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Efficacy of Per-oral Methylene Blue Formulation for Screening Colonoscopy[a]

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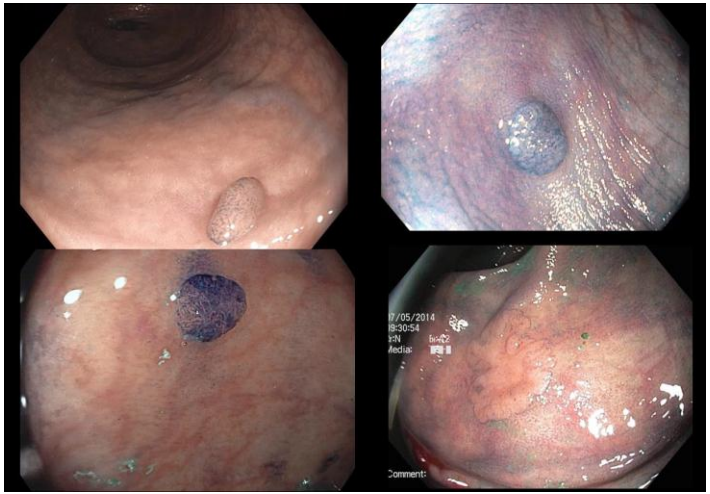
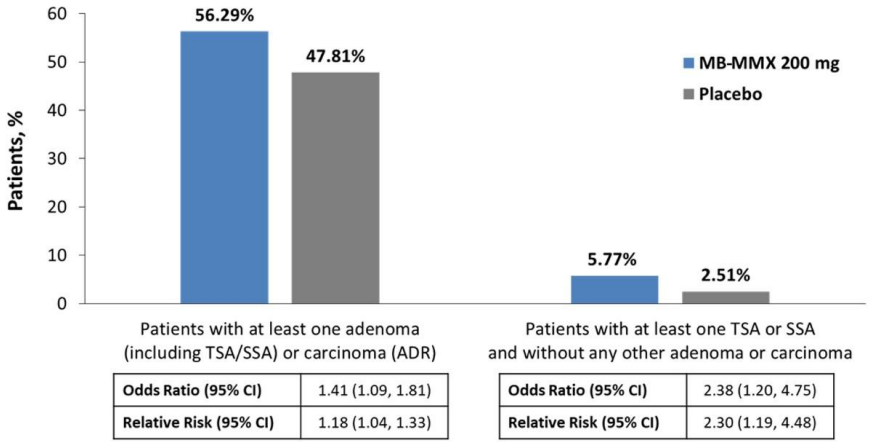
A per oral formulation of Methylene blue increases the adenoma detection rate compared to placebo in patients undergoing colorectal cancer surveillance or screening, without increasing the removal of non-neoplastic lesions.





Odds Ratio (95% CI)	1.41 (1.09, 1.81)
Relative Risk (95% CI)	1.18 (1.04, 1.33)

Odds Ratio (95% CI)	2.38 (1.20, 4.75)
Relative Risk (95% CI)	2.30 (1.19, 4.48)

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Efficacy of Per-oral Methylene Blue Formulation for Screening Colonoscopy[a]

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Author contributions: AR, MW, PS, JE, CH: study concept and design; drafting of the manuscript; all authors: acquisition of data; MRO: statistical analysis; all authors: analysis and interpretation of data.

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Abstract

Background & Aims: Topically applied methylene blue dye chromoendoscopy is effective in improving detection of colorectal neoplasia. When combined with a pH- and time-dependent multi-matrix structure, a per-oral methylene blue formulation (MB-MMX) can be delivered directly to the colorectal mucosa.

Methods: We performed a phase 3 study of 1205 patients scheduled for colorectal cancer screening or surveillance colonoscopies (50–75 years old) at 20 sites in Europe and the United states, from December 2013 through October 2016. Patients were randomly assigned to groups given 200 mg MB-MMX, placebo, or 100 mg MB-MMX (ratio of 2:2:1). The 100 mg MB-MMX group included for masking purposes. MB-MMX and placebo tablets were administered with a 4-liter polyethylene glycol-based bowel preparation. The patients then underwent colonoscopy by an experienced endoscopist with centralized double-reading. The primary endpoint was the proportion of patients with 1 adenoma or carcinoma (adenoma detection rate [ADR]). We calculated odds ratio (OR) and 95% CIs for differences in detection between the 200 mg MB-MMX and placebo groups. False-positive (resection rate for non-neoplastic polyps) and adverse events were assessed as secondary endpoints.

Results: The ADR was higher for the MB-MMX group (273/485 patients, 56.29%) than the placebo group (229/479 patients, 47.81%) (OR, 1.46; 95% CI, 1.09–1.96). The proportion of patients with nonpolypoid lesions was higher in the MB-MMX group (213/485 patients, 43.92%) than the placebo group (168/479 patients, 35.07%) (OR, 1.66; 95% CI, 1.21–2.26). The proportion of patients with adenomas $\leq$5 mm was higher in the MB-MMX group (180/485 patients, 37.11%) than the placebo group (148/479 patients, 30.90%) (OR, 1.36; 95% CI, 1.01–1.83), but there was no difference between groups in detection of polypoid or larger lesions. The false-positive rate did not differ significantly between groups (83/356 patients with non-neoplastic lesions, 23.31% in the MB-MMX vs 97/326 patients with non-neoplastic lesions, 29.75% in the placebo group). Overall, 0.7% of patients had severe adverse events but there was no significant difference between groups.

Conclusions: In a phase 3 trial of patients undergoing screening or surveillance colonoscopies, we found MB-MMX led to an absolute 8.5% increase in ADR, compared to placebo, without increasing the removal of non-neoplastic lesions. Clinicaltrials.gov no: NCT01694966

KEY WORDS: colon cancer; chromoendoscopy; endoscopy; visualization

Background

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and second most common cause of death from cancer worldwide.[1, 2] Colonoscopy with polypectomy has been shown to reduce CRC incidence and mortality.[3, 4] Thus, its use as first tier screening test is recommended,[5,6] The degree of CRC prevention by screening colonoscopy has been closely associated with adenoma detection rate (ADR),[7-9] with higher rates being associated with lower interval cancers.[7, 8,10]

Widespread application of blue dye to the mucosal surface of the colon has been shown to increase detection of colorectal neoplasia in patients at average or increased risk of CRC due to selective staining of subtle and non-polypoid lesions, both adenomas and sessile serrated adenomas (SSA).[11-17] While recommended for high-risk patients, i.e. ulcerative colitis or hereditary CRC syndromes,[18, 19] use of blue dye has been considered too time consuming for average-risk subjects, and it is currently not recommended.[19]

A case series using dye-powder given with the bowel preparation showed variable dye-distribution and staining.[20] To overcome such limitation, we combined methylene blue with a per oral, colon-release, pH- and time-dependent multimatrix structure (MB-MMX) able to directly deliver the agent in the colon lumen. We hypothesized that, when orally administered with bowel preparation, MB-MMX tablets may increase ADR by staining and contrast-enhancement of the colorectal mucosa.

We conducted this multicenter, placebo-controlled, randomized, double-blind, phase III study to assess the efficacy and safety of MB-MMX for CRC screening and surveillance.

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Methods

Study Population

Twenty clinical sites participated in this multicenter FDA-registration trial conducted in Europe and United States between December 2013 and October 2016. Approval was obtained from all institutional review boards, and study participants signed written informed consent (NCT01694966). The target population included 50 to 75-years-old subjects scheduled for CRC screening or surveillance colonoscopy. Exclusion criteria are listed in Appendix 1. In detail, patients with cardiovascular or other comorbidities were excluded, as well as those with deficiency of glucose-6-phosphate dehydrogenase or nicotinamide adenine dinucleotide phosphate reductase, and those treated with fluoxetine or selective serotonin reuptake inhibitors. All authors had access to the study data and reviewed and approved the final manuscript.

