 (59.9)	142 (57.5)	307 (61.6)	751 (60.1)
Race					
White/Caucasian	N (%)	451 (89.5)	226 (91.5)	462 (92.8)	1139 (91.2)
Black or African American	N (%)	38 (7.5)	15 (6.1)	24 (4.8)	77 (6.2)
Hispanic or Latino	N (%)	5 (1.0)	3 (1.2)	3 (0.6)	11 (0.9)
Asian	N (%)	6 (1.2)	1 (0.4)	5 (1.0)	12 (1.0)
Japanese	N (%)	1 (0.2)	0 (0.0)	0 (0.0)	1 (0.1)
Native Hawaiian or other Pacific Islander	N (%)	0 (0.0)	1 (0.4)	1 (0.2)	2 (0.2)
Other	N (%)	3 (0.6)	1 (0.4)	3 (0.6)	7 (0.6)
Age (years)[1]	N	504	247	498	1249
	Mean (SD)	61.2 (6.8)	61.0 (6.5)	61.6 (6.8)	61.3 (6.7)
	Median	61.0	61.0	62.0	62.0

	[Range]	[50 to 75]	[50 to 75]	[50 to 75]	[50 to 75]
Reason for Colonoscopy Screening	N (%)	243 (48.2)	116 (47.0)	239 (48.0)	598 (47.9)
Surveillance within 2 years from previous colonoscopy	N (%)	28 (5.6)	18 (7.3)	32 (6.4)	78 (6.2)
Surveillance after more than 2 years from previous colonoscopy	N (%)	233 (46.2)	113 (45.7)	227 (45.6)	573 (45.9)

Medical History

No clinically relevant differences regarding medical history were observed when comparing the different treatment groups. Similar results were seen for the Safety Set.

Prior and Concomitant Medications

No clinically relevant differences regarding prior and concomitant medications were observed when comparing the different treatment groups. Similar results were seen for the Safety Set.

Numbers analysed

Table 11-1: Analysis Sets (ITT Set)

	Methylene Blue Full Dose N=504 N (%)	Methylene Blue Low Dose N=247 N (%)	Placebo N=498 N (%)	Overall N=1249 N (%)
Safety Set	488 (96.8)	241 (97.6)	479 (96.2)	1208 (96.7)
Full Analysis Set	485 (96.2)	241 (97.6)	479 (96.2)	1205 (96.5)
Per Protocol Set	455 (90.3)	225 (91.1)	457 (91.8)	1137 (91.0)

Patients are summarised according to the product they actually received. The number and the proportion of patients included in any analysis set are reported. The denominator for calculating the proportions was the number of patients assigned to each product and overall in the ITT Set. N=Number of patients.

Outcomes and estimation

Primary Efficacy Endpoint

A total of 626/1205 patients (51.95%) in the overall population for the FAS had at least one histologically proven Adenoma or Carcinoma found during colonoscopy (Table 11-6). The proportion of patients was highest in the Methylthioninium chloride Cosmo full dose group (56.29% of patients) when compared to the Methylthioninium chloride Cosmo low dose group (51.45% of patients) and the placebo group (47.81% of patients). Results showed that the odds ratio [95% CI] of 1.41 [1.09, 1.81] between the Methylthioninium chloride Cosmo full dose group and the placebo in the proportion of patients with at least one histologically proven Adenoma or Carcinoma was statistically significant

(using Fisher's Exact test) (pvalue=0.0099), indicating a significantly higher detection rate by the Methylthioninium chloride Cosmo full dose when compared with the placebo group. The odds ratio (odds ratio [95% CI] of 1.16 [0.85; 1.58]) for the Methylthioninium chloride Cosmo low dose group compared to the placebo group in the proportion of patients achieving the primary endpoint was close to 1 and not statistically significant (p-value=0.3851). Similarly, the odds ratio [95% CI] of 1.22 [0.89, 1.66] for the Methylthioninium chloride Cosmo full dose group compared to the Methylthioninium chloride Cosmo low dose group in the proportion of patients achieving the primary endpoint was not statistically significant (p-value=0.2353). It is important to consider that the Methylthioninium chloride Cosmo low dose was not powered to show any statistically significant difference from placebo or the full dose. Nevertheless, a numerical dose-related increase in ADR was observed in the sequence placebo - Methylthioninium chloride Cosmo low dose - Methylthioninium chloride Cosmo full dose. The analysis of the PP Set confirms the evidence resulting from the FAS analysis (Table 11-7): Similar to the ADR reported for FAS, a total of 605/1137 patients (53.21%) in the overall population for the PP Set had at least one histologically proven Adenoma or Carcinoma found during colonoscopy, with the highest ADR recorded in the Methylthioninium chloride Cosmo full dose group (58.24% of patients) when compared to the Methylthioninium chloride Cosmo low dose group (53.78% of patients) and the placebo group (47.92% of patients). In the PP Set the difference in ADR between the Methylthioninium chloride Cosmo full dose group and the placebo group was 10.32%, a higher value than the 8.48% difference in ADR recorded in the FAS. Results for the PP Set showed the odds ratio [95% CI] of 1.52 [1.17, 1.97] for the Methylthioninium chloride Cosmo full dose group compared to the placebo group in the proportion of patients achieving the primary endpoint was highly statistically significant (Fisher's Exact test) (p-value=0.0018).

In the PP Set, the difference in ADR between the Methylthioninium chloride Cosmo low dose group and the placebo group, even though not powered for this comparison, was 5.86%, also a higher value than the 3.64% difference in ADR recorded in the FAS, supporting the indication of a dose related trend of the dye as already indicated from the analysis in the FAS.

Table 14.2.1.1 - Proportion of subjects with at least one histologically proven Adenoma or Carcinoma (Full analysis set)

		Full Analysis Set			
		Methylene Blue Full Dose N=485 n (%)	Methylene Blue Low Dose N=241 n (%)	Placebo N=479 n (%)	Overall N=1205 n (%)
Subjects with at least one histologically proven Adenoma or Carcinoma	Y	273 (56.29)	124 (51.45)	229 (47.81)	626 (51.95)
	N	212 (43.71)	117 (48.55)	250 (52.19)	579 (48.05)
Odds Ratio and 95% CI of Methylene Blue Full Dose vs. Placebo		1.41	[1.09, 1.81]		
Relative Risk and 95% CI of Methylene Blue Full Dose vs. Placebo		1.18	[1.04, 1.33]		
Difference in Proportions and 95% CI of Methylene Blue Full Dose vs. Placebo		8.48	[2.20, 14.77]		
p-value of Fisher's exact test of Methylene Blue Full Dose vs. Placebo		0.0099			
Odds Ratio and 95% CI of Methylene Blue Low Dose vs. Placebo		1.16	[0.85, 1.58]		
Relative Risk and 95% CI of Methylene Blue Low Dose vs. Placebo		1.08	[0.92, 1.26]		
Difference in Proportions and 95% CI of Methylene Blue Low Dose vs. Placebo		3.64	[-4.09, 11.38]		
p-value of Fisher's exact test of Methylene Blue Low Dose vs. Placebo		0.3851			
Odds Ratio and 95% CI of Methylene Blue Full Dose vs. Methylene Blue Low Dose		1.22	[0.89, 1.66]		
Relative Risk and 95% CI of Methylene Blue Full Dose vs. Methylene Blue Low Dose		1.09	[0.95, 1.27]		
Difference in Proportions and 95% CI of Methylene Blue Full Dose vs. Methylene Blue Low Dose		4.84	[-2.86, 12.54]		
p-value of Fisher's exact test of Methylene Blue Full Dose vs. Methylene Blue Low Dose		0.2353			

Note: Histologically proven Adenomas = Vienna categories 3, 4.1 and 4.2, traditional serrated adenomas and sessile serrated adenomas
Histologically proven Carcinomas = Vienna categories 4.3, 4.4, 5.a and 5.b
Subjects are summarised according to the product they actually received
The number and the proportion of subjects achieving and not achieving the endpoint are reported
The denominator for calculating the proportions is the number of subjects treated with each product and overall in the full analysis set

Table 11-8: Proportion of Patients with at least One Histologically Proven Adenoma or Carcinoma (FAS; Methylene Blue Full Dose versus Placebo only)

Methylene Blue Full Dose N=485, Placebo N=479

	Type 3 Analysis of Effects					Adjusted Odds Ratio	
Effects	**Degrees of Freedom**	**Wald Chi-Square**	**p-value**	**Comparison**	**Comparison p-value[3]**	**Point Estimate**	**95% Wald Confidence Limits**
Treatment	1	6.5231	0.0106	Methylene Blue Full Dose vs. Placebo	0.0106	1.46	[1.09, 1.96]
Analysis Centre[1]	18	24.1518	0.1501				
Age	1	6.1824	0.0129				
Sex	1	18.6655	<0.0001				
Reason for Colonoscopy	2	5.1142	0.0775				
Number of Excisions[2]	2	98.6387	<0.0001				

Patients were analysed according to the product they actually received. The proportion of patients were analysed through a logistic regression with treatment, analysis centre, age, sex, reason for colonoscopy and number of excisions as fixed effects. Histologically proven Adenomas = Vienna categories 3, 4.1 and 4.2, traditional serrated adenomas and sessile serrated adenomas. Histologically proven Carcinomas = Vienna categories 4.3, 4.4, 5.a and 5.b.

[1]In order to avoid quasi-complete separation of data points, the centres 430 and 431 (Prof. Kiesslich) were pooled together.

[2]Classified as '≤3', '4 - 6' and '>6'

[3]Null hypothesis to be rejected H_0: Adjusted Odds Ratio$_{\text{Methylene Blue Full Dose vs. Placebo}} \leq 1$

N=Number of patients.

Primary Efficacy Endpoint Sensitivity Analyses

Multiple Imputation under MAR Assumption Analysis

When the MAR assumption was used for imputing the missing values for the ITT Set, results by means of the logistic regression model continued to show that there was a statistically significantly higher detection rate of at least one histologically proven Adenoma or Carcinoma in the Methylthioninium chloride full dose group when compared to the placebo group (adjusted odds ratio [95% CI] of 1.46 [1.09, 1.95]) (p-value=0.0114). All variables included in the regression model as fixed effects (i.e., treatment, centre, patients' age and sex, reason for the colonoscopy and the number of excisions) were statistically significant variables in the proportion of patients with at least one histologically proven Adenoma or Carcinoma when comparing differences between the Methylthioninium chloride full dose group and the placebo group (p-values of 0.0111, <0.0001, 0.0120, <0.0001, 0.0066 and <0.0001, respectively).

The results based on MI method were generally consistent with those for the primary analysis based on the FAS, with the exceptions of statistical significance of centre and reason for the colonoscopy, indicating that missing data had a minimal impact on the validity of the primary analysis.

Table 11-9: Proportion of Patients with at least One Histologically Proven Adenoma or Carcinoma (ITT Set, Multiple Imputation under MAR Assumption)

Methylene Blue Full Dose N=504, Placebo N=498

	Type 3 Analysis of Effects					Adjusted Odds Ratio	
Effects	**Degrees of Freedom**	**Wald Chi-Square**	**p-value**	**Comparison**	**Comparison p-value[3]**	**Point Estimate**	**95% Wald Confidence Limits**
Treatment	0.97	6.3391	0.0111	Methylene Blue Full Dose vs. Placebo	0.0114	1.46	[1.09, 1.95]
Analysis Centre[1]	17.25	435.1535	<0.0001				
Age	0.96	6.1785	0.0120				
Sex	0.99	19.1048	<0.0001				
Reason for Colonoscopy	1.87	9.7407	0.0066				
Number of Excisions[2]	2.00	204.4889	<0.0001				

Patients were analysed according to the product they were assigned to. The proportion of patients were analysed through a logistic regression with treatment, analysis centre, age, sex, reason for colonoscopy and number of excisions as fixed effects. Histologically proven Adenomas = Vienna categories 3, 4.1 and 4.2, traditional serrated adenomas and sessile serrated adenomas. Histologically proven Carcinomas = Vienna categories 4.3, 4.4, 5.a and 5.b. Missing data were replaced using a logistic regression method for a binary variable with a monotone missing pattern (100 imputations were performed).

[1]In order to avoid quasi-complete separation of data points, the centres 430 and 431 (Prof. Kiesslich) were pooled together.

[2]Classified as '≤3', '4 - 6' and '>6'

[3]Null hypothesis to be rejected H_0: Adjusted Odds $Ratio_{\text{Methylene Blue Full Dose vs. Placebo}} \leq 1$

N=Number of patients; MAR= Missing at random.

Worst Case Analysis

For the worst case, success is ascribed with ⩽3 excisions for placebo and failure with >6 excisions for Methylene Blue. Nineteen (19) patients assigned to Methylene Blue full dose and excluded from the FAS were analysed as having >6 excisions and no histologically proven Adenoma or Carcinoma; 18 patients assigned to placebo and excluded from the FAS were analysed as having ⩽3 excisions and at least one histologically proven Adenoma or Carcinoma; Patient 131-124, assigned to placebo and excluded from the FAS due to failure to take at least one dose of IMP, underwent colonoscopy and was analysed according to the actual findings (i.e. ⩽3 excisions and at least one histologically proven Adenoma or Carcinoma). When the missing data were imputed by the worst case method for the ITT Set, results by means of the logistic regression model showed that any difference between the Methylene Blue full dose group and the placebo in the proportion of patients with at least one histologically proven Adenoma or Carcinoma (point estimate [95% CI] of 1.13 [0.86, 1.49]) was no longer statistically significant (p-value=0.3862). When used as fixed effects, patients' age and sex, reason for the colonoscopy and the number of excisions were all statistically significant variables in the proportion of patients with at least one histologically proven Adenoma or Carcinoma when comparing differences between the Methylene Blue full dose group and the placebo group (p-values of 0.0134, <0.0001, 0.0244 and <0.0001, respectively). Results showed that neither the treatment nor the centre, were statistically significant variables in the proportion of patients with at least one histologically proven Adenoma or Carcinoma when comparing the Methylene Blue full dose group and the placebo group (p-values of 0.3862 and 0.0941, respectively).

Post-hoc Analyses of the Primary Efficacy Endpoint

In line with relevant ICH guidelines, the number of excisions was removed as a covariate in the logistic regression model among the primary analysis covariates.

A post-hoc logistic regression analysis was conducted where the number of excisions was not included into the model as a covariate. The other pre-specified covariates as per SAP Version 1.0 (treatment, centre, age, sex, and reason for colonoscopy) were maintained in the analysis. The results are summarised in Table 4 and were even more favourable to Methylthioninium chloride Cosmo 200 mg with respect to placebo, with an adjusted OR of 1.51 [1.15, 1.97] and p = 0.0027. Age, sex, and reason for colonoscopy were confirmed to be statistically significant variables, whereas the analysis centre is not significant.

Table 4: Primary Efficacy Analysis: Proportion of Subjects with at Least One Histologically Proven Adenoma or Carcinoma, Methylthioninium chloride Cosmo 200 mg versus Placebo (FAS; Logistic Regression Without Number of Excisions as Covariate) (Study CB-17-01/06)

Methylthioninium chloride Cosmo **200 mg N = 485, Placebo N = 479**							
	Type 3 Analysis of Effects					**Adjusted Odds Ratio**	
Effects	**Degrees of Freedom**	**Wald Chi-Square**	**p-value**	**Comparison**	**Comparison p-value[a]**	**Point Estimate**	**95% Wald Confidence Limits**
Treatment	1	8.9870	0.0027	Methylthioninium chloride Cosmo 200 mg vs. Placebo	0.0027	1.51	[1.15, 1.97]
Analysis Centre	18	27.8141	0.0649				
Age	1	12.9377	0.0003				
Sex	1	26.1826	< 0.0001				
Reason for Colonoscopy	2	11.2987	0.0035				

FAS = Full Analysis Set; MMX = Multi-Matrix; N = number of subjects.

[a] Null hypothesis to be rejected H_0: adjusted odds ratio $_{\text{Methylthioninium chloride Cosmo 200 mg versus placebo}} \leqslant 1$

Note: Subjects were analysed according to the study drug they actually received. The proportion of subjects were analysed through a logistic regression with treatment, analysis centre, age, sex and reason for colonoscopy as fixed effects. Histologically proven adenomas = Vienna categories 3, 4.1, and 4.2, traditional serrated adenomas and sessile serrated adenomas. Histologically proven carcinomas = Vienna categories 4.3, 4.4, 5.a, and 5.b.

