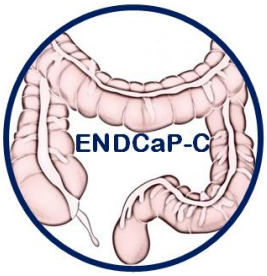
# Enhanced Neoplasia Detection and Cancer Prevention in Chronic Colitis (ENDCaP-C)

## A Multicentre test accuracy study




**ENDCaP-C STUDY PROTOCOL**

**Version 5.0, 21st February 2016**

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| | |
|---|---|
| **Sponsor:** | **University of Birmingham** |
| **Chief Investigator:** | **Professor Tariq Iqbal** |
| **Coordinating Unit:** | **Birmingham Clinical Trials Unit (BCTU)** |
| **Funder:** | **Efficacy and Mechanism Evaluation Programme (EME)** |
| **ISRCTN:** | **81826545** |
| **Main REC Ref. No.:** | **14/LO/1842** |
| **Sponsor Protocol Number** | **RG_12-074** |

UNIVERSITY OF BIRMINGHAM | BCTU Birmingham Clinical Trials Unit

# ENDCaP-C Study Management Committee

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**FOR SAFETY REPORTING**

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**Current Protocol Version**
5.0, 21st February 2017

**Previous Protocol Versions**
4.0, 11th February 2016
3.0, 28th July 2015
2.0, 16th October 2014 (First Version released to sites)
1.0, 17th September 2014 (submitted to Ethics Committee, not released to sites)

**ISRCTN**
81826545

**Funding Body**
Efficacy and Mechanism Evaluation Programme (EME)

This protocol is part of the overall project, EME Ref: 11/100/29 - Enhanced Neoplasia Detection and Cancer Prevention in Chronic Colitis (ENDCaP-C), funded by EME and awarded to Dr Glenn Matthews as the Chief Investigator for the overall project. The role of Chief Investigator for the overall project has been transferred to Professor Dion Morton.

**Sponsor**
University of Birmingham

**Signatures**
The investigators and the sponsor have discussed this protocol. The investigators agree to perform the investigation and to abide by this protocol except in case of medical emergency or where departures from it are mutually agreed in writing.

**Chief investigator**
Professor Tariq Iqbal

| | | |
|---|---|---|
| University of Birmingham | Signature | Date |

## Protocol Amendments

| Amendment number | Date of amendment | Protocol version number | Type of amendment | Date of Ethics approval |
|---|---|---|---|---|
| 1 | 28th July 2015 | 3.0 | Substantial | 19th August 2015 |

**Summary of amendment**

Substantial changes:

- Addition of blood and stool sample collection to test for hypermethylation of secreted Wnt antagonists in serum and faecal samples, and if feasible, to assess their accuracy.
- Change to the timing of the repeat colonoscopy, increasing from 4-6 months to 6-9 months after the initial colonoscopy.
- Update to the Patient Information Sheet to reflect the addition of the blood and stool sample collection and the change to the timing of the repeat colonoscopy.
- Update to the Consent Form to reflect the addition of the blood and stool sample collection.
- Update to the GP Letter to reflect the addition of the condition Primary Sclerosing Cholangitis as per the eligibility criteria clarification.

Summary of non-substantial changes:

- Changes to the research team; some members of the Trial Management Group have changed address.
- Confirmation of DMEC and TSC member details.
- Clarifications to the study design to provide a more detail in section 3.1.
- Clarifications to eligibility criteria by reordering of inclusion criteria to make clear patients are eligible if they have either Ulcerative Colitis (UC) of over 10 years duration and disease beyond the splenic flexure, or known Primary Sclerosing Cholangitis alone.
- Clarifications to the sample collection requirements and procedures at colonoscopy:
  - Routine biopsies are required for the trial rather than five additional biopsies specifically for the study.
  - The process for histological analysis and methylation testing.

| Other modified documents approved | Previous version | New version |
|---|---|---|
| Consent form | v2.0 (16th October 2014) | v3.0 (28th July 2015) |
| Patient Information Sheet | v2.0 (16th October 2014) | v3.0 (28th July 2015) |
| GP letter | v2.0 (16th October 2014) | v3.0 (28th July 2015) |

| Amendment number | Date of amendment | Protocol version number | Type of amendment | Date of Ethics approval |
|---|---|---|---|---|
| 2 | 11th February 2015 | 4.0 | Substantial | 15th April 2016 |

