NL51461.078.15 / NCT02328664 Scar biopsies after malignant colorectal polypectomy of uncertain radicality

SCAPURA STUDY

Version April, 2016

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PROTOCOL TITLE 'The sensitivity of scar-biopsies for residual colorectal adenocarcinoma after endoscopic resection with uncertain radicality.'

Protocol ID	SCAPURA Study
Short title	**SCar-biopsies After malignant colorectal Polypectomy of Uncertain RAdicality.**
Short title (Dutch)	Litteken-biopten na maligne colorectale poliepectomie met onzekere radicaliteit
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Date	April 2016
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List of abbreviations and relevant definitions

AE	Adverse Event
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
DSMB	Data Safety Monitoring Board
EudraCT	European drug regulatory affairs Clinical Trials
IC	Informed Consent
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
(S)AE	(Serious) Adverse Event
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEM	Transanal Endoscopic Microsurgical Excision
TAMIS	Transanal Minimal Invasive Surgery
eFTR	Endoscopic Full-Thickness Resection
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

Summary

Rationale: colorectal polyps may harbor adenocarcinoma. Numbers are increasing due to the nationwide colorectal screening program. After endoscopic removal, rescue surgery is often performed because radicality can not be guaranteed by the pathologist. However, in 85% of surgical specimen no residual malignancy is found. Given morbidity and mortality associated with surgery a method to diagnose residual cancer is needed.
Biopsies from the polypectomy site are variably used to reduce the likelihood of residual tumor at the polypectomy site under these circumstances. However, the sensitivity of such biopsies is unknown.
Many patients are operated upon because of risk factors for dissemination such as lymfangioinvasion, despite a radical local resection. There has never been a prospective registration of these patients with regard to surgical outcome.
Objectives: 1 to evaluate the sensitivity of second-look endoscopic biopsies from the polypectomy site for residual tumor. 2. To register patients who do not fulfill inclusion criteria in order to evaluate their surgical outcomes.
Study design: prospective cross-sectional design using a multi-center approach.
Study population: patients in whom a malignant colonic polyp has been removed. For the biopsy part of the study (1) they should be planned for rescue surgery (including transmural excision) for the sole reason of (potentially) irradical endoscopic resection of a colorectal adenocarcinoma without poor differentiation, lymphovascular invasion or tumor budding and without other signs of dissemination.
Intervention (biopsy part of the study): endoscopic biopsies from the polypectomy site before operation. Patients who are excluded will only be registered.
Main study parameters/endpoints: Biopsy part of the study: sensitivity of second-look biopsies from the polypectomy site for residual tumor in the resected bowel and postoperative mortality. Various other factors will be assessed that might be associated with residual cancer. Observational part of the study: The prevalence of patients with exclusion criteria and outcome of surgery in this group (observational part of the study).
Nature and extent of the burden and risks associated with participation and benefit (only applicable for biopsy-part of the study): Depending on the situation: a): In case a tattoo needs to be done of the polypectomy site before surgery, or an endoscopic full-thickness resection is done, taking biopsies will be of no extra burden; b): In case no endoscopy is indicated before surgery (including TEM / TAMIS), a sigmoidoscopy (lesion distal to the splenic flexure) or occasionally colonoscopy (proximal to the splenic flexure) needs to be arranged for the purpose of this study. A sigmoidoscopy takes 10-20 minutes. Preparation consists of two enemas or flavored polyethylene glycol solution, depending on

the local protocol. A colonoscopy takes 20-30 minutes. Preparation consists of drinking flavored polyethylene glycol solution, both usually done at home. Notice that the patient has recent experience with colonoscopy. If necessary, both investigations can be arranged under conscious sedation (the rule in colonoscopy), which also implies day-care admission.
The risk of complications of a second endoscopy is estimated < 1:5000. The benefit of a 2nd colonoscopy is the discovery of new polyps in 10-25% of cases.

1 Introduction and rationale

Colorectal carcinoma (CRC) is the second most important cause of cancer mortality in the Netherlands with 15.003 new cases (2014) and 4940 deaths in 2013[1]. Colorectal cancer usually develops from an adenomatous polyp, which is primarily resected via colonoscopy. The number of colonoscopies has dramatically increased with the introduction of population-based screening for colorectal cancer (2014). One or more polyps are roughly found in 1 out of 3 colonoscopies, and may harbor adenocarcinoma in 3-9%[2, 3]. This is sometimes unsuspected: Kudo *et al* reported that 2.8% of benign looking colonic polyps harbor invasive cancer[4].