Cosmo has performed additional statistical analysis of the primary endpoint using the definition of adenoma, as per protocol Version 1.2 (Amendment 1, dated 16 September 2013):

The primary endpoint of this study is to assess the detection efficacy of chromoendoscopy performed with 200mg Methylthioninium chloride Cosmo 25 mg tablets versus placebo tablets (white light endoscopy) in terms of the proportion of subjects with at least one histologically proven adenoma or carcinoma.

Histologically proven adenoma is defined as Vienna Grade 3 to 4.2.

Histologically proven carcinoma is defined as Vienna Grade 4.3 to 5b.

The analysis of the primary endpoint with the exclusion of SSAs and TSAs was carried out through a logistic regression with treatment, centre, age, sex, reason for colonoscopy (screening, surveillance

within 2 years from previous colonoscopy, and surveillance after more than 2 years from previous colonoscopy), and number of excisions as fixed effects (Table 5).

Table 5: Primary Efficacy Analysis as per Protocol Version 1.2: Proportion of Subjects with at least One Histologically Proven Adenoma or Carcinoma, Excluding TSAs and SSAs, Methylthioninium chloride Cosmo 200 mg versus Placebo (FAS; Logistic Regression Analysis) (Study CB-17-01/06)

Methylene Blue MMX 200 mg N = 485, Placebo N = 479							
	Type 3 Analysis of Effects					**Adjusted Odds Ratio**	
Effects	**Degrees of Freedom**	**Wald Chi-Square**	**p-value**	**Comparison**	**Comparison Two-sided p-value**	**Point Estimate**	**95% Wald Confidence Limits**
Treatment	1	3.8551	0.0496	Methylene Blue MMX 200 mg vs. Placebo	0.0496	1.3088	[1.0005, 1.7120]
Analysis Centre	18	28.9488	0.0490				
Age	1	15.0369	0.0001				
Sex	1	25.4349	< 0.0001				
Reason for Colonoscopy	2	14.9656	0.0006				

FAS = Full Analysis Set; MMX = Multi-Matrix; N = number of subjects; SSA = sessile serrated adenoma; TSA = traditional serrated adenoma.
Note: Null hypothesis to be rejected H_0: adjusted odds ratio $_{\text{Methylene Blue MMX 200 mg versus placebo}} \leqslant 1$
Subjects were analysed according to the study drug they actually received. The proportion of subjects was analysed through a logistic regression with treatment, analysis centre, age, sex, reason for colonoscopy and number of excisions as fixed effects. Histologically proven adenomas = Vienna categories 3, 4.1, and 4.2. Histologically proven carcinomas = Vienna categories 4.3, 4.4, 5.a, and 5.b.

The analysis confirmed the results of the primary analysis for this dossier as per SAP.

Secondary Efficacy Endpoints

False positive Rate (FPR) (at the individual level)

Table 11-12: False Positive Rate (FAS)

	Methylene Blue Full Dose N=485 n (%)	Methylene Blue Low Dose N=241 n (%)	Placebo N=479 n (%)	Overall N=1205 n (%)
Patients with excisions[1]	356 (73.40)	168 (69.71)	326 (68.06)	850 (70.54)
Patients with excisions and without any histologically proven Adenoma or Carcinoma[2]	83 (23.31)	44 (26.19)	97 (29.75)	224 (26.35)
Difference in FPRs (%) and 95% CI of Methylene Blue Full Dose vs. Placebo	-6.44	[-13.07, 0.19]		
p-value of difference in FPRs[3]	<0.0001			
Difference in FPRs (%) and 95% CI of Methylene Blue Low Dose vs. Placebo	-3.56	[-11.86, 4.73]		
p-value of difference in FPRs[4]	<0.0001			
Difference in FPRs (%) and 95% CI of Methylene Blue Full Dose vs. Methylene Blue Low Dose	-2.88	[-10.84, 5.09]		
p-value of difference in FPRs[5]	<0.0001			

Patients are summarised according to the product they actually received. Histologically proven Adenomas = Vienna categories 3, 4.1 and 4.2, traditional serrated adenomas and sessile serrated adenomas. Histologically proven Carcinomas = Vienna categories 4.3, 4.4, 5.a and 5.b.

[1]The denominator for calculating the proportions was the number of patients treated with each product and overall in the FAS.

[2]The number of false positive patients and the FPRs are reported. FPR=Patients with excisions and without any histologically confirmed Adenoma or Carcinoma/Patients with excisions

[3]Null hypothesis to be rejected H_0: $FPR_{\text{Methylene Blue Full Dose}} - FPR_{\text{Placebo}} \geq 15\%$.

[4]Null hypothesis to be rejected H_0: $FPR_{\text{Methylene Blue Low Dose}} - FPR_{\text{Placebo}} \geq 15\%$.

[5]Null hypothesis to be rejected H_0: $FPR_{\text{Methylene Blue Full Dose}} - FPR_{\text{Methylene Blue Low Dose}} \geq 15\%$.

N=Number of patients; CI=Confidence interval; %=Percentage; FPR=False positive rate.

Post-hoc Analyses of the False Positive Rate (at excision level)

Following a request by another Agency, an additional post-hoc analysis of FPR was performed at the excision level, to compare treatment with Methylthioninium chloride Cosmo 200 mg versus placebo with regard to the number of excised lesions identified as false positives.

Overall, there were 1356/2780 (48.8%) false positives (histology negative lesions) in the FAS, with slightly higher proportions reported in both the Methylthioninium chloride Cosmo 200 mg group (592/1189; 49.8%) and the Methylthioninium chloride Cosmo 100 mg group (283/571; 49.6%) compared to the placebo group (481/1020; 47.2%).

The upper limit of the 95% CI for the difference in the FPRs between the Methylthioninium chloride Cosmo 200 mg group and the placebo group was 6.81%, less than the threshold of 15% for rejecting the null hypothesis that the difference is higher than 15%. The corresponding p-value for testing the null hypothesis was < 0.0001. Similar results were observed for the PP Population.

Table 7: False Positive Rate (Excision Level; FAS) (Study CB-17-01/06)

	Methylthioninium chloride Cosmo 200 mg N = 1189	Methylthioninium chloride Cosmo 100 mg N = 571	Placebo N = 1020	Overall N = 2780
	n (%)			
False Positives (Histology Negative Lesions)[a]	592 (49.79)	283 (49.56)	481 (47.16)	1356 (48.78)
True Positives (Histology Positive Lesions)[a]	597 (50.21)	288 (50.44)	539 (52.84)	1424 (51.22)
Difference in FPRs (%) and 95% CI of Methylthioninium chloride Cosmo 200 mg vs. Placebo[b]	2.63	[-1.55, 6.81]		
p-value of Difference in FPRs[b]	<0.0001			

CI = confidence interval; FAS = Full Analysis Set; FPR = false positive rate; MMX = Multi-Matrix; N/n = Number of subjects.

14 The denominator for calculating the proportions was the number of excised lesions in each treatment group and overall in the FAS

15 Null hypothesis to be rejected H_0: $FPR_{\text{Methylthioninium chloride Cosmo 200 mg}} - FPR_{\text{Placebo}} \geq 15\%$

Note: Subjects are summarised according to the study drug they actually received. Histologically proven adenomas = Vienna categories 3, 4.1, and 4.2, traditional serrated adenomas and sessile serrated adenomas. Histologically proven carcinomas = Vienna categories 4.3, 4.4, 5.a, and 5.b.

Post hoc analysis of FPR excluding SSA and TSA

Following the request by the DKMA to perform the statistical analysis on the primary endpoint according to the initial definition of adenoma per protocol Version 1.2 (Amendment 1, dated 16 September 2013), Cosmo also investigated the outcome of the main secondary endpoint of the trial, the FPR, using the initial definition of adenoma. The result of this exploratory post-hoc analysis for the FAS is reported in Table 8.

Table 8: False Positive Rate as per Protocol Version 1.2, Excluding TSAs and SSAs (Subject Level; FAS) (Study CB-17-01/06)

	Methylene Blue MMX 200 mg N = 485	Methylene Blue MMX 100 mg N = 241	Placebo N = 479	Overall N = 1205
	n (%)			
Subjects with Excisions[a]	356 (73.40)	168 (69.71)	326 (68.06)	850 (70.54)
Subjects with Excisions and Without Any Histologically Proven Adenoma or Carcinoma (excluding TSA and SSA)[b]	111 (31.18)	52 (30.95)	109 (33.44)	272 (32.00)
Difference in FPRs (%) and 95% CI of Methylene Blue MMX 200 mg vs. Placebo	-2.26	[-9.28, 4.77]		
p-value of Difference in FPRs[c]	< 0.0001			

CI = confidence interval; FAS = Full Analysis Set; FPR = false positive rate; MMX = Multi-Matrix; N/n = Number of subjects; SSA = sessile serrated adenoma; TSA = traditional serrated adenoma.

[a] The denominator for calculating the proportions was the number of subjects treated with each study drug and overall in the FAS

[b] The number of false positive subjects and the FPRs are reported. FPR = Subjects with excisions and without any histologically confirmed adenoma or carcinoma/Subjects with excisions

[c] Null hypothesis to be rejected H_0: $FPR_{Methylene\ Blue\ 200\ mg} - FPR_{Placebo} \geqslant 15\%$
Note: Subjects are summarised according to the study drug they actually received. Histologically proven adenomas = Vienna categories 3, 4.1, and 4.2 (excludes traditional serrated adenomas and sessile serrated adenomas). Histologically proven carcinomas = Vienna categories 4.3, 4.4, 5.a, and 5.b.
Data Source: Study CB-17-01/06 Post-hoc Analysis Table 14.2.3.1a

Proportion of Patients with at least One Histologically Proven Adenoma

Overall, 621/1205 patients (51.54%) in the FAS had at least one histologically proven Adenoma, with a higher percentage of patients (55.88%) in the Methylthioninium chloride Cosmo full dose group when compared to the Methylthioninium chloride Cosmo low dose group (51.45%) and the placebo group (47.18%). There was a higher detection rate of patients with at least one histologically proven Adenoma (odds ratio [95% CI] of 1.42 [1.10, 1.83]) in the Methylthioninium chloride Cosmo full dose group than the placebo group and the difference was statistically significant (Fisher's Exact test p-value=0.0082).

There were only small differences in the proportion of patients with at least one histologically proven Adenoma between the Methylthioninium chloride Cosmo low dose group and the placebo group (odds ratio [95% CI] of 1.19 [0.87; 1.62]); also between the Methylthioninium chloride Cosmo full dose group and the Methylthioninium chloride Cosmo low dose group (odds ratio [95% CI] of 1.19 [0.88, 1.63]), neither was statistically significant.

Proportion of Patients with at least One Histologically Proven Carcinoma;

Only a small proportion of patients in the FAS (17/1205 patients; 1.41%) had at least one histologically proven Carcinoma, whilst the majority (1188/1205 patients; 98.59%) did not have any carcinoma. The proportion of patients with at least one histologically proven Carcinoma fell between 1% and 2% for the three treatment groups with no statistically significant differences recorded.

Number of histologically proven Adenomas or a histologically proven TSA, or a histologically proven SSA and Carcinomas detected per patient;

Overall, a mean number of 1.2 (SD=1.8) histologically proven Adenomas and Carcinomas were detected per patient in the FAS, individually ranging from 0 to 13. Mean values were similar for all three treatment groups: 1.2 (SD=1.8) for the Methylthioninium chloride Cosmo full dose group; 1.2 (SD=1.7) for the Methylthioninium chloride Cosmo low dose group and 1.1 (SD=1.8) for the placebo group with differences not regarded statistically significant.

Number of histologically proven hyperplastic polyps, fibroblastic polyps and mixed polyps detected per patient;

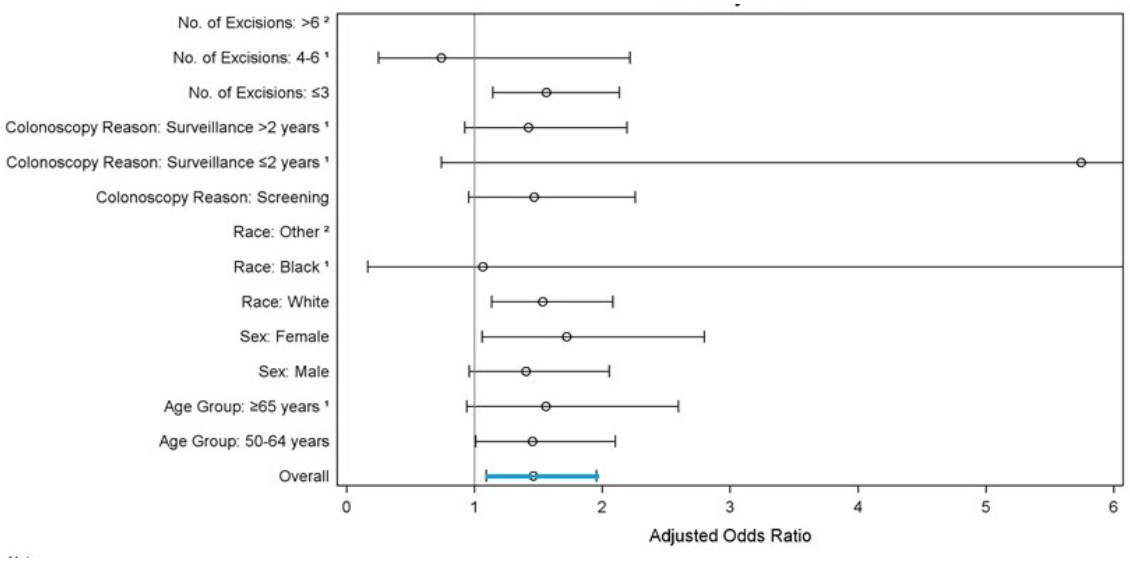
A mean number of 0.6 (SD=1.2) hyperplastic, fibroblastic and mixed polyps were detected per patient for the overall population in the FAS (ranging from 0 to 9), with the highest number in the Methylthioninium chloride Cosmo full dose group with a mean of 0.7 (SD=1.3) (ranging from 0 to 9). The Methylthioninium chloride Cosmo low dose group had a mean of 0.6 (SD=1.2) (ranging from 0 to 8) and the placebo group, 0.5 (SD=1.2) (ranging from 0 to 7). The difference in means (0.16 [95% CI: 0.01; 0.30]) between the Methylthioninium chloride Cosmo full dose group and the placebo group was statistically significant (p-value=0.0326).

Ancillary analyses

Comparison of Results in Subpopulations

As part of the SCE analyses of data, subgroup analyses of the adenoma or carcinoma detection rate, FPR, ADR, and non-polypoid lesion detection rate were performed by age group (18 to 49 years, 50 to 64 years, ⩾ 65 years), sex (male, female), race (White, Black, Other), reason for colonoscopy (screening, surveillance within 2 years from previous colonoscopy, surveillance after more than 2 years from previous colonoscopy), and number of excisions (⩽ 3; 4 to 6; > 6) for Studies CB-17-01/06 and CB-17-01/05. Although Study CB-17-01/05 was included in the SCE analyses, very limited conclusions could be drawn based on the low numbers of subjects treated in the preliminary efficacy study. Thus, data discussed herein are limited to Study CB-17-01/06. Forest plots are presented for Study CB-17-01/06 for overall FAS and by subgroups for the efficacy for the primary endpoint. Similar figures (not shown) were completed for the secondary endpoints. All statistical models used for subgroups had the same factors as in the primary efficacy endpoint analysis, except for the factor relating to the subgroup being presented (i.e., that term was not included in the model). The other difference is that the primary analysis included Methylthioninium chloride Cosmo 200 mg and placebo only in the analysis model for the treatment group term, and the secondary analyses included Methylthioninium chloride Cosmo 200 mg, Methylthioninium chloride Cosmo 100 mg, and placebo.

Figure : Adenoma or Carcinoma Detection Rate, Overall and by Subgroups (FAS; Study CB-17-01/06)



CI = confidence interval; FAS = Full Analysis Set; MMX = Multi-Matrix.
Adjusted odds ratio and CI may not be accurate due to quasi-complete separation of data points being detected in model fit.
Adjusted odds ratio and CI not presented due to poor model fit.
Note: Figure presents the adjusted odds ratio and associated 95% CI for comparison of Methylthioninium chloride Cosmo 200 mg and placebo.
Statistical models for subgroups use the following factors, except the factor relating to the subgroup being considered is not included in the model: treatment group (Methylthioninium chloride Cosmo 200 mg and placebo only), centre, age, sex, reason for colonoscopy, and number of excisions. The overall analysis included all terms.
For Race: Black, upper limit of the 95% CI extends to 6.84.
For Colonoscopy Reason: Surveillance ⩽ 2 years, upper limit of the 95% CI extends to 44.51. No subjects were aged between 18 and 49 years.

