Clinical trial results:
Preoperative endoscopic treatment with fosfomycin and metronidazole in patients with right-sided colon cancer and colon adenoma: a clinical proof-of-concept intervention study
MEFO trial

Summary

EudraCT number	2019-000131-51
Trial protocol	DK
Global end of trial date	01 July 2023

Results information

Result version number	v1 (current)
This version publication date	18 December 2024
First version publication date	18 December 2024

Trial information

Trial identification

Sponsor protocol code	1921

Additional study identifiers

ISRCTN number	-
ClinicalTrials.gov id (NCT number)	NCT04312360
WHO universal trial number (UTN)	-
Other trial identifiers	Research Ethics Committee number: 66694

Notes:

Sponsors

Sponsor organisation name	Zealand University Hospital
Sponsor organisation address	Lykkebækvej 1, Køge, Denmark, 4600
Public contact	Zealand University Hospital, Zealand University Hospital, +45 28526432, aslb@regionsjaelland.dk
Scientific contact	Zealand University Hospital, Zealand University Hospital, +45 28526432, aslb@regionsjaelland.dk

Notes:

Paediatric regulatory details

Is trial part of an agreed paediatric investigation plan (PIP)	No
Does article 45 of REGULATION (EC) No 1901/2006 apply to this trial?	No
Does article 46 of REGULATION (EC) No 1901/2006 apply to this trial?	No

Notes:

Results analysis stage

Analysis stage	Final
Date of interim/final analysis	17 June 2024
Is this the analysis of the primary completion data?	Yes
Primary completion date	01 July 2023
Global end of trial reached?	Yes
Global end of trial date	01 July 2023
Was the trial ended prematurely?	No

Notes:

General information about the trial

Main objective of the trial:

The aim of this study is to investigate the effect of local antibiotic treatment with fosfomycin and metronidazole on tumor characteristics and the colonic biofilm in patients with right-sided colon cancer or right-sided colon adenomas.

Protection of trial subjects:

In case of serious side effects or adverse events, the data will be monitored and the trial stopped if there is any evidence of lack of safety regarding the intervention and the study drugs. Included are unexpected complications in relation to colon cancer surgery.

Background therapy:

All patients included in this trial received a bowel preparation by PicoPrep before the intervention colonoscopies.

All patients in track 1 received perioperative administration of 500mg of metronidazole during the colon cancer surgery (post-trial-intervention).

All patient in track 2 received a bowel preparation by PicoPrep before the endosckopic mucosal resection (post-trial-intervention).

Evidence for comparator:

For comparators, we will compare tissue and blood samples at baseline with samples after the invervention.
The surgical resection of colon adenomas are usually performed as an endoscopic mucosal resection (EMR). This is a delicate procedure to perform as only the superficial layers of the colon is affected, but the patients avoid surgical incisions of the abdominal wall. Biopsies are not taken before EMR procedures as the inflammation and scaring of the tissue may make the EMR impossible to perform. In order to have precursor tissue that has not been treated with the antibiotic intervention we will have a retrospective cohort of 28 patients with colon precursor lesions (track 2b). We will retrieve FFPE tissue from the resected mucosa bearing adenoma. They will have had endoscopic mucosal resection performed in 2018 at Department of Surgery, Zealand University Hospital.

Actual start date of recruitment	17 January 2020
Long term follow-up planned	No
Independent data monitoring committee (IDMC) involvement?	No

Notes:

Population of trial subjects

Subjects enrolled per country

Country: Number of subjects enrolled	Denmark: 28
Worldwide total number of subjects	28
EEA total number of subjects	28

Notes:

Subjects enrolled per age group	
In utero	0
Preterm newborn - gestational age < 37 wk	0
Newborns (0-27 days)	0
Infants and toddlers (28 days-23 months)	0
Children (2-11 years)	0
Adolescents (12-17 years)	0
Adults (18-64 years)	4
From 65 to 84 years	24
85 years and over	0

Subject disposition

Recruitment

Recruitment details:

Recruitment at the Surgical Department, Zealand University Hospita was from January 2020 until April 2022. Recruitment at the Gastro Unit, Herlev Hospital, was from September 2020 until April 2022.

Pre-assignment

Screening details:

Consecutive patients with endoscopically diagnosed right-sided colon adenomas/precursor lesions (≥2cm in diameter) or right-sided colon cancer tumors were eligible for inclusion in the study.