Unfortunately, the pathologist is sometimes unable to guarantee a radical endoscopic resection from an oncological point of view. The bowel wall is thin and margins for endoscopic resection are limited to the submucosal space, often less than 1 mm. En-bloc resection is often impossible or too dangerous (perforation). Thus, the majority of endoscopic resection specimens show a tumor-free resection margin < 1 mm or a resection margin that is impossible to evaluate due to piecemeal resection (multiple fragments)[5, 6]. For these patients, additional surgery is advised[5]. However, residual tumor at the polypectomy site in surgical rescue specimens is found in only 5 (uncertain radical) to 30% (certainly not radical) of patients[7, 8, 9].

Affected locoregional lymph nodes are also of concern, even when imaging studies are normal. They are, however, rarely encountered in case of a moderately to well-differentiated adenocarcinoma without risk factors for lymph node metastasis (deep (> 1mm) submucosal invasion[10], tumor budding, a poorly differentiated histology and/or invasion of lymphatic or blood vessels[11, 12, 13, 14]). If one or more of these risk factors for lymph node metastases are present, metastases can be found in 11-15% of these surgical specimens. In such cases, the decision to additional surgery is made independently of local radicality.

According to the Dutch Surgical Colorectal Audit (DSCA)[15], 30-day mortality of surgery for colonic cancer in 2013 was 1.9% and it also has been demonstrated that 90-day mortality is 1,51 times the 30-day mortality[16]. Thus, 90-days postoperative mortality of surgery for colonic cancer is estimated to be 2.8% overall and 12% for patients aged 70 and above. With the advance of endosurgical (TEM, TAMIS) or endoscopic full-thickness bowel wall resection (eFTR) techniques the risks of additional surgery (especially mortality) are reportedly reduced. Given these risks, a test to diagnose the presence of residual cancer before deciding to surgery would be ideal. In practice, biopsies are often taken from the polypectomy site to see whether tumor has remained. However, no data exist on the sensitivity of endoscopic biopsies from the polypectomy site for residual cancer.

Many patients in whom a malignant polyp has been removed endoscopically are operated for because of the presence of risk factors for lymphovascular invasion, even if the endoscopic resection has been radical.

In such patients, biopsies are not helpful.

These patients will be registered in the study database, as there is no prospective evaluation in the Netherlands of surgical outcomes of these patients.

2 Objectives

2.1 Primary Objective:

To study the diagnostic properties, expressed by the sensitivity, of biopsies from the site of a malignant polypectomy for residual adenocarcinoma, as compared to surgical rescue specimen in patients with (potentially) irradical endoscopic resection of a malignant colonic polyp.