**Summary of amendment**

Substantial changes:

- Patient Stool Collection Instructions have been written for those patients coming in for a repeat colonoscopy alongside the stool sample collection kit.
- A patient information poster has been designed to be displayed in clinics and patient waiting areas to raise awareness of the ENDCaP-C Study amongst the patient population and also to promote the study.
- Removal of EQ-5D are quality of life data was to be collected from 100 study patients, which has been achieved.
- Clarification to the procedure for Standard Colonoscopy to include guidance on instances where symptoms of fulminant colitis or bowel obstruction are not detected or reported by the patient and these diagnoses are only found at the time of colonoscopy.
- Clarification on blinding and the risk of bias
- Update to the Patient Information Sheet to reflect the removal of the quality of life questionnaire.
- Update to the Consent Form to reflect the removal of the quality of life questionnaire.

Summary of non-substantial changes:

- Correction of the eligibility criteria in the protocol synopsis to match the criteria in the main body of the protocol.
- Increase to the number of recruitment sites.
- Clarification to the decision tool to be used to assess methylation status.

| Other modified documents approved | Previous version | New version |
|---|---|---|
| Consent form | v3.0 (28th July 2015) | v4.0 (11th February 2016) |
| Patient Information Sheet | v3.0 (28th July 2015) | v4.0 (11th February 2016) |
| Stool Collection Patient Instructions | n/a | v1.0 (11th February 2016) |
| Patient Poster | n/a | v1.0 (11th February 2016) |

| Amendment number | Date of amendment | Protocol version number | Type of amendment | Date of Ethics approval |
|---|---|---|---|---|
| 3 | 21st February 2017 | 5.0 | Substantial | TBC |

**Summary of amendment**

Substantial changes:

- Expansion of the late reference standard (repeat) colonoscopy window to 4-12 months after the initial colonoscopy.
- Clarification and relaxation of eligibility criteria:
  - UC diagnosisdiagnose “with symptoms” for 10 years.
  - Removal of requirement for UC disease extent to be “beyond the splenic flexure”.
  - Clarification that patients must be on surveillance programme and undergoing a routine colonoscopy during study period rather than the baseline procedure having to be specifically for surveillance.
  - Clarification the patients must be able to “undergo” complete colonoscopies
  - Clarification that certain exclusion criteria apply only to UC only patients; fulminant colitis, Crohn’s colitis, unclassified IBD and microscopic colitis.
- Clarification of End of study definition.
- Alteration to allow more than one colonoscopist at a site to perform the late reference standard colonoscopy.
- Clarification of data handling and record keeping.

Summary of non-substantial changes:

- Changes to the research team and address.
- BCTU logo replaced with new version
- Addition of protocol amendment summaries.
- Trial duration updated.
- Correction of Assessment Schedule – Blood and stool “test” have been corrected to “sample collection”.
- Clarification of informed consent and eligibility confirmation requirements.
- Minor clarification of screening log, histology and withdrawal data requirements.
- Clarification that blood and stool samples collection is optional.
- Addition of an expected SAE that does not need reporting.
- Removal of requirement for local PI to provide expectedness assessments.
- Clarification of long-term storage of data requirements.
- Minor clarification to the responsibilities of the PI, Research Co-ordinator and ENDCaP-C Study Office.
- Inclusion of specific ethics committee for the study.

| Other modified documents approved | Previous version | New version |
|---|---|---|
| Consent form | v4.0 (11th February 2016) | V5.0 (23th January 2017) |
| Patient Information Sheet | v4.0 (11th February 2016) | V5.0 (23th January 2017) |

## Abbreviations

| | |
|---|---|
| AE | Adverse event |
| AR | Adverse reaction |
| BCTU | Birmingham Clinical Trials Unit at the University of Birmingham |
| CI | Chief Investigator |
| DMEC | Data Monitoring and Ethics Committee |
| GCP | Good Clinical Practice |
| ISRCTN | International Standard Randomised Controlled Study Number |
| REC | Research Ethics Committee |
| NICE | National Institute for Health and Care Excellence |
| PI | Principal Investigator |
| PIS | Participant Information Sheet |
| RR | Relative Risk |
| SAE | Serious Adverse Event |
| SOP | Standard Operating Procedure |
| TMG | Trial Management Group |
| TSC | Trial Steering Committee |
| UC | Ulcerative Colitis |

**CONTENTS**

# STUDY SYNOPSIS

**Trial Title**

Enhanced Neoplasia Detection and Cancer Prevention in Chronic Colitis (ENDCaP-C): A Multicentre test accuracy study

**Trial Design**

ENDCaP-C is multi-centre cohort test accuracy study.