Period 1

Period 1 title	Overall trial (overall period)
Is this the baseline period?	Yes
Allocation method	Not applicable
Blinding used	Not blinded

Arms

Arm title	Experimental

Arm description:

Therapeutic endoscopy

Arm type	Experimental
Investigational medicinal product name	Fosfomycin
Investigational medicinal product code	J01 XX 01
Other name	Fosfomycin disodium, Infectofos
Pharmaceutical forms	Powder and gel for gel
Routes of administration	Local use

Dosage and administration details:

During the therapeutic endoscopy the right hemicolon was sprayed with 100ml of the gel (8mg/ml of fosfomycin), yielding a dose of 800mg of fosfomycin. For this study, the following dosages was chosen: 800mg of fosfomycin and 200mg of metronidazole in 100ml of gel. We estimated that this dosage of gel is sufficient to cover the inner surface of the right hemicolon. The gel consisted of two components. One component consisted of fosfomycin, metronidazole and a solution. The other component was a gel. During administration, the two components were delivered through a dual channel colono-videoscope. The gel formed on the bowel wall and adhered to the mucosa. This was a one-time application at least five days before the surgical tumour resection.

Investigational medicinal product name	Metronidazole
Investigational medicinal product code	J01 XD 01
Other name	metronidazole "B. Braun"
Pharmaceutical forms	Powder and gel for gel
Routes of administration	Local use

Dosage and administration details:

During the therapeutic endoscopy the right hemicolon was sprayed with 100ml of the gel (2mg/ml of metronidazole), yielding a dose of 200mg of metronidazole. For this study, the following dosages was chosen: 800mg of fosfomycin and 200mg of metronidazole in 100ml of gel. We estimated that this dosage of gel is sufficient to cover the inner surface of the right hemicolon. The gel consisted of two components. One component consisted of fosfomycin, metronidazole and a solution. The other component was a gel. During administration, the two components were delivered through a dual channel colono-videoscope. The gel formed on the bowel wall and adhered to the mucosa. This was a one-time application at least five days before the surgical tumour resection.

Number of subjects in period 1	Experimental
Started	28
Completed	22
Not completed	6
Consent withdrawn by subject	5
Protocol deviation	1

Baseline characteristics

Reporting groups	
Reporting group title	Overall trial

Reporting group description: -

Reporting group values	Overall trial	Total	
Number of subjects	28	28	
Age categorical			
Age at inclusion			
Units: Subjects			
Adults (18-64 years)	4	4	
From 65-84 years	24	24	
85 years and over	0	0	
Gender categorical			
Units: Subjects			
Female	17	17	
Male	11	11	

Subject analysis sets	
Subject analysis set title	Track 1
Subject analysis set type	Full analysis

Subject analysis set description:

Patients with colon cancer

Subject analysis set title	Track 2
Subject analysis set type	Full analysis

Subject analysis set description:

Patients with colon adenoma/precursor lesion

Subject analysis set title	Track 1 post-intervention
Subject analysis set type	Full analysis

Subject analysis set description:

Same patients as track 1, post-intervention samples

Subject analysis set title	Track 2 post-intervention
Subject analysis set type	Full analysis

Subject analysis set description:

Same patients as track 2, post-intervention samples

Subject analysis set title	Track 2b retrospective controls
Subject analysis set type	Full analysis

Subject analysis set description:

Each patient from track 2 was matched by age and gender with two historical controls (track 2b) with an EMR procedure in 2018, in order to obtain neoplastic tissue samples that were not exposed to the antibiotics.

Reporting group values	Track 1	Track 2	Track 1 post-intervention
Number of subjects	14	14	14
Age categorical			
Age at inclusion			
Units: Subjects			

Adults (18-64 years)	2	2	2
From 65-84 years	12	12	12
85 years and over	0	0	0
Gender categorical Units: Subjects			
Female	9	8	9
Male	5	6	5

Reporting group values	Track 2 post-intervention	Track 2b retrospective controls	
Number of subjects	14	24	
Age categorical			
Age at inclusion			
Units: Subjects			
Adults (18-64 years)	2		
From 65-84 years	12		
85 years and over	0		
Gender categorical Units: Subjects			
Female	8		
Male	6		

End points

End points reporting groups

Reporting group title	Experimental
Reporting group description: Therapeutic endoscopy	
Subject analysis set title	Track 1
Subject analysis set type	Full analysis
Subject analysis set description: Patients with colon cancer	
Subject analysis set title	Track 2
Subject analysis set type	Full analysis
Subject analysis set description: Patients with colon adenoma/precursor lesion	
Subject analysis set title	Track 1 post-intervention
Subject analysis set type	Full analysis
Subject analysis set description: Same patients as track 1, post-intervention samples	
Subject analysis set title	Track 2 post-intervention
Subject analysis set type	Full analysis
Subject analysis set description: Same patients as track 2, post-intervention samples	
Subject analysis set title	Track 2b retrospective controls
Subject analysis set type	Full analysis
Subject analysis set description: Each patient from track 2 was matched by age and gender with two historical controls (track 2b) with an EMR procedure in 2018, in order to obtain neoplastic tissue samples that were not exposed to the antibiotics.	

