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Methylene Blue-MMX for Screening Colonoscopy

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Abstract:	<p>Background and Aims</p> <p>Topically applied methylene blue dye chromoendoscopy is effective in improving detection of colorectal neoplasia. When combined with a pH- and time-dependent multimatrix structure, a per-oral formulation methylene blue formulation (MB-MMX) is directly delivered to colorectal mucosa.</p> <p>Methods</p> <p>In a Phase III study, 50-to75-year-old patients scheduled for colorectal cancer screening or surveillance colonoscopy were randomized between 200 mg MB-MMX, placebo, or 100 mg MB-MMX in a ratio of 2:2:1. The 100 mg MB-MMX arm was only for masking purposes. MB-MMX and placebo tablets were administered with a 4 liters polyethylene glycol-based bowel preparation. The primary endpoint was the proportion of patients with one adenoma or carcinoma (adenoma detection rate [ADR]) expressed as odds ratio (OR) with 95% CI between the 200 mg MB-MMX and placebo groups, while false-positive (resection rate for non-neoplastic polyps) and adverse event rates were secondary endpoints.</p> <p>Results</p> <p>Across 1,205 randomized patients, ADR was higher with MB-MMX (273/485[56.29%]) than the placebo (229/479[47.81%]; OR: 1.46[1.09, 1.96]). The proportion of patients with nonpolypoid lesion was higher with MB-MMX than the placebo (213/485[43.92%] vs. 168/479 [35.07%]; OR: 1.66[1.21, 2.26]), as was that for <5 mm adenomas (180/485[37.11%] vs 148/479 [30.90%]; OR: 1.36[1.01, 1.83]), while no difference for those with polypoid or larger lesions was observed. The false-positive rate was similar across the study arms (MB-MMX:83/356[23.31%] vs placebo: 97/326[29.75%]). Overall, 0.7% of patients had severe adverse events with no difference between the two arms.</p> <p>Conclusions</p> <p>MB-MMX led to an 18% ADR improvement without increasing the removal of non-neoplastic lesions.</p> <p>Trial Registration</p> <p>NCT01694966</p>

[Category: Original Article]

Methylene Blue-MMX for Screening Colonoscopy^a

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Abstract

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

Methods: In a Phase III study, 50-to75-year-old patients scheduled for colorectal cancer screening or surveillance colonoscopy were randomized between 200 mg MB-MMX, placebo, or 100 mg MB-MMX in a ratio of 2:2:1. The 100 mg MB-MMX arm was only for masking purposes. MB-MMX and placebo tablets were administered with a 4 liters polyethylene glycol-based bowel preparation. The primary endpoint was the proportion of patients with one adenoma or carcinoma (adenoma detection rate [ADR]) expressed as odds ratio (OR) with 95% CI between the 200 mg MB-MMX and placebo groups, while false-positive (resection rate for non-neoplastic polyps) and adverse event rates were secondary endpoints.

Results: Across 1,205 randomized patients, ADR was higher with MB-MMX (273/485[56.29%]) than the placebo (229/479[47.81%]; OR: 1.46[1.09, 1.96]). The proportion of patients with nonpolypoid lesion was higher with MB-MMX than the placebo (213/485[43.92%] vs. 168/479 [35.07%]; OR: 1.66[1.21, 2.26]), as was that for ≤ 5 mm adenomas (180/485[37.11%] vs 148/479 [30.90%]; OR: 1.36[1.01, 1.83]), while no difference for those with polypoid or larger lesions was observed. The false-positive rate was similar across the study arms (MB-MMX:83/356[23.31%] vs placebo: 97/326[29.75%]). Overall, 0.7% of patients had severe adverse events with no difference between the two arms.

Conclusions: MB-MMX led to an 18% ADR improvement without increasing the removal of non-neoplastic lesions.

Trial Registration: NCT01694966

Keywords: adenoma detection rate; chromoendoscopy; colorectal cancer screening; endoscopist.

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.

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.

Randomization

Details of study visits are reported in Appendix 2. Eligible patients were randomized to MB-MMX 200 mg, placebo, or MB-MMX 100 mg in a 2:2:1 ratio. The MB-MMX 100 mg group was included only for masking purposes to reduce acquisition and ascertainment bias due to lack of investigator and patient blinding between placebo and MB-MMX 200 mg. Randomization was stratified by center and indication for colonoscopy (screening, surveillance within 2 years from previous colonoscopy, and surveillance after more than 2 years). Study endoscopists were blinded to randomization allocation.

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.

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 ($\geq 75\%$) 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/LST²²) and removed (biopsy for nonresectable lesions). Time to reach the caecum and clean withdrawal time, excluding intervention time, if any, were recorded. Bowel preparation was scored according to

Boston Bowel Preparation Scale (BBPS).²³ At least 6 minutes of withdrawal time was required. Sedation was carried out according to the local practice.