Randomization

Eligible subjects were allocated to 200 mg MB-MMX arm, placebo arm or 100 mg MB-MMX arm according to a 2:2:1 ratio. Randomization was stratified by center and reason for colonoscopy (screening, surveillance <2 years or ≥2 years from previous colonoscopy). The randomization algorithm – by permuted block, block size 5 – was generated by computer programme by an independent CRO. The patients were randomised using a central IWRS (Interactive Web Response System) system. The study was in double-blind (patient and endoscopists), and all treatment kits were visually identical. Due to the characteristic of the product and to the trial design, the endoscopist were able to ascertain whether a patient had been administered with tablets containing MB or with placebo tablets. In order to minimize this unavoidable source of bias, a masking arm with a low dose of methylene blue (100 mg MB-MMX) was included in the study, under request by the FDA (confounding arm, i.e. not powered for statistical analysis). The 2 central study histopathologists, whose histological classifications

of the resected/biopsied specimens were used for the primary and secondary endpoint assessment, remained blinded throughout the study. The Sponsor remained blinded throughout the study, up to the database lock. A study centre staff member at each site dispensed the individual clinical supplies according to the randomisation number assigned by the IWRS. Details of study visits are reported in Appendix 2.

Colonoscopy Bowel Preparation and Drug Administration

Polyethylene Glycol

Following a low residue diet for 3 days prior to colonoscopy, all patients received a standard 4 L polyethylene glycol-based preparation (Selg-Esse 1000, Alfasigma; NuLytely, Braintree Laboratories), starting in the late afternoon before the colonoscopy day. Patients drank at least 250 mL of bowel preparation every 15 minutes, to complete the administration 4 hours after commencement.

MB-MMX or Placebo

MB-MMX 200 mg. Patients received an oral dose of 8 tablets of 25 mg MB-MMX: 3 tablets (75 mg) after 2 L of bowel preparation, 3 tablets (75 mg) after 3 L, and 2 tablets (50 mg) after all 4 L had been consumed.

MB-MMX 100 mg. Patients received an oral dose of 4 tablets of 25 mg MB-MMX: 1 MB-MMX tablet and 2 placebo tablets after 2 L of bowel preparation, 2 MB-MMX tablets (50 mg) and 1 placebo tablet after 3 L, and 1 MB-MMX tablet and 1 placebo tablet after all 4 L had been consumed. Based on Phase II study, such reduced dose was still expected to offer some staining of the colorectal mucosa, creating a confounding effect on the operator between the active and the masking arm, while being at least 40% less effective than the active arm in staining the colorectal mucosa. (Editor)

Placebo. Patients received an oral dose of 8 tablets of 25 mg placebo: 3 tablets after 2 L

of bowel preparation, 3 tablets after 3 L, and 2 tablets after all 4 L had been consumed.

Before colonoscopy, compliance with study drug (≥75%) and occurrence of adverse events (AEs) was assessed.

Colonoscopy

Before enrollment, all endoscopists (1-2 per centre) completed an online training course with qualifying examination on chromoendoscopy. Study colonoscopies were to be performed in the morning using high-definition (HD) endoscopes. Use of narrow-band imaging and other electronic chromoendoscopy techniques were not permitted as not recommended at that time.[21] Lesions were classified (location, size, morphology: polypoid, Ip/Is and non-polypoid, IIa/IIb/IIc/LST[22]) and removed (biopsy for nonresectable lesions). Time to reach the caecum and clean withdrawal time, excluding intervention time, if any, were recorded. For this purpose, a dedicated computer to measure the clean withdrawal time was supplied to the sites: endoscopists were instructed to stop the withdrawal time before initiating a resection and to restart the timer when the resection was completed. In addition, a red signal on the screen alerted the endoscopist when the 6-minute required threshold was reached. Bowel preparation was scored according to Boston Bowel Preparation Scale (BBPS).[23] Sedation was carried out according to the local practice.

Central Reading

Recording. For each patient, the whole endoscopy was digitally recorded in HD without any loss of quality and stored at both local and remote (cloud) setting, and areas with polypoid or nonpolypoid lesions were digitally annotated. The videos were then randomly allocated to the central viewers by the CRO.

Double-reading. The recorded endoscopy was reviewed by a central endoscopist for concordance

between investigator and reviewer interpretation of the need to remove the lesion (i.e., obvious elevation or depression, mucosal nodular irregularity, interruption of the course of superficial vascular network) as well as to assess if the area where the lesion/polyp had been identified was stained or not stained, if mucosal lesions/polyps had been missed, and if cecal intubation was successful. Consensus between local and central reading with regards to need for excision of identified lesions was compared using Cohen's κ (Appendix 3). In case of disagreement, the local endoscopist reviewed the case and the required corrections were done.

Central and Local Pathology

Each lesion was stored in a separate specimen bottle. Histologic assessment was made by two regional, blinded central laboratory pathologists (1 in Europe, 1 in America), who individually reviewed additional slides prepared at the local center laboratory, based on Vienna category and serrated lesion classification.[24, 25] For the study endpoint, only the central read pathology was considered. For the purpose of this study, adenoma was not limited to histologically proven Vienna Grade 3 to 4.2 lesions, but also histologically proven traditional serrated adenomas (TSAs) or sessile serrated adenomas (SSAs), as required by FDA.

Compliance and Safety

The site investigator assessed the compliance of the patient to allocated treatment, determining the amount of study medication dispensed to the subject and that of unused medication returned. Physical examination with vital signs and blood check were performed, and AEs were assessed by the investigator at each pre- and endoscopic-visits and 3 to 7 days after colonoscopy (Appendix 2).

Statistical Analysis

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Study Endpoints

The primary endpoint of this study was to assess the efficacy of 200 mg of MB-MMX versus placebo in terms of the proportion of patients with at least one histologically proven adenoma (including TSA and SSA) (R1-1) or carcinoma (ADR). The main secondary endpoint was the false-positive rate (FPR) defined as the proportion of patients with no adenoma within any excised lesions who had undergone at least one excision with histopathological examination (R1-3); FPR was required by FDA to avoid indiscriminate removal of clinically irrelevant lesions. Other secondary endpoints were the proportion of patients with either adenoma or carcinoma (also according to size, location, and morphology); and the rate and type of AEs (according to Medical Dictionary for Regulatory Activities). As methylene blue is well-known to cause chromaturia, feces discoloration, and blue sclera, which are all clinically irrelevant AE, we calculated rate of AEs after excluding these cases.

Analyses Sets

Intention-to-treat (ITT) Set. This set was used for sensitivity analyses and included all randomized patients, regardless of study drug intake, colonoscopy execution, and colon cleansing.

Full analysis set (FAS). This set was used for primary efficacy analysis and included all randomized patients who received at least one dose of the study drug and underwent colonoscopy (regardless of completion status).

Per-protocol (PP) set. This set was used for sensitivity analyses and included all randomized patients who fulfilled study protocol requirements in terms of study drug intake (≥75%) and collection of primary efficacy data (full colonoscopy successfully executed), had an acceptable colon cleansing, and did not have inclusion/exclusion criteria violation and no major deviations.

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Safety set. All patients who received at least one dose of the study drug.

Primary analysis was a logistic regression on the proportion of patients with at least one histologically proven adenoma (including TSA and SSA) (R1-1) or carcinoma colonoscopy in the FAS population, expressed as odds ratio (OR_{LR}), between the 200 mg MB-MMX and placebo groups. The 100 mg MB-MMX arm was excluded as only for masking purposes (100 mg MB-MMX data are reported in Appendix 4). Treatment, center, age, sex, indication for colonoscopy and number of excisions were included in the regression model as fixed effects. Unadjusted relative risks (RR) were also assessed as directly related to the clinical efficacy of the drug. FPR was compared between the two groups according to the following hypothesis test: the null hypothesis was rejected if the upper bound of the 95% CI of the difference, $FPR_{Full\ Dose} - FPR_{placebo}$, was less than the proportion, $P_{Threshold}$. A $P_{Threshold}$ of 15% for $FPR_{Full\ Dose} - FPR_{placebo}$ was established.

Sample Size

The superiority of 200 mg MB-MMX versus placebo was tested in terms of the adjusted odds ratio derived from the logistic regression model according to the following hypothesis test: $H_0 = OR_{LR} \leq 1$; $H_a = OR_{LR} > 1$. The null hypothesis was rejected if the lower bound of the 95% CI of the adjusted odds ratio was greater than 1. Sensitivity analyses were performed on the PP and ITT sets. Considering an exclusion rate from the FAS around 5%, a sample size of at least 1,270 patients was selected to achieve at least 1,203 evaluable patients.