Primary: Change in bacterial biomass (non-tumour bearing mucosa)

End point title	Change in bacterial biomass (non-tumour bearing mucosa)
End point description: A quantitative change in the bacterial biomass adherent to the colonic epithelium of the resected colon adjusted for baseline level found before the intervention through fluorescence in situ (FISH) technique. The background tissue biomass volume was used to adjust the bacterial biomass volume. The specific measurement variable is 3D biofilm mass. The method of aggregation will be means.	
End point type	Primary
End point timeframe: Baseline to surgical tumour resection	

End point values	Track 1	Track 2	Track 1 post-intervention	Track 2 post-intervention
Subject group type	Subject analysis set	Subject analysis set	Subject analysis set	Subject analysis set
Number of subjects analysed	9[1]	10[2]	9[3]	10[4]
Units: Percentage				
number (not applicable)	9	10	9	10

Notes:

[1] - 4 patients dropped out. One patient was excluded for analyses due to staining artefacts.

[2] - 1 pt dropped out. 1 pt was a screening failure. 2 patients exluded: artefacts and no post-tissue.

[3] - 4 patients dropped out. One patient was excluded for analyses due to staining artefacts.

[4] - 1 pt dropped out. 1 pt was a screening failure. 2 patients exluded: artefacts and no post-tissue.

Statistical analyses

Statistical analysis title	Change in bacterial biomass (track 1)

Statistical analysis description:

Percentage bacterial biomass (mean (range)):
Baseline: 5.390e-03 (2.997e-05 – 2.050e-02)
Post-intervention: 6.081e-04 (8.465e-04 – 1.151e-02)

Comparison groups	Track 1 v Track 1 post-intervention
Number of subjects included in analysis	18
Analysis specification	Pre-specified
Analysis type	other
P-value	= 0.674 [5]
Method	Wilcoxon (Mann-Whitney)
Parameter estimate	Mean difference in bacterial biomass

Notes:

[5] - Paired one-tailed wilcoxon test

Statistical analysis title	Change in bacterial biomass (track 2)

Statistical analysis description:

Percentage bacterial biomass (mean (range)):
Baseline: 3.0e-04 (2.1e-05 - 7.4e-04)
Post-intervention: 9.8e-05 (3.3e-06 – 2.4e-04)

Comparison groups	Track 2 v Track 2 post-intervention
Number of subjects included in analysis	20
Analysis specification	Pre-specified
Analysis type	other
P-value	= 0.025 [6]
Method	t-test, 1-sided
Parameter estimate	Mean difference in bacterial biomass

Notes:

[6] - Paired one-tailed t-test

Primary: Change in gene expression (non-tumour bearing mucosa)

End point title	Change in gene expression (non-tumour bearing mucosa)

End point description:

The determination of differences in translational markers present in the resected tumor tissue adjusted for the level seen at the baseline (the therapeutic colonoscopy) through a principal component analysis (PCA). The translational markers of interest are tumor markers, and markers of the microenvironment and the immune response. The analyses will be performed using the PanCancer IO 360TM Gene Expression Panel (nanoString).

End point type	Primary

End point timeframe:

Baseline to surgical tumour resection

End point values	Track 1	Track 2	Track 1 post-intervention	Track 2 post-intervention
Subject group type	Subject analysis set	Subject analysis set	Subject analysis set	Subject analysis set
Number of subjects analysed	10[7]	11[8]	10[9]	11[10]
Units: log2 Fold Change				
number (not applicable)	10	11	10	11

Notes:

[7] - 4 patients dropped out before the intervention

[8] - 1 patient dropped out. 1 patient was a screening failure. 1 patient had no post-intervention tissue

[9] - 4 patients dropped out before the intervention

[10] - 1 patient dropped out. 1 patient was a screening failure. 1 patient had no post-intervention tissue

Statistical analyses

Statistical analysis title	Differentially expressed genes (track 1)

Statistical analysis description:

Differentially expressed genes in the non-tumour bearing mucosa samples after the intervention using the Wald significance test with an adjusted p-value <0.05 using the Benjamini-Hochberg approach: we observed one gene down-regulated and 27 genes up-regulated.

Comparison groups	Track 1 v Track 1 post-intervention
Number of subjects included in analysis	20
Analysis specification	Post-hoc
Analysis type	other
P-value	< 0.05
Method	Wald significance test
Parameter estimate	log2FoldChange

Statistical analysis title	Differentially expressed genes (track 2)

Statistical analysis description:

Non-tumour bearing mucosa samples before and after the antibiotic application were compared for differentially expressed (DE) genes using the Wald significance test with an adjusted p-value <0.05 using the Benjamini-Hochberg approach one gene was found to be downregulated, however, this is likely an effect of the multiple testing due to the nearly uniform distribution of the p-value histogram.

Comparison groups	Track 2 v Track 2 post-intervention
Number of subjects included in analysis	22
Analysis specification	Post-hoc
Analysis type	other
P-value	< 0.05
Method	Wald significance test
Parameter estimate	log2FoldChange

Primary: Change in bacterial composition (non tumour bearing)

End point title	Change in bacterial composition (non tumour bearing)

End point description:

The determination of a change in the bacterial composition adjusted for the bacteria found at baseline

before the intervention through fluorescence in situ (FISH) technique and sequencing with Metagenome.