Analysis of Clinical Information Relevant to Dosing recommendation

A Methylthioninium chloride Cosmo 100 mg group was included in the pivotal Study CB-17-01/06 for masking purposes, in order to reduce the potential acquisition bias due to the possible lack of investigator (endoscopist) and subject blinding between placebo and Methylthioninium chloride Cosmo 200 mg groups after dosing. Fewer subjects were enrolled in that dose group, and the study was not powered to show statistically significant differences between the Methylthioninium chloride Cosmo 100 mg group and placebo, or between the Methylthioninium chloride Cosmo 100 mg group and the 200 mg group. However, overall, 241 subjects were randomised to the 100 mg group in the FAS and this allowed a comparison of efficacy among the doses.

Table 36: Overview of Results by Dose (FAS; Study CB-17-01/06)

Endpoint	Methylthioninium chloride Cosmo 200 mg	Methylthioninium chloride Cosmo 100 mg	Placebo (N = 479)
Primary Efficacy Endpoint: Subjects with at least one histologically proven adenoma or carcinoma, %	56.3	51.5	47.8
False Positives: Subjects with excisions and without any histologically proven adenomas or carcinomas, %	23.3	26.2	29.8
Subjects with at least one histologically proven adenoma, %	55.9	51.5	47.2
Subjects with at least one histologically proven adenoma or carcinoma (subjects with 0 to 1 excisions), %	26.2	20.8	18.9
Subjects with at least one histologically proven adenoma or carcinoma (subjects with ⩽ 3 excisions), %	45.3	38.1	35.7
Subjects with at least one histologically proven adenoma and without any histologically proven carcinoma, %	55.3	50.2	45.9
Subjects with at least one non-polypoid lesion, %	43.9	42.7	35.1
Subjects with at least one proven adenoma or carcinoma (< 5 mm), %	37.1	33.2	30.9

FAS = Full Analysis Set; N = number of subjects

Summary of main efficacy results

The following tables summarise the efficacy results from the main study supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy and the benefit risk assessment (see later sections).

Summary of efficacy for trial CB-17-01/06

Title: The safety and efficacy of Methylthioninium chloride Cosmo prolonged release tablets administered to patients undergoing screening or surveillance colonoscopy		
Study identifier	Sponsor code: CB-17-01/06 EudraCT Number:2012-003983-32 ClinicalTrials.gov identifier (NCT number): NCT01694966	
Design	The study was a multi-centre, multinational, placebo controlled, randomised, double-blind at randomisation, parallel-group Phase III study of Methylthioninium chloride Cosmo (multimatrix structure) 'MMX®' prolonged release tablets compared to placebo in the treatment of patients undergoing full colonoscopy for colorectal cancer (CRC) screening or surveillance. Patients were randomised to the treatment group 200 mg of Methylthioninium chloride Cosmo tablets (Group One) or placebo tablets (Group Two) or the reduced dose group 100 mg of Methylthioninium chloride Cosmo tablets (Group Three) in a ratio of 2:2:1. The reduced dose group was included only for masking purposes in order to reduce the acquisition bias due to the lack of investigator and patient blinding between placebo and Methylthioninium chloride MMX 200 mg groups.	
	Duration of main phase:	First patient enrolled: 15 December 2013 Last patient enrolled: 20 October 2016
Hypothesis	Superiority of Methylthioninium chloride Cosmo (200mg arm) compared to placebo (High Definition White Light endoscopy) in terms of proportion of patients with at least one histologically proven Adenoma or Carcinoma detected. Low dose (100 mg) arm was included for confounding purposes to reduce potential acquisition bias only.	
Treatments groups	Methylthioninium chloride Cosmo 200 mg (MB MMX 200 mg)	Treatment: 8 tablets of Methylthioninium chloride Cosmo 25 mg tablets taken (total dose of methylthioninium chloride: 200 mg) Duration: single administration *per os* during the intake of the bowel cleansing preparation the day before the colonoscopy Number randomized: 504
	High Definition White Light (HDWL) Endoscopy	Treatment: 8 tablets of Methylthioninium chloride Cosmo placebo tablets Duration: single administration *per os* during the intake of the bowel cleansing preparation the day before the colonoscopy Number randomized: 498
	Methylthioninium chloride Cosmo 100 mg (MB MMX 100 mg)	Treatment: 4 tablets of Methylthioninium chloride Cosmo 25 mg tablets and 4 tablets of Methylthioninium chloride Cosmo placebo tablets (total dose of methylthioninium chloride: 100 mg) Duration: single administration *per os* during the intake of the bowel cleansing preparation the day before the colonoscopy Number randomized: 247

Endpoints and definitions	Primary endpoint	Adenoma Detection Rate (ADR)	Proportion of subjects with at least one histologically proven Adenoma or Carcinoma: i.e., proportion of subjects who were found with at least one lesion during study colonoscopy, which was excised or biopsied and was subsequently histologically confirmed to be an Adenoma or Carcinoma Histologically proven Adenoma was defined as Vienna Grade 3 to 4.2, or Traditional Serrated Adenoma (TSA), or Sessile Serrated Adenoma (SSA). Histologically proven Carcinoma was defined as Vienna Grade Vienna Grade 4.3 to 5b.
	Secondary	False Positive Rate (FPR)	Proportion of subjects with no histologically confirmed Adenoma or Carcinoma within any of the subjects' excised lesions and the subject having undergone at least one excision
	Secondary	Proportion of subjects with at least one Adenoma	Proportion of subjects who were found with at least one lesion during study colonoscopy, which was excised or biopsied and was subsequently histologically confirmed to be an Adenoma Histologically proven Adenomas were defined as above
	Secondary	Nr. of hyperplastic, fibroblastic and mixed polyps per subject	Number of histologically proven hyperplastic, fibroblastic and mixed polyps detected per subject
	Exploratory	Proportion of subjects with at least one Adenoma and without any Carcinoma	Proportion of subjects who were found with at least one lesion during study colonoscopy, which was excised or biopsied and was subsequently histologically confirmed to be a Adenoma, and had no histologically proven Carcinoma in any of the other subjects' excised lesions Histologically proven Adenomas or Carcinomas were defined as above
	Exploratory	Proportion of subjects with at least 1 non-polypoid lesion detected	Proportion of subjects with at least one lesion with non-polypoid morphology detected. Lesion indicates an abnormal area at the surface of the mucosa that was detected by any of the following elements: (1) obvious elevation or depression, (2) mucosal nodular irregularity, (3) interruption of the course of superficial vascular network, regardless of the final histological classification Morphology of the lesions was assessed during colonoscopy according to Paris classification; non-polypoid lesions were defined as either class IIa, IIb, IIc or III

	Exploratory	Nr. of non-polypoid lesions per subject	Number of non-polypoid lesions detected per subject Morphology and lesion were defined as above
	Exploratory	Proportion of subjects with at least one Adenoma or Carcinoma <5 mm	Proportion of subjects who were found with at least one lesion less than 5 mm in size during study colonoscopy, which was excised or biopsied and was subsequently histologically confirmed to be an Adenoma or Carcinoma Histologically proven Adenomas or Carcinomas were defined as above
	Exploratory	Proportion of Subjects with at least One Histologically Proven Adenoma or Carcinoma for Subjects with $\leqslant$3 Excisions	Subgroup analysis of ADR (as defined above) in the subgroup of subjects who had 3 or less lesions excised. Subjects with no lesions excised are included in this subgroup
	Exploratory	Proportion of Subjects with at least One Histologically Proven Adenoma or Carcinoma for Subjects with 0-1 excisions	Subgroup analysis of ADR (as defined above) in the subgroup of subjects who had 1 or no lesions excised.
	Post-hoc	Nr. of excised lesions	Number of lesions which were excised or biopsied Lesion was defined as above
	Post-hoc	Proportion of subjects with at least one excised lesion	Proportion of subjects who had at least one lesion excised or biopsied Lesion was defined as above
	Post-hoc	Proportion of subjects with at least one Adenoma or Carcinoma <10 mm	Proportion of subjects who were found with at least one lesion less than 10 mm in size during study colonoscopy, which was excised or biopsied and was subsequently histologically confirmed to be an Adenoma or Carcinoma Histologically proven Adenoma and Carcinoma were defined as above Morphology and lesion were defined as above

	Post-hoc	Proportion of subjects with at least one non-polypoid Adenoma or Carcinoma	Proportion of subjects who were found with at least one non-polypoid lesion during study colonoscopy, which was excised or biopsied and was subsequently histologically confirmed to be an Adenoma or Carcinoma Histologically proven Adenoma and Carcinoma were defined as above Morphology and lesion were defined as above
	Post-hoc	Proportion of subjects with at least one non-polypoid Adenoma or Carcinoma <10 mm	Proportion of subjects who were found with at least one non-polypoid lesion less than 10 mm in size during study colonoscopy, which was excised or biopsied and was subsequently histologically confirmed to be an Adenoma or Carcinoma Histologically proven Adenoma and Carcinoma were defined as above Morphology and lesion were defined as above
	Post-hoc	Scheduled surveillance <10 years	Proportion of subjects who went into a surveillance program shorter than 10 years based on the recommendations of the US guidance of surveillance after screening and polypectomy (Lieberman et al. Gastroenterology 2012). Subjects who were found to have a histologically proven Carcinoma at their study colonoscopy were excluded from the analysis
	Post-hoc	Dose-response relationship analysis for ADR	Analysis of the dose-response relationship for the primary endpoint ADR (as defined above)
	Post-hoc	Dose-response relationship analysis for proportion of subjects with at least one excised non-polypoid lesion	Analysis of the dose-response relationship for the primary endpoint for proportion of subjects with at least one excised non-polypoid lesion (as defined above)
	Post-hoc	Dose-response relationship analysis for proportion of subjects with at least one excised non-polypoid lesion <10 mm	Analysis of the dose-response relationship for the primary endpoint for proportion of subjects with at least one excised non-polypoid lesion <10 mm (as defined above)

	Post-hoc	Dose-response relationship analysis for proportion of subjects with at least one histologically proven Adenoma or Carcinoma <10 mm	Analysis of the dose-response relationship for the primary endpoint for proportion of subjects with at least one histologically proven Adenoma or Carcinoma <10 mm (as defined above)
	Post-hoc	Dose-response relationship analysis for proportion of subjects with at least one non-polypoid histologically proven Adenoma or Carcinoma <10 mm	Analysis of the dose-response relationship for the primary endpoint for proportion of subjects with at least one non-polypoid histologically proven Adenoma or Carcinoma <10 mm (as defined above)
Database lock	November 4th 2016		

Results and Analysis				
Analysis description	**Primary Analysis**			
Analysis population and time point description	Full Analysis Set (FAS): All randomised subjects who received at least one dose of the IMP and underwent colonoscopy (regardless of the completion status). This analysis set was used for the primary efficacy analysis. Reasons for exclusion from the FAS were: - Failure to have taken at least one dose of the IMP; - Lack of any data post randomisation (i.e. colonoscopy not performed). The primary analysis was performed on the subjects included in the FAS.			
Descriptive statistics and estimate	Treatment group	MB MMX 200 mg (Full dose)	MB MMX 100 mg (Low Dose)	HDWL Endoscopy
	Number of subjects	485	241	479
	Primary endpoint: Adenoma Detection Rate (ADR)	273 (56.29%)	124 (51.45%)	229 (47.81%)
	Secondary endpoint: False Positive Rate (FPR)	83 (23.31%)	44 (26.19%)	97 (29.75%)
	Secondary endpoint: Proportion of subjects with at least one Adenoma	271 (55.88%)	124 (51.45%)	226 (47.18%)
	Secondary endpoint: Nr. of hyperplastic, fibroblastic and mixed polyps per subject	0.7	0.6	0.5
	SD CV% Min. Median Max.	1.3 186.9 0 0.0 9	1.2 197.5 0 0.0 8	1.0 195.9 0 0.0 7
	Exploratory endpoint: Proportion of subjects with at least one Adenoma and without any Carcinoma	268 (55.26%)	121 (50.21%)	220 (45.93%)
	Exploratory endpoint: Proportion of subjects with at least 1 non-polypoid lesion detected	213 (43.92%)	103 (42.74%)	168 (35.07%)
	Exploratory endpoint: Nr. of non-polypoid lesions detected per subject	1.5	1.2	1.2
	SD CV% Min. Median Max.	2.9 190.4 0 0.0 27	2.4 190.8 0 0.0 18	2.9 249.6 0 0.0 29

	Exploratory endpoint: Proportion of subjects with at least one Adenoma or Carcinoma <5 mm	180 (37.11%)	80 (33.20%)	148 (30.90%)
	Exploratory endpoint: Proportion of Subjects with at least One Histologically Proven Adenoma or Carcinoma for Subjects with ⩽3 Excisions	164 (45.30%)	69 (38.12%)	134 (35.73%)
	Number of subjects in the subgroup	362	181	375
	Exploratory endpoint: Proportion of Subjects with at least One Histologically Proven Adenoma or Carcinoma for Subjects with 0-1 Excisions	61 (26.18%)	25 (20.83%)	50 (18.94%)
	Number of subjects in the subgroup	233	120	264
	Post-hoc: Nr. of excised lesions	1189	571	1020
	Post-hoc: Proportion of subjects with at least one Adenoma or Carcinoma <10 mm	256 (52.78%)	114 (47.30%)	207 (43.22%)
	Post-hoc: Proportion of subjects with at least one non-polypoid Adenoma or Carcinoma	125 (25.77%)	51 (21.16%)	92 (19.21%)
	Post-hoc: Proportion of subjects with at least one non-polypoid Adenoma or Carcinoma <10 mm	116 (23.92%)	48 (19.92%)	80 (16.70%)
	Post-hoc: Scheduled surveillance <10 years	250 (52.1%)	109 (45.8%)	209 (44.5%)
	Number of subjects in the analysis	480	238	470
	Post-hoc: Dose-response relationship analysis for ADR	273 (56.29%)	124 (51.45%)	229 (47.81%)
	Post-hoc: Dose-response relationship analysis for proportion of subjects with at least one excised non-polypoid lesion	212 (43.71%)	102 (42.32%)	167 (34.86%)

	Post-hoc: Dose-response relationship analysis for proportion of subjects with at least one excised non-polypoid lesion <10 mm	205 (42.27%)	99 (41.08%)	158 (32.99%)
	Post-hoc: Dose-response relationship analysis for proportion of subjects with at least one histologically proven Adenoma or Carcinoma <10 mm	256 (52.78%)	114 (47.30%)	207 (43.22%)
	Post-hoc: Dose-response relationship analysis for proportion of subjects with at least one non-polypoid histologically proven Adenoma or Carcinoma <10 mm	116 (23.92%)	48 (19.92%)	80 (16.70%)

Clinical studies in special populations

Only patients age 50-75 were included in the pivotal study (i.e. patients eligible for entering a colonoscopy screening programme). A limited number of patients between 75 and 80 years were included in the supportive studies. All patients with ALT, AST, GGT, Bilirubin, Creatinine or Urea greater than 2.5 x the upper limit for normal range were excluded from the pivotal study, thus patients with more severe hepatic and/or renal impairment were not studied.

	Age 65-74 (Older subjects number /total number)	Age 75-84 (Older subjects number /total number)	Age 85+ (Older subjects number /total number)
Controlled Trials (CB-17-01/06)	419/1208	13/1208	0/1208
Non Controlled trials*	77/358	4/358	0/358

All subjects included in the NDA ISS, i.e. those in the Safety Analysis Set
*Non-controlled trials CB-17-01/02, CB-17-01/03, CB-17-01/04, CB-17-01/05 and CB-17-01/08

Supportive study(ies)

Study CB-17-01/05: Polyp detection rate after single oral dose of Methylthioninium chloride Cosmo prolonged release tablets administered to subjects undergoing outpatients colonoscopy

Open label, uncontrolled, efficacy, exploratory, descriptive study. Patients undergoing screening or surveillance colonoscopy were treated with Methylthioninium chloride Cosmo during the cleansing

procedure prior to endoscopy. Subsequent colonoscopy was performed as usual removing all identified polyps.