Results

Study Population

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Out of 1,346 screened patients, a total of 1,249 (ITT) were randomized. Of these, 1,205 (96.5%), 1,137 (91.0%), and 1,208 (96.7%) were entered in Full Analysis Set, Per Protocol, and Safety analysis, respectively (Figure 1). As shown in Table 1, no differences in demographics, clinical indications, or other baseline clinical characteristics was observed across the study arms. A mean compliance to the study drug of 99.6%±4.8% was achieved, with similar proportions of compliance across the treatment groups. A total of 1,198/1,205 (99.4%) patients achieved a compliance of ≥75% (Appendix 5).

Adenoma Detection Rate (ADR)

A total of 626/1,205 (51.95%) patients had at least one adenoma or carcinoma at colonoscopy. ADR was higher in the 200 mg MB-MMX arm (273/485 [56.29%]) than the placebo arm (229/479 [47.81%]), corresponding to an OR_{LR} of 1.41 (1.09, 1.81) (Figure 2). The difference further increased at PP analysis (58.24% vs. 47.92%; OR_{LR}, 1.52 [1.17, 1.97]), and it was not affected by study centers at regression analysis (Appendix 6). In addition, the proportion of patients with at least one TSA or SSA was higher in the 200 mg MB-MMX arm than the placebo arm (5.8% vs. 2.5%; OR_{LR}, 2.38 [1.20, 4.75]) (Figure 2). Regarding morphology (Figure 3), the rate of patients with nonpolypoid lesions was higher in the 200 mg MB-MMX arm (213/485 [43.92%]) than the placebo arm (168/479 [35.07%]; OR_{LR}, 1.45 [1.12, 1.88]), while no difference was found for those with polypoid lesions (50.52% vs. 49.69%; OR_{LR}, 1.03 [0.80, 1.33]). Regarding polyp size (Figure 4), the proportion of patients with ≤5 mm adenomas (Table 2) was higher in the 200 mg MB-MMX group (180/485 [37.11%]) than in the placebo group (148/479, 30.90%; OR_{LR}, 1.32 [1.01, 1.72]), whilst no difference for those with 6-9 mm or ≥10 mm as largest lesion was observed (Table 2). Corresponding RR are provided in Table 2 for all analysis.

When relating the detection rate with the absolute number of resections performed, the proportion of adenoma-bearing patients with ≤3 polyps resected was higher in the 200 mg MB-

MMX group (164/362 [45.30%]) than in the placebo group (134/375 (35.73%); OR_{LR}, 1.56 [1.14, 2.13]), while no difference was observed for those patients with ≥4 polyps removed.

FPR

Overall, 850/1,205 (70.54%) patients had polyp resections. Of these, 224 (26.35%) did not have histologically proven adenoma or carcinoma, with similar proportions reported across the treatment groups. As shown in Appendix 7, the placebo group had the highest proportion of patients with excisions of non-neoplastic lesions (97/326 [29.75%]), while the lowest proportion was reported in the 200 mg MB-MMX group (83/356 [23.31%]), excluding a higher FPR rate in the MB-MMX full dose group (*P* value for testing the null hypothesis: <.0001).

Centralized Reading of Colonoscopy, Bowel Cleansing, and Withdrawal Times

At centralized reading of colonoscopy, 962/1,205 (79.83%) lesions detected in the 200 mg MB-MMX arm were in stained areas (Appendix 8). BBPS was locally recorded for 1,201/1,205 (99.67%) patients, with a mean total score – for the non-split regimen adopted in our study – of 6.7±1.7 and similar total scores reported across the treatment groups (Appendix 8). By subgrouping the 200 mg MB-MMX and the placebo groups according to the level of cleansing, the therapeutic effect of MB-MMX – defined as difference in ADR between the active and control groups – was limited to those with adequate cleansing level (BBPS≥6), while no effect was obtained in those with BBPS<6 (Δ ADR 200 mg MB-MMX-placebo: 7.5% vs 1%; p<0.01). Time to reach the caecum was reported for 1,161/1,205 (96.35%) patients, with a mean of 10.3 ±6.5 minutes and similar values between the groups. The (clean) withdrawal time was reported for 1,129/1,205 (93.69%) patients. Overall, it was ≥6 minutes in 90.9% and 90.8% in the 200 mg MB-MMX and placebo arms, respectively (p=1), with a mean of 11.5±5 minutes (200 mg MB-MMX: 12.2±5.6 minutes vs. placebo: 10.7±4.4 minutes).

Safety

In total, 49.4% of patients in the Safety Set had AEs (992 events) during the study (Table 3**)**. The proportion of patients with AEs was higher in the 200 mg MB-MMX (64.3%), mainly chromaturia and discolored feces, which are related to the presence of a vital dye in the drug formulation, when compared to the placebo (29.2%). When excluding these cases, the rate of AEs was similar between the two arms (200 mg MB-MMX: 145/488 [29.71%] vs placebo: 135/479 [28.18%]; *P*=.27), and mainly consisted of nausea, vomiting and headache. Overall, 0.7% of patients had severe AEs, including 4/488 patients (0.82%) in the 200 mg MB-MMX group and 2/479 patients (0.42%) in the placebo group. Few minor changes (16/1208; 1.32%) were found at blood meaasurements throughout the study with no difference in distribution between 200 mg MB-MMX (3/16), 100 mg MB-MMX (4/16) and placebo (13/16) groups.

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Discussion

Oral administration of MB-MMX was associated with a clinically relevant increase in the ADR during screening/surveillance colonoscopy, corresponding to an absolute increase of 8.5% and 10% at FAS and PP analysis, respectively. This appeared to be mainly related to the detection of ≤5 mm and nonpolypoid adenomatous lesions in patients with only one or few lesions, as expected when using chromoendoscopy.[11-17] In addition, use of MB-MMX also resulted in a two-fold increase in the proportion of patients with SSA and TSA, a result also expected when using chromoendoscopy.[11-17] The evidence that most of the detected lesions in the 200 mg MB-MMX arm were classified as stained gives plausibility to the observed efficacy of the drug, and suggests that MB-MMX works effectively as a contrast-enhancement technique.

Such ADR increase was not associated with a higher FPR, i.e., useless removal of non-neoplastic polyps, as the rate was not higher with 200 mg MB-MMX compared to the placebo, excluding an operator-related bias, i.e. an artificial ADR increase due to an indiscriminate resection policy in the active arm (R1-3). We also showed that MB-MMX was well tolerated , as the most frequently reported AEs, chromaturia and fecal discoloration, were merely due to the staining effect of the vital dye. (Editor) Of note, we excluded patients on selective serotonin reuptake inhibitors (SSRIs), as the concomitant intravenous use of methylene blue – a potent monomonoamine oxidase (MAO) inhibitor – has been associated with serotonine toxicity. However, the widespread use of MB-MMX in average-risk screening setting could result in its inappropriate administration to patients on SSRIs. At this regard, it may be relevant to note that no serotonin toxicity occurred to those very few patients who were included in our trial, despite the concomitant use of SSRIs. This was not unexpected as the intravenous concentration after MB-MMX is of one magnitude inferior as compared to that achieved after the intravenous administration of methylene blue. Preliminary evidence suggested the possibility of DNA damage when using methylene blue for chromoendoscopy.[26, 27] For this reason, we assessed, in a

previous Phase II study, possible DNA damage in colorectal biopsies of patients treated with a single dose of MB-MMX 200 mg, showing the lack of any genotoxicity.[28] In addition, it should be noticed that, differently from traditional chromoendoscopy, there is no possibility to use a non-vital alternative as indigo carmine for a per oral formulation due to different pharmacodynamic properties.

The clinical relevance of this study is strictly associated with the long-term implications of a nearly 10% absolute increase in ADR achieved by MB-MMX on the subsequent risk of post-colonoscopy CRC. When considering that a 1% absolute increase in ADR has been associated with a relative 3% reduction in the risk of CRC, the contribution of MB-MMX to reduce the risk of post-colonoscopy CRC may be relevant.[8] In addition, very high ADR values, as those reached by MB-MMX, have been associated with the most profound reduction of such post-colonoscopy risk.[7, 8, 10] Secondly, the approximately two-fold increase in detection of clinically relevant serrated lesions, SSA and TSA, may contribute to reduction in risk of proximal CRC.[29, 30,31, 32,33] When considering the high ADR in the control group, we cannot exclude that MB-MMX may have additional benefits when applied to 'low-detectors', and further studies are needed.