End point type	Primary

End point timeframe:

Baseline to surgical tumour resection

End point values	Track 1	Track 2	Track 1 post-intervention	Track 2 post-intervention
Subject group type	Subject analysis set	Subject analysis set	Subject analysis set	Subject analysis set
Number of subjects analysed	10[11]	12[12]	10[13]	12[14]
Units: R2 (proportion)				
number (not applicable)	10	12	10	12

Notes:

[11] - 4 patients dropped out before the intervention

[12] - 1 patient dropped out before the intervention. 1 patient was a screening failure

[13] - 4 patients dropped out before the intervention

[14] - 1 patient dropped out before the intervention. 1 patient was a screening failure

Statistical analyses

Statistical analysis title	Beta diversity (track 1)

Statistical analysis description:

The beta diversity was compared between pre- and post-samples using the PERMANOVA test: R2=13%, F=2.3, p=0.002.

Comparison groups	Track 1 v Track 1 post-intervention
Number of subjects included in analysis	20
Analysis specification	Post-hoc
Analysis type	other
P-value	= 0.002
Method	PERMANOVA

Statistical analysis title	Beta diversity (track 2)

Statistical analysis description:

The beta diversity was compared between pre- and post-samples using the PERMANOVA test: R2=11%, F=2.1, p=0.021.

Comparison groups	Track 2 v Track 2 post-intervention
Number of subjects included in analysis	24
Analysis specification	Post-hoc
Analysis type	other
P-value	= 0.021
Method	PERMANOVA

Statistical analysis title	Bacterial presence (Track 1)

Statistical analysis description:

Tissue slides pre- and post-intervention were manually evaluated for the presence of Bacteroides fragilis, Fusobacterium.

*Bacteroides fragilis (N (%)):
Baseline: 10 (100)
Post-intervention: 10 (100)
*Fusobacterium (N (%)):
Baseline: 8 (80)
Post-intervention: 7 (70)

Comparison groups	Track 1 v Track 1 post-intervention
Number of subjects included in analysis	20
Analysis specification	Pre-specified
Analysis type	other
P-value	= 1 [15]
Method	Mcnemar

Notes:

[15] - McNemar's Chi-squared test with continuity correction.

Statistical analysis title	Bacterial presence (Track 2)

Statistical analysis description:

Tissue slides pre- and post-intervention were manually evaluated for the presence of Bacteroides fragilis, Fusobacterium.
*Bacteroides fragilis (N (%)):
Baseline: 8 (66.7)
Post-intervention: 9 (81.8)
*Fusobacterium (N (%)):
Baseline: 7 (58.3)
Post-intervention: 7 (64.6)

Comparison groups	Track 2 v Track 2 post-intervention
Number of subjects included in analysis	24
Analysis specification	Pre-specified
Analysis type	other
P-value	≥ 0.64 [16]
Method	Mcnemar

Notes:

[16] - McNemar's Chi-squared test with continuity correction

Primary: Change in bacterial biomass (tumour centre)

End point title	Change in bacterial biomass (tumour centre)

End point description:

A quantitative change in the bacterial biomass adherent to the colonic epithelium of the resected colon adjusted for baseline level found before the intervention through fluorescence in situ (FISH) technique. The background tissue biomass volume was used to adjust the bacterial biomass volume. The specific measurement variable is 3D biofilm mass. The method of aggregation will be means.

End point type	Primary

End point timeframe:

Baseline to surgical tumour resection.

End point values	Track 1	Track 1 post-intervention		
Subject group type	Subject analysis set	Subject analysis set		
Number of subjects analysed	9[17]	9[18]		
Units: Percent				
number (not applicable)	9	9		

Notes:

[17] - Four patients dropped out before the intervention. One patient had no tumour centre tissue available
[18] - Four patients dropped out before the intervention. One patient had no tumour centre tissue available

Statistical analyses

Statistical analysis title	Change in bacterial biomass (tumour centre)

Statistical analysis description:

Percentage of bacterial biomass (mean (range)):
Baseline: 9.216e-03 (4.4e-4 – 1.0)
Post-intervention: 7.453e-02 (1.3e-5 – 0.4)

Comparison groups	Track 1 v Track 1 post-intervention
Number of subjects included in analysis	18
Analysis specification	Post-hoc
Analysis type	other
P-value	= 0.5 [19]
Method	Wilcoxon (Mann-Whitney)

Notes:

[19] - Paired one-tailed wilcoxon

Primary: Change in bacterial biomass (tumour periphery)

End point title	Change in bacterial biomass (tumour periphery)

End point description:

A quantitative change in the bacterial biomass adherent to the colonic epithelium of the resected colon adjusted for baseline level found before the intervention through fluorescence in situ (FISH) technique. The background tissue biomass volume was used to adjust the bacterial biomass volume. The specific measurement variable is 3D biofilm mass. The method of aggregation will be means.