Study CB-17-01/04: Intraepithelial neoplasia detection rate after single oral dose of Methylthioninium chloride Cosmo prolonged release tablets administered to patients with long standing ulcerative colitis undergoing colonoscopy

This was an open label, efficacy, exploratory, descriptive study. Patients with long standing ulcerative colitis who, as per guideline recommendations underwent screening colonoscopy for CRC, were treated with Methylthioninium chloride Cosmo during the cleansing procedure prior to endoscopy. During subsequent colonoscopy, as per guideline, 2 biopsies were taken randomly from each segment of the colon (ascending colon, transverse colon, descending colon and rectum/sigmoid) as well as from any identified lesion.

Due to the design (open label, uncontrolled) and the study population (for study CB-17-01/04) the results of these studies are of very limited support for the proposed indication, and not presented.

2.7.3. Discussion on clinical efficacy

Design and conduct of clinical studies

Demonstration of clinical efficacy and safety of Methylthioninium chloride Cosmo in the proposed indication is based on a single pivotal study (study CB 17 01/06). As stated during previous scientific advice given by the CHMP, this could be acceptable provided that the study is well conducted and provides statistically and clinically significant and robust results.

The objective of study CB 17 01/06 was to investigate if Methylthioninium chloride Cosmo, compared to placebo, increases ADR (adenoma detection rate) which is a generally accepted measure of the quality of the screenings and surveillance colonoscopies. This objective is considered relevant and adequately reflected in the primary endpoint and the primary efficacy analysis.

Study CB 17 01/06 was a randomised, double-blind, placebo controlled study comparing Methylthioninium chloride Cosmo to placebo in terms of ADR in subjects scheduled for screening or surveillance colonoscopy. Overall this design is considered acceptable.

The inclusion and exclusion criteria selected a population with very little concomitant disease whose main medical problem was age-defined screening for colorectal cancer or surveillance for previously identified colorectal polyps. Considering the proposed indication, this is acceptable.

Patients were randomised to receive either test drug (Methylthioninium chloride Cosmo), 200 mg or 100 mg or matching placebo. Treatment was given as add-on to the usual standard PEG based cleansing procedure prior to colonoscopy. Both study medication (Methylthioninium chloride COsmo or placebo) and background treatment is adequately described. The efficacy of the drug was investigated in combination with 4 L PEG based bowel preparation procedure. Use of Methylthioninium chloride Cosmo is restricted to this volume and type of bowel preparation**.**

The primary endpoint was the proportion of patients with at least one histologically proven Adenoma or Carcinoma. Adenoma was defined as a histologically proven Vienna Grade 3 to 4.2 or a histologically proven Traditional Serrated Adenoma (TSA), or a histologically proven Sessile Serrated Adenoma (SSA). Histologically proven Carcinoma was defined as Vienna Grade 4.3 to 5b. It is generally accepted that adenoma detection rate is a surrogate for the risk reduction (for colorectal cancer) which can be achieved by colonoscopy. The higher the ADR (and subsequent removal of identified adenomas), the

higher the reduction in colorectal cancer risk. In that respect, ADR is considered an appropriate primary endpoint. However, while the inclusion of adenomas and cancers follow the generally accepted definition of ADR, the inclusion of sessile serrated adenomas (SSA) and traditional serrated adenomas (TSA) is at least debatable. It is fully acknowledged that both these types of lesions are associated with increased risk of cancer. However, the data on the effect of removal of such lesions on the subsequent colorectal cancer risk is less robust than for the classical adenomas. Thus, the proposed primary endpoint (including SSA and TSA as well as classic adenomas) was considered acceptable and backed up by secondary endpoints investigating the effect on ADR using the previously applied definition of ADR, i.e. only including classic adenomas and excluding TSA and SSA. The change in primary endpoint is considered scientifically justified and is not considered to invalidate the internal validity of the study as the change of the primary endpoint occurred prior to unblinding, thus precluding that the decision to change endpoints could have been data driven. Furthermore, as endoscopist from the very start of the study (i.e. before the change in endpoint) were required to remove all lesions detected, the potential effect of the change of endpoint on endoscopists' selection of lesions to be removed can be considered negligible. In addition, the applicant has stated that a Central Endoscopy review provided an independent confirmation that each colonoscopy was complete (caecum reached), that biopsies and excisions had been appropriately performed, that all the lesions had been detected and appropriately managed and whether or not the sampled area was a stained region. This lends additional support to the conclusion that the above-mentioned change of primary endpoint did not induce bias in terms of changes in selection of lesions to be removed.

As regards bias introduced in subsequent handling and reading of biopsies, the applicant has explained that the study Histology Charter for the classification of the biopsied or resected lesions did not change as a consequence of the revision in the definition of adenoma during the study. From study start, the central histologists were required to classify the lesions according to the Vienna classification or to the serrated lesions classification (including SSAs and TSAs). Pathologist were blinded to the treatment received and during the blinded processing of biopsies/lesions prior to histological evaluation residual methylthioninium chloride staining was removed/covered by the sample preparation and staining. Thus, pathologists were blinded to treatment and consequently any bias as a result of the change of primary endpoint is unlikely. In conclusion, the change in primary endpoint is not considered to have a detrimental effect on the internal validity of the study.

The sample size calculations were based on results of previous studies. The Applicant considered that a 10 % increase in detection of lesions to be clinically significant. The sample size calculations are endorsed. Patients were randomization according to an adequately described and conducted strategy.

Due to the colour of the product, it is not feasible to assure a complete blinding of the colonoscopists. Thus, the Applicant included a third arm in the study, where patients received a low dose of Methylthioninium chloride Cosmo to mitigate this effect. The rest of the staff involved was blinded to the treatment assignment. The blinding strategy is endorsed. However, it is noted that in essence neither endoscopist nor patient could remain blinded.

In general, the statistical analysis plan is endorsed. The use of the FAS (full analysis set) for the primary analysis is agreed.

The primary endpoint is related to difference in proportion of subjects with at least one histologically proven Adenoma or Carcinoma found during colonoscopy between methylthioninium chloride Cosmo and placebo. The use of logistic regression to calculate the odds ratio is endorsed. The inclusion of the stratification variables used at randomization (centre and reason for colonoscopy) in the logistic model is agreed. The use of age and sex in the logistic model are not agreed. The main reason to include a covariate in the analysis of a trial is the existence of strong or moderate association between the covariate and the primary outcome measure. According to the Guideline on adjustment for baseline

covariates (EMA/295050/2013), known or expected associations with the primary outcome variable should be justified on the basis of previous evidence (possibly data from previous or other current trials) and/or on clinical grounds. When there is a lack of such established prior knowledge, it is safer to use a simple model with no, or only a few, covariates. The applicant also included a post-baseline variable "number of excisions". This is not endorsed since this variable is affected by treatment. However, the applicant has performed additional analyses for the primary endpoint where only the stratification variables (centre and reason for colonoscopy) and treatment were included in the model and these support the primary analysis.

The applicant was also requested to perform additional analysis for the primary endpoint using the Fisher Exact test and FAS according to the original randomization list, regardless of the treatment actually received by the patients and excluding TSAs and SSAs from the primary endpoint

The sensitivity analysis planned for the primary endpoint are agreed. The calculations for the secondary endpoints are endorsed.

A number of protocol violations have been noted. However, neither the number nor the nature of these violations raises any concerns about the conduct of the study. As previously mentioned, the primary efficacy endpoint was changed to include (in addition to "classical" adenomas) TSA and SSA. The rationale for this change is supported as TSA and SSA do have potential to develop into cancer.

Efficacy data and additional analyses

Included patients were representative of the proposed indication. The three study groups were well balanced in term of demographics, previous medical history and previous and concomitant medication. Treatment compliance was high and similar across the three treatment groups.

The applicant defined three analyses sets: the safety set, the full analysis set (FAS) and the Per Protocol Set (PP). Subjects randomized to a wrong stratum or receiving the wrong treatment were analysed by the applicant according to the treatment they actually received and to their actual reason for colonoscopy (regardless of the stratum they were assigned to) in the FAS and PP (per protocol) analyses. This is methodologically not appropriate. However, the applicant provided sensitivity analyses demonstrating that using the (correct) assigned stratum/population for these patients did not affect results significantly. The definitions of the safety and per protocol sets are considered acceptable.

The study met its primary objective. Methylthioninium chloride Cosmo 200 mg given in conjunction with standard bowel preparation prior to colonoscopy, increased the ADR significantly (in both FAS and PP set). The significance was evident for both Fischer exact test and logistic regression. The difference in ADR between Methylthioninium chloride COSMCosmoO and placebo was 8.48 (95 % CI (2.20 - 14.77)) percentage points.

As noted, the study was not powered to detect differences in ADR between the low dose Methylthioninium chloride Cosmo (100 mg) group and the high dose Methylthioninium chloride Cosmo group (200 mg) or placebo. The low dose group had numerically lower ADR than the high dose group but numerically higher than placebo.

The statistical robustness was investigated in a series of sensitivity analyses that to some extent supported the robustness of the results. The multiple imputation under MAR (missing at random) assumption did support the primary efficacy. The worst-case analysis did not. Subgroup analyses demonstrated that the effect on ADR (both original definition excluding SSA/TSA and updated definition including SSA/TSA) was maintained across relevant subgroups (age, sex, reason for colonoscopy (screening or surveillance)) although number of patients of non-Caucasian ethnicity was

too limited to allow meaningful analysis. Following post-hoc analysis of the data, the effect of Methylthioninium chloride Cosmo on ADR was calculated to be statistically significant in non-US (EU and Canada) subgroup only. When considering the regional difference, it was considered that within US centres, non-polypoid lesion constituted approximately 25% of the lesions whereas the corresponding figure for the EU/Canada centres was approximately 50%. Given that the effect of Methylthioninium chloride Cosmo is mainly on the non-polypoid lesions (see below), this difference in fraction of non-polypoid lesions could explain the apparent lower effect size in the US stratum (difference in proportion between active and placebo 8.01 % US vs 11.13 % EU/Canada). Several causes were postulated which could have contributed to the observed difference in non-polypoid lesion between the US and RoW study subpopulations. While the cause of the difference could not be determined, differences in clinical practice and training in the detection of these lesions, as well as, limited inter-rater agreement may have influenced the results. Using the original definition of the primary endpoint (i.e. excluding TSA and SSA and only including classical adenomas and carcinomas) the results just barely reached significance (p=0.0496).

As regards the clinical relevance of the observed effect, it is well-known that the impact of colonoscopy screening in terms of reduction in risk of CRC is critically dependent on a sufficiently high ADR and there appears to be a correlation between the magnitude of the ADR and the reduction of risk for colorectal cancer. Thus, while any effect on ADR in principle is beneficial, the exact size of the effect on the clinically relevant endpoint, i.e. risk of CRC, is difficult to ascertain accurately as the risk of cancer differs according to size, location and histological characteristics of the removed adenomas. As expected, the effect of Methylthioninium chloride Cosmo was primarily driven by increased detection of adenomas. The effect was statistically significant for adenomas but not for cancers. It is fully acknowledged that the number of cancers was very low and consequently the power correspondingly low. Thus, it is considered acceptable and expected that Methylthioninium chloride Cosmo did not increase the number of cancers detected.

The exploratory endpoint differentiating effect on polypoid versus non-polypoid lesions clearly demonstrates that the benefits of Methylthioninium chloride Cosmo is limited to the non-polypoid lesions. This is to be expected as polypoid lesions generally are easier detectable than flat lesions. Although small lesions generally have lower risk of transformation to malignancy, the risk is not zero as they may, albeit rarely, harbour frank malignancy in spite of the size. Furthermore, by nature, smaller polyps are more often missed and these missed lesions account for a substantial part of the interval cancers observed. This supports the clinical benefit of finding and removing even small adenomas/cancers.

The effect of Methylthioninium chloride Cosmo on ADR was in part due to an increase in smaller and flat, non-polypoid lesions. However, the applicant has provided analyses excluding small TSA and SSA less than 5 mm, demonstrating that the effect of Methylthioninium chloride Cosmo on ADR remained statistically significant even after exclusion of small (less than 5 mm) TSA/SSA. Although the differences between Methylthioninium chloride Cosmo and placebo in terms of ADR were numerically slightly smaller than for comparisons including all polyps (irrespectively of size), the analyses indicate that the beneficial effect of Methylthioninium chloride Cosmo in terms of increase in ADR is not solely dependent on increase in detection of small SSA/TSA (with a presumably lesser risk of subsequent development of cancer) thus supporting the clinical relevance of the observed increase in ADR.

Whereas an increase in the number of patients in whom an adenoma is detected (ADR) is of interest and a generally accepted measure of the quality of an endoscopist/an endoscopy screening program (and thus the reduction in risk of CRC), the individual patient it is also interested in achieving a truly "clean colon" (i.e. no relevant polyps left). As the truth (when is the colon clean) remains unknown, it is reasonable to assume that number of polyps removed per patient is a surrogate for achieving a truly clean colon. Number of histology confirmed adenomas and carcinomas per patient did not differ

between Methylthioninium chloride Cosmo treated patients and placebo. This is somewhat unexpected, as a treatment that increases the detection of polyps on a population level would also be expected to increase the detection on an individual level. The applicant argues that this is expected and consequently does not question the clinical relevance of the observed effect on ADR. Chromoendoscopy most likely does not increase the detection of polypoid lesion, only (otherwise hard to detect) flat, non-polypoid lesions. Thus, an effect on number of polyps removed per patient would only be evident for the non-polypoid lesion, not for the polypoid lesions. As the polypoid lesions make up 50-75% of all lesions, the effect on the non-polypoid lesions may "drown" in the lack of effect on the detection of the large number of polypoid lesions. This interpretation is supported by analyses indicating that, compared to placebo, the number of non-polypoid lesions is indeed increased by Methylthioninium chloride Cosmo (acknowledging that no formal correction for multiplicity was performed, please see above). Whereas, increased detection and removal of lesions associated with risk of CRC indisputably is considered beneficial, increased detection and removal of lesions that are not associated with any risk of CRC is not beneficial and could potentially be harmful. Thus, the number of patients with false positive excision (i.e. excisions made assuming that the lesion was an adenoma but where subsequent histological evaluation could not confirm that the removed lesion was indeed an adenoma/TSA/SSA) compared to total number of patients with excisions (FPR), is an important secondary endpoint. Any intervention aiming at increasing detection of lesions in need of excision should not cause a disproportionate increase in false positive excisions as every excision is associated with a risk (albeit very small for the smallest lesions). The data presented shows that Methylthioninium chloride Cosmo did not cause a clinically relevant increase in FPR at the subject level. In fact, compared to placebo, the FPR in the high dose, Methylthioninium chloride Cosmo was statistically significantly lower although the difference was not clinically relevant. While this to some extent is reassuring, it is of more interest to evaluate the FPR at the excision level. At the excision level, the FPR was numerically greater in the 200 mg Methylthioninium chloride Cosmo group. However, the upper boundary of the confidence interval around the difference was below the predefined limit for non-inferiority and not considered clinically significant.

Excluding SSA and TSA, FPR at the subject level was numerically (but not statistically significantly) greater in the two Methylthioninium chloride Cosmo groups. However, the upper boundary of the confidence interval around the difference was well below the predefined limit for clinical significance. The FPR at excision level (excluding SSA/TSA) in the Methylthioninium chloride Cosmo full dose group was similar to the placebo group (54.08% vs 51.27%; difference [95% CI]: 2.80 [-1.37, 6.98]). The difference is below 15 %. These post hoc analyses using the originally proposed definition of adenomas (i.e. excluding SSA and TSA) confirms the results of the analysis presented using the later definition of adenomas (i.e. including SSA and TSA). Thus, Methylthioninium chloride Cosmo does not increase the FPR, neither at subject nor at excision level, in a clinical meaningful way. Thus, there is no indication that Methylthioninium chloride Cosmo increase the detection and removal of mucosa that does not represent a lesion that should be removed (FPR).

Number of histology confirmed Hyperplastic, Fibroblastic and Mixed Polyps was slightly but statistically significantly higher in the Methylthioninium chloride Cosmo 200 mg treated patients compared to the placebo treated patients. As these lesions generally are considered without malignant potential, the increased detection and removal cannot be considered of benefit but actually potentially harmful to the patient and unwelcomed for the colonoscopists as it unnecessarily prolongs the procedure. However, considering the safety and ease of removal of these lesions as well as the lack of clinically relevant effect on overall FPR, the increased detection and removal of the before mentioned, non-neoplastic lesions is not considered to pose a clinically relevant risk to the patient.