2.2 Secondary Objectives:

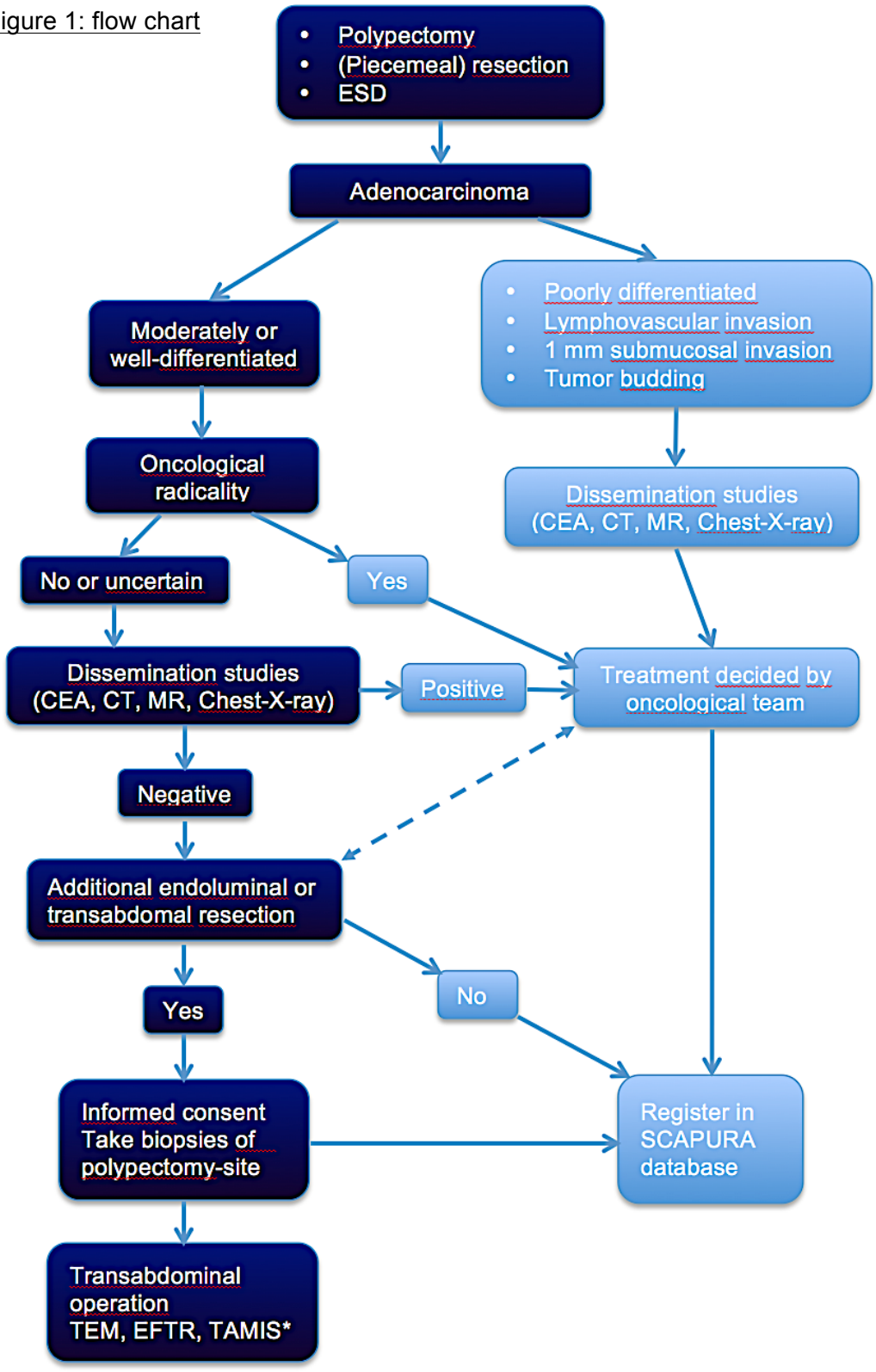
- 90-day mortality after rescue surgery: the number of patients that died within 91 day after the operation for presumed residual adenocarcinoma
- The sensitivity of biopsies for residual cancer in the bowel wall: the number of patients with endoscopic biopsies containing adenocarcinoma divided by the number of patients with adenocarcinoma in the resected bowel wall (regardless of regional lymph nodes)
- The number of complications (defined according to GCP and the Dutch Association of Gastroenterology) after biopsies from the polypectomy: the number of patients with bleeding or perforation after taking biopsies from the polypectomy scar, requiring at least prolongation of treatment, or admission to hospital, or delay or speeding up rescue surgery. This up until the moment of surgery.
- The sensitivity of global endoscopic assessment of initial polypectomy as well as scar biopsies for residual cancer: the number of patients in whom the endoscopic resection was assessed as incomplete and who also have residual cancer in the surgically resected specimen divided by the total number of patients in whom the endoscopic resection was judged to be incomplete.
- The proportion of patients with residual cancer in the resected specimen if malignancy was unsuspected during the endoscopic polypectomy: the number of patients in whom the malignancy was initially unsuspected during endoscopic polypectomy and who also have residual cancer in the surgical specimen divided by the total number of patients in whom the malignancy was initially unsuspected during endoscopic polypectomy.
- The proportion of patients in whom exclusion criteria apply and the results of surgery / complications of surgery in these patients.

3 Study design

The study has a multicenter approach and a cross-sectional observational design. There is no comparison to a placebo-group or control group; the study merely intends to evaluate the

sensitivities of biopsies from the malignant colorectal polypectomy site for residual adenocarcinoma. A flow chart of the study procedures is given in figure 1:

3.1 Figure 1: flow chart



* Endoluminal resection includes: Transanal Endoscopic Microsurgical excision (TEM), Transanal Minimal Invasive Surgery (TAMIS) or Endoscopic Full-thickness Resection (eFTR).

4 Study population

4.1 Population

The number of reported T1 colorectal carcinomas from the Dutch Cancer Registration in 2011 was 1065[17].
Data from the Integral Cancer Registration in the South of the Netherlands (covering 2.3 million inhabitants) are not included. Extrapolated to the whole population of the Netherlands (16.8 million inhabitants), the nationwide incidence of T1 colorectal carcinoma is estimated to be 1200-1250 cases. These numbers are expected to increase due to the implementation of a nationwide screening program for colorectal cancer.
An endoscopically removed polyp with uncertain radicality is not equivalent to a T1 carcinoma, as it is actually unknown if the tumor extends into the muscularis propria layer. However, if the latter is the case, endoscopic removal is usually impossible because of fixation and lack of elevation with submucosal injection (non-lifting sign).
According to the data of Cooper[18] and Ikamatsu[19] *et al*, 30-33% is treated by endoscopic removal without rescue surgery. On the other hand, we assume that in 20% of these tumors no attempt for endoscopic resection is undertaken (direct referral for surgery). Accordingly, 50% of all patients (nationwide 600 patients per year) with a T1 colorectal carcinoma are eligible for the study. Assuming patients who are unwilling or unable to participate, we estimate a nationwide number of 500 evaluable patients per year.