The main strength of our study is the level of bias controls, mainly through utilization of a centralized histopathology and double-reading for endoscopic procedures, as well as a masking arm. The main limitations were represented by the impossibility to fully blind the operator to the allocated arm, similarly to all the previous chromoendoscopy studies. In order to minimize this bias, we utilized double reading in order to assure an adequate quality of the procedure in both arms. In addition, we reduced such bias by incorporating a masking arm, with a reduced dose of MB-MMX, so that the operator could have never been fully confident that the patient was actually enrolled in the active arm. Of note, the masking arm with 100mg MB-MMX dose resulted only in an intermediate ADR between placebo and 200 mg MB-MMX arms, excluding that the 200 mg MB-MMX benefit was due to the unblinding of the operator. We also

marginalized this operator-bias by excluding a higher rate of false-positive resection in the active as compared with the placebo arms. The second limitation was the lack of a split regimen in the study protocol. However, when the study was designed, most centres had not yet adopted split bowel preparation as standard of care. This was further mitigated by the fact that study colonoscopies were to be performed only in the morning according to our protocol. At this regard, the therapeutic effect of MB-MMX was limited in those with adequate cleansing level (BBPS≥6), while no effect was obtained in the minority with BBPS<6 (data not shown). As a split regimen has been associated with an increase in ADR,[34] a possible synergism with MB-MMX – to be yet administered the day before colonoscopy – cannot be excluded. Third, withdrawal time was slightly but significantly (R2-1) longer in both the 200 mg and 100 mg MB-MMX arms than in the placebo group. However, the rate of procedures fulfilling the main key quality threshold of at least 6 minutes of withdrawal time[35, 36] – the only criteria required by our protocol – was similar across the study arms, assuring the lack of influence of such difference on the main study result. In addition, the slight difference in withdrawal time has been frequently reported in chromoendoscopy studies when using both dye-spray and electronic chromoendoscopy techniques,[11, 3337] presumably due to the need of additional washing and the combined effect of a darker and more contrast endoscopic image. At this regard, only interventions, but not washing procedures, were excluded when calculating the clean withdrawal time, and this may be considered as an additional limitation of our study. However, in both arms, clean withdrawal time was ≥10 minutes, excluding the risk of a suboptimal withdrawal technique.

In conclusion, our study showed the efficacy of orally administered MB-MMX dye with bowel preparation in increasing the ADR, a clinically relevant endpoint of screening and surveillance colonoscopy (R1-4).

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Legends

Figure 1. Flowchart of the Study

Figure 2. Distribution of Patients in the Two Groups According to Final Diagnosis. ADR indicates adenoma detection rate; MB-MMX, methylene blue-multimatrix structure; SSA, sessile serrated adenoma; TSA, traditional serrated adenoma.

Figure 3. Distribution of Patients in the Two Groups According to Morphology of the Detected Lesion. MB-MMX indicates methylene blue-multimatrix structure.

Figure 4. Distribution of Patients in the Two Groups According to Size of the Detected Lesion. MB-MMX indicates methylene blue-multimatrix structure.

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Table 1. Patient Baseline Demographics and Reason for Colonoscopy (ITT Population)

Characteristic	MB-MMX 200 mg (n=504)	MB-MMX 100 mg (n=247)	Placebo (n=498)	Overall (N=1,249)
Sex, No. (%)				
Female	202 (40.1)	105 (42.5)	191 (38.4)	498 (39.9)
Male	302 (59.9)	142 (57.5)	307 (61.6)	751 (60.1)
Race, No. (%)				
Asian	6 (1.2)	1 (0.4)	5 (1.0)	12 (1.0)
Black or African American	38 (7.5)	15 (6.1)	24 (4.8)	77 (6.2)
Hispanic or Latino	5 (1.0)	3 (1.2)	3 (0.6)	11 (0.9)
Native Hawaiian or other Pacific Islander	0 (0.0)	1 (0.4)	1 (0.2)	2 (0.2)
White	451 (89.5)	226 (91.5)	462 (92.8)	1139 (91.2)
Other	3 (0.6)	1 (0.4)	3 (0.6)	7 (0.6)
Age, y				
Mean (SD)	61.2 (6.8)	61.0 (6.5)	61.6 (6.8)	61.3 (6.7)
Median (range)	61.0 (50-75)	61.0 (50-75)	62.0 (50-75)	62.0 (50-75)
Reason for colonoscopy, No. (%)				
Screening	243 (48.2)	116 (47.0)	239 (48.0)	598 (47.9)
Surveillance within 2 y from previous colonoscopy	28 (5.6)	18 (7.3)	32 (6.4)	78 (6.2)
Surveillance after more than 2 y	233 (46.2)	113 (45.7)	227 (45.6)	573 (45.9)

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from previous colonoscopy

Abbreviations: MB-MMX, methylene blue-multimatrix structure; ITT, intention-to-treat.

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Table 2. Efficacy Results at Per-Patient Analysis (FAS Analysis)

Proportion of patients with:	**MB-MMX 200 mg (n=485), No. (%)**	**Placebo (n=479), No. (%)**	**Odds Ratio (95% CI)**	**Relative Risk (95% CI)**
Histology				
At least 1 adenoma (including TSA/SSA) or carcinoma (ADR)	273 (56.29)	229 (47.81)	1.41* (1.09, 1.81)	1.18* (1.04, 1.33)
At least 1 adenoma (including SSA/TSA), without carcinoma	268 (55.26)	220 (45.93)	1.45* (1.13, 1.87)	1.20* (1.06, 1.36)
At least 1 adenoma (excluding SSA/TSA), without carcinoma	230 (47.42)	186 (38.83)	1.42* (1.10, 1.84)	1.22* (1.06, 1.41)
At least 1 TSA or SSA, without any other adenoma or carcinoma	28 (5.77)	12 (2.51)	2.38 (1.20, 4.75)	2.30 (1.19, 4.48)
At least 1 carcinoma	5 (1.03)	9 (1.88)	0.54 (0.18, 1.64)	0.55 (0.19, 1.63)
Morphology				
At least 1 nonpolypoid lesion	213 (43.92)	168 (35.07)	1.45* (1.12, 1.88)	1.25* (1.07, 1.47)
At least 1 polypoid lesion	245 (50.52)	238 (49.69)	1.03 (0.80, 1.33)	1.02 (0.92, 1.27)

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Size				
At least 1 adenoma or carcinoma ≤5 mm	180 (37.11)	148 (30.90)	1.32** (1.01, 1.72)	1.20** (1.01, 1.43)
At least 1 adenoma or carcinoma 6-9 mm	62 (12.78)	56 (11.69)	1.11 (0.75, 1.63)	1.09 (0.78, 1.53)
At least 1 adenoma or carcinoma ≥10 mm	67 (13.81)	67 (13.99)	0.99 (0.68, 1.42)	0.99 (0.72, 1.35)

Abbreviations: FAS, full analysis set; MB-MMX, methylene blue-multimatrix structure; SSA, sessile serrated adenoma; TSA, traditional serrated adenoma.