A dose response relationship was evident for the data presented. Methylthioninium chloride Cosmo 200 mg was numerically superior to Methylthioninium chloride Cosmo 100 mg. The 100 mg dose was included for masking purposes, as the blue colouring of mucosa/faeces/urine makes it impossible to maintain blinding. The inferior results of the lower dose do suggest a drug effect, not merely an increased awareness of the endoscopist because of the unblinding as a result of any blue colouring.

Efficacy has not been studied in elderly patients and patients with severe hepatic and/or renal impairment. While, efficacy is in these populations is expected to be similar to the efficacy in the target population (i.e. otherwise healthy subjects between 50 and 75 eligible for entering colposcopy based colorectal cancer screening program), the lack of data has been noted in the SPC. As regards the safety in these populations, please refer to subsequent section.

In addition to the above mentioned pivotal, randomised, placebo-controlled phase three study (Study CB-17-01/06) the applicant has submitted two uncontrolled studies in otherwise healthy subjects (Study CB-17-01/05) and patients with long standing ulcerative colitis (Study CB-17-01/04) who underwent screening/surveillance and surveillance, respectively. Due to the nature of these studies (un-blinded and uncontrolled) the results of these studies are difficult to interpret.

2.7.4. Conclusions on clinical efficacy

Overall, the study met its primary endpoint, i.e. the ADR was significantly higher in Methylthioninium chloride Cosmo treated patients as compared to placebo treated patients. The primary endpoint was changed during the study to include SSA and TSA but as the change was well justified from a scientific point of view and as there are no indications that this change has had a negative effect on the internal validity of the study, this is considered acceptable. Overall the results are considered statistically robust and compelling. No impact on the robustness of the efficacy results is anticipated and the issue is not pursued further. Compared to placebo, Methylthioninium chloride Cosmo caused an increase in ADR of 8.48 (95 % CI (2.20 -14.77) percentage points. Previous studies indicate inverse relationship between ADR and risk of CRC supporting the clinical relevance of the observed effect. However, the before mentioned relationship is not linear and influenced by size, location and histology of the removed adenomas, precluding an accurate translation of the observed effect on ADR in terms of improvement of reduction of CRC risk. The data presented is considered to support the conclusion that Methylthioninium chloride Cosmo can improve ADR in screening/surveillance colonoscopy.

2.7.5. Clinical safety

The clinical development program includes seven studies in humans. All seven studies contribute safety data. They are tabulated below:

Study Number	Phase	Study Design	Subject Population	Number of Subjects Treated per Group Enrolled/Treated	Safety Endpoints	Included in ISS Pooled Safety Analyses?
CB-17-01/01	1	Single centre, randomised, open-label, safety, and bioavailability study Part 1: randomised, 2-period cross-over design Part 2: single dose, one-period design	Male and post-menopausal female healthy subjects, aged 18 to 65 years	Part 1: 200 mg Methylthioninium chloride Cosmo: 10 subjects; 100 mg methylthioninium chloride 1% solution: 10 subjects. 10/10 Part 2: 400 mg Methylthioninium chloride Cosmo: 12 subjects. 12/12	AEs Laboratory parameters Vital signs ECG	No[a]
CB-17-01/02	1	Single centre, open-label, single ascending dose, efficacy, safety, and bioavailability study	Healthy male subjects aged 18 to 65 years	Part 1: 100 mg Methylthioninium chloride Cosmo: 5 subjects. 6/5 Part 2: 200 mg Methylthioninium chloride Cosmo: 18 subjects. 18/18	AEs Laboratory parameters Vital signs ECG	Yes

Study Number	Phase	Study Design	Subject Population	Number of Subjects Treated per Group Enrolled/Treated	Safety Endpoints	Included in ISS Pooled Safety Analyses?
CB-17-01/03	2	Single centre, open-label, exploratory efficacy and safety study	Subjects aged 18 to 70 years, with indication for colonoscopy including faecal occult blood test positive CRC screening, polypectomy follow-up, and inflammatory bowel disease check	150 mg Methylthioninium chloride Cosmo: 24 subjects. 200 mg Methylthioninium chloride Cosmo: 90 subjects. 122/114	AEs Vital signs	Yes
CB-17-01/04	2	Single centre, open-label, exploratory, descriptive efficacy and safety study	Subjects aged 18 years or older, with clinically and endoscopically verified ulcerative colitis with a diagnosis of at least 8 years	200 mg Methylthioninium chloride Cosmo: 53 subjects. 59/53	AEs Vital signs	Yes

Study Number	Phase	Study Design	Subject Population	Number of Subjects Treated per Group Enrolled/Treated	Safety Endpoints	Included in ISS Pooled Safety Analyses?
CB-17-01/05	2	Single centre, open-label, exploratory, descriptive efficacy and safety study	Subjects aged 18 years or older undergoing screening or surveillance colonoscopy who met the joint guideline from the American Cancer Society, the US Multi-Society Task Force on CRC and the American College of Radiology (Levin Gastroenterology 2008)[b]	Part 1: 200 mg Methylthioninium chloride Cosmo: 96 subjects. 100/96 Part 2: 200 mg Methylthioninium chloride Cosmo: 62 subjects. 70/62	AEs Vital signs	Yes
CB-17-01/06	3	Multicentre, multinational, randomised, double-blind, placebo-controlled, parallel-group efficacy and safety study	Subjects aged 50 to 75 years, scheduled for screening or surveillance colonoscopy for CRC detection	200 mg Methylthioninium chloride Cosmo: 488 subjects; 100 mg Methylthioninium chloride Cosmo: 241 subjects; Placebo group: 479 subjects. 1346/1208	AEs Selected laboratory parameters Vital signs	Yes

Study Number	Phase	Study Design	Subject Population	Number of Subjects Treated per Group Enrolled/Treated	Safety Endpoints	Included in ISS Pooled Safety Analyses?
CB-17-01/08	2	Single centre, open-label safety study	Subjects aged 18 to 75 years, scheduled for screening or surveillance colonoscopy and identified as having the clinical requirement for a second colonoscopy within 2 weeks of the initial colonoscopy	200 mg Methylthioninium chloride Cosmo: 10 subjects. 13/10	Effect on colonic epithelial double stranded DNA (primary endpoint) AEs Selected laboratory parameters Vital signs	Yes

All studies are included in the pooled dataset except from the phase 1 study CB-17-01/01 as this study employed a cross-over design, administered a prototype form of Methylthioninium chloride Cosmo (200 mg tablets) rather than the 8 × 25 mg tablets used in later studies and i.v. injections of Methylthioninium chloride Cosmo. Safety data from this study are considered separately.

Patient exposure

Exposure in the pooled group is presented below. In the phase 1 study CB-17-01/01, 22 patients were included.

Pooled Analysis: Study Treatment Exposure (Safety Analysis Set)

	Methylthioninium chloride Cosmo 200 mg (N = 817) n (%)	Methylthioninium chloride Cosmo 150 mg (N = 24) n (%)	Methylthioninium chloride Cosmo 100 mg (N = 246) n (%)	Placebo (N = 479) n (%)	Methylthioninium chloride Cosmo Any Dose (N = 1087) n (%)
Total Dose (mg)					
25	0	0	0	0	0
50	1 (0.1)	0	0	0	1 (0.1)
75	4 (0.5)	0	0	1 (0.2)	4 (0.4)

	Methylthioninium chloride Cosmo 200 mg (N = 817) n (%)	**Methylthioninium chloride Cosmo 150 mg (N = 24) n (%)**	**Methylthioninium chloride Cosmo 100 mg (N = 246) n (%)**	**Placebo (N = 479) n (%)**	**Methylthioninium chloride Cosmo Any Dose (N = 1087) n (%)**
100	1 (0.1)	0	244 (99.2)	0	245 (22.5)
125	7 (0.9)	0	N/A	0	7 (0.6)
150	5 (0.6)	24 (100.0)	N/A	1 (0.2)	29 (2.7)
175	0	N/A	N/A	1 (0.2)	0
200	798 (97.7)	N/A	N/A	476 (99.4)	798 (73.4)
Not evaluable[a,b]	1 (0.1)	0	2 (0.8)	0	3 (0.3)

ISS = Integrated Summary of Safety; MMX = Multi-Matrix; N/A = not applicable.

[a] Two subjects in the 100 mg group of Study CB-17-01/06 were recorded as not having taken all 8 tablets (Subject 310-138 took 4/8 tablets; Subject 610-037 took 7/8 tablets); since this study was blinded, it was not possible to distinguish between placebo and Methylthioninium chloride Cosmo tablets taken.

[b] One subject (131-114) in the 200 mg group of Study CB-17-01/06 was lost to follow-up, without confirmation as to how many tablets were taken.

Note: The pooled analysis comprises Studies CB-17-01/02, CB-17-01/03, CB-17-01/04, CB-17-01/05, CB-17-01/06, and CB-17-01/08.

Demographics of the study populations

Pooled Analysis: Demographic and Baseline Characteristics (Safety Analysis Set)

	Methylthioninium chloride Cosmo 200 mg (N = 817)	**Methylthioninium chloride Cosmo 150 mg (N = 24)**	**Methylthioninium chloride Cosmo 100 mg (N = 246)**	**Placebo (N = 479)**	**Methylthioninium chloride Cosmo Any Dose (N = 1087)**
Sex [n (%)]					
Male	479 (58.6)	11 (45.8)	143 (58.1)	295 (61.6)	633 (58.2)
Female	338 (41.4)	13 (54.2)	103 (41.9)	184 (38.4)	454 (41.8)
Age at Screening (years)					
Mean ± SD	58.4 ± 9.8	50.4 ± 11.0	60.4 ± 7.0	61.7 ± 6.8	58.7 ± 9.4
Median (range)	60.0 (21 to 80)	51.5 (28 to 69)	61.0 (37 to 75)	62.0 (50 to 75)	60.0 (21 to 80)

	Methylthioninium chloride Cosmo 200 mg (N = 817)	**Methylthioninium chloride Cosmo 150 mg (N = 24)**	**Methylthioninium chloride Cosmo 100 mg (N = 246)**	**Placebo (N = 479)**	**Methylthioninium chloride Cosmo Any Dose (N = 1087)**
18 to 49 [n (%)]	111 (13.6)	11 (45.8)	5 (2.0)	0	127 (11.7)
50 to 64 [n (%)]	456 (55.8)	9 (37.5)	163 (66.3)	298 (62.2)	628 (57.8)
≥ 65 [n (%)]	250 (30.6)	4 (16.7)	78 (31.7)	181 (37.8)	332 (30.5)
Race [n (%)]					
White	463 (56.7)	NA	225 (91.5)	450 (93.9)	688 (63.3)
Black	38 (4.7)	NA	15 (6.1)	17 (3.5)	53 (4.9)
Other	15 (1.8)	NA	6 (2.4)	12 (2.5)	21 (1.9)
Not collected	301 (36.8)	24 (100.0)	0	0	325 (29.9)

ISS = Integrated Summary of Safety; MMX = Multi-Matrix; NA = not available; SD = standard deviation.

Note: Race: White = White/Caucasian; Black = Black or African American; Other = all other categories.

Note: Race was not provided for Studies CB-17-01/03, CB-17-01/04, and CB-17-01/05.

Note: The pooled analysis comprises Studies CB-17-01/02, CB-17-01/03, CB-17-01/04, CB-17-01/05, CB-17-01/06, and CB-17-01/08.

Phase I non-pooled study

Table 11.2.1 Summary of sex distribution, mean±SD of age, height and BW (Safety and PK population)

Sex		Age	Height	BW
Males	Females	(y)	(cm)	(kg)
12 (54.5%)	10 (45.5%)	46.0±12.4	168.7±11.1	72.1±7.2

Adverse events

Adverse events were monitored from Screening to the final study visit. For the phase 3 placebo-controlled study, Study CB-17-01/06, monitoring took place within 3 to 7 days from colonoscopy. Monitoring of adverse for the other studies were as follows: Day 4 for Study CB-17-01/02; Day 2 for Studies CB-17-01/03, CB-17-01/04, and CB-17-01/05; day of second colonoscopy for Study CB-17-01/08.

Phase 3 placebo-controlled trial:

Study CB-17-01/06: Treatment-emergent Adverse Events Reported in ≥ 1% of Subjects in the Methylthioninium chloride Cosmo 200 mg Group (Safety Analysis Set)

	Methylthioninium chloride Cosmo 200 mg (N = 488)	**Methylthioninium chloride Cosmo 100 mg (N = 241)**	**Placebo (N = 479)**
PT	**n (%)**		
Chromaturia	234 (48.0)	102 (42.3)	7 (1.5)
Faeces discoloured	95 (19.5)	43 (17.8)	0
Haemorrhoids	29 (5.9)	15 (6.2)	36 (7.5)
Nausea	29 (5.9)	9 (3.7)	17 (3.5)
Diverticulum intestinal	24 (4.9)	8 (3.3)	29 (6.1)
Vomiting	23 (4.7)	2 (0.8)	13 (2.7)
Headache	13 (2.7)	8 (3.3)	8 (1.7)
Diverticulum	10 (2.0)	5 (2.1)	11 (2.3)
Abdominal pain	6 (1.2)	2 (0.8)	2 (0.4)
Hypotension	5 (1.0)	3 (1.2)	3 (0.6)

ISS = Integrated Summary of Safety; MMX = Multi-Matrix; PT = preferred term.

Pooled data:

Due to the very low AEs reported in the 150 mg group, the applicant provided pooled safety analysis with and without the 150mg dose group.

Table 2: Pooled Analysis: Treatment-emergent Adverse Events Reported in ≥ 2 Subjects in the Methylene Blue Multi Matrix 200 mg Group (Safety Analysis Set) with and without the inclusion of the 150mg dose group.

SOC PT	Methylene Blue MMX 200 mg (N = 817)	Methylene Blue MMX 150 mg (N = 24)	Methylene Blue MMX 100 mg (N = 246)	Placebo (N = 479)	Methylene Blue MMX Any Dose (with 150mg) (N = 1087)	Methylene Blue MMX Any Dose (without 150mg) (N = 1063)
		n (%)				
Number of subjects with any TEAE	363 (44.4)	1 (4.2)	144 (58.5)	140 (29.2)	508 (46.7)	507 (47.7)
Renal and urinary disorders	**247 (30.2)**	**0**	**102 (41.5)**	**8 (1.7)**	**349 (32.1)**	**349 (32.8)**
Chromaturia	242 (29.6)	0	102 (41.5)	7 (1.5)	344 (31.6)	344 (32.4)
Polyuria	4 (0.5)	0	0	0	4 (0.4)	4 (0.4)
Dysuria	3 (0.4)	0	0	0	3 (0.3)	3 (0.3)
Gastrointestinal disorders	**232 (28.4)**	**1 (4.2)**	**76 (30.9)**	**107 (22.3)**	**309 (28.4)**	**308 (29.0)**
Faeces discoloured	99 (12.1)	0	43 (17.5)	0	142 (13.1)	142 (13.4)
Nausea	58 (7.1)	0	9 (3.7)	17 (3.5)	67 (6.2)	67 (6.3)
Vomiting	30 (3.7)	1 (4.2)	2 (0.8)	13 (2.7)	33 (3.0)	32 (3.0)
Haemorrhoids	29 (3.5)	0	15 (6.1)	36 (7.5)	44 (4.0)	44 (4.1)
Diverticulum intestinal	24 (2.9)	0	8 (3.3)	29 (6.1)	32 (2.9)	32 (3.0)
Diverticulum	10 (1.2)	0	5 (2.0)	11 (2.3)	15 (1.4)	15 (1.4)
Abdominal pain	6 (0.7)	0	2 (0.8)	2 (0.4)	8 (0.7)	8 (0.8)
Abdominal discomfort	4 (0.5)	0	1 (0.4)	1 (0.2)	5 (0.5)	5 (0.5)
Abdominal distension	2 (0.2)	0	2 (0.8)	2 (0.4)	4 (0.4)	4 (0.4)
Diarrhoea	2 (0.2)	0	2 (0.8)	0	4 (0.4)	4 (0.4)
Haematemesis	2 (0.2)	0	0	0	2 (0.2)	2 (0.2)
Rectal haemorrhage	2 (0.2)	0	0	2 (0.4)	2 (0.2)	2 (0.2)
Nervous system disorders	**21 (2.6)**	**0**	**9 (3.7)**	**13 (2.7)**	**30 (2.8)**	**30 (2.8)**
Headache	14 (1.7)	0	9 (3.7)	8 (1.7)	23 (2.1)	23 (2.2)
Migraine	3 (0.4)	0	0	1 (0.2)	3 (0.3)	3 (0.3)
Presyncope	2 (0.2)	0	0	2 (0.4)	2 (0.2)	2 (0.2)
General disorders and administration site conditions	**14 (1.7)**	**0**	**8 (3.3)**	**5 (1.0)**	**22 (2.0)**	**22 (2.1)**
Chills	2 (0.2)	0	0	0	2 (0.2)	2 (0.2)
Fatigue	2 (0.2)	0	0	1 (0.2)	2 (0.2)	2 (0.2)
Pain	2 (0.2)	0	1 (0.4)	0	3 (0.3)	3 (0.3)
Pyrexia	2 (0.2)	0	0	2 (0.4)	2 (0.2)	2 (0.2)
Vascular disorders	**9 (1.1)**	**0**	**4 (1.6)**	**4 (0.8)**	**13 (1.2)**	**13 (1.2)**
Hypotension	5 (0.6)	0	3 (1.2)	3 (0.6)	8 (0.7)	8 (0.8)
Flushing	2 (0.2)	0	0	1 (0.2)	2 (0.2)	2 (0.2)
Infections and infestations	**5 (0.6)**	**0**	**6 (2.4)**	**8 (1.7)**	**11 (1.0)**	**11 (1.0)**
Nasopharyngitis	2 (0.2)	0	3 (1.2)	1 (0.2)	5 (0.5)	5 (0.5)
Musculoskeletal and connective tissue disorders	**4 (0.5)**	**0**	**1 (0.4)**	**2 (0.4)**	**5 (0.5)**	**5 (0.5)**
Skin and subcutaneous tissue disorders	**3 (0.4)**	**0**	**4 (1.6)**	**1 (0.2)**	**7 (0.6)**	**7 (0.7)**
Injury, poisoning and procedural complications	**3 (0.4)**	**0**	**1 (0.4)**	**4 (0.8)**	**4 (0.4)**	**4 (0.4)**