4.2 Inclusion criteria (biopsy part of the study)

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Aged 18 or above.
- Endoscopically removed colorectal lesion with the following pathological characteristics:
    - A moderately-to-well differentiated adenocarcinoma.
    - If possible to judge: distance between adenocarcinoma and vertical or lateral resection margin is less than 1 mm.
    - In case of piecemeal resection: radicality cannot be assessed (mostly due to loss of orientation and multiple fragments).
    - Absence of / not assessable lymphatic / vascular invasion.
    - No tumor budding (only if specifically mentioned in the pathology report).
    - No deep submucosal invasion (> 1 mm)

- No suspicion of dissemination on the following investigations: serum carcino-embryonic antigen, a computer tomographic (CT) scan of the abdomen and a chest X-ray; in case of a rectal tumor (less than 15 cm from the anal verge): an additional magnetic resonance imaging of the rectum.
- Operation or endoscopic full-thickness resection (including Transanal Endoscopic Microsurgical excision (TEM), Transanal Minimal Invasive Surgery (TAMIS) or Endoscopic Full-thickness Resection (eFTR)) is advised in agreement with the Dutch Guideline on Colorectal cancer, planned and agreed on by the patient.
- Written informed consent is obtained.

4.3 Exclusion criteria (observation part of the study)

A potential subject who meets any of the following criteria will be excluded from participation in this study. Noticeably, these patients should be registered in the database to identify outcomes in this group (observational registration):

- Pathology shows one or more of the following characteristics:
    - A radical en-bloc resection with a free vertical and lateral margin of ≧ 1 mm.
    - A poorly differentiated or signet-cell containing adenocarcinoma.
    - Lymphatic or vascular invasion (if this feature is not assessable due to piecemeal resection, no exclusion is done).
    - Tumor budding (only if specifically mentioned in the pathology report)
    - Deep submucosal invasion (> 1 mm).
- Suspicion of dissemination on investigations as mentioned in the inclusion criteria.
- Patients already receiving anti-tumor treatment for another tumor or a synchronous colorectal cancer or patients pretreated with radiotherapy.
- Patients in whom a second-look endoscopy would require major and unacceptable effort and / or resources, for instance clinical admission for bowel preparation, long travel, general anesthesia, difficult to reach polypectomy site. This decision is left to the discretion of the patient and / or treating physician.
- Patient is not planned for surgery or additional full-thickness resection.
- Patient is pregnant.
- Patient does not provide written informed consent or is unable to provide such.

4.4 Sample size calculation

The overarching goal is to investigate whether second-look endoscopic biopsies after resection of a malignant polyp with uncertain pathological completeness can be used as a screening instrument to aid in the clinical decision whether rescue surgery should be performed or not. The specificity of second-look biopsies from the polypectomy site for residual malignancy is estimated to be near 100%, as it is reasonable to state that no person without residual malignancy in the bowel wall is likely to have tumor in second look biopsies, in other words, the false-positive rate will be approximately zero.

Accordingly, sample size calculations will be done on the basis of sensitivity, i.e. the number of patients with positive second-look biopsies divided by the number of patients who do have residual tumor in the resected specimen. We state that sensitivity should be at least 95% to be clinically relevant. A non-inferiority approach can be used to calculate sample size[20]. In that case, the null-hypothesis states that sensitivity is significantly inferior to 95%. Because proof of exact equality to 95% is impossible, a pre-stated margin of non-inferiority of -5% (90% in our study) is accepted.

The distributions of the probabilities belonging to the null- (around $p = 0.90$) and alternative (around $p = 0.95$) hypothesis is binomial but may be approximated by a normal distribution for sample size calculation.

Using the normal approximation, it can be calculated that 184 patients need to be included to achieve a power of 0.80 with and a probability of falsely accepting biopsies as non-inferior (α) of 0.05.

Using binomial distributions, it can be shown that 194 patients need to be included to achieve this goal. We will use the latter number for a robust estimate of sample size.

We estimate incomplete data and / or refusal to participate in 1 out of 10 patients invited. This calculates to a number of 216 patients with residual tumor to be invited. Depending on the prevalence of residual tumor, which is estimated to vary between 10 and 30% (average 20%) of all patients, a total of 1080 patients fulfilling the inclusion criteria need to be invited to accomplish this number.

We aim at participation of all eight Academic Centers and all community hospitals. In case of full participation, approximately 520 patients could be acquired each year. We aim at a maximum inclusion period of four years, but preferably shorter.

5 Treatment of subjects

Not applicable

5.1 Investigational product

Not applicable

5.2 Use of co-intervention

Not applicable

5.3 Escape medication

Not applicable

6 Investigational product

Not applicable.