**Objectives**

**Primary objective**

- To prospectively evaluate the ability of the methylation assay to detect pre-cancerous lesions (dysplasia) missed by histology within a surveillance programme for colitis associated neoplasia (CAN).

**Secondary objectives:**

- To estimate the incremental accuracy of methylation testing over histology within a CAN surveillance programme and gain experience of its applicability in the clinical setting
- To evaluate the feasibility of testing for hypermethylation of secreted Wnt antagonists in serum and faecal samples, and if feasible, to assess their accuracy

**Outcomes**

**Primary Outcome Measures**

- The presence of dysplasia in a mucosal biopsy taken at follow up colonoscopy at 4-12 months
- The presence of hypermethylation and dysplasia in a mucosal biopsy taken at follow up colonoscopy at 4-12 months

**Secondary Outcome Measures**

- Complications during or following colonoscopy

**Patient Population and Sample Size**

ENDCaP-C will aim to recruit 1000 patients from up to 37 centres in the UK. Eligible centres will be those having active chronic IBD endoscopic surveillance programmes.

**Patient Inclusion and Exclusion Criteria**

**Inclusion criteria**

- Diagnosis of either:
    - Chronic ulcerative colitis with symptoms for over 10 years
    - Primary Sclerosing Cholangitis
- On the surveillance programme and undergoing a routine colonoscopy during the study period
- Willing to accept the possibility of an additional colonoscopy between 4 and 12 months after registration
- No previous history of colorectal cancer
- Aged 18 years or over
- Be able and willing to provide written informed consent for the study

## Exclusion criteria

- Bowel obstruction
- Patients in whom it is not possible to undergo complete colonoscopies
- Patients with proctitis only
- Unable to give written informed consent
- Less than 18 years of age
- For UC only patients:
    - Patients with fulminant colitis
    - Crohns colitis patients
    - Patients with unclassified IBD
    - Patients with microscopic colitis

### Study duration

It is anticipated that recruitment will last approximately 29 months (or until sufficient analyses have been performed to provide a clear result).

### End of study

The end of the study is defined as the date of the last visit of the last patient undergoing protocol based treatment and collection of associated data. Within ENDCaP-C, this is 12-months after the last participant has undergone the late reference procedure at 4-12 months. This will allow sufficient time for the completion of protocol procedures, data collection and data input.

Follow-up at 4-12 months post-study entry will constitute the end of the non-interventional phase of the ENDCaP-C study. It is possible that the patient will be eligible to continue with further follow up in another study and consent for this possibility will be obtained along with consent to participate in the ENDCaP-C study.

# 1. BACKGROUND

## 1.1. Ulcerative Colitis

Over 30,000 patients in the UK are affected by chronic ulcerative colitis (UC) and, as a consequence are at increased risk of colorectal cancer. The risk of cancer rises with duration of disease, reaching 18% after 30 years and results in over 1,000 colectomies being performed each year in the UK for colorectal cancer or cancer prevention. Despite intensive colonoscopic surveillance, as many as 50% of cases progress to invasive cancer before neoplasia is detected (Shu 2011, Wildt 2010). This can prevent safe restoration of bowel continuity, require adjuvant chemotherapy and result in incurable disease and death (in >40% of patients with colitis associated large bowel cancer).