Respiratory, thoracic and mediastinal disorders	**3 (0.4)**	**0**	**0**	**1 (0.2)**	**3 (0.3)**	**3 (0.3)**
Cough	2 (0.2)	0	0	0	2 (0.2)	2 (0.2)
Investigations	**2 (0.2)**	**0**	**3 (1.2)**	**5 (1.0)**	**5 (0.5)**	**5 (0.5)**
Alanine aminotransferase increased	2 (0.2)	0	2 (0.8)	2 (0.4)	4 (0.4)	4 (0.4)

ISS = Integrated Summary of Safety; MMX = Multi-Matrix; PT = preferred term; SOC = system organ class; TEAE = treatment-emergent adverse event.
Note: The pooled analysis comprises Studies CB-17-01/02, CB-17-01/03, CB-17-01/04, CB-17-01/05, CB-17-01/06, and CB-17-01/08.

Severity of the adverse events

The severity of the adverse events of the phase 3 placebo-controlled trial is tabulated below.

Study CB-17-01/06: Overall Summary of Treatment-emergent Adverse Events (Safety Analysis Set)

	Methylthioninium chloride Cosmo 200 mg (N = 488)	**Methylthioninium chloride Cosmo 100 mg (N = 241)**	**Placebo (N = 479)**
	n (%)		
Number of subjects with any:			
TEAE	314 (64.3)	143 (59.3)	140 (29.2)
Severe TEAE	4 (0.8)	2 (0.8)	2 (0.4)
Serious TEAE	1 (0.2)	1 (0.4)	0
Related[a] TEAE	256 (52.5)	111 (46.1)	21 (4.4)
TEAE leading to discontinuation from the study	4 (0.8)	0	2 (0.4)
TEAE leading to discontinuation of study treatment	3 (0.6)	0	1 (0.2)

ISS = Integrated Summary of Safety; MMX = Multi-Matrix; TEAE = treatment-emergent adverse event.

[a] 'Related' = causal relationship of 'Possible', 'Probable', 'Definite', 'Unassessable' or not recorded.

Adverse events of special interest

Some studies have suggested that methylthioninium chloride can induce DNA damage when exposed to white light in CRC cell lines and *in vivo* (Barrett's oesophagus and ulcerative colitis biopsy samples) (Davies Gut 2007, Olliver Biomarkers 2003), although safety data in patients with ulcerative colitis and gastric intestinal metaplasia suggest that these observations regarding DNA damage are unlikely to have biological consequences (Kiesslich Gastrointestinal Endoscopy 2004, Dinis-Ribeiro Gastrointest Endosc 2008).

The primary endpoint in Study CB-17-01/08 was evaluation, by γH2AX analysis, of the effect of a total oral dose of 200 mg of Methylthioninium chloride Cosmo tablets on colonic epithelial double stranded DNA in colonic biopsy samples collected during chromoendoscopy as compared to control biopsies

collected during standard white light colonoscopy without Methylthioninium chloride Cosmo in the same subject. Effect on double stranded DNA was evaluated by counting the cells that stained positive for the DNA damage marker, γH2AX, and based on the mean fluorescence intensity (MFI) of staining for γH2AX. The threshold for both variables was set according to the negative and positive control staining of human HT29 cell line samples. The biopsy was considered negative if the average of MFI and of the percentage of gated cells was ≤ 1.5%, considered as an acceptable level of deviation, and positive if it was > 1.5%.

Results of the γH2AX analysis are summarised in the table below.

Table 12.2.1 Results of the γH2AX analysis by colonic region and by colonoscopy (N=10)

Colonic region	γH2AX analysis result	
	White light colonoscopy n (%)	Colonoscopy with methylene blue staining n (%)
All regions	**Negative**	**Negative**
Caecum	Negative	Negative
Ascending colon	Negative	Negative
Transverse colon	Negative	Negative
Descending colon	Negative	Negative
Sigmoid and rectum	Negative	Negative

The γH2AX assay was negative for all the colonic regions for all the analysed subjects (N=10 – safety set) and at both colonoscopies. The occurrence of any DNA double strand damage could be excluded at each of the performed colonoscopies on the basis of the γH2AX analysis result performed in all colonic regions.

Serious adverse event/deaths/other significant events

Deaths

No subjects died during the studies.

Serious adverse events

Serious TEAEs were reported in a total of 3 events subjects across the pooled analysis. The serious TEAEs were events of gastrointestinal haemorrhage and bronchitis viral in 1 (0.1%) subject each in the Methylthioninium chloride Cosmo 200 mg treatment group and an event of haematochezia in 1 (0.4%) subject in the Methylthioninium chloride Cosmo 100 mg group. No serious TEAEs were reported in subjects who were treated with placebo.

The serious adverse events are tabulated first for the pooled data. No SAEs or any other significant AE occurred during the non-pooled phase 1 study.

The pooled studies:

Table 4.4.1
Pooled Analysis: Treatment Emergent Serious Adverse Events by System Organ Class and Preferred Term
Safety Analysis Set

	Treatment Group				
System Organ Class/ Preferred Term [1]	Methylene Blue 200 mg (N=817) n (%)	Methylene Blue 150 mg (N=24) n (%)	Methylene Blue 100 mg (N=246) n (%)	Placebo (N=479) n (%)	Methylene Blue Any Dose (N=1087) n (%)
Number of Serious Events	2	0	1	0	3
Number of Subjects with any Serious Adverse Event	2 (0.2)	0	1 (0.4)	0	3 (0.3)
Gastrointestinal disorders	1 (0.1)	0	1 (0.4)	0	2 (0.2)
Gastrointestinal haemorrhage	1 (0.1)	0	0	0	1 (0.1)
Haematochezia	0	0	1 (0.4)	0	1 (0.1)
Infections and infestations	1 (0.1)	0	0	0	1 (0.1)
Bronchitis viral	1 (0.1)	0	0	0	1 (0.1)

Laboratory findings

A panel of clinical laboratory assessments were conducted in Studies CB-17-01/01 and CB-17-01/02, including haematology, coagulation (Study CB-17-01/01 only), blood chemistry, and urinalysis parameters. In Studies CB-17-01/06 and CB-17-01/08, clinical laboratory assessments were limited to creatinine, urea, aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT), and total bilirubin (all assessed in both studies) and haemoglobin (Study CB-17-01/08 only). No clinical laboratory assessments were conducted in Studies CB-17-01/03, CB-17-01/04, and CB-17-01/05.

Results of liver and renal function laboratory tests are tabulated for the pooled analysis below:

Table 10: Pooled Analysis: Renal and Liver Function Parameters Shift Table (Safety Analysis Set)

Parameter	Shift (baseline to post-baseline)	Methylene Blue MMX 200 mg (N = 516)	Methylene Blue MMX 100 mg (N = 246)	Placebo (N = 479)	Methylene Blue MMX Any Dose (N = 762)
		n (%)			
Creatinine	Low/normal to high	15 (2.9)	6 (2.4)	13 (2.7)	21 (2.8)
	High/normal to low	12 (2.3)	7 (2.8)	7 (1.5)	19 (2.5)
Urea	Low/normal to high	31 (6.0)	12 (4.9)	28 (5.9)	43 (5.7)
	High/normal to low	23 (4.5)	11 (4.5)	15 (3.1)	34 (4.5)
GGT	Low/normal to high	8 (1.6)	4 (1.6)	3 (0.6)	12 (1.6)
	High/normal to low	4 (0.8)	5 (2.0)	14 (2.9)	9 (1.2)
AST	Low/normal to high	25 (4.9)	13 (5.3)	28 (5.9)	38 (5.0)
	High/normal to low	1 (0.2)	0	0	1 (0.1)
ALT	Low/normal to high	28 (5.4)	13 (5.3)	16 (3.4)	41 (5.4)
	High/normal to low	1 (0.2)	1 (0.4)	4 (0.8)	2 (0.3)
Total bilirubin	Low/normal to high	61 (11.8)	27 (11.0)	57 (11.9)	88 (11.6)
	High/normal to low	6 (1.2)	0	2 (0.4)	6 (0.8)

ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyltransferase; ISS = Integrated Summary of Safety; MMX = Multi-Matrix.
Post baseline: Worst result for all scheduled and unscheduled post-baseline visits, including follow-up. Highest result for 'low/normal to high', lowest result for 'high/normal to low'.
Note: Percentages are based on available data for subjects in the Safety Analysis Set who have both a baseline and post-baseline assessment, for the parameter and treatment group being considered.
Note: The pooled analysis comprises Studies CB-17-01/02, CB-17-01/03, CB-17-01/04, CB-17-01/05, CB-17-01/06, and CB-17-01/08.

Vital signs

There were no clinically significant changes from baseline in systolic or diastolic blood pressure, heart rate, and oxygen saturation by treatment group. Median measurements, minimum change, and maximum change from baseline in systolic or diastolic blood pressure, heart rate, and oxygen saturation were similar across treatment groups, including placebo.

ECG evaluations were performed in Studies CB-17-01/01 and CB-17-01/02. No clinically relevant changes in ECG parameters or clinically significant ECG abnormalities were observed in either study.

A thorough QT interval corrected for heart rate (QTc) study has not been performed with Methylthioninium chloride Cosmo, but such a study was performed previously with an IV formulation of methylthioninium chloride. ProvayBlue® is an injectable form of methylene blue and is currently approved in the US for the treatment of paediatric and adult patients with acquired methaemoglobinaemia. A randomised, open-label, single-dose, cross-over, thorough QTc study (Protocol PVP-2014002) was performed in 48 healthy volunteers who received 2 mg/kg IV ProvayBlue, moxifloxacin 400 mg orally, and placebo (sodium chloride) IV, with a 7-day wash-out period between each treatment. The sample size had 90% power to exclude a QTc effect > 10 msec if the true effect was up to 3 msec, based on the published effect size for placebo. In that study, no significant QTc prolongation effect was detected for a single 2 mg/kg IV dose of ProvayBlue.

Safety in special populations

Age

Adverse events by age group

MedDRA Terms	**Age <65*** **number (percentage)**	**Age ≥ 65number (percentage)**
Total AEs	65.7%	61.8 %
Gastrointestinal disorders, total	42.5%	33.5%
- Faeces discoloured	19.7%	19.1%
- Haemorrhoids	8.6%	1.2%
- Nausea	6.7%	4.6%
- Diverticulum intestinal	5.7%	3.5%

Gender

Adverse events by gender

MedDRA Terms	**Males** **number (percentage)**	**Females** **number (percentage)**
Total AEs	59.7%	71.5%
Gastrointestinal disorders, total	34.2%	47.2%

- Faeces discoloured	20.3%	18.1%
- Haemorrhoids	4.4%	8.3%
- Nausea	3.4%	9.8%
- Diverticulum intestinal	3.7%	6.7%
- Diverticulum	1.0%	3.6%
Nervous system disorders, total	34.2%	47.2%
General/administration side disorders, total	1.4%	3.6%

Race

Overall, in the Methylthioninium chloride Cosmo 200 mg treatment group in Study CB-17-01/06, TEAEs were reported in 287/435 (66.0%) White subjects, 17/38 (44.7%) Black subjects, and 10/15 (66.7%) subjects with race categorised as "Other". However, the small numbers of subjects in the Black (N = 38) and "Other" subgroups (N = 15) precludes meaningful comparisons between these subgroups.

Renal Impairment

It is estimated that approximately 40% of methylthioninium chloride is excreted by the kidneysProvepharm SAS 2016). In order to provide an assessment of the safety profile of Methylthioninium chloride Cosmo in subjects with renal impairment, TEAEs were summarised in a retrospective analysis for the pivotal Phase 3 study (CB-17-01/06) according to possible renal impairment.

For the purposes of the current retrospective analysis, subjects were considered to have evidence of renal impairment if they had:

• A high (> ULN) clinical laboratory result for creatinine at Screening; **and/or**

• Reported medical history(ies) with PTs within the Standardised MedDRA Query (SMQ)

of "Acute renal failure" (broad scope) that was ongoing at the start of treatment.

Based on this definition, a total of 68 subjects were identified with some degree of renal impairment in Study CB-17-01/06, comprising 38 subjects in the Methylthioninium chloride Cosmo 200 mg group, 9 subjects in the Methylthioninium chloride Cosmo 100 mg group, and 21 subjects in the placebo group.

Table 12: Study CB-17-01/06: Treatment-emergent Adverse Events Reported in ≥ 2 Subjects in the Methylene Blue Multi-Matrix 200 mg Renal Impairment Group (Safety Analysis Set)

SOC / PT	Subjects with Renal Impairment			Subjects Without Renal Impairment		
	Methylene Blue MMX 200 mg (N = 38)	Methylene Blue MMX 100 mg (N = 9)	Placebo (N = 21)	Methylene Blue MMX 200 mg (N = 450)	Methylene Blue MMX 100 mg (N = 232)	Placebo (N = 458)
	n (%)					
Number of subjects with any TEAE	**14 (36.8)**	**6 (66.7)**	**13 (61.9)**	**300 (66.7)**	**137 (59.1)**	**127 (27.7)**
Renal and urinary disorders	**10 (26.3)**	**5 (55.6)**	**1 (4.8)**	**224 (49.8)**	**97 (41.8)**	**7 (1.5)**
Chromaturia	10 (26.3)	5 (55.6)	1 (4.8)	224 (49.8)	97 (41.8)	6 (1.3)
Gastrointestinal disorders	**10 (26.3)**	**4 (44.4)**	**10 (47.6)**	**182 (40.4)**	**72 (31.0)**	**97 (21.2)**
Diverticulum	3 (7.9)	2 (22.2)	1 (4.8)	7 (1.6)	3 (1.3)	10 (2.2)
Faeces discoloured	3 (7.9)	2 (22.2)	0	92 (20.4)	41 (17.7)	0
Vomiting	2 (5.3)	0	0	21 (4.7)	2 (0.9)	13 (2.8)
Nervous system disorders	**2 (5.3)**	**0**	**0**	**17 (3.8)**	**8 (3.4)**	**13 (2.8)**

MMX = Multi-Matrix; PT = preferred term; SOC = system organ class; TEAE = treatment-emergent adverse event.

Hepatic impairment

Methylthioninium chloride is extensively metabolised in the liverProvepharm SAS 2016), and is an inhibitor and inducer, respectively, of various cytochrome P450 (CYP) enzymes (inhibitor: CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4/5; inducer: CYP1A2, CYP2B6).

In order to provide an assessment of the safety profile of Methylthioninium chloride Cosmo in subjects with hepatic impairment, TEAEs were summarised in a retrospective analysis for the pivotal Phase 3 study (CB-17-01/06) according to possible hepatic impairment.