7 Non-investigational product

Not applicable

8 Methods

8.1 Study parameters/endpoints

8.1.1 Main study parameter

The sensitivity of endoscopic biopsies from the polypectomy-site for residual adenocarcinoma, as compared to the surgically resected specimen.

8.1.2 Secondary study parameters

The postoperative mortality of the surgical resection (for the purpose of a possibly incompletely removed malignant polyp), as occurring within 91 days after surgery, irrespective of the cause of death as well as of the relation to the surgery (in order to evaluate competing risks).

8.1.3 Other study parameters

The following parameters will be recorded:

8.1.3.1 General information

- Center name, name of treating physician
- Gender, date of birth, initials, first letter of family name.
- Comorbidity according to the American Association of Anesthesiology (ASA status).
- Has the patient an elevated risk of having Lynch syndrome[26]?

8.1.3.2 Data from endoscopic procedure during which the malignant polyp has been removed

- Date of the procedure.
- Estimated location of the tumor (distal rectum (before the first haustric fold), proximal rectum (proximal to the first haustric fold, but within 15 cm of the anal verge), sigmoid, descending colon, splenic flexure, transverse colon, hepatic flexure, ascending colon, cecum.
- Distance from the anal verge, measured during withdrawal of the endoscope. Distance is estimated using the centimeter scale on the shaft of endoscope
- Endoscopically estimated size of the polyp as compared to a closed snare (3 mm) or opened biopsy forceps (7 mm).
- Expected pathology (adenoma, serrated adenoma, carcinoma).
- Polyp shape according to Paris[21].
- En-bloc resection.
- Use of a snare.
- Use of submucosal injection.
- Use of electrosurgical current.
- Tattoo.
- Estimated endoscopic radicality.
- Circumferential incision (partial or complete).
- Submucosal dissection.
- Bleeding (immediate or delayed).
- Perforation (immediate or delayed).
- Intervention for complication (endoscopic, angiographic, surgical).

- Severity of complication according to the standard of the dutch association of gastroenterology.

  Advanced characteristics (not obligatory):
- Presence of spontaneous bleeding
- NICE[22]- and Hiroshima[23]-classification.
- Pit-pattern according to Kudo[24].
- Lifting classification graded according to Kato[25].

8.1.3.3 Pathology of the endoscopically removed malignant polyp[26]

- T-number.
- Date of the report.
- Specimen: en-bloc or fragmented.
- Size of the (largest) fragment.
- Primary lesion: hyperplastic polyp, tubular adenoma, tubulovillous adenoma, villous adenoma, traditional serrated adenoma, sessile serrated lesion, with invasive malignancy.
- Finding: with low-grade dysplasia, with high-grade dysplasia, suspicion of carcinoma, with invasive malignancy, with pseudoinvasion
- Tumor type: adenocarcinoma, other.
- Differentiation grade: well, moderate, poor.
- Depth of invasion.
- Resection margin (free, not free of tumor, unable to judge, ….mm).
- Lymphangioinvasion (yes, no, unable to judge).

Additional pathology findings (not obligatory).

- Tumor budding[27].
- Haggit Level[28].
- Kikuchi Level[29].

8.1.3.4 Data from biopsies from the polypectomy site (second investigational endoscopy)

- Date of the endoscopic procedure.
- Type of procedure (sigmoidoscopy or colonoscopy).
- Finding: scar, tattoo, ulcer, polypous remnant, suspicion of residual cancer, no reliable identification of the polypectomy site.
- Number of biopsies.
- Bleeding (immediate, delayed).
- Perforation (immediate, delayed).

- Intervention for complication (endoscopic, angiographic, surgical).
- Impact of complication on scheduled surgical resection: no change, antedation, postponation, cancellation.
- Severity of complication according to the Dutch Society of Gastroenterology.

8.1.3.5 Pathology of scar biopsies[26]

- T-number.
- Date of pathology report.
- Number of biopsies.
- Primary finding: normal mucosa, granulation tissue, ulcer, scar tissue, low-grade dysplasia, hig-grade dysplasia, suspicion of carcinoma, invasive carcinoma.
- Type of tumor: adenocarcinoma, other.

8.1.3.6 Data from the surgical intervention or endoluminal full-thickness resection

- Date of the operation.
- Type of operation: TEM, TAMIS, eFTR, abdominoperineal resection, low anterior resection, sigmoid resection, Hartmann procedure, left hemicolectomy, transverse resection, right hemicolectomy, ileocecal resection, total proctocolectomy, subtotal colectomy.
- Ostomy: temporarily, definitive.
- Conversion to open procedure.
- Complications (within 93 days): Anastomotic leakage, rebleeding, abdominal sepsis, other infection, relaparotomy, deceased.
- Intensive care ….days.