Patients are currently stratified into low (quiescent or left-sided), intermediate (mild inflammation or a family history of colorectal cancer) or high risk (extensive moderate or severe active inflammation, primary sclerosing cholangitis, a history of dysplasia/colonic stricture, or a family history) and surveillance in these groups is performed by colonoscopy at 5, 3 or 1 yearly intervals respectively, enabling identification and biopsy of suspected neoplasia. Standard colonoscopic surveillance involves 24 hour bowel preparation preceded by 36 hours of liquid diet, and is performed under intravenous sedation or nitrous oxide. The colonoscope is advanced to the small bowel junction and then withdrawn slowly. Biopsy protocol varies: most endoscopists perform a series of random biopsies. The 2002 BSG guidelines (Cairns and Scholefield 2002) suggested 2-4 biopsies taken every 10cm. More recently (Cairns et al 2011) it has been recommended that the colonic mucosa should be dye-sprayed with targeted biopsy of any abnormalities. Current biopsy practice varies between hospitals. The biopsies are formalin fixed for histological analysis. Well circumscribed dysplastic adenomas may be amenable to local endoscopic resection. In the event of there being dysplastic change in flat mucosa or adenomatous change in a background of abnormal mucosa, the patient is offered prophylactic total colectomy.

There is a pressing need to enhance the effectiveness of surveillance and early selection for prophylactic resection. This was highlighted in the recent NICE guidelines (NICE 2011), which recommend the identification of epigenetic and genetic biomarkers to aid more accurate patient identification. An ideal test would complement colonoscopy and biopsy by providing enhanced detection of pre-cancerous lesions (dysplasia) thereby delivering better patient selection for prophylactic resection.

## 1.2. Epigenetic change in UC progression

Tumour development requires loss of tumour suppressor gene function and gain of oncogenic drivers. Historically, the role of chromosomal loss and genetic mutation of tumour suppressor gene has been well documented but more recent data demonstrate the importance of epigenetic silencing particularly in early tumourigenesis. DNA hypermethylation associated with epigenetic silencing provides an attractive diagnostic target as it reflects functional change, is stable in DNA obtained from biological fluids and can be detected reliably in DNA extracted from formalin fixed paraffin embedded biopsies. A number of studies over the past decade have shown frequent epigenetic silencing of tumour suppressor genes associated with promotor hypermethylation in the development of colitis-associated neoplasia (CDH1, Azarschab et al, 2002; HPP1, Sato et al, 2002; ESR1, Tominaga et al, 2005; EYA4, Osborn et al, 2006; p14ARF , Moriyama et al 2007; p16, Kukitsu et al 2007).

Combining multiple methylation markers can provide a sensitive estimation of the presence of neoplasia. Dhir et al (2008) investigated Wnt-pathway associated genes and found a combination of APC1A, APC2, SFRP1 and SFRP2 gave a ROC value of over 77% for prediction of neoplasia using a logistic regression model. Similarly, Garrity-Park et al (2010) found a high degree of association between methylation of RUNX3, MINT1 and COX2 in non-neoplastic mucosa and the presence of neoplasia: Multivariate logistic regression indicated an odds ratio of 12.6 for RUNX3 and 9.1 for MINT1.

Studies of sporadic and genetic colorectal cancer have shown that hypermethylation and epigenetic silencing of secreted Wnt antagonists such as SFRP1 occurs early in tumour development (Caldwell et al 2004/6/8/10), and suggest that this methylation and silencing could be a 'gatekeeper' event, essential for large bowel neoplastic change. Wnt antagonist silencing, therefore, provides a sensitive marker for the development of neoplasia. More recent studies have examined the Wnt antagonist genes in chronic ulcerative colitis: hypermethylation patterns of 6 Wnt pathway-associated genes were assessed in a discovery cohort of UC-associated cancers compared to age-matched non-neoplastic colitic control biopsies. This identified significant hypermethylation in sFRP1, sFRP2 and WIF-1. These data were then validated using a series of dysplastic and cancer biopsies, compared with further non-neoplastic UC controls. Mucosal hypermethylation was absent in the controls for all genes, but was invariably present for at least one, and frequently (70% of cases) all three in both the dysplastic and cancer cases, indicating that these loci can identify neoplastic change in a UC background.

A study of hypermethylation of these genes in *non-neoplastic* biopsies from patients with synchronous large bowel dysplasia or cancer identified hypermethylation of these same genes in the non-neoplastic biopsies in 19/21 patients tested. A similar field change effect was suggested by Garrity-Park et al (2010) for 3 other gene loci; RUNX3, COX2 and MINT1, supporting these data.