End point type	Primary

End point timeframe:

Baseline to surgical tumour resection

End point values	Track 1	Track 1 post-intervention		
Subject group type	Subject analysis set	Subject analysis set		
Number of subjects analysed	10[20]	10[21]		
Units: percent				
number (not applicable)	10	10		

Notes:

[20] - 4 patients dropped out before the intervention.

[21] - 4 patients dropped out before the intervention.

Statistical analyses

Statistical analysis title	Change in bacterial biomass (tumour periphery)

Statistical analysis description:

Percentage of bacterial biomass (mean (range)):
Baseline: 0.1 (9.8e-5 – 0.6)
Post-intervention: 1.1e-02 (8.0e-04 – 4.2e-2)

Comparison groups	Track 1 v Track 1 post-intervention
Number of subjects included in analysis	20
Analysis specification	Pre-specified
Analysis type	other
P-value	= 0.161 [22]
Method	Wilcoxon (Mann-Whitney)

Notes:

[22] - Paired one-tailed wilcoxon test

Primary: Change in bacterial biomass (adenoma)

End point title	Change in bacterial biomass (adenoma)

End point description:

Bacterial biomass of the adenoma tissue after the surgical tumour resection from patients who received the intervention was compared with bacterial biomass of adenoma tissue in a retrospective cohort of patients.
A quantitative change in the bacterial biomass adherent to the colonic epithelium of the resected colon adjusted for baseline level found before the intervention through fluorescence in situ (FISH) technique. The background tissue biomass volume was used to adjust the bacterial biomass volume. The specific measurement variable is 3D biofilm mass. The method of aggregation will be means.

End point type	Primary

End point timeframe:

Retrospective control group and adenomas from patients who received the intervention after surgical tumour resection

End point values	Track 2	Track 2b retrospective controls		
Subject group type	Subject analysis set	Subject analysis set		
Number of subjects analysed	12[23]	22[24]		
Units: percent				
number (not applicable)	12	22		

Notes:

[23] - 2 patients excluded (tissue unavailable+artefact).

[24] - 2 controls were excluded due to staining artefacts and size of neoplasms

Statistical analyses

Statistical analysis title	Change in bacterial biomass (adenoma)

Statistical analysis description:

Percentage of bacterial biomass (mean (range)):
Control-group: 8.6e-03 (1.1e-05 - 7.5e-02)
Post-intervention, case-group: 1.1e-02 (3.4e-04 - 5.5e-02)

Comparison groups	Track 2 v Track 2b retrospective controls

Number of subjects included in analysis	34
Analysis specification	Pre-specified
Analysis type	other
P-value	= 0.386 [25]
Method	Wilcoxon (Mann-Whitney)

Notes:

[25] - One-tailed mann-whitney u test

Primary: Change in bacterial composition (tumour centre)

End point title	Change in bacterial composition (tumour centre)

End point description:

The determination of a change in the bacterial composition adjusted for the bacteria found at baseline before the intervention through fluorescence in situ (FISH) technique and sequencing with Metagenome.

End point type	Primary

End point timeframe:

Baseline to surgical tumour resection.

End point values	Track 1	Track 1 post-intervention		
Subject group type	Subject analysis set	Subject analysis set		
Number of subjects analysed	10[26]	10[27]		
Units: R2 (proportion)				
number (not applicable)	10	10		

Notes:

[26] - 4 patients dropped out before the intervention

[27] - 4 patients dropped out before the intervention

Statistical analyses

Statistical analysis title	Beta diversity

Statistical analysis description:

The beta diversity was compared between pre- and post-samples using the PERMANOVA test: R2=14%, F=2.5, p=0.006.

Comparison groups	Track 1 v Track 1 post-intervention
Number of subjects included in analysis	20
Analysis specification	Pre-specified
Analysis type	other
P-value	= 0.006
Method	PERMANOVA

Statistical analysis title	Bacterial presence

Statistical analysis description:

Tissue slides pre- and post-intervention were manually evaluated for the presence of Bacteroides. fragilis, Fusobacterium.
*Bacteroides fragilis (N (%)):
Baseline: 9 (90)
Post-intervention: 8 (89)
*Fusobacterium (N (%)):

Baseline: 7 (70)
Post-intervention: 7 (78)

Comparison groups	Track 1 v Track 1 post-intervention
Number of subjects included in analysis	20
Analysis specification	Pre-specified
Analysis type	other
P-value	= 1 [28]
Method	Mcnemar

Notes:

[28] - McNemar's Chi-squared test with continuity correction

Primary: Change in bacterial composition (tumour periphery)

End point title	Change in bacterial composition (tumour periphery)

End point description:

The determination of a change in the bacterial composition adjusted for the bacteria found at baseline before the intervention through fluorescence in situ (FISH) technique and sequencing with Metagenome.