8.1.3.7 Pathology of the surgical specimen[30]

- T-number.
- Date of the pathology report.
- Tumor present.
- Type of tumor: adenocarcinoma, other.
- Differentiation grade: well/moderate, poor, unable to judge.
- Depth of invasion: intramucosal, lamina propria, submucosal, muscularis propria, pericolic fat / tissue, peritoneal surface, other organ.
- Angioinvasion: not found, lymphatic vessel, venous invasion.
- Total number of lymph nodes in the resected specimen.
- Number of lymph nodes with metastases.

- Number of lymph nodes with isolated tumor cells.

8.2 Randomization, blinding and treatment allocation

Not applicable

8.3 Study procedures

8.3.1 Investigational endoscopy

For the purpose of the study, preoperative biopsy of the polypectomy site will be done. To allow for sufficient healing of the polypectomy site, an arbitrary period of at least 14 days will exist between the endoscopic resection of the malignant polyp and the second endoscopy. In daily practice, however, it is hard to imagine that the time interval between the two endoscopies could be shorter. First, the pathology report has to be completed, the patient has to be informed, dissemination studies have to be arranged, and the results have to be discussed in a multidisciplinary oncological meeting. If no dissemination has been found and additional resection has been decided on, the patient has to be seen by the surgeon for final planning of the operation. Only after this moment, participation in the study can be offered. The time between the biopsies and surgery should not exceed 4 weeks.
It is convenient to take biopsies from the polypectomy site when a second endoscopy is done for tattooing the polypectomy site or during endoscopic full-thickness resection. In this situation, the burden of participation in the study is zero.
If tattooing has already been done during the first endoscopy (when the malignant polyp was removed), a second endoscopy has to be arranged for the sole reason of participation in the study.
If the malignant polyp was located in the recto-sigmoid colon, a sigmoidoscopy will suffice. Otherwise, the colonoscopy has to be repeated. The details of these investigations are explained in the patient information form. Of cause, it remains the decision of the patient whether or not to participate in case a separate endoscopy needs to be arranged. It should be realized, that the patient recently underwent a colonoscopy, so he or she is “experienced” and this is an optimal situation to decide whether or not to participate. It should be kept in mind, that in back-to-back colonoscopy studies, new polyps have been found in 10-25% of cases[31]. This extra colonoscopy check may be of advantage for the patient.

8.3.2 Investigational biopsies

Biopsies will be taken through the endoscope with a standard biopsy forceps. The number of biopsies depends on the size of the lesion / scar. In case of a small scar, the neo-epithelial surface of the lesion should be removed by biopsies. However, if more than ten biopsies would be necessary to do this, five biopsies will be taken from the center in a mesh-like fashion at approximately 5 mm intervals. In addition, biopsies will be taken from the edge (if discernible) at 5 mm intervals. Any mucosal irregularities in or around the scar will be biopsied separately. Biopsies should be collected according to pathology standards in formalin.

8.3.3 Other procedures

Pathology should be reported according to current standards[26,30]
After time, all pathology will be independently assessed by Prof. dr. Gerrit Meijer, pathologist at the National Cancer Institute / Antoni van Leeuwenhoekziekenhuis. The patient needs to consent to this procedure.
Reference assessment will be done without knowledge of the clinical situation.

Local endoluminal, laparoscopic or open resection of the affected colon will take place according to the current standards of care.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. Specific criteria for withdrawal are not applicable

8.5 Replacement of individual subjects after withdrawal

The study will continue until the requested amount of subjects is included.

8.6 Follow-up of subjects withdrawn from treatment

No treatment is given, so no follow-up will be instigated. These patients are subjected to normal treatment (operation) as has already been planned.

8.7 Premature termination of the study

It is possible that at an earlier inclusion stage it may be seem that the sensitivity of biopsies is low. However, a low sensitivity, e.g. 70%, may still be useful as it may substantially reduce the post-test probability to below the probability of postoperative death in high risk groups (this is currently difficult to predict, as it is dependent on the observed prevalence of residual tumor, the sensitivity of the biopsy, and the postoperative mortality in the study group). Thus a low observed sensitivity at an earlier inclusion stage shall be no reason to stop the inclusion prematurely.
It can be contemplated to stop inclusion if the observed sensitivity is very high, “definitely” not inferior to 95%. In that case, the negative predictive value of biopsies is so high that is may seem to be unethical to continue operating on these patients if biopsies show no residual cancer. However, the risk of a false conclusion (type I-error) should be extremely small: we pre-state a probability less than 0.0005. For such limits, table 1 is applicable. Results will be evaluated after every inclusion of 20 patients with residual tumor in biopsy. The study will be stopped if the limit has been reached at two evaluations.