Central Reading

Recording. Each endoscopy was digitally recorded in HD, and areas with polypoid or nonpolypoid lesions was digitally documented.

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

Histologic assessment was made by two regional, blinded central laboratory pathologists (1 in Europe, 1 in America), who 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.

Safety

Physical examination with vital signs and blood check were performed and AEs were assessed at each pre- and endoscopic-visits and 3 to 7 days after colonoscopy (Appendix 2).

Statistical Analysis

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 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; 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

($\geq 75\%$) 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.

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 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

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 $99.6\% \pm 4.8\%$ 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 $\geq 75\%$ (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 to 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 of 6.7 ± 1.7 and similar total scores reported across the treatment groups (Appendix 8). 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, 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%]; $P=.27$). 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.

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 small 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. We also excluded clinically relevant safety issues, as the most frequently reported AEs, chromaturia and fecal discoloration, were merely due to the staining effect of the vital dye, with no other significant safety signals. This further reassures on the safety of MB-MMX, following the exclusion of DNA damage by white-light photosensitization in a previous Phase II study.²⁶

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

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4 lesions, SSA and TSA, may contribute to reduction in risk of proximal CRC.^{27, 28,29, 30,31} When
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6 considering the high ADR in the control group, we cannot exclude that MB-MMX may have
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8 additional benefits when applied to ‘low-detectors’, such as an increase detection of small to large
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10 lesions.
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13 The main strength of our study is the level of bias controls, mainly through utilization of a
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15 centralized histopathology and double-reading for endoscopic procedures, as well as a masking
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17 arm. Double reading was utilized to prevent possible bias, as is required by the FDA for a
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19 registration study. While it was impossible to fully blind the operator to the allocated arm, we
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21 reduced such bias by incorporating a masking arm, with a reduced dose of MB-MMX. When the
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23 study was designed, most centres had not yet adopted split bowel preparation as standard of care.
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25 As a split regimen has been associated with an increase in ADR,³² a possible synergism with MB-
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27 MMX – to be yet administered the day before colonoscopy – cannot be excluded. In both arms,
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29 clean withdrawal time was ≥ 10 minutes, presumably contributing to the high mean ADR of the
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31 study. However, only interventions, but not washing procedures, were excluded when calculating
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33 the clean withdrawal time. It was also slightly longer in both the 200 mg and 100 mg MB-MMX
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35 arms than in the placebo,^{11, 33} as already reported when using both dye-spray and electronic
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37 chromoendoscopy techniques,^{11, 33,34} presumably due to the need of additional washing and the
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39 combined effect of a darker and more contrast endoscopic image. Despite not included in the
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41 statistical analysis, the masking arm with 100mg MB-MMX dose resulted only in an intermediate
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43 ADR between placebo and 200 mg MB-MMX arms, excluding that the 200 mg MB-MMX benefit
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45 was due to the unblinding of the operator.
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55 In conclusion, our study showed the efficacy and safety of orally administered MB-MMX
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57 dye with bowel preparation in increasing the ADR, a clinically relevant endpoint of screening and
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59 surveillance colonoscopy, due to selectively enhanced detection of colorectal neoplasia, without
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4 requiring useless removal of non-neoplastic lesions.
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References