End point type	Primary

End point timeframe:

Baseline to surgical tumour resection

End point values	Track 1	Track 1 post-intervention		
Subject group type	Subject analysis set	Subject analysis set		
Number of subjects analysed	10[29]	10[30]		
Units: R2 (proportion)				
number (not applicable)	10	10		

Notes:

[29] - 4 patients dropped out before the intervention

[30] - 4 patients dropped out before the intervention

Statistical analyses

Statistical analysis title	Beta diversity

Statistical analysis description:

The beta diversity was compared between pre- and post-samples using a PERMANOVA test: R2=15%, F=3.0, p=0.004 for the tumour periphery

Comparison groups	Track 1 v Track 1 post-intervention
Number of subjects included in analysis	20
Analysis specification	Post-hoc
Analysis type	other
P-value	= 0.004
Method	PERMANOVA

Statistical analysis title	Bacterial presence

Statistical analysis description:

Tissue slides pre- and post-intervention were manually evaluated for the presence of Bacteroides fragilis, Fusobacterium.
*Bacteroides fragilis (N (%)):

Baseline: 10 (100)
Post-intervention: 10 (100)
*Fusobacterium (N (%)):
Baseline: 9 (90)
Post-intervention: 10 (100)

Comparison groups	Track 1 v Track 1 post-intervention
Number of subjects included in analysis	20
Analysis specification	Pre-specified
Analysis type	other
P-value	= 1 [31]
Method	Mcnemar

Notes:

[31] - McNemar's Chi-squared test with continuity correction

Primary: Change in gene expression (tumour centre)

End point title	Change in gene expression (tumour centre)

End point description:

The determination of differences in translational markers present in the resected tumor tissue adjusted for the level seen at the baseline (the therapeutic colonoscopy) through a principal component analysis (PCA). The translational markers of interest are tumor markers, and markers of the microenvironment and the immune response. The analyses will be performed using the PanCancer IO 360TM Gene Expression Panel (nanoString).

End point type	Primary

End point timeframe:

Baseline to surgical tumour resection

End point values	Track 1	Track 1 post-intervention		
Subject group type	Subject analysis set	Subject analysis set		
Number of subjects analysed	7[32]	7[33]		
Units: Log2fold change				
number (not applicable)	7	7		

Notes:

[32] - 4 patients dropped out. 3 patients were excluded due to no post-intervention samples.

[33] - 4 patients dropped out. 3 patients were excluded due to no post-intervention samples.

Statistical analyses

Statistical analysis title	Differentially expressed genes

Statistical analysis description:

Tumour centre samples before and after the antibiotic application were compared for differentially expressed (DE) genes using the Wald significance test with a log2 fold change ≥0.5 and an adjusted p-value <0.05 using the Benjamini-Hochberg approach: six genes were down-regulated and one gene was upregulated.

Comparison groups	Track 1 v Track 1 post-intervention
Number of subjects included in analysis	14
Analysis specification	Post-hoc
Analysis type	other
P-value	< 0.05
Method	Wald significance test
Parameter estimate	log2FoldChange

Primary: Change in gene expression (tumour periphery)

End point title	Change in gene expression (tumour periphery)

End point description:

The determination of differences in translational markers present in the resected tumor tissue adjusted for the level seen at the baseline (the therapeutic colonoscopy) through a principal component analysis (PCA). The translational markers of interest are tumor markers, and markers of the microenvironment and the immune response. The analyses will be performed using the PanCancer IO 360TM Gene Expression Panel (nanoString).

End point type	Primary

End point timeframe:

Baseline to surgical tumour resection

End point values	Track 1	Track 1 post-intervention		
Subject group type	Subject analysis set	Subject analysis set		
Number of subjects analysed	10[34]	10[35]		
Units: Log2foldchange				
number (not applicable)	10	10		

Notes:

[34] - 4 patients dropped out before the intervention

[35] - 4 patients dropped out before the intervention

Statistical analyses

Statistical analysis title	Differentially expressed genes

Statistical analysis description:

Tumour periphery samples before and after the antibiotic application were compared for differentially expressed (DE) genes using the Wald significance test with an adjusted p-value <0.05 using the Benjamini-Hochberg approach: 12 genes were down-regulated and 10 genes were up-regulated.

Comparison groups	Track 1 v Track 1 post-intervention
Number of subjects included in analysis	20
Analysis specification	Post-hoc
Analysis type	other
P-value	< 0.05
Method	Wald significance test
Parameter estimate	log2FoldChange

Primary: Change in gene expression (adenomas)

End point title	Change in gene expression (adenomas)

End point description:

Gene expression of the adenoma tissue after the surgical tumour resection from patients who received the intervention was compared with gene expression of adenoma tissue in a retrospective cohort of patients.