For the purposes of the current retrospective analysis, subjects were considered to have evidence of hepatic impairment if they had:

- A high (> ULN) clinical laboratory result for ALT, bilirubin, and/or GGT at Screening; **and/or**
- Reported medical history(ies) with PTs within the SMQ of "Hepatic disorders" (narrow scope) that was ongoing at the start of treatment.

Based on this definition, a total of 241 subjects were identified with some degree of hepatic impairment in Study CB-17-01/06, comprising 90 subjects in the Methylthioninium chloride Cosmo 200 mg group, 44 subjects in the Methylthioninium chloride Cosmo 100 mg group, and 107 subjects in the placebo group. It is worth noting that of these 241 subjects, 40 had elevated bilirubin alone (i.e., without any reported ongoing medical history within the "Hepatic disorders" SMQ and without any elevation in ALT or GGT at Screening), including 13/90 (14.4%), 7/44 (15.9%), and 20/107 (18.7%) subjects in the Methylthioninium chloride Cosmo 200 mg, 100 mg, and placebo groups, respectively.

Table 13: Study CB-17-01/06: Treatment-emergent Adverse Events Reported in ≥ 2 Subjects in the Methylene Blue Multi-Matrix 200 mg Hepatic Impairment Group (Safety Analysis Set)

SOC PT	Subjects with Hepatic Impairment			Subjects Without Hepatic Impairment		
	Methylene Blue MMX 200 mg (N = 90)	Methylene Blue MMX 100 mg (N = 44)	Placebo (N = 107)	Methylene Blue MMX 200 mg (N = 398)	Methylene Blue MMX 100 mg (N = 197)	Placebo (N = 372)
	n (%)					
Number of subjects with any TEAE	**57 (63.3)**	**31 (70.5)**	**33 (30.8)**	**257 (64.6)**	**112 (56.9)**	**107 (28.8)**
Renal and urinary disorders	**45 (50.0)**	**22 (50.0)**	**1 (0.9)**	**189 (47.5)**	**80 (40.6)**	**7 (1.9)**
Chromaturia	45 (50.0)	22 (50.0)	1 (0.9)	189 (47.5)	80 (40.6)	6 (1.6)
Dysuria	2 (2.2)	0	0	0	0	0
Gastrointestinal disorders	**38 (42.2)**	**18 (40.9)**	**26 (24.3)**	**154 (38.7)**	**58 (29.4)**	**81 (21.8)**
Faeces discoloured	27 (30.0)	12 (27.3)	0	68 (17.1)	31 (15.7)	0
Nausea	5 (5.6)	2 (4.5)	5 (4.7)	24 (6.0)	7 (3.6)	12 (3.2)
Diverticulum intestinal	3 (3.3)	3 (6.8)	3 (2.8)	21 (5.3)	5 (2.5)	26 (7.0)
Haemorrhoids	3 (3.3)	1 (2.3)	7 (6.5)	26 (6.5)	14 (7.1)	29 (7.8)
Vomiting	3 (3.3)	0	5 (4.7)	20 (5.0)	2 (1.0)	8 (2.2)
Abdominal pain	2 (2.2)	0	2 (1.9)	4 (1.0)	2 (1.0)	0
General disorders and administration site conditions	**3 (3.3)**	**3 (6.8)**	**1 (0.9)**	**8 (2.0)**	**5 (2.5)**	**4 (1.1)**
Nervous system disorders	**2 (2.2)**	**4 (9.1)**	**1 (0.9)**	**17 (4.3)**	**4 (2.0)**	**12 (3.2)**
Migraine	2 (2.2)	0	0	0	0	1 (0.3)

MMX = Multi-Matrix; PT = preferred term; SOC = system organ class; TEAE = treatment-emergent adverse event.

Glucose-6-phosphate Dehydrogenase Deficiency

Methylthioninium chloride belongs to a group of drugs which have the potential to cause haemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency, although there is controversy regarding the clinical importance of this (Youngster Drug Saf 2010; Lu BMC Med 2018). There are case reports of methylthioninium chloride being associated with severe haemolytic reactions in neonatal G6PD deficiency (Gauthier J Matern Fetal Med 2000).

Subjects with G6PD deficiency were excluded from participation in Studies CB-17-01/01, CB-17-01/02, CB-17-01/06, and CB-17-01/08, and while this was not an exclusion criterion in Studies CB-17-01/03, CB-17-01/04, and CB-17-01/05, no subjects enrolled in those studies had a known medical history of G6PD deficiency (Listing 16.2.10.3 [CB-17-01/03 CSR]; Listing 16.2.10.1 [CB-17-01/04 CSR]; Listing 16.2.10.1 [CB-17-01/05 CSR]). Thus, the effect of single-dose oral methylthioninium chloride in adult patients with G6PD deficiency is unknown, but caution is warranted.

Based on experience of the use of methylthioninium chloride in the treatment of malaria and IV use (Lu BMC Med 2018), Methylthioninium chloride Cosmo is contraindicated in patients with known G6PD deficiency.

Pregnancy and lactation

Methylthioninium chloride Cosmo has not been tested in pregnant or lactating women. In the clinical development programme for Methylthioninium chloride Cosmo, there were 464 females who received any dose of the drug, of which 34 were aged 15 to 44 years. No pregnancies and no AEs related to pregnancy, lactation, or fertility occurred in any of the studies. Pregnancy tests at screening were required for women of childbearing potential and the results had to be negative for enrolment. Since Methylthioninium chloride Cosmo has not yet been approved or marketed in any country, potential use rates of this drug amongst females of reproductive age are not known.

Historically, epidemiological studies have shown an increased risk of neonatal intestinal atresia and foetal death after intra-amniotic injection of a methylthioninium chloride class product during the second trimester (Cragan Teratology 1999). Intra-amniotic injection of a methylthioninium chloride class product hours to days prior to birth has been reported to cause hyperbilirubinemia, haemolytic anaemia, skin staining, methaemoglobinaemia, respiratory distress, and photosensitivity in newborns (Cragan Teratology 1999).

Studies with methylthioninium chloride in animals and in vitro have shown reproductive toxicity. Based on this animal experience, it has been concluded that methylthioninium chloride may cause foetal harm when administered to a pregnant woman and should therefore not be used during pregnancy (Coddington Fertil Steril 1989; National Toxicology Program 1993; National Toxicology Program 1994; Cragan Teratology 1999).

There is no information regarding the presence of methylthioninium chloride in human milk, the effects on the breastfed infant, or the effects on milk production. Studies in animals suggests methylthioninium chloride will pass into breast milk. Nonetheless, it is recommended that Methylthioninium chloride Cosmo not be given to breastfeeding or lactating females (Ziv J Vet Pharmacol Ther 1984).

Immunological events

No immunological events occurred.

Safety related to drug-drug interactions and other interactions

Serotonergic Drugs

Serotonin syndrome has been reported with the use of methylthioninium chloride class products (Provepharm SAS 2016). Safety announcements released by the US FDA and the UK Medicines and Healthcare products Regulatory Agency have indicated that most cases of central nervous system (CNS) toxicity/serotonin syndrome occurred in the context of IV administration of methylthioninium chloride as a visualising agent in preparation for parathyroid or thyroid surgery (US FDA 2011; UK Medicines and Healthcare products Regulatory Agency 2009). Most reports of serotonin syndrome with IV methylthioninium chloride have been associated with concomitant use of serotonergic drugs, some tricyclic antidepressants and other psychiatric medications, and monoamine oxidase inhibitors (Provepharm SAS 2016). Not all serotonergic drugs have equal capacity to cause serotonin syndrome with IV methylthioninium chloride. The cases of serotonin syndrome with IV methylthioninium chloride occurred in patients taking specific serotonergic psychiatric drugs, namely a selective serotonin reuptake inhibitor (SSRI), a serotonin-norepinephrine reuptake inhibitor (SNRI), or clomipramine (US FDA 2011).

Serotonin syndrome may include the following signs and symptoms: mental status changes (e.g., agitation, hallucinations, delirium, and coma), autonomic instability (e.g., tachycardia, labile blood pressure, dizziness, diaphoresis, flushing, and hyperthermia), neuromuscular symptoms (e.g., tremor, rigidity, myoclonus, hyperreflexia, and incoordination), seizures, and/or gastrointestinal symptoms (e.g., nausea, vomiting, and diarrhoea) (Provepharm SAS 2016).

There was no report of serotonin syndrome in the Methylthioninium chloride Cosmo clinical programme, although a small number of subjects who received Methylthioninium chloride Cosmo 200 mg (8 × 25 mg tablets) were also receiving concomitant psychiatric serotonergic medications. It is not known if there is a risk of serotonin syndrome when methylthioninium chloride is administered orally in preparation for colonoscopy. However, maximal systemic exposure to methylthioninium chloride (maximum plasma concentration [C_{max}]) was lower for orally administered Methylthioninium chloride

Cosmo than for IV methylthioninium chloride, suggesting that there may be a lower risk of systemic CNS effects such as serotonin syndrome occurring with oral Methylthioninium chloride Cosmo than for IV methylthioninium chloride. Nonetheless, appropriate warnings regarding serotonin syndrome are included in the proposed labelling.

Agents Metabolised by Cytochrome P450 Enzymes

Methylthioninium chloride is an inhibitor and inducer, respectively, of various CYP enzymes (inhibitor: CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4/5; inducer: CYP1A2, CYP2B6) (Module 2.7.2). These interactions could be more pronounced with narrow therapeutic index drugs that are metabolised by one of these enzymes (e.g., digoxin, warfarin, phenytoin, alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, and tacrolimus). Across the clinical studies, a total of 11 subjects in the Safety Analysis Set were receiving 1 or more of these narrow therapeutic index drugs upon study entry, including 7 subjects receiving warfarin, 2 subjects receiving digoxin, and 2 subjects receiving. Among these 11 subjects, there were no safety concerns raised, with TEAEs including chromaturia in 5 subjects, faeces discoloured and diverticulum in 3 subjects each, haemorrhoids in 2 subjects, and vomiting, alanine aminotransferase increased, gamma-glutamyltransferase increased, and aspartate aminotransferase increased in 1 subject each.

Overall, there is limited clinical information regarding the concomitant use of Methylthioninium chloride Cosmo with drugs that are metabolised by CYP isoenzymes.

Transporter Interactions

Based on in vitro studies, methylthioninium chloride was found to be a substrate of the membrane transport proteins P-glycoprotein (P-gp) and organic anion transporter (OAT) 3 (Module 2.7.2); therefore, drugs which are inhibitors of these transporters have the potential to decrease the excretion efficiency of Methylthioninium chloride Cosmo. Caution should be taken when Methylthioninium chloride Cosmo is co-administered with agents such as cyclosporine A, ritonavir, saquinavir, amiodarone, alectinib, probenecid and novobiocin. Across the clinical studies, a total of 2 subjects in the Safety Analysis Set were receiving 1 or more of these drugs upon study entry, both of whom were in Study CB-17-01/06.. No safety concerns were raised in these 2 subjects; one subject experienced a single TEAE of diverticulum intestinal.

Overall, there is limited clinical information regarding the concomitant use of Methylthioninium chloride Cosmo with drugs that are inhibitors of P-gp and OAT3.

Methylthioninium chloride was also found to potentially act as a mild inhibitor of P-gp. The clinical relevance of this *in vitro* interaction is unknown; however, there is the potential for co-administration to increase the plasma concentrations of drugs which are substrates of this transporter (digoxin, topotecan, sirolimus, everolimus, nilotinib and lapatinib). Across the clinical studies, a total of 2 subjects were receiving 1 or more of these drugs upon study entry, both of whom were receiving digoxin. No safety concerns were raised in these 2 subjects; one subject experienced a single TEAE of chromaturia.

Overall, there is limited clinical information regarding the concomitant use of Methylthioninium chloride Cosmo with drugs that are substrates of P-gp. Appropriate monitoring is recommended.

Discontinuation due to adverse events

TEAEs leading to study discontinuation were reported in a total of 7 (0.9%) subjects in the Methylthioninium chloride Cosmo 200 mg treatment group (pooled analysis) and no subjects in any other Methylthioninium chloride Cosmo group. All TEAE PTs that led to study discontinuation were reported in individual subjects (1 [0.1%] subject each in the 200 mg group), with the events including gastrointestinal haemorrhage (SAE), haematemesis, vomiting, adhesion, adverse reaction (verbatim: 'adverse reaction during colonoscopy'), presyncope, and bradycardia. The events of gastrointestinal haemorrhage and adverse reaction were assessed as severe in intensity, while all other events were assessed as mild or moderate. One event, vomiting, was assessed as related to study treatment while all other events were assessed as not related.

Table 4.8.1
Pooled Analysis: Treatment Emergent Adverse Events Leading to Discontinuation from the Study
by System Organ Class and Preferred Term
Safety Analysis Set

	Treatment Group				
System Organ Class/ Preferred Term [1]	Methylene Blue 200 mg (N=817) n (%)	Methylene Blue 150 mg (N=24) n (%)	Methylene Blue 100 mg (N=246) n (%)	Placebo (N=479) n (%)	Methylene Blue Any Dose (N=1087) n (%)
Number of Events	7	0	0	2	7
Number of Subjects with any Adverse Event	7 (0.9)	0	0	2 (0.4)	7 (0.6)
Gastrointestinal disorders	3 (0.4)	0	0	1 (0.2)	3 (0.3)
Gastrointestinal haemorrhage	1 (0.1)	0	0	0	1 (0.1)
Haematemesis	1 (0.1)	0	0	0	1 (0.1)
Vomiting	1 (0.1)	0	0	0	1 (0.1)
Abdominal pain upper	0	0	0	1 (0.2)	0
General disorders and administration site conditions	2 (0.2)	0	0	0	2 (0.2)
Adhesion	1 (0.1)	0	0	0	1 (0.1)
Adverse reaction	1 (0.1)	0	0	0	1 (0.1)
Nervous system disorders	1 (0.1)	0	0	1 (0.2)	1 (0.1)
Presyncope	1 (0.1)	0	0	1 (0.2)	1 (0.1)
Cardiac disorders	1 (0.1)	0	0	0	1 (0.1)
Bradycardia	1 (0.1)	0	0	0	1 (0.1)

2.7.6. Post marketing experience

No post-marketing data are available. The medicinal product has not been marketed in any country.

2.7.7. Discussion on clinical safety

Seven studies are included in the main safety analyses set, hereof one placebo-controlled phase 3 study of 1208 patients. In total, 798 subjects have been exposed to the proposed dose of 200 mg (8x25 mg) and 244 subjects have been exposed to 100 mg (4x25 mg); the 100 mg group was included in the phase 3 study to assure blinding of the physician conducting the colonoscopy. Since some patients didn't receive/take the full intended dose, a minority of subjects received a variety of doses between 25 mg to 175 mg. Additionally, 22 healthy volunteers have been exposed to 100 mg methylthioninium chloride i.v. as part of a phase I study. These studies together are considered to contribute sufficient data to assess the safety profile. An additional study on the possible long-term effects of Methylthioninium chloride Cosmo has been investigated in a single study of 10 patients, in whom possible colonic epithelial DNA damage was investigated two weeks after exposure to

Methylthioninium chloride Cosmo. While the design of the study does not provide firm evidence of possible long-term risks, the likelihood of any long-term risks is considered very low due to the single dose exposure to Methylthioninium chloride Cosmo. Furthermore, it should be acknowledged that methylthionium chloride is approved for i.v. injection in the treatment of different other conditions.

More males than females were included and more patients of 64 year or less than patients of 65 years or more were included. However, a sufficient number of elderly (≥ 65 years) have been included to evaluate the safety profile in this group. In the Phase 3 study, the Methylthioninium chloride Cosmo and placebo groups were balanced with regard to the age, gender and race of the patients. The demographics of the pooled studies including the phase 3 study is sufficiently representative of the populations expected to be undergoing colonoscopy.

Discontinuations

Seven subjects discontinued the studies; however, none of the reasons for discontinuations were recorded in more than one patient. Hence, there was not a pattern of adverse events leading to discontinuations. Furthermore, only one of the cases were assessed as related to the Methylthioninium chloride Cosmo, which was vomiting. There were no deaths during the studies. Three patients experienced serious adverse events; two patients in the 200 mg Methylthioninium chloride Cosmo group and one patient in the 100 mg Methylthioninium chloride Cosmo group consisting of gastrointestinal haemorrhage, viral bronchitis, and haematochezia. However, the narratives clarified that these serious adverse events were not related to Methylthioninium chloride Cosmo. Overall, the rate of discontinuation due to AEs in subjects treated with Methylthioninium chloride Cosmo was acceptable.