1. Edwards BK, Noone AM, Mariotto AB, et al. Annual Report to the Nation on the status of cancer, 1975-2010, featuring prevalence of comorbidity and impact on survival among persons with lung, colorectal, breast, or prostate cancer. *Cancer* 2014;120:1290-1314.
2. Ferlay J, Autier P, Boniol M, et al. Estimates of the cancer incidence and mortality in Europe in 2006. *Ann Oncol* 2007;18:581-92.
3. Winawer SJ, Zauber AG, Ho MN, et al. Prevention of colorectal cancer by colonoscopic polypectomy. The National Polyp Study Workgroup. *N Engl J Med* 1993;329:1977-81.
4. Zauber AG, Winawer SJ, O'Brien MJ, et al. Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. *N Engl J Med* 2012;366:687-96.
5. Rex DK, Boland CR, Dominitz JA, et al. Colorectal Cancer Screening: Recommendations for Physicians and Patients From the U.S. Multi-Society Task Force on Colorectal Cancer. *Gastroenterology* 2017;153:307-323.
6. Klabunde CN, Joseph DA, King JB, et al. Vital signs: colorectal cancer screening test use - United States, 2012. *MMWR Morb Mortal Wkly Rep* 2013;62:881-888.
7. Kaminski MF, Regula J, Kraszewska E, et al. Quality indicators for colonoscopy and the risk of interval cancer. *N Engl J Med* 2010;362:1795-803.
8. Corley DA, Jensen CD, Marks AR, et al. Adenoma detection rate and risk of colorectal cancer and death. *N Engl J Med* 2014;370:1298-306.
9. Hassan C, Repici A. Defeating Cancer by Boosting the Adenoma Detection Rate: The Circle of Life. *Gastroenterology* 2017;153:8-10.
10. Kaminski MF, Wieszczyn P, Rupinski M, et al. Increased Rate of Adenoma Detection Associates With Reduced Risk of Colorectal Cancer and Death. *Gastroenterology* 2017;153:98-105.
11. Pohl J, Schneider A, Vogell H, et al. Pancolonial chromoendoscopy with indigo carmine versus standard colonoscopy for detection of neoplastic lesions: a randomised two-centre trial. *Gut* 2011;60:485-90.
12. Kiesslich R, von Bergh M, Hahn M, et al. Chromoendoscopy with indigocarmine improves the detection of adenomatous and nonadenomatous lesions in the colon. *Endoscopy* 2001;33:1001-6.
13. Kiesslich R, Fritsch J, Holtmann M, et al. Methylene blue-aided chromoendoscopy for the detection of intraepithelial neoplasia and colon cancer in ulcerative colitis. *Gastroenterology* 2003;124:880-8.
14. Chiu HM, Chang CY, Chen CC, et al. A prospective comparative study of narrow-band imaging, chromoendoscopy, and conventional colonoscopy in the diagnosis of colorectal neoplasia. *Gut* 2007;56:373-9.
15. Kahi CJ, Anderson JC, Waxman I, et al. High-definition chromocolonoscopy vs. high-definition white light colonoscopy for average-risk colorectal cancer screening. *Am J Gastroenterol* 2010;105:1301-7.
16. Huneburg R, Lammert F, Rabe C, et al. Chromocolonoscopy detects more adenomas than white light colonoscopy or narrow band imaging colonoscopy in hereditary nonpolyposis colorectal cancer screening. *Endoscopy* 2009;41:316-22.
17. Lecomte T, Cellier C, Meatchi T, et al. Chromoendoscopic colonoscopy for detecting preneoplastic lesions in hereditary nonpolyposis colorectal cancer syndrome. *Clin Gastroenterol Hepatol* 2005;3:897-902.
18. Laine L, Kaltenbach T, Barkun A, et al. SCENIC international consensus statement on surveillance and management of dysplasia in inflammatory bowel disease. *Gastroenterology* 2015;148:639-651 e28.
19. Kaminski MF, Hassan C, Bisschops R, et al. Advanced imaging for detection and differentiation of colorectal neoplasia: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. *Endoscopy* 2014;46:435-49.
20. Mitooka H, Fujimori T, Ohno S, et al. Chromoscopy of the colon using indigo carmine dye with electrolyte lavage solution. *Gastrointest Endosc* 1992;38:373-4.
21. Levin B, Lieberman DA, McFarland B, et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *Gastroenterology* 2008;134:1570-95.
22. The Paris endoscopic classification of superficial neoplastic lesions: esophagus, stomach, and colon: November 30 to December 1, 2002. *Gastrointest Endosc* 2003;58:S3-43.
23. Lai EJ, Calderwood AH, Doros G, et al. The Boston bowel preparation scale: a valid and reliable instrument for colonoscopy-oriented research. *Gastrointest Endosc* 2009;69:620-5.
24. Dixon MF. Gastrointestinal epithelial neoplasia: Vienna revisited. *Gut* 2002;51:130-1.

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- 4 25. Schlemper RJ, Riddell RH, Kato Y, et al. The Vienna classification of gastrointestinal epithelial neoplasia. *Gut* 2000;47:251-5.
- 5
- 6 26. Repici A, Ciscato C, Wallace M, et al. Evaluation of genotoxicity related to oral methylene blue chromoendoscopy. *Endoscopy* 2018.
- 7
- 8 27. Hassan C, Rex DK, Cooper GS, et al. Primary prevention of colorectal cancer with low-dose aspirin in combination with endoscopy: a cost-effectiveness analysis. *Gut* 2012;61:1172-9.
- 9
- 10 28. East JE, Atkin WS, Bateman AC, et al. British Society of Gastroenterology position statement on serrated polyps in the colon and rectum. *Gut* 2017;66:1181-1196.
- 11
- 12 29. Kahi CJ, Hewett DG, Norton DL, et al. Prevalence and variable detection of proximal colon serrated polyps during screening colonoscopy. *Clin Gastroenterol Hepatol* 2011;9:42-6.
- 13
- 14 30. Payne SR, Church TR, Wandell M, et al. Endoscopic detection of proximal serrated lesions and pathologic identification of sessile serrated adenomas/polyps vary on the basis of center. *Clin Gastroenterol Hepatol* 2014;12:1119-26.
- 15
- 16
- 17 31. Nishihara R, Wu K, Lochhead P, et al. Long-term colorectal-cancer incidence and mortality after lower endoscopy. *N Engl J Med* 2013;369:1095-105.
- 18
- 19 32. Radaelli F, Paggi S, Hassan C, et al. Split-dose preparation for colonoscopy increases adenoma detection rate: a randomised controlled trial in an organised screening programme. *Gut* 2017;66:270-277.
- 20
- 21 33. Brown SR, Baraza W, Din S, et al. Chromoscopy versus conventional endoscopy for the detection of polyps in the colon and rectum. *Cochrane Database Syst Rev* 2016;4:CD006439.
- 22
- 23 34. Adler A, Aschenbeck J, Yenerim T, et al. Narrow-band versus white-light high definition television endoscopic imaging for screening colonoscopy: a prospective randomized trial. *Gastroenterology* 2009;136:410-6 e1; quiz 715.
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Table 1. Patient Baseline Demographics and Reason for Colonoscopy (ITT Population)