End point type	Primary

End point timeframe:

Retrospective control group and adenomas from patients who received the intervention after surgical tumour resection

End point values	Track 2	Track 2b retrospective controls		
Subject group type	Subject analysis set	Subject analysis set		
Number of subjects analysed	12[36]	21[37]		
Units: Log2foldchange				
number (not applicable)	12	21		

Notes:

[36] - 1 pt dropped out, 1 patient was screening failure.

[37] - 1 control removed due to tumor size, 2 controls removed due to very low gene count.

Statistical analyses

Statistical analysis title	Differentially expressed genes

Statistical analysis description:

DE genes between pre- and post-intervention samples (time A vs B) were analysed using the Wald significance test with adjustment for multiple testing by the Benjamini-Hochberg approach: three genes down-regulated and seven genes up-regulated.

Comparison groups	Track 2 v Track 2b retrospective controls
Number of subjects included in analysis	33
Analysis specification	Post-hoc
Analysis type	other
P-value	< 0.05
Method	Wald significance test
Parameter estimate	log2FoldChange

Primary: Change in bacterial composition (adenomas)

End point title	Change in bacterial composition (adenomas)

End point description:

End point type	Primary

End point timeframe:

Retrospective control group and adenomas from patients who received the intervention after surgical tumour resection

End point values	Track 2	Track 2b retrospective controls		
Subject group type	Subject analysis set	Subject analysis set		
Number of subjects analysed	12	24		
Units: Numbers	12	24		

Statistical analyses

Statistical analysis title	Bacterial presence

Statistical analysis description:

Tissue slides from control group patients (not exposed for antibiotics) and post-intervention tissue slides were manually evaluated for the presence of Bacteroides
fragilis, Fusobacterium.
*Bacteroides fragilis (N (%)):
Control group: 22 (92)
Post-intervention: 10 (83)
*Fusobacterium (N (%)):
Control group: 13 (54)
Post-intervention: 8 (67)

Comparison groups	Track 2 v Track 2b retrospective controls
Number of subjects included in analysis	36
Analysis specification	Pre-specified
Analysis type	other
P-value	≥ 0.588 [38]
Method	Mcnemar

Notes:

[38] - Test for Bacteroides fragilis p=0.588.
Test for Fusobacterium p=0.721.
McNemar's Chi-squared test with continuity correction.

Adverse events

Adverse events information	
Timeframe for reporting adverse events:	
From the day of intervention (therapeutic endoscopy) until 14 days postoperatively (at least 19 days after the intervention) and then 12 months postoperatively'	
Adverse event reporting additional description:	
CTCAE v5	
Assessment type	Systematic

Dictionary used	
Dictionary name	CTCAE
Dictionary version	5

Reporting groups	
Reporting group title	Track 1
Reporting group description:	
Patients with colon cancer	
Reporting group title	Track 2
Reporting group description:	
Patients with colon adenoma/precursor lesion	

Serious adverse events	Track 1	Track 2	
Total subjects affected by serious adverse events			
subjects affected / exposed	0 / 10 (0.00%)	1 / 13 (7.69%)	
number of deaths (all causes)	0	0	
number of deaths resulting from adverse events	0	0	
Infections and infestations			
Fever after a surgical procedure leading to readmission	Additional description: One patient had a SAE grade 3: the patient developed fever in the hours after the surgical tumour resection, and had to be readmitted to hospital.		
subjects affected / exposed	0 / 10 (0.00%)	1 / 13 (7.69%)	
occurrences causally related to treatment / all	0 / 0	0 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	

Frequency threshold for reporting non-serious adverse events: 0 %

Non-serious adverse events	Track 1	Track 2	
Total subjects affected by non-serious adverse events			
subjects affected / exposed	7 / 10 (70.00%)	13 / 13 (100.00%)	
Neoplasms benign, malignant and unspecified (incl cysts and polyps)			
Adenoma recurrence	Additional description: Patients reporting adenoma recurrence within 12 months postoperatively		