*p<0.01
**p<0.05

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Table 3. Treatment-Emergent Adverse Events

Adverse events	MB-MMX 200 mg (n=488), No. (%)	MB-MMX 100 mg (n=241), No. (%)	Placebo (n=479), No. (%)	Overall (N=1208), No. (%)
Incidence	314 (64.3)	143 (59.3)	140 (29.2)	597 (49.4)
Related	256 (52.5)	111 (46.1)	21 (4.4)	388 (32.1)
Not related	129 (26.4)	72 (29.9)	124 (25.9)	325 (26.9)
Gastrointestinal disorders	192 (39.3)	76 (31.5)	107 (22.3)	375 (31.0)
Discolored feces	95 (19.5)	43 (17.8)	0 (0.0)	138 (11.4)
Hemorrhoids	29 (5.9)	15 (6.2)	36 (7.5)	80 (6.6)
Nausea	29 (5.9)	9 (3.7)	17 (3.5)	55 (4.6)
Vomiting	23 (4.7)	2 (0.8)	13 (2.7)	38 (3.1)
Renal and urinary disorders	234 (48.0)	102 (42.3)	8 (1.7)	344 (28.5)
Chromaturia	234 (48.0)	102 (42.3)	7 (1.5)	343 (28.4)
Nervous system disorders	19 (3.9)	8 (3.3)	13 (2.7)	40 (3.3)
Headache	13 (2.7)	8 (3.3)	8 (1.7)	29 (2.4)
Intensity				
Mild	293 (60.0)	137 (56.8)	128 (26.7)	558 (46.2)
Moderate	39 (8.0)	9 (3.7)	17 (3.5)	65 (5.4)
Severe	4 (0.8)	2 (0.8)	2 (0.4)	8 (0.7)
Life-threatening	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Death	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

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Leading to discontinuation	4 (0.8)	0 (0.0)	2 (0.4)	6 (0.5)

Abbreviation: MB-MMX, methylene blue-multimatrix structure

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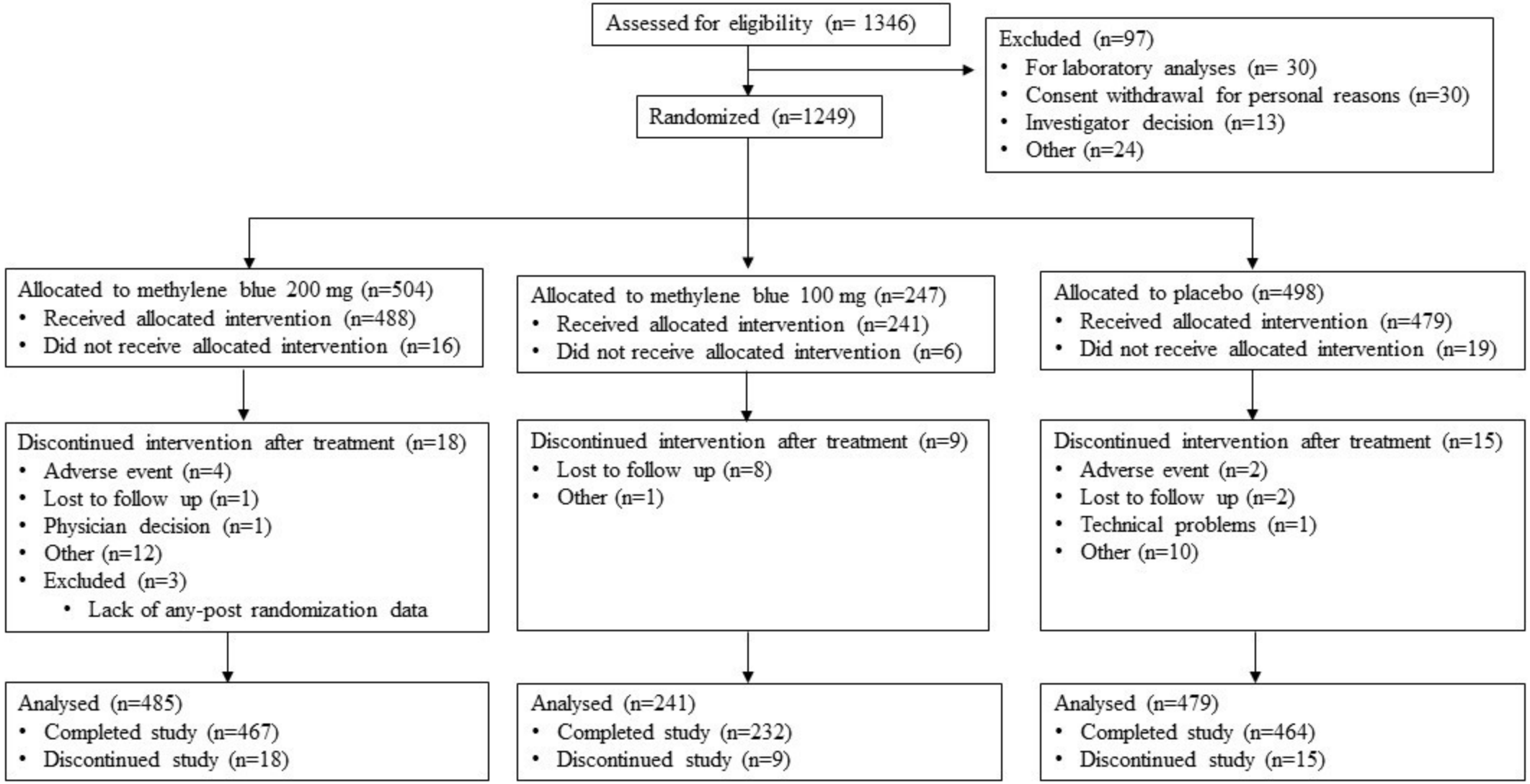
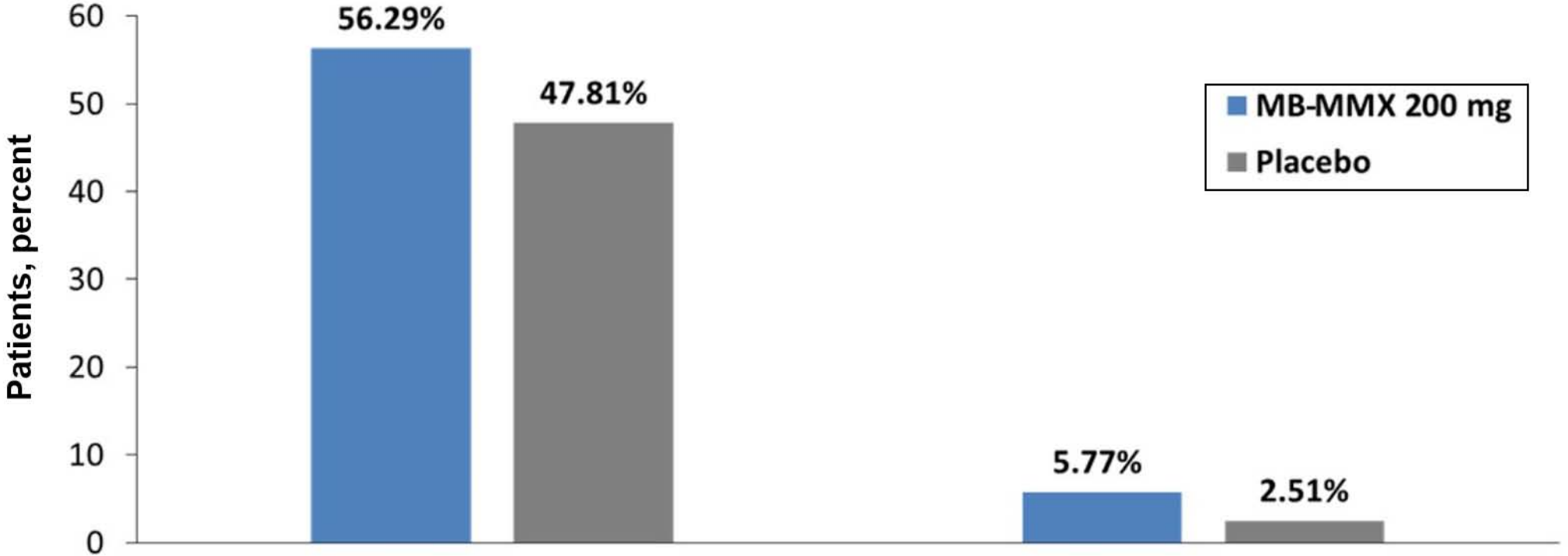