	MB-MMX	MB-MMX		
	200 mg	100 mg	Placebo	Overall
Characteristic	(n=504)	(n=247)	(n=498)	(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)
from previous colonoscopy				

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

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

	MB-MMX 200 mg	Placebo	Odds Ratio	Relative Risk
Proportion of patients with:	(n=485), No. (%)	(n=479), No. (%)	(95% CI)	(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.

* $p < 0.01$
** $p < 0.05$

Table 3. Treatment-Emergent Adverse Events

	MB-MMX 200	MB-MMX 100	Placebo	Overall
	mg (n=488),	mg (n=241),	(n=479),	(N=1208),
Adverse events	No. (%)	No. (%)	No. (%)	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)
Leading to discontinuation	4 (0.8)	0 (0.0)	2 (0.4)	6 (0.5)

Abbreviation: MB-MMX, methylene blue-multimatrix structure

Legends

Figure 1. Flowchart of the Study

Figure 2. Distribution of Patients in the Two Groups According to Final Diagnosis. ADR

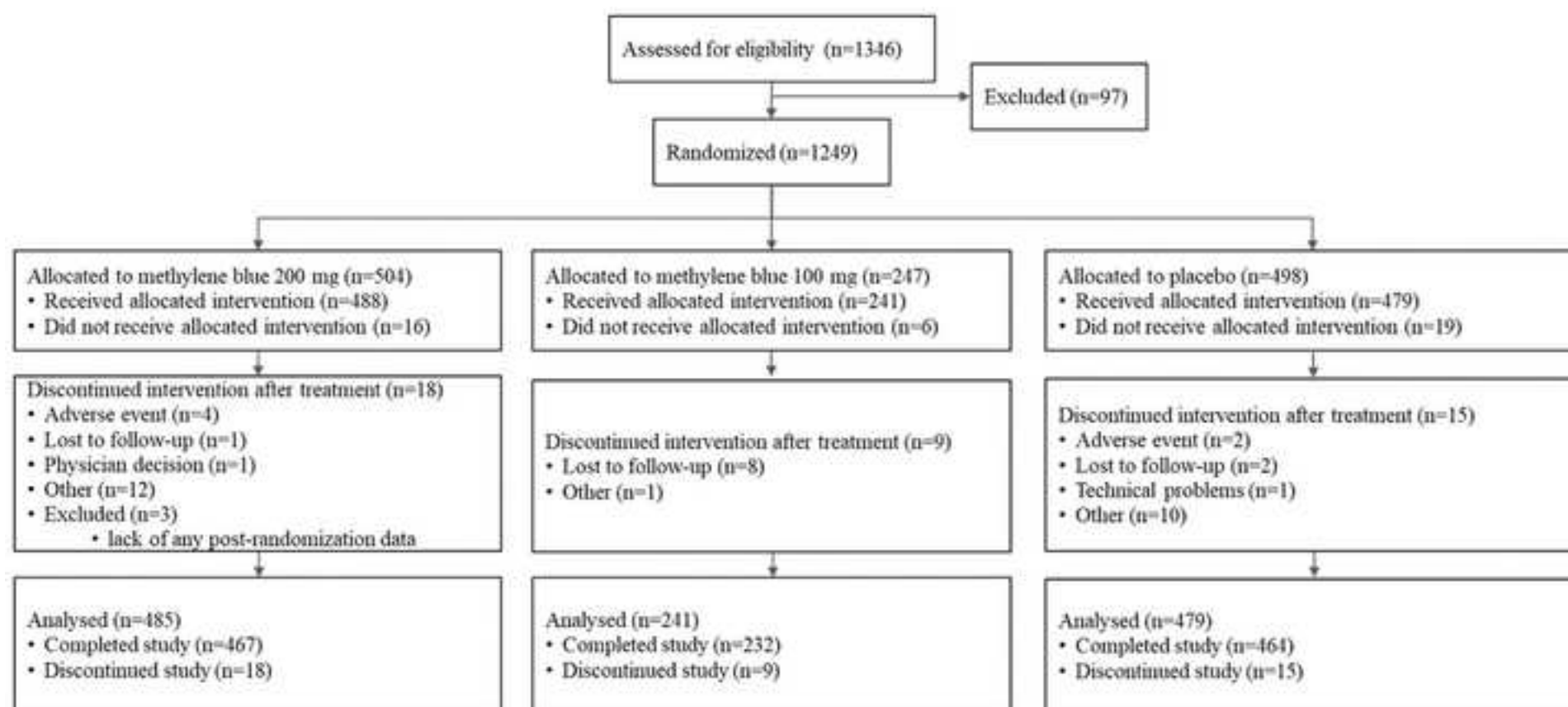
indicates adenoma detection rate; MB-MMX, methylene blue-multimatrix structure; SSA, sessile serrated adenoma; TSA, traditional serrated adenoma.