Number of patients with residual cancer at operation	Limit for rejecting the 0-hypothesis with a type I error risk < 0.0005
20	19 (95%) (all are positive)
40	39 (97.5%) (all are positive)
60	59 (98.3%) (all are positive)
80	78 (97,5%)
100	97 (97%)
120	116 (96.67%)
140	135 (96.43%)
160	155 (96.89%)
180	173 (96.11%)

Table 1: numbers of patients with tumor in the resection specimen and observed sensitivity limits at which it can be concluded that sensitivity is no inferior to 95% with a probability of a type I error < 0.0005. If these limits are reached on two evaluation points, the study will be prematurely terminated.

9 SAFETY REPORTING

9.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardize the subjects' health. The investigator will take care that all subjects are kept informed.

9.2 AEs and SAEs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the experimental intervention. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that:

- results in death;
- is life-threatening (at the time of the event);
- requires hospitalization or prolongation of existing inpatients' hospitalization;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject or may require an intervention to prevent one of the outcomes listed above.

According to the guidelines of the Dutch Association of Gastroenterology and Hepatology, adverse events will be categorized as follows:

Slight: no (prolongation of) admission, no intervention, no change in scheduled surgical operation date;
Mild: (prolongation of) admission less than 4 days, otherwise as mentioned under "slight"

Moderate: (prolongation of) admission less than 11 days, and/or transfusion of no more than 4 units of blood and/or endoscopic or percutaneous (non angiographic) intervention, and/or delay of the scheduled surgical operation.
Serious: (prolongation of) admission of more than 10 days, and/or transfusion of more than four units of blood, and/or angiographic intervention and/or intensive care admission and/or antedating or cancellation of the scheduled surgical operation, and/or death.

Although not specifically addressed in previous studies, the risk of taking biopsies from a healed polypectomy wound is considered virtually zero, as is our own experience as well. It is even unnecessary to discontinue systemic anticoagulants such as coumarins or double thrombocyte aggregation inhibition[32].
The risk of as sigmoidoscopy and colonoscopy are roughly the same and are associated with polypectomy, which can cause perforation and bleeding, both immediately or delayed. In a mixed population of diagnostic and therapeutic colonoscopies, both are approximately one to two in 1000 procedures[33,34]. The intent of the investigational procedures in this study is only to take biopsies. The risk if the investigational procedure is to our best guess considered very small, less than 1 out of 5000.
All patients will be followed for ninety-two days after the operation to assess for (S)AE's. In case of any suspected (S)AE, all appropriate medical action will be taken to ensure damage control from the patient's health perspective. There is a 7 x 24 service at the participating centers, which is easy amendable to the trial subjects in case anything might happen.
The trial center will report the (S)AEs through the web portal "*ToetsingOnline*" to the accredited METC that approved the protocol, within 15 days after the trial center has first knowledge of the serious adverse events.
SAEs that result in death or are life threatening should be reported expedited. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse event. This is for a preliminary report with another 8 days for completion of the report.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable (no investigational product)

9.3 Annual safety report

Not applicable (no investigational product)

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

9.5 Safety Committee

A Data Safety Monitoring Board is not needed. Adverse events will be reported to the trial center and the safety committee. This committee consists of Dr. Hans-Peter van Jonbergen, orthopedic surgeon and head of the scientific committee at the Deventer Hospital, and mrs. Esther van ‘t Riet, epidemiologist at the Deventer Hospital. They will be informed about adverse events after the endoscopic procedures and may decide to take specific measurements such as detailed examination of the AE.

10 Statistical analysis

All statistical analyses will be done in the Deventer Hospital, with support of the local department of clinical epidemiology (E. van ‘t Riet). Final approval will be obtained from the department of biostatistics and epidemiology at the Erasmus Medical Center (B. Hansen).
In principle, sensitivity will be calculated by dividing the number of patients with positive biopsies by the number of patients with tumor in the bowel wall and / or resected lymph nodes. Separate analysis will be done for bowel wall affection (as opposed to lymphatic spread) only.
The above-mentioned factors of potential influence will be studied on associations with the presence or absence of tumor in the bowel wall and / or resected lymph nodes. This will be done by using an independent samples *t*-test in the case of continuous normally distributed variables; by nonparametric test (e.g. Mann-Whitney U-test) in case of non-normally distributed ordinal variables; by Chi-Square statistics in case of categorical variables and by multiple logistic regression to investigated multiple associations.
Missing data will be dismissed from the analysis: the main issue is, whether there are preoperative biopsies and a resection specimen. If one of both fails, the loss will be compensated by the inclusion of another patient, as long as the number of evaluable patients is not reached.

10.1 Primary study parameter(s)

As is described in chapter 8.1.1

10.2 Secondary study parameter(s)

As is described in chapter 8.1.2

10.3 Interim analysis

As is described in chapter 8.7.