Assessed for eligibility (n= 1346)
Excluded (n=97)
• For laboratory analyses (n= 30)
• Consent withdrawal for personal reasons (n=30)
• Investigator decision (n=13)
• Other (n=24)
Randomized (n=1249)
Allocated to methylene blue 200 mg (n=504)
• Received allocated intervention (n=488)
• Did not receive allocated intervention (n=16)
Allocated to methylene blue 100 mg (n=247)
• Received allocated intervention (n=241)
• Did not receive allocated intervention (n=6)
Allocated to placebo (n=498)
• Received allocated intervention (n=479)
• Did not receive allocated intervention (n=19)
Discontinued intervention after treatment (n=18)
• Adverse event (n=4)
• Lost to follow up (n=1)
• Physician decision (n=1)
• Other (n=12)
• Excluded (n=3)
• Lack of any-post randomization data
Discontinued intervention after treatment (n=9)
• Lost to follow up (n=8)
• Other (n=1)
Discontinued intervention after treatment (n=15)
• Adverse event (n=2)
• Lost to follow up (n=2)
• Technical problems (n=1)
• Other (n=10)
Analysed (n=485)
• Completed study (n=467)
• Discontinued study (n=18)
Analysed (n=241)
• Completed study (n=232)
• Discontinued study (n=9)
Analysed (n=479)
• Completed study (n=464)
• Discontinued study (n=15)

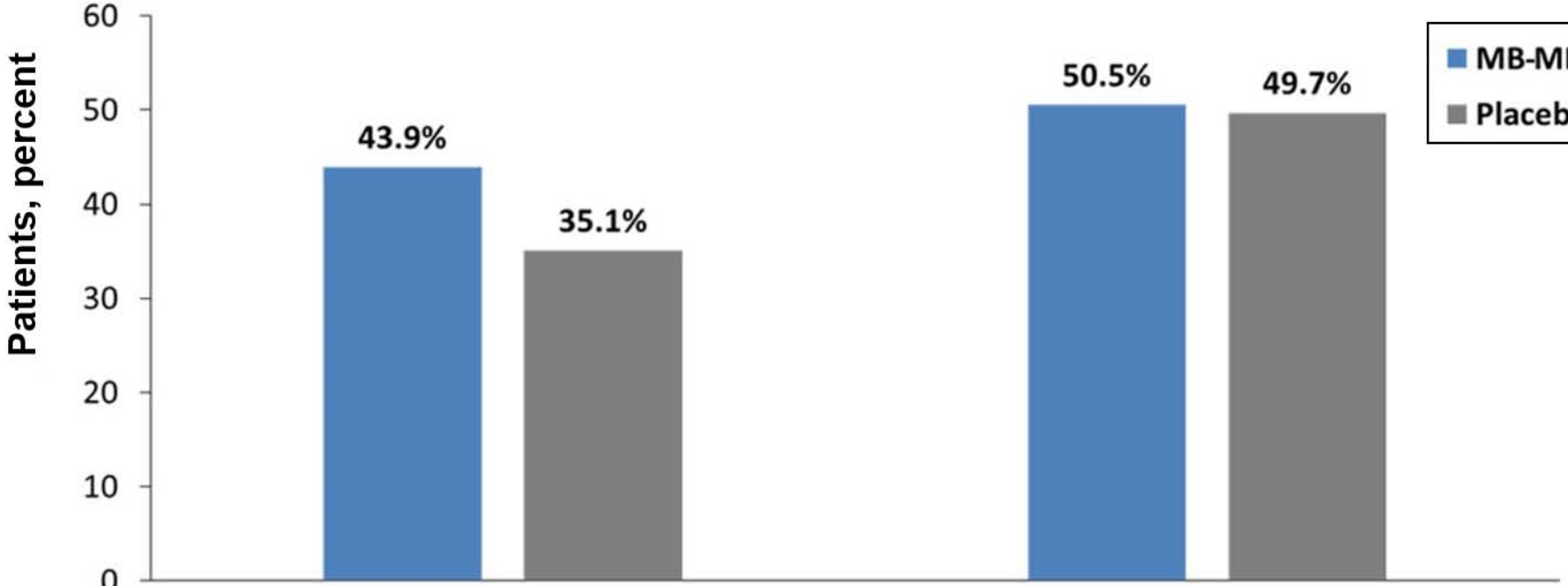


Patients with at least one adenoma (including TSA/SSA) or carcinoma (ADR)

Odds Ratio (95% CI)	1.41 (1.09, 1.81)
Relative Risk (95% CI)	1.18 (1.04, 1.33)

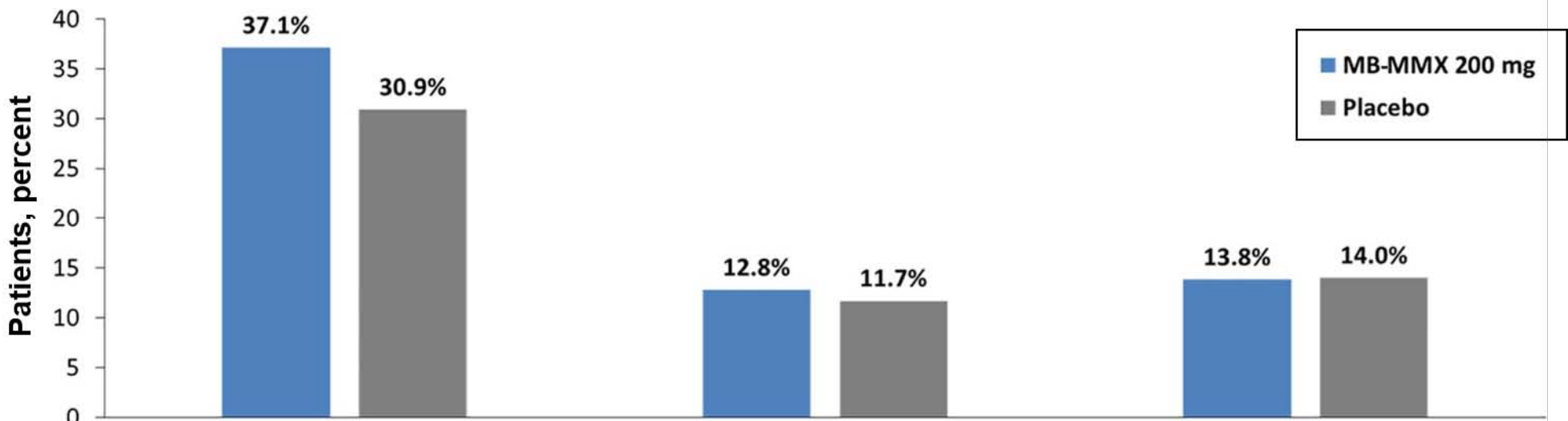
Patients with at least one TSA or SSA and without any other adenoma or carcinoma

Odds Ratio (95% CI)	2.38 (1.20, 4.75)
Relative Risk (95% CI)	2.30 (1.19, 4.48)



Odds Ratio (95% CI)	1.45 (1.12, 1.88)
Relative Risk (95% CI)	1.25 (1.07, 1.47)

Odds Ratio (95% CI)	1.03 (0.80, 1.33)
Relative Risk (95% CI)	1.02 (0.92, 1.27)



Odds Ratio (95% CI)	1.32 (1.01, 1.72)
Relative Risk (95% CI)	1.20 (1.01, 1.43)

Odds Ratio (95% CI)	1.11 (0.75, 1.63)
Relative Risk (95% CI)	1.09 (0.78, 1.53)

Odds Ratio (95% CI)	0.99 (0.68, 1.42)
Relative Risk (95% CI)	0.99 (0.72, 1.35)

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Appendix 1. Study exclusion criteria.

Patients were excluded for high-risk of CRC (i.e., inflammatory bowel diseases and familial cancer syndromes), pregnancy or lactation, previous hypersensitivity to methylene blue or polyethylene glycol, history of either gastrointestinal obstruction, perforation, severe diverticulitis or major colonic resection. Patients with cardiovascular or other comorbidities were also excluded, as well as those with deficiency of glucose-6-phosphate dehydrogenase or nicotinamide adenine dinucleotide phosphate reductase, and those treated with fluoxetine or selective serotonin reuptake inhibitors.

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Appendix 2. Details on Study Visits Performed Within the Study.