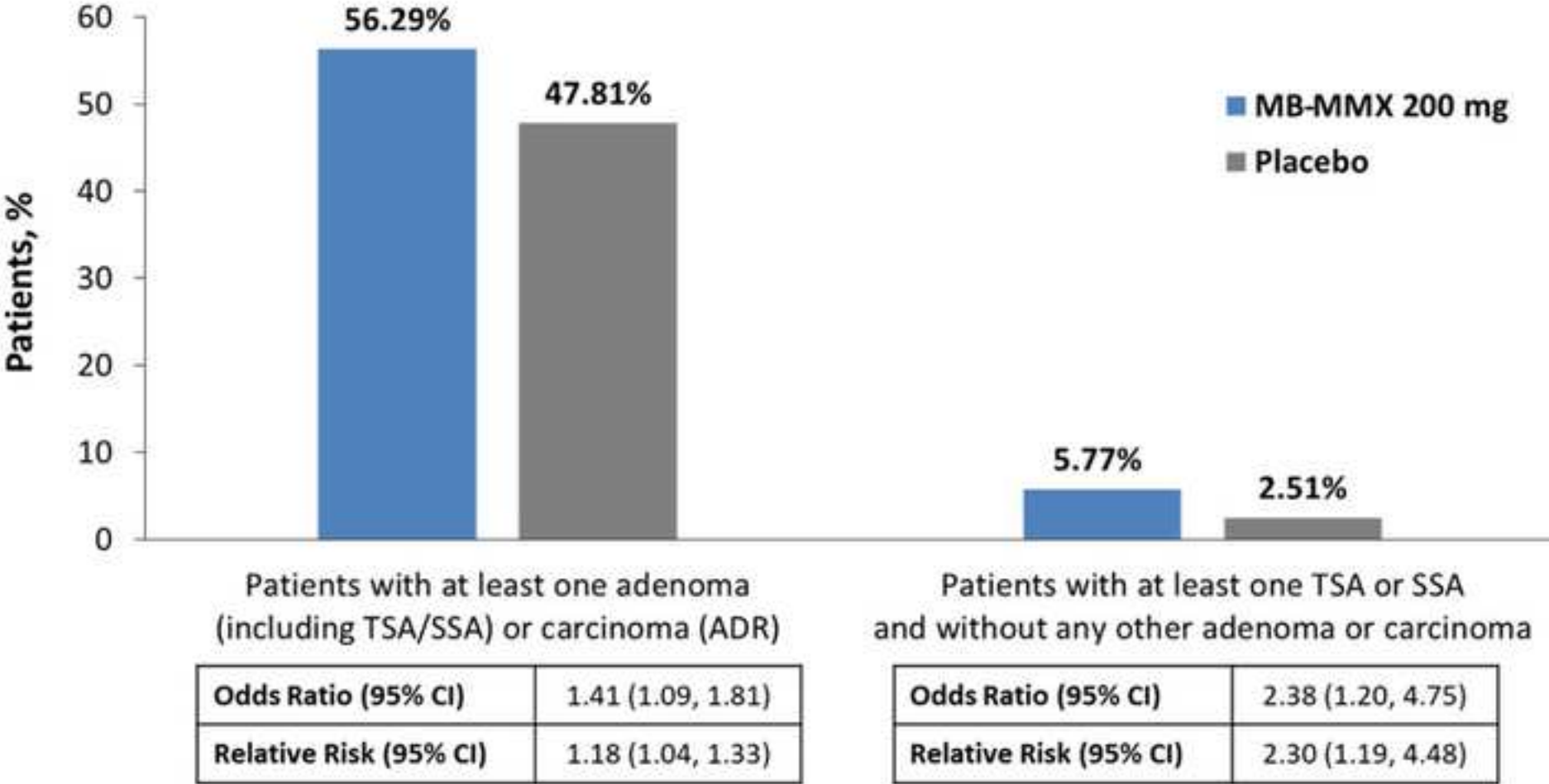
Figure 3. Distribution of Patients in the Two Groups According to Morphology of the Detected