subjects affected / exposed	0 / 10 (0.00%)	1 / 13 (7.69%)	
occurrences (all)	0	1	
Nervous system disorders			
Interrupted sleep	Additional description: Patients reporting interrupted sleep within 14 days postoperatively		
subjects affected / exposed	0 / 10 (0.00%)	1 / 13 (7.69%)	
occurrences (all)	0	1	
Fatigue	Additional description: Patients reporting fatigue within 14 days postoperatively		
subjects affected / exposed	0 / 10 (0.00%)	1 / 13 (7.69%)	
occurrences (all)	0	1	
Blood and lymphatic system disorders			
Anaemia	Additional description: Patients experiencing anaemia within 14 days postoperatively		
subjects affected / exposed	1 / 10 (10.00%)	0 / 13 (0.00%)	
occurrences (all)	1	0	
Eye disorders			
Light sensitivity	Additional description: Patients reporting light sensitivity within 14 days postoperatively		
subjects affected / exposed	0 / 10 (0.00%)	1 / 13 (7.69%)	
occurrences (all)	0	1	
Gastrointestinal disorders			
Loose stools	Additional description: Patients reporting stools with a more loose consistency than before the intervention within 14 days postoperatively		
subjects affected / exposed	4 / 10 (40.00%)	10 / 13 (76.92%)	
occurrences (all)	4	10	
Diarrhoea	Additional description: Patients reporting diarrhoea within 14 days postoperatively		
subjects affected / exposed	0 / 10 (0.00%)	4 / 13 (30.77%)	
occurrences (all)	0	4	
Constipated diarrhoea	Additional description: Patients who reported constipated diarrhoea within 14 days postoperatively		
subjects affected / exposed	0 / 10 (0.00%)	1 / 13 (7.69%)	
occurrences (all)	0	1	
Constipation	Additional description: Patients who reported constipation within 14 days postoperatively		
subjects affected / exposed	0 / 10 (0.00%)	1 / 13 (7.69%)	
occurrences (all)	0	1	
Abdominal pain	Additional description: Patients reporting abdominal pain within 14 days postoperatively		
subjects affected / exposed	4 / 10 (40.00%)	5 / 13 (38.46%)	
occurrences (all)	4	5	
Dysgeusia	Additional description: Patients who reported dysgeusia within 14 days postoperatively		

subjects affected / exposed	0 / 10 (0.00%)	1 / 13 (7.69%)	
occurrences (all)	0	1	
Dry mouth	Additional description: Patients reporting dry mouth within 14 days postoperatively		
subjects affected / exposed	0 / 10 (0.00%)	1 / 13 (7.69%)	
occurrences (all)	0	1	
Rectal bleeding	Additional description: Patients reporting rectal bleeding within 14 days postoperatively		
subjects affected / exposed	2 / 10 (20.00%)	1 / 13 (7.69%)	
occurrences (all)	2	1	
Arterial bleeding during the endoscopic mucosal resection	Additional description: Patients developing arterial bleeding during the endoscopic mucosal resection (only track 2)		
subjects affected / exposed	0 / 10 (0.00%)	1 / 13 (7.69%)	
occurrences (all)	0	1	
Change in stool odour	Additional description: Patients reporting change in stool odour within 14 days postoperatively		
subjects affected / exposed	1 / 10 (10.00%)	0 / 13 (0.00%)	
occurrences (all)	1	0	
Yellow coloured stool	Additional description: Patients reporting yellow coloured stool within 14 days postoperatively		
subjects affected / exposed	1 / 10 (10.00%)	0 / 13 (0.00%)	
occurrences (all)	1	0	
Faecal incontinence	Additional description: Patients reporting faecal incontinence within 12 months postoperatively		
subjects affected / exposed	1 / 10 (10.00%)	0 / 13 (0.00%)	
occurrences (all)	1	0	
Nausea	Additional description: Patients reporting nausea within 14 days postoperatively		
subjects affected / exposed	1 / 10 (10.00%)	0 / 13 (0.00%)	
occurrences (all)	0	0	
Skin and subcutaneous tissue disorders			
Hot flashes	Additional description: Patients reporting hot flashes within 14 days postoperatively		
subjects affected / exposed	1 / 10 (10.00%)	1 / 13 (7.69%)	
occurrences (all)	1	1	
Musculoskeletal and connective tissue disorders			
Myalgia	Additional description: Patients reporting myalgia within 14 days postoperatively		
subjects affected / exposed	0 / 10 (0.00%)	1 / 13 (7.69%)	
occurrences (all)	0	1	
Weight loss 1-2 kg	Additional description: Patients reporting weight loss of 1-2 kg within 14 days postoperatively		

subjects affected / exposed	1 / 10 (10.00%)	0 / 13 (0.00%)	
occurrences (all)	1	0	
Infections and infestations			
Fever	Additional description: Patients reporting fever within 14 days postoperatively		
subjects affected / exposed	1 / 10 (10.00%)	0 / 13 (0.00%)	
occurrences (all)	1	0	
CRP elevation	Additional description: Patients experiencing CRP elevation within 14 days postoperatively		
subjects affected / exposed	1 / 10 (10.00%)	0 / 13 (0.00%)	
occurrences (all)	1	0	

More information

Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? Yes

Date	Amendment
12 March 2019	Addition of the exclusion criteria "fenemal usage".
02 July 2020	Change in PI
22 October 2020	Removal of the exclusion criteria "preoperative iron infusion" Prolonged study period.
22 September 2022	An update of the known side-effects of Fosfomycin and Metronidazole was written in the patient information. Change in PI
05 January 2023	Change in PI

Notes:

Interruptions (globally)

Were there any global interruptions to the trial? No

Limitations and caveats

None reported