11 Ethical considerations

11.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (version, date, see for the most recent version: www.wma.net) and in accordance with the Medical Research Involving Human Subjects Act (WMO)

11.2 Recruitment and consent

After polypectomy, the patient is seen on the outpatient clinic to be informed by the treating physician, usually a gastroenterologist, about the fact that the polyp contained adenocarcinoma, and that it is uncertain whether the resection is complete from an oncological point of view. Staging procedures are arranged and the patient is discussed in a multidisciplinary oncological meeting.
A second visit on the outpatient clinic is planned after the meeting, in order to inform the patient about the results of staging and the outcome of the oncological multidisciplinary meeting. So far, this is the standard of care in patients with this problem.
If additional surgical resection was proposed in the multidisciplinary oncological meeting, and the patient fulfills the inclusion criteria (chapter 4.2), participation in the study is proposed by the treating physician (usually a gastroenterologist).
In case a tattoo needs to be done for laparoscopic surgery, the endoscopic procedure will be planned and the patient will be asked to consent to take biopsies from the polypectomy site. Otherwise, a separate endoscopic procedure needs to be planned as is explained in chapter 8.3.1.

11.3 Objection by minors or incapacitated subjects (if applicable)

Not applicable. Patients who are not able to provide written informed consent are excluded

11.4 Benefits and risks assessment, group relatedness

Participation in the study is in principle of no benefit to the patient. It should be kept in mind, however, that in case a second colonoscopy needs to be done, back-to-back colonoscopy studies have shown the discovery of additional polyps in 10-25% of cases[31], which implies some benefit in this subgroup, as the removal of these polyps may prevent cancer.

Although not specifically addressed in studies, the risk of taking biopsies from a healed polypectomy wound is considered virtually zero, as is our own experience as well. It is even unnecessary to discontinue systemic anticoagulants such as coumarins or double thrombocyte aggregation inhibition[33].
The risk of as sigmoidoscopy and colonoscopy are roughly the same and are associated with polypectomy, which can cause perforation and bleeding, both immediately or delayed. In a mixed population of diagnostic and therapeutic colonoscopies, both are approximately one to two in 1000 procedures[33,34]. The intent of the investigational procedures in this study is only to take biopsies. The risk if the investigational procedure is to our best guess considered very small, less than 1 out of 5000.
The study is specifically related to the group of patients described in the inclusion criteria.

11.5 Compensation for injury

All participating centers have a liability insurance which is in accordance with article 7, subsection 9 of the WMO.
This insurance provides cover for damage to research subjects through injury or death caused by the study.

€ 450,000 (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;

€ 3.500,000 (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;

€ 5.000,000 (i.e. five million Euro) for the total damage incurred by the organization for all damage disclosed by scientific research for the Sponsor as ‘verrichter’ in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives (if applicable)

Not applicable

12 Administrative aspects, monitoring and publication

12.1 Handling and storage of data and documents

Patients will be coded by a numeric code (anonymous). The code is only only accessible to the main investigators (A.D. Koch and F. ter Borg), and the safety committee (E. van 't Riet and HP Jonbergen).
The treating physician enters data at the participating center. In case of data incompleteness, correspondence will be made between study coordinator and treating physician on the basis of trial number; verification of the patient will be done on the basis of date of birth and gender. The same principle will be done in case the safety committee needs clarification on adverse events.
The project leader will keep the source data for 20 years.

12.2 Monitoring and Quality Assurance

The safety committee and the principal investigators will monitor the study.

12.3 Amendments

Amendments are changes made to the research after a favorable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favorable opinion.
Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 End of study report

The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.
In case the study is ended prematurely, the investigator will notify the accredited METC within 15 days, including the reasons for the premature termination.
Within one year after the end of the study, the investigator/sponsor will submit a final study

report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

The results of this study will be published. The principal investigators (F. ter Borg) will be first author and A.D. Koch will be last author. In case a fellowship will be granted during the course of the study, the fellow will be first author, A.D. Koch will be second author and F. ter Borg will be the last author. In case of multiple publications, the positions above mentioned of F. ter Borg and A.D. Koch will rotate. For each participating center we have calculated a realistic number of patients that should be included, based on the number of outpatient visits. This number is discussed and agreed on with the principal investigator. All participants who include at least 80 percent of this estimated number of patients will be granted co-authorship, regardless the total number of authors. If necessary, this will be explained to the editorial board of a peer-reviewed journal. The order of authors will be based on (1) scientific input and (2) number of inclusions per center. For purposes of abstract presentation and publication, any secondary publication will be delegated to the appropriate principal authors.

13 Structured risk analysis

Not applicable (no investigational product or device). For risk associated with endoscopy please refer to chapter 9.2.2

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