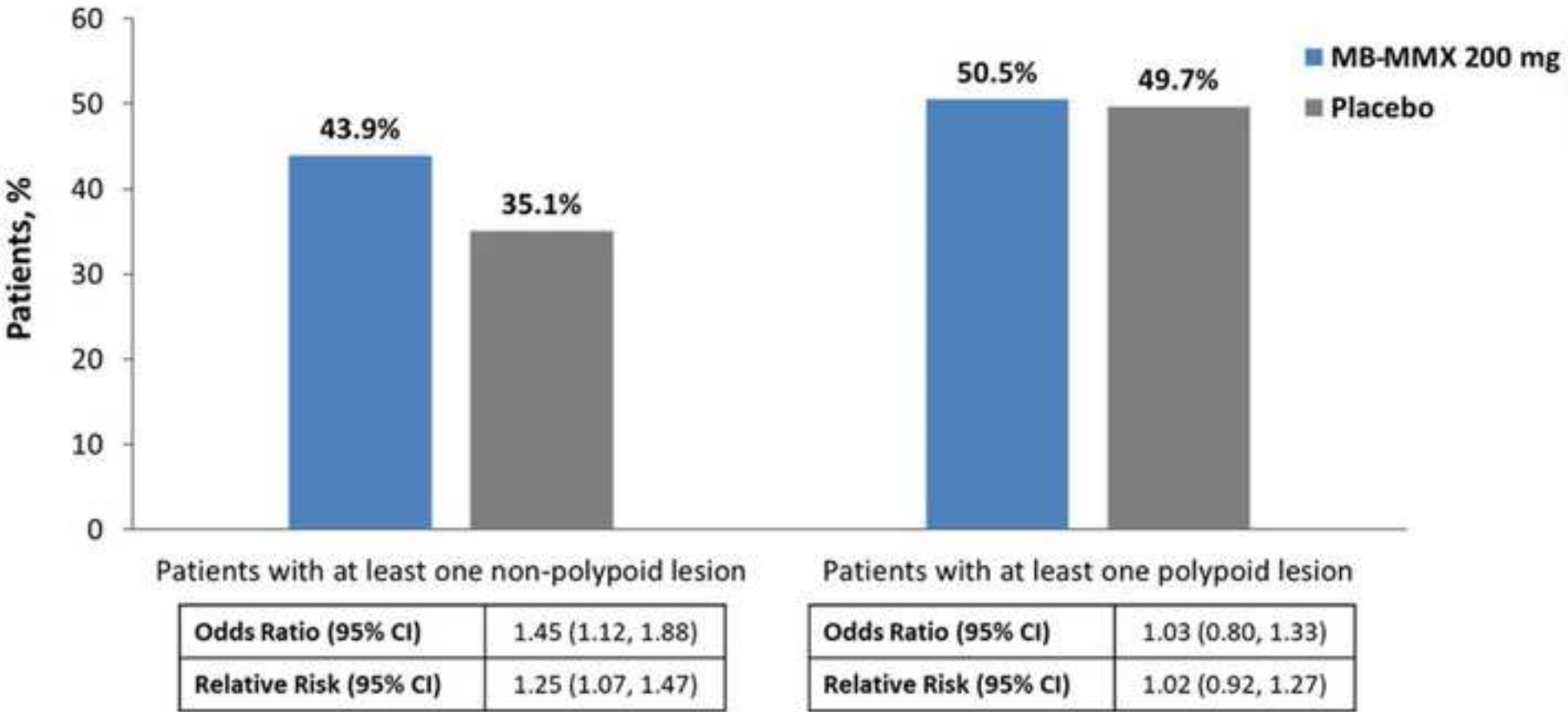
Lesion. MB-MMX indicates methylene blue-multimatrix structure.