VISIT #	VISIT 01	VISIT 01 A*	-	-	-	VISIT 02	VISIT 03
TIMELINE	DAY -32 to 0	DAY -29 to 0	DAY 01	DAY 02	DAY 03	DAY 04	WITHIN 3-7 DAYS FROM COLONOSCOPY
PROCEDURE	SCREENING	RANDOMISATION	LOW RESIDUE DIET BEGINS	SECOND DAY OF LOW RESIDUE DIET	IMP INTAKE BEGINS	COLONOSCOPY PROCEDURE DAY	FOLLOW-UP
Issue patient instructions for diet, bowel preparation and IMP intake	X	X					
Book date for colonoscopy	X	X					
Blood evaluation	X	X					
Randomisation	X	X					
Reported AE check		X				X	X
Dispensation of study medication[2]	X	X					
Complete patient's eCRF [3]	X	X				X	X
Low residue diet for patient			X	X	X		
Intake of bowel cleansing preparation					X		
Fasting (non-gaseous water intake only)					X	X	

VISIT #	VISIT 01	VISIT 01 A*	-	-	-	VISIT 02	VISIT 03
TIMELINE	DAY -32 to 0	DAY -29 to 0	DAY 01	DAY 02	DAY 03	DAY 04	WITHIN 3-7 DAYS FROM COLONOSCOPY
PROCEDURE	SCREENING	RANDOMISATION	LOW RESIDUE DIET BEGINS	SECOND DAY OF LOW RESIDUE DIET	IMP INTAKE BEGINS	COLONOSCOPY PROCEDURE DAY	FOLLOW-UP
Obtain written informed consent	X						
Confirm Inclusion/ Exclusion Criteria met	X	X					
Record concomitant medications	X	X				X	
Record demographics	X						
Record medical history	X	X				X	
Physical examination	X						
Record vital signs	X					X	
Pregnancy test (women)[4]	X				X	X	
Blood collection[1]	X					X	X

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VISIT #	VISIT 01	VISIT 01 A*	-	-	-	VISIT 02	VISIT 03
TIMELINE	**DAY -32 to 0**	**DAY -29 to 0**	**DAY 01**	**DAY 02**	**DAY 03**	**DAY 04**	**WITHIN 3-7 DAYS FROM COLONOSCOPY**
PROCEDURE	**SCREENING**	**RANDOMISATION**	**LOW RESIDUE DIET BEGINS**	**SECOND DAY OF LOW RESIDUE DIET**	**IMP INTAKE BEGINS**	**COLONOSCOPY PROCEDURE DAY**	**FOLLOW-UP**
Intake of IMP					X		
Return of study medication						X	X
Assess colon cleansing score (Boston Bowel Prep)						X	
Colonoscopy (with excisions as required)						X	

[1]Blood test included liver and renal function testing (creatinine, urea, GGT, AST, ALT, total bilirubin, serum pregnancy test for females of childbearing potential).

[2]Dispensation of study medication (IMP and bowel preparation) performed after the assignment of the randomisation number.

[3]All visits data was to be entered into the eCRF within 72 h.

[4]For women of childbearing potential a negative serum pregnancy test result was obtained during screening, and a negative self-administered home urine pregnancy test result was required prior to commencing intake of the study drug (IMP and bowel preparation).

Not more than 3-7 days, elapsed between the colonoscopy day and the follow-up visit.

*Visit 01A was applicable only if the required information was not available at Visit 01. Where the centre had the capability to obtain rapid blood results, then Visits 01 and 01A could have been combined.

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Appendix 3. Consensus between local and central reading of the endoscopy video with regards to the need for excision of the identified lesions was compared using Cohen's Kappa (K) (−1.0 represented "complete disagreement", 0.0 represented "agreement expected by chance", and 1.0 represented "complete agreement"). The percentage of chance findings was determined by calculating p values for each K statistic for each attribute of the endoscopy examination (n=3) and histology examination. p values below 0.05 indicated that the observed agreement between appraisers was not due to chance alone. The K values were interpreted as suggested by Fleiss: K values below 0.40 indicated poor agreement, values from 0.40 to 0.75 indicated fair to good agreement, and values above 0.75 indicated excellent agreement.

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			Lesions that should have been excised during the colonoscopy?		
			Y	**N**	**NA**
Methylene Blue Full Dose	Lesions excised during the colonoscopy?	Y	1134	10	45
		N	16	11	2
Methylene Blue Low Dose	Lesions excised during the colonoscopy?	Y	555	7	9
		N	13	7	0
Placebo	Lesions excised during the colonoscopy?	Y	974	10	36
		N	9	24	0
Overall	Lesions excised during the colonoscopy?	Y	2663	27	90
		N	38	42	2

Methylene Blue Full Dose	Cohen's Kappa, 95% CI and p-value[1]	0.4472	[0.2699, 0.6244]	<0.0001
Methylene Blue Low Dose	Cohen's Kappa, 95% CI and p-value[1]	0.3946	[0.1827, 0.6066]	<0.0001
Placebo	Cohen's Kappa, 95% CI and p-value[1]	0.7068	[0.5812, 0.8323]	<0.0001
Overall	Cohen's Kappa, 95% CI and p-value[1]	0.5518	[0.4545, 0.6490]	<0.0001

Patients are summarised according to the product they actually received. The numbers of lesions that were excised/not excised vs. the ones that should have excised/not excised are reported. Lesions not revised during the central reading of the endoscopy video (the ones reported as being 'Not Applicable') were not included in the calculation of Cohen's Kappa. Kappa value below 0.40 indicates poor agreement, value from 0.40 to 0.75 indicates fair to good agreement and value above 0.75 indicates excellent agreement.

[1]Null hypothesis to be rejected H_0: Cohen's Kappa = 0.

N=Number of patients; CI=Confidence interval; %=Percentage; Y=Yes; N=N; NA=Not applicable.

Appendix 4. Patients with at least one histologically confirmed adenoma or carcinoma in the FAS and PP groups in all study arms and overall.

	FAS			
	MB MMX 200 mg (n=485) n (%)	**MB MMX 100 mg (n=241) n (%)**	**Placebo (n=479) n (%)**	**Overall (n=1205) n (%)**
Patients with at least one histologically confirmed adenoma or carcinoma	273 (56.29)	124 (51.45)	229 (47.81)	626 (51.95)
Odds ratio vs placebo [95% CI]	1.41 [1.09, 1.81]			
Relative risk vs placebo [95% CI]	1.18 [1.04, 1.33]			
P value	0.0099			
	PP			
	MB 200 mg (n=455) n (%)	**MB 100 mg (n=225) n (%)**	**Placebo (n=457) n (%)**	**Overall (n=1137) n (%)**
Patients with at least one histologically confirmed adenoma or carcinoma	265 (58.24)	121 (53.78)	219 (47.92)	605 (53.21)
Odds ratio vs placebo [95% CI]	1.52 [1.17, 1.97]			
Relative risk vs placebo [95% CI]	1.22 [1.07, 1.37]			
P value	0.0018			

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Appendix 5. Compliance to the study drug in the three arms. Proportions indicate the number of patients treated with each product and overall in the FAS. Compliance is defined as [expressed as percentage] = (Number of dispensed tablets - Number of returned unused tablets)/Number of dispensed tablets.

Parameter		Methylene Blue Full Dose N=485	Methylene Blue Low Dose N=241	Placebo N=479	Overall N=1205
Number of dispensed tablets	N	485	241	479	1205
	Mean (SD)	8.0 (0.0)	8.0 (0.0)	8.0 (0.0)	8.0 (0.0)
	CV%	0.0	0.0	0.0	0.0
	Median [Range]	8.0 [8 to 8]	8.0 [8 to 8]	8.0 [8 to 8]	8.0 [8 to 8]
Number of returned unused tablets	N	485	241	479	1205
	Mean (SD)	0.1 (0.5)	0.0 (0.3)	0.0 (0.2)	0.0 (0.4)
	CV%	838.3	1552.4	1682.5	1122.4
	Median [Range]	0.0 [0 to 6]	0.0 [0 to 4]	0.0 [0 to 5]	0.0 [0 to 6]
Compliance[1] (%)	N	485	241	479	1205
	Mean (SD)	99.2 (6.5)	99.7 (3.3)	99.8 (3.1)	99.6 (4.8)
	CV%	6.5	3.3	3.1	4.8
	Median [Range]	100.0 [25 to 100]	100.0 [50 to 100]	100.0 [38 to 100]	100.0 [25 to 100]
Compliance[1]	≥75%	480 (99.0)	240 (99.6)	478 (99.8)	1198 (99.4)
	<75%	5 (1.0)	1 (0.4)	1 (0.2)	7 (0.6)

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Appendix 6. Logistic regression analysis, including the MB MMX 200 mg arm (n=485) and the placebo arm (n=479) (FAS). ADR was analyzed through a logistic regression with treatment, center, age, sex, reason for colonoscopy, and number of excisions as fixed effects. This model demonstrates the effect of each variable on the final result. In particular, study entre was not associated with the main study result.