Taken together, these data indicate that it should be possible to perform *molecular identification* of neoplastic change, even if the neoplastic lesion(s) were missed at colonoscopy. This should complement surveillance colonoscopy by improved early tumour identification, enabling early treatment for these patients.

A diagnostic test has been developed to identify IBD patients at high risk for development of colon cancer based on the methylation of an array of relevant genes. Briefly, to get to this stage, a retrospective diagnostic case-control study was performed. A large cohort of formalin fixed paraffin embedded (FFPE) biopsies obtained from biopsies obtained at routine surveillance colonoscopies for patients with chronic ulcerative colitis were identified from histology archives in six hospitals. The cohort was structured to include adequate samples from cases recorded as having neoplasia and dysplasia were identified, as well as those from controls without either neoplasia or dysplasia, to be able to create a reliable classifier. DNA was extracted from these blocks and then bisulphite pyrosequencing was used to measure methylation at multiple CpG positions within various wnt antagonists. As hypermethylation and epigenetic silencing of secreted Wnt antagonists occurs early in tumour development and is thought to be a 'gatekeeper' event, essential for large bowel neoplastic change, this provides a sensitive marker for the development of neoplasia. This was followed by the development and validation of a quality-assured, clinically applicable test for a panel of biomarkers.

The aim of this study is to assess the validity of this test in patients with ulcerative colitis undergoing surveillance colonoscopy. The accuracy of the methylation assay will be established through evaluation of its ability to detect dysplasia missed by histology within a prospective cohort study allowing prediction of its likely clinical utility. This study will also establish the generalisability of the assay as it is introduced alongside routine surveillance across at least 8 different hospitals The feasibility of testing for hypermethylation of secreted Wnt antagonists in serum and faecal samples will also be investigated.

### 1.3. Potential benefits to patients and the NHS

If sufficiently accurate, the diagnostic methylation test will direct the frequency of future colonoscopies according to risk status. It is not anticipated the diagnostic methylation test will replace, or triage the need for, histological examination of colorectal mucosa biopsies. It is however envisaged that methylation status will modify the need for colonoscopic assessment, and so the frequency of histological assessment.

### 1.4. The need for ENDCaP-C

Improved outcomes from colitis associated neoplasia are dependent on early identification and treatment however current screening techniques are not adequate to address this need and the mortality from late-detected colitis associated cancers remains high. This study will assess the applicability and outcome of a novel methylation-based screening test as an adjunct to current practice across multiple sites and, if successful, would result in a significant change to clinical practice which would be widely applicable.

## 2. OBJECTIVES AND OUTCOMES

### 2.1. Objectives

ENDCaP-c is multi-centre cohort study with the following objectives:

**Primary objective:**

- To estimate prospectively the ability of the methylation assay to detect pre-cancerous lesions (dysplasia) missed by histology within a surveillance programme for colitis associated neoplasia (CAN).

**Secondary objectives:**

- To estimate the incremental accuracy of methylation testing over histology within a CAN surveillance programme and gain experience of its applicability in the clinical setting.
- To evaluate the feasibility of testing for hypermethylation of secreted Wnt antagonists in serum and faecal samples, and if feasible, to assess their accuracy

### 2.2. Outcome Measures

#### 2.2.1 Primary Outcome Measures

The primary outcome measures are:

- The presence of dysplasia in a mucosal biopsy taken at follow up colonoscopy at 4-12 month
- The presence of hypermethylation and dysplasia in a mucosal biopsy taken at follow up colonoscopy at 4-12 months

#### 2.2.2 Secondary Outcome Measures

The secondary outcome measures for the study are:

- Complications from colonoscopy.