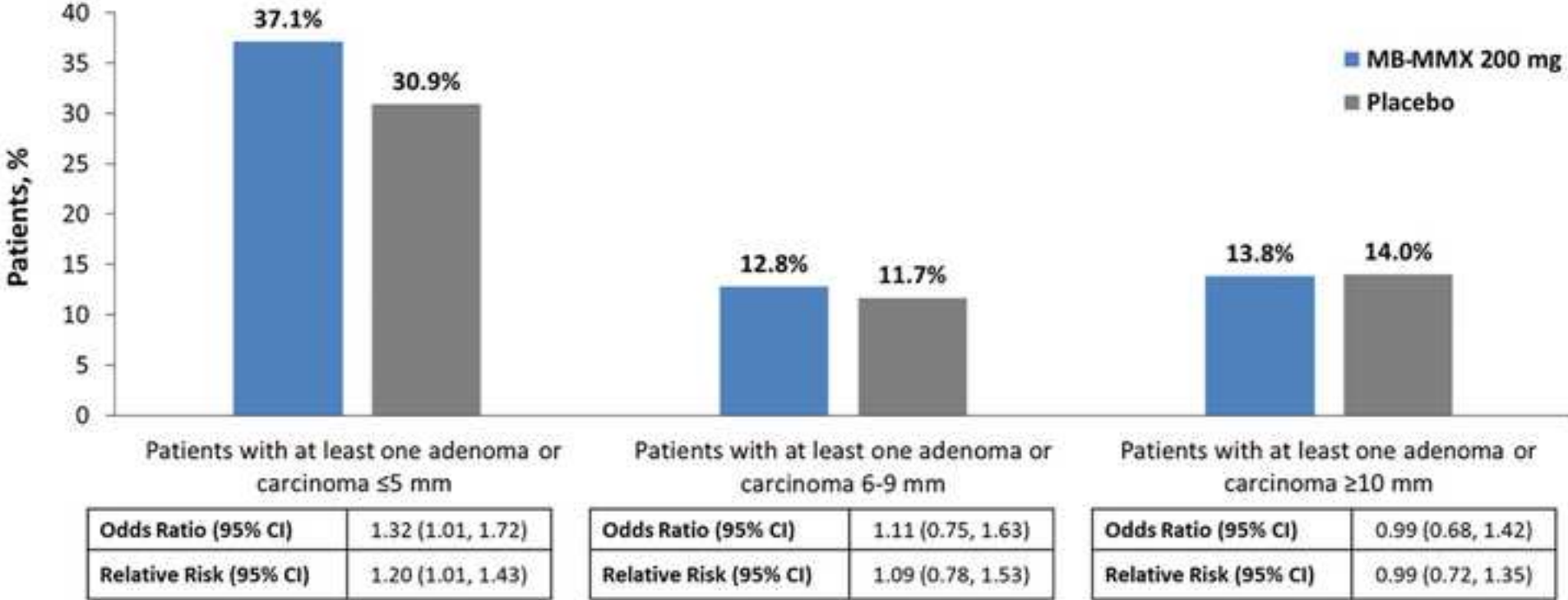
Figure 4. Distribution of Patients in the Two Groups According to Size of the Detected Lesion.

MB-MMX indicates methylene blue-multimatrix structure.











CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	8,9
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	6
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	6
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	6
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	6

		assessing outcomes) and how	
Statistical methods	11b	If relevant, description of the similarity of interventions	6,7,8
	12a	Statistical methods used to compare groups for primary and secondary outcomes	9
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	10
Recruitment	14a	Dates defining the periods of recruitment and follow-up	10
	14b	Why the trial ended or was stopped	10
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	10,11,12
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	10,11,12
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	10,11,12
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	10,11,12
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	12
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	13
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	14
Other information			
Registration	23	Registration number and name of trial registry	5
Protocol	24	Where the full trial protocol can be accessed, if available	TBD
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.



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Clinical Trial Protocol

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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.

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 ² 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

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	

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

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

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

⁴For 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.

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.

			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.

¹Null 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]			
<i>P</i> 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]			
<i>P</i> value	0.0018			

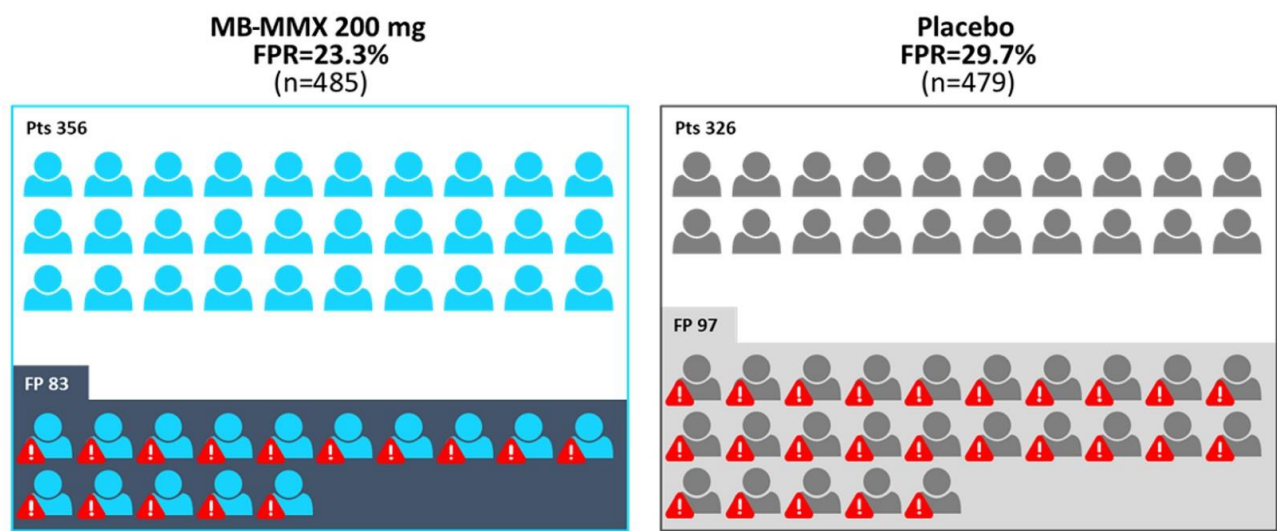
Appendix 5. Compliance to the study drug in the three arms. Proportions indicate the number of patients treated with each product and overall in the FAS. Compliance is defined as [expressed as percentage] = (Number of dispensed tablets - Number of returned unused tablets)/Number of dispensed tablets.