Analysis of adverse events

Overall, AEs were reported as mild or moderate and 46.7% patients in Methylthioninium chloride Cosmo treatment group experienced at least 1 AE compared to 29.2% in the placebo group.

The most commonly reported adverse events were chromaturia (48%) and discoloured faeces (20%), followed by nausea (6%), vomiting (5%), headache (3%), and abdominal pain (1%), percentages as reported in the phase 3 placebo-controlled trial. These common adverse events are adequately addressed in the SmPC and are also labelled for the already approved Methylthioninium chloride Proveblue (which, however, has additional common AEs as it is given as i.v. injection). The phase 1 study, in which healthy volunteers received i.v. injections of methylthioninium chloride, did not indicate other common adverse events.

Despite some evidence on carcinogenic activity of methylthioninium chloride has been shown in male mice and male rats, and female mice, it has been clarified that the proposed use of Methylthioninium chloride Cosmo cannot be expected to be carcinogenic in humans.

PK assessment for Methylthioninium chloride Cosmo was conducted in healthy volunteers with an intact colonic mucosa. However, a thorough discussion indicate that colonic mucosa lesions are not expected to affect the rate and extent of methylthioninium chloride systemic absorption, and hence do not influence the overall safety profile.

Serious adverse events

The rate of SAEs was low. Overall, only 3 severe TEAEs were reported in the Methylthioninium chloride Cosmo group: 2 in 200 mg group and 1 in 100 mg group. No one TEAE was considered to be IMP related. Of note, one patient experienced haematemesis and melaena the day after colonoscopy, while another patient reported haematochezia. The narratives indicated that the SAEs were most likely related to underlying conditions and not to exposure to Methylthioninium chloride Cosmo. Furthermore, there was no indications of hypersensitivity reactions or anaphylaxis. Drug-drug interactions, which are

well-described for i.v. methylthioninium chloride products are adequately addressed in the SmPC, even though the frequency and severity is expected to be modified by the relatively low serum concentrations obtained with Methylthioninium chloride Cosmo.

Laboratory findings

There were no clinically significant differences in the incidence of any renal or liver function laboratory test results across treatment groups. It has been clarified that an observed increase in ALT from baseline was higher in Methylthioninium chloride Cosmo group (5.4%) compared to placebo (3.4%), as well as increase of GGT from baseline was slightly higher in Methylthioninium chloride Cosmo group (1.6% versus 0.6 in placebo group) were most likely not related to the Methylthioninium chloride Cosmo exposure but rather to the patients' underlying conditions.

The phase I and II clinical studies did not reveal any abnormal laboratory findings that were considered related to Methylthioninium chloride Cosmo exposure. This is considered an adequate justification for the absence of e.g. haematology in the phase 3 controlled trial and the limited number of days, during which clinical laboratory assessments were conducted.

It can be expected that methylthioninium chloride will be used more than once in patients undergoing more than one colonoscopy over years, however the use of Methylthioninium chloride Cosmo on repeated occasions is not expected to lead to immunological reactions.

Special populations

Age

The representativeness of the age groups is considered acceptable. It has been concluded that the pattern of AEs was generally similar across the age subgroups. Difference in the proportion of subjects of ≥ 2% between subgroups for the Methylthioninium chloride Cosmo 200 mg group (study CB-17-01/06) were gastrointestinal disorders, reported in 42.5% of subjects aged 50 to 64 years and 33.5% of subjects aged ≥ 65 years. Overall, slightly lower incidence of AEs was reported in the age group ≥ 65 years.

Sex

Enrolment of female subjects was lower compared to male subjects. A higher proportion of TEAEs was reported in females compared to males, though the pattern of events was generally similar regardless of sex. It is therefore proposed that neither warnings nor dose adjustments are required in respect of patient's sex.

Race

The proportion of non-white subjects was extremely low. Therefore, any definite conclusions in relation to safety assessment by race/ethnicity cannot be driven based on the available data set.

Renal impairment

Although renal impairment was listed as an exclusion criterion, looking retrospectively, it has been found that some patients included in the study had some degree of renal impairment at the time of Methylthioninium chloride Cosmo administration.
It has been concluded that these data do not imply to the higher frequency of any TEAEs in the subjects with renal impairment compared to those without. However, it was also concluded that based upon these limited data it is not possible to further extrapolate the safety profile of the product stratified by GFR. Nonetheless, the SmPC has been revised to reflect the lack of data in patients with moderate to severe renal impairment

Hepatic impairment

Other methylthioninium chloride medicinal products are restricted for use in patients with severe hepatic impairment. During the studies no increased risk of adverse events were observed in patients with hepatic impairment. Furthermore, the single dose exposure is not expected to lead to a significantly different safety profile in patients with hepatic impairment. However, the SmPC has been updated with information of higher proportion of "faeces discoloured" in the hepatic impairment group than other patient groups.

Patients with glucose-6-phosphate dehydrogenase deficiency

There are case reports of methylthioninium chloride being associated with severe haemolytic reactions in neonatal G6PD deficiency, therefore known G6PD deficiency is included as a contraindication. As the Methylthioninium chloride Cosmo will be used predominantly in middle aged or elderly patients, it is not considered necessary to require genotyping for G6PD deficiency before use of Methylthioninium chloride Cosmo; it is considered that patients with severe G6PD deficiency are aware of their condition at this time in life and/or that the systemic exposure to doses Methylthioninium chloride Cosmo will be too low to cause severe haemolytic reactions.

2.7.8. Conclusions on clinical safety

In total, 798 subjects have been exposed to the proposed dose of 200 mg (8x25 mg), 244 subjects have been exposed to 100 mg (4x25 mg), while a minority of subjects received a variety of doses between 25 mg to 175 mg. Additionally, 22 healthy volunteers have been exposed to 100 mg methylthioninium chloride i.v. as part of a phase I study. Less than 1% experienced serious adverse events and/or discontinued the studies, no subjects died during the studies.

The main safety issues consist of chromaturia (48%) and discoloured faeces (20%), followed by nausea (6%), vomiting (5%), headache (3%), and abdominal pain (1%). Drug-drug interactions were not observed in the studies but are labelled for concomitant treatment with seronergic medicinal products (serotonin syndrome) or with medicinal products with a narrow therapeutic index since methylthioninium chloride both inhibits and induces various CYP-enzymes (reflected in the PI).

2.8. Risk Management Plan

Safety concerns

Table SVIII.1: Summary of safety concerns

Summary of safety concerns	
Important identified risks	• None
Important potential risks	• Serotonin syndrome • Reproductive toxicity
Missing information	• Use in patients with cardiovascular disease • Use in patients with gastrointestinal disease

Pharmacovigilance plan

Routine pharmacovigilance only.

Risk minimisation measures

Table Part V.1: Description of routine risk minimisation measures by safety concern

Safety concern	Routine risk minimisation activities
Serotonin syndrome	Routine risk communication: Information is included in Section 4.4 and 4.5 of the SmPC and Section 2 and 4 of the PIL. Routine risk minimisation activities recommending specific clinical measures to address the risk: Serotonin syndrome is more likely to occur when methylthioninium chloride is following concomitant use of serotonergic drugs. Other routine risk minimisation measures beyond the Product Information: None
Reproductive toxicity	Routine risk communication: Information is included in Section 4.3 and 4.6 of the SmPC and Section 2 and 4 of the PIL. Routine risk minimisation activities recommending specific clinical measures to address the risk: It is recommended in Section 4.6 that methylthioninium chloride is not used during pregnancy and breastfeeding should be discontinued prior to and after treatment with Methylthioninium chloride MMX. Other routine risk minimisation measures beyond the Product Information: None
Use in patients with cardiovascular disease	Routine risk communication: None Routine risk minimisation activities recommending specific clinical measures to address the risk: None Other routine risk minimisation measures beyond the Product Information: None
Use in patient with gastrointestinal disease	Routine risk communication: None Routine risk minimisation activities recommending specific clinical measures to address the risk: Other routine risk minimisation measures beyond the Product Information:

	None

Conclusion

The CHMP and PRAC considered that the risk management plan version 0.2 is acceptable.

2.9. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the Guideline on the readability of the label and package leaflet of medicinal products for human use.

3. Benefit-risk balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Methylthioninium chloride Cosmo is indicated as a diagnostic agent enhancing visualisation of colorectal lesions in adult patients undergoing screening or surveillance colonoscopy.

3.1.2. Available therapies and unmet medical need

Colonoscopy with identification and removal of suspected precancerous lesions is the standard screening method for CRC. The quality of the colonoscopy has been sought improved by improving bowel preparation methods (e.g. split dosing), improving education of endoscopists and technical improvements such as high definition white light imaging and NBI. Chromoendscopy, i.e. the direct application of dye (e.g. methylthioninium chloride or indigo carmine) is to improve characterization, differentiation and diagnosis of endoscopically detected suspicious lesions. However, this procedure is time consuming or not feasible in a high-volume screening programme. Currently it is only routinely used in patient groups with very high risk (e.g. long-standing ulcerative colitis).

Evidenced by a high number of interval cancers (i.e. cancers being identified in between two screening/surveillance endoscopies), current methods are far from perfect and the need to improve the detection of relevant colonic lesions can be considered to constitute an unmet medical need. The need encompasses both the need to improve detection of lesions as well as the ability to differentiate between relevant (precancerous) and non-relevant (not associated with subsequent risk of CRC) lesions. While an intervention ideally should affect both factors, an intervention only improving overall

detection can be considered beneficial provided that it does not do so primarily by increasing the detection of non-relevant lesions.

3.1.3. Main clinical studies

The confirmatory evidence of Methylthioninium chloride Cosmo in the proposed indication is based on a single pivotal study. Study CB-17-01/06 was a phase 3, multicentre, randomised, controlled study in patients eligible for inclusion in a general screening/surveillance programme for colorectal cancer comparing 200 mg and 100 mg Methylthioninium chloride Cosmo to placebo in terms of adenoma detection rate which is considered a surrogate for the quality of the screenings/surveillance programme and a surrogate for the reduction of risk of colorectal cancer.

3.2. Favourable effects

Compared to placebo (47.81 % of patients), Methylthioninium chloride Cosmo 200 mg statistically significantly increased the fraction of patients in whom at least one adenoma (including SSA and TSA) or carcinoma was detected (56.29% of patients). In the 100 mg Methylthioninium chloride Cosmo group, the corresponding figure was 51.45%. The difference between placebo and 200 mg Methylthioninium chloride Cosmo was statistically significant (p=0.0099). As regards the original primary endpoint, i.e. fraction of patients in whom at least one adenoma (excluding TSA and SSA) or carcinoma was detected, the Odds Ratio between 200 mg Methylthioninium chloride Cosmo and placebo was 1.3088 [95% confidence interval 1.0005, 1.7120], P=0.0496. The effect was maintained across important predefined subgroups such as age, sex and reason for colonoscopy. The increase in detection rate was secondary to increased detection of adenomas whereas the rate of detection of cancers, as expected, was low and similar in both groups. Compared to placebo, Methylthioninium chloride Cosmo did not increase FPR (False positive rate= fraction of all polyps removed in which histological assessment did not confirm the diagnosis "adenoma (including TSA and SSA)" or "adenocarcinoma"), neither at subject level, nor at incision level.

3.3. Uncertainties and limitations about favourable effects

Previous studies indicate inverse relationship between ADR and risk of CRC supporting the clinical relevance of the observed effect. However, the before mentioned relationship is not linear and influenced by size, location and histology of the removed adenomas, precluding an accurate translation of the observed effect on ADR in terms of improvement of reduction of CRC risk. Adding to the difficulty of translating the improvement in ADR into improved CRC risk reduction is the fact that the increased detection rate associated with Methylthioninium chloride Cosmo was mainly a result of an increased detection of small polyps with a lower potential for malignant transformation. Furthermore, whereas Methylthioninium chloride Cosmo (compared to placebo) increased the fraction of patients with at least one adenoma or carcinoma, there was no difference between placebo and Methylthioninium chloride Cosmo in terms of number of adenomas and carcinomas detected per patient. However, the number of non-polypoid lesions detected per patient was statistically significantly greater in the Methylthioninium chloride Cosmo group compared to placebo.

3.4. Unfavourable effects

Chromaturia (48%) and discoloured faeces (20%) were the most commonly reported adverse events, followed by nausea (6%), vomiting (5%), headache (3%), and abdominal pain (1%), percentages as reported in the phase 3 placebo-controlled trial.

No deaths occurred and no serious adverse events appeared related to Methylthioninium chloride Cosmo.

With regard to drug-drug interactions, it cannot be excluded that serotonin syndrome may occur if Methylthioninium chloride Cosmo is administered concomitantly with serotonergic medicinal products. Adverse events of other medicinal products, especially medicinal products with a narrow therapeutic index, may occur following Methylthioninium chloride Cosmo administration, since Methylthioninium chloride Cosmo is both an inducer and inhibitor, respectively, of various CYP enzymes (reflected in SmPC sections 4.5 and 4.5).

3.5. Uncertainties and limitations about unfavourable effects

The long-term risks related to the use of Methylthioninium chloride Cosmo are unknown. There was not a minimum time of follow-up after administration of Methylthioninium chloride Cosmo, the last safety evaluation took place at a not specified time between the second and the seventh day after colonoscopy. However, long-term adverse events are not labelled for i.v. methylthioninium chloride containing products.

3.6. Effects Table

Effects Table for Methylthioninium chloride Cosmo (Study CB-17-01/06).

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	Ref.
Favourable Effects						
ADR new definition	Adenoma detection rate including SSA and TSA	%	56.29	47.81	P=0.0099	
ADR original definition	Adenoma detection rate excluding SSA and TSA	%	50.52	45.30	P=0.0496 OR 1.3088 [1.0005, 1.7120]	
Unfavourable Effects (as observed in the phase 3 placebo-controlled trial)						
Chromaturia		Number (%)	234 (48.0)	7 (1.5)		SCS Table 8
Faeces discoloured		Number (%)	95 (19.5)	0		SCS Table 8
Nausea		Number (%)	29 (5.9)	17 (3.5)		SCS Table 8
Vomiting		Number (%)	23 (4.7)	13 (2.7)		SCS Table 8
Headache		Number (%)	13 (2.7)	8 (1.7)		SCS Table 8

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	Ref.
Adominal pain		Number (%)	6 (1.2)	2 (0.4)		SCS Table 8

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

ADR is a generally accepted indicator of the quality, and hence the effect in terms of risk reduction, of a programme for screening for CRC. An increase in ADR is likely to result in improvement of the CRC risk reduction although the relationship is not linear. Thus, the exact size of the CRC risk reduction cannot be accurately determined based on this study. Although, the increase in ADR is considered robust, it is primarily the result of detection of smaller, flat lesions. On one hand these generally have a lower risk of transformation than larger lesions, but on the other hand small, flat and thus hard to detect lesions account for a substantial proportion missed polyps/interval cancers (cancers occurring between endoscopies). Thus, while it is acknowledged that observed increase in ADR will reduce risk of CRC, the exact effect size in terms of CRC risk reduction cannot precisely defined.

The safety profile of Methylthioninium chloride Cosmo is adequately characterised and comprise mainly mild or moderate transient symptoms. The safety profile of methylthioninium chloride given iv is well known from previously approved products using this method of administration, albeit for another indication, and do not indicate long-term risks.

3.7.2. Balance of benefits and risks

The benefits of Methylthioninium chloride Cosmo in terms of increase in ADR has been convincingly demonstrated. The safety profile is adequately characterised and there are no major safety issues associated with the drug. The benefit risk balance of Methylthioninium chloride Cosmo is considered positive.

3.7.3. Additional considerations on the benefit-risk balance

The indication reflects that Methylthioninium chloride Cosmo can enhance visualisation and detection of colorectal lesions without any specification of histology. It has not been demonstrated that Methylthioninium chloride Cosmo enhances visualisation of suspected (pre-) cancerous lesions.

3.8. Conclusions

The overall B/R of Methylthioninium chloride Cosmo is positive.

4. Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus decision that the benefit-risk balance of Methylthioninium chloride Cosmo is favourable in the following indication:

Methylthioninium chloride Cosmo is indicated as a diagnostic agent enhancing visualisation of colorectal lesions in adult patients undergoing screening or surveillance colonoscopy.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.