	Type 3 Analysis of Effects		
Effect	**Degree of Freedom**	**Wald Chi-Square**	***P* value**
Treatment	1	6.5231	0.0106
Analysis Center	18	24.1518	0.1501
Age	1	6.1824	0.0129
Sex	1	18.6655	<.0001
Reason for Colonoscopy	2	5.1142	0.0775
Number of Excision	2	98.6387	<.0001
		Adjusted Odds Ratio	
Comparison	**Comparison *P* value**	**Point Estimate**	**95% Wald Confidence Limits**
MB MMX 200 mg vs Placebo	0.0106	1.46	[1.09, 1.96]

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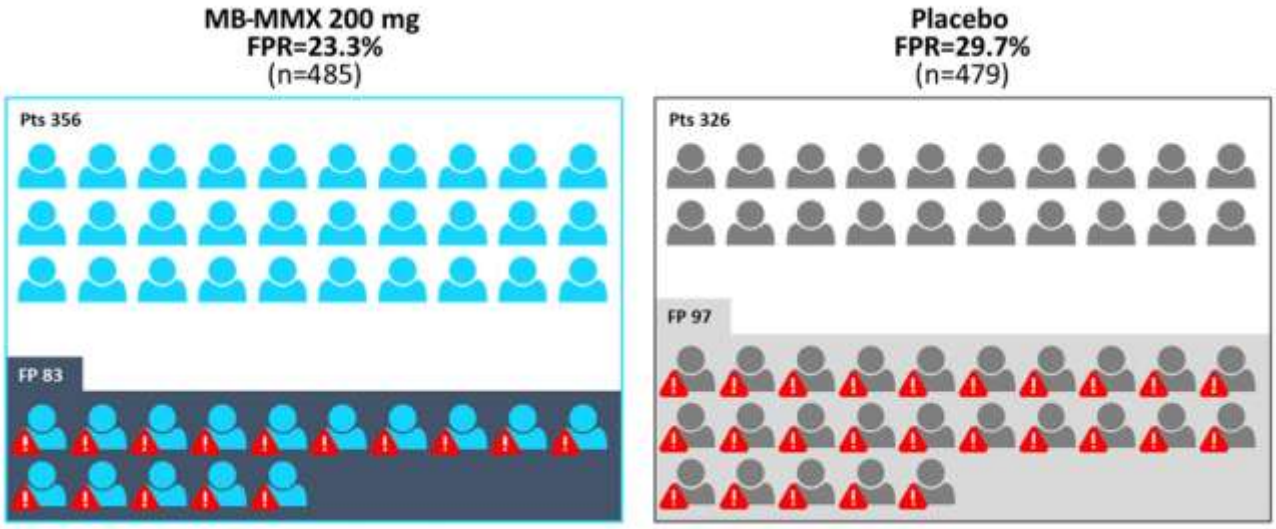
Appendix 7. Distribution of FPR in the Two Groups (Per-Patient Analysis). FFR indicates false-positive results; MB-MMX, methylene blue-multimatrix structure.



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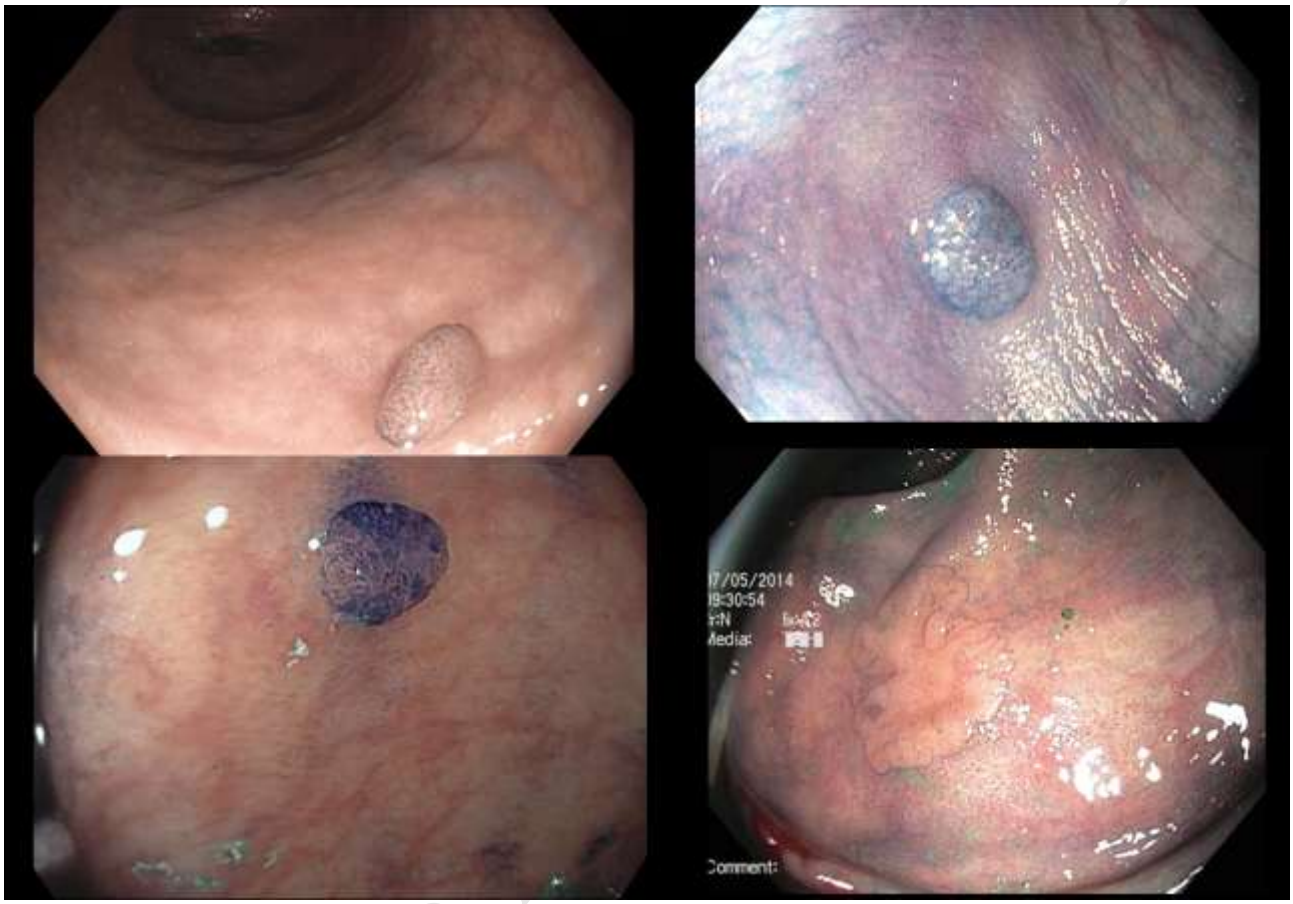
Appendix 8. Examples of stained lesions. Overall, 80% of the lesions detected in the MB-MMX full-dose arm were classified as stained by the central readers; MB-MMX, methylene blue-multimatrix structure.



Appendix 9. Quality parameters: Boston Bowel Preparation Score (FAS), in all study arms and overall.

Boston Bowel Preparation Score	**MB MMX 200 mg (n=485)**	**MB MMX 100 mg (n=241)**	**Placebo (n=479)**	**Overall (n=1205)**
Left Colon (Including descending and sigmoid colon and rectum), mean	2.3	2.3	2.4	2.3
Transverse colon (Including hepatic and splenic flexures), mean	2.3	2.3	2.4	2.3
Right colon (Including cecum and ascending colon), mean	2.0	2.0	2.2	2.1
Total score, mean	6.5	6.6	6.9	6.7