		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	8.0	8.0	8.0	8.0
	[Range]	[8 to 8]	[8 to 8]	[8 to 8]	[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	0.0	0.0	0.0	0.0
	[Range]	[0 to 6]	[0 to 4]	[0 to 5]	[0 to 6]
Compliance¹ (%)	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	100.0	100.0	100.0	100.0
	[Range]	[25 to 100]	[50 to 100]	[38 to 100]	[25 to 100]
Compliance¹	≥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)

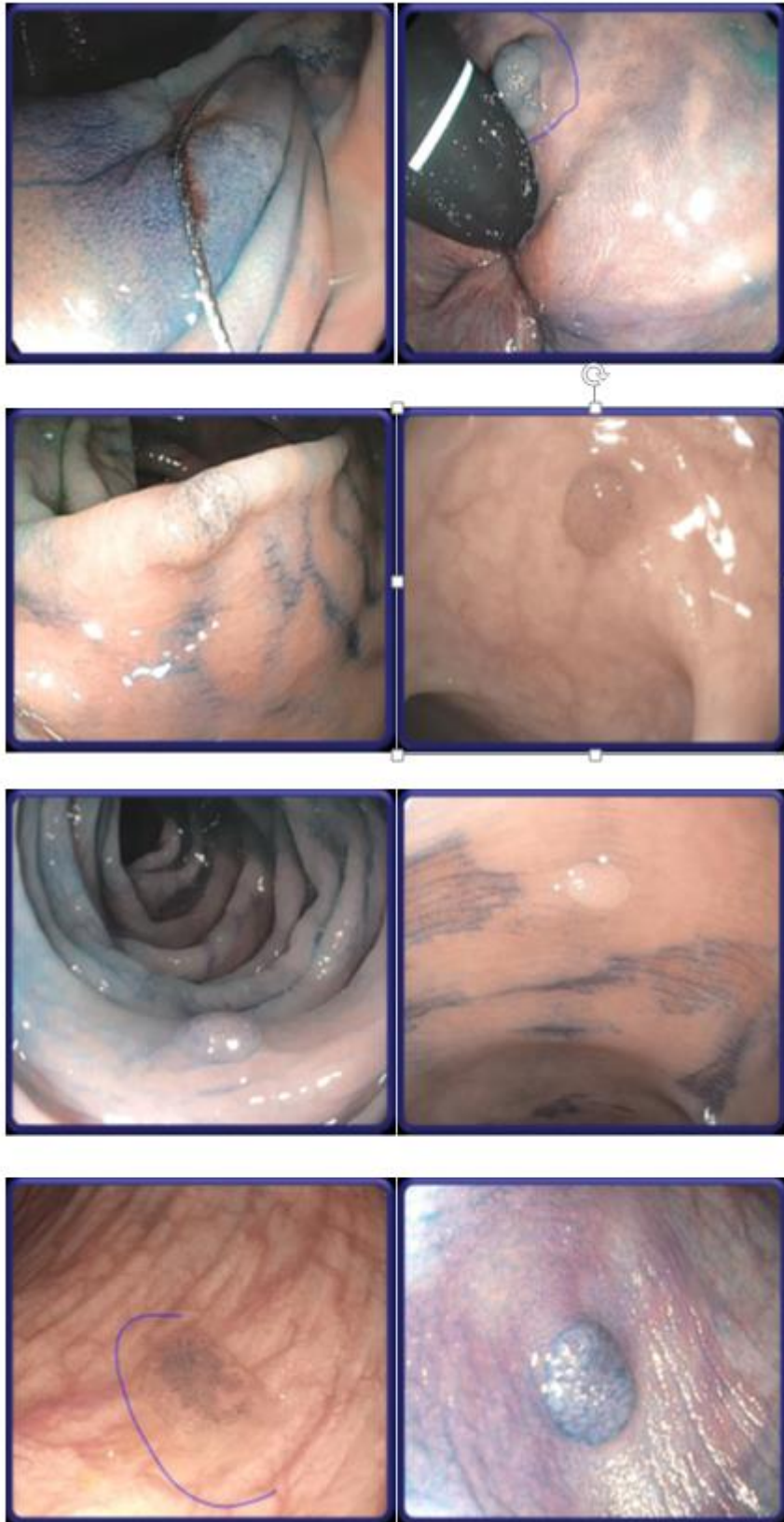
Appendix 6. Logistic regression analysis, including the MB MMX 200 mg arm (n=485) and the placebo arm (n=479) (FAS). ADR was analyzed through a logistic regression with treatment, center, age, sex, reason for colonoscopy, and number of excisions as fixed effects. This model demonstrates the effect of each variable on the final result. In particular, study centre 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]

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



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