## 3. STUDY DESIGN

### 3.1. Test Accuracy Study Design

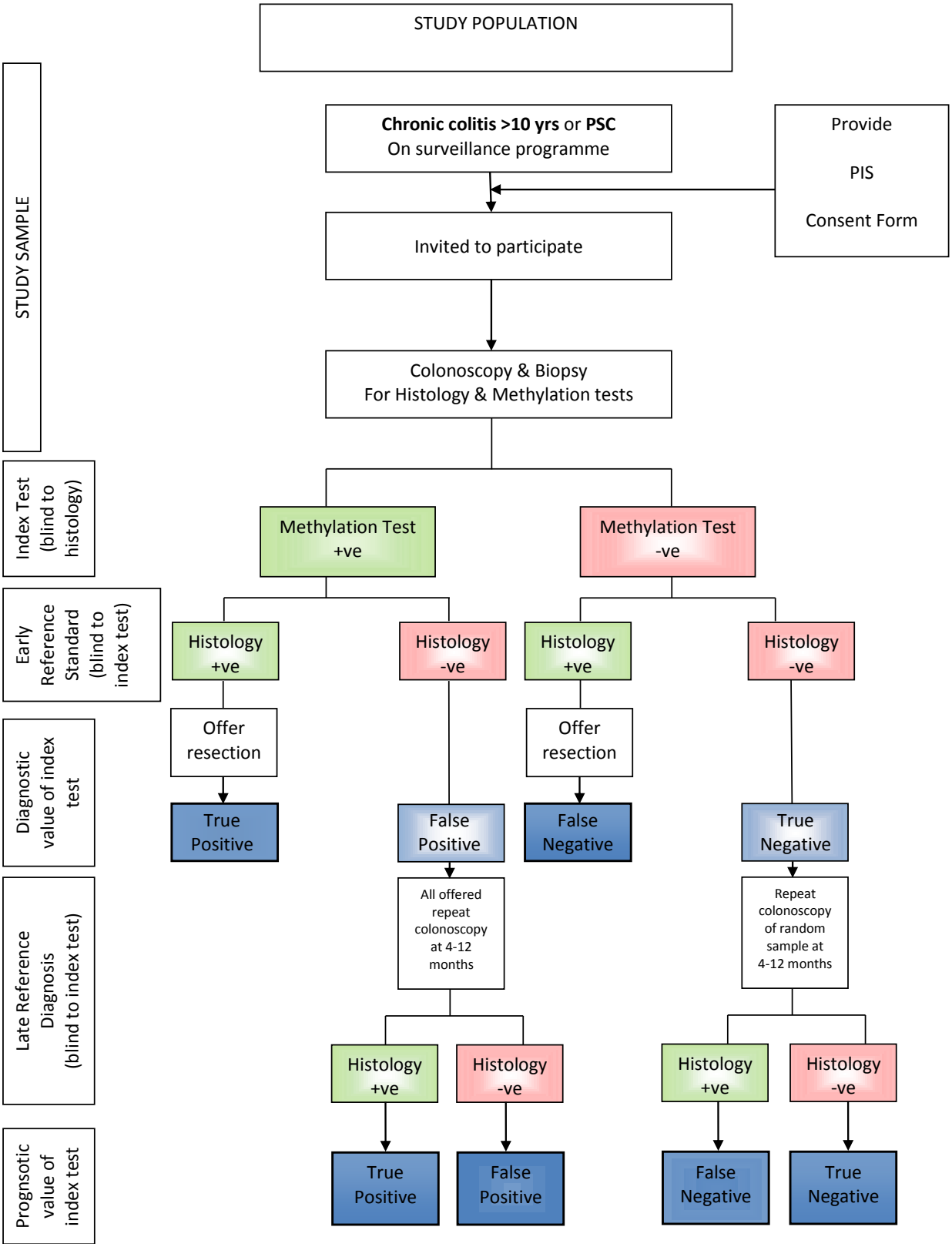
ENDCaP-C is a prospective diagnostic test accuracy study. An outline of the test accuracy study is shown in the study schema. It is designed to generate a comparison of measurements obtained by the index test with those obtained by reference standard. In this way the accuracy of the index test can be estimated. A reference standard is a test that confirms or refutes the presence or absence of disease beyond reasonable doubt. Therefore it is sometimes also known as the gold standard. The methylation test is the index test whereas the reference standard will be histology at 4-12 months after the methylation test.

In the study the results of the index test will first be compared with histology of biopsy samples taken at the first standard surveillance colonoscopy (labelled “diagnostic value of the test” in the study schema

below) and then with the reference standard colonoscopy (labelled “prognostic value of the test” in the study schema). The second comparison is the primary comparison for the study as the reference standard colonoscopy used at this point will be of the highest quality possible, utilising dye spray to increase dysplasia detection and restricting to nominated, experienced colonoscopists at each site. In addition this colonoscopy will occur 4-12 months after the initial colonoscopy, which will increase the chances of finding dysplasia that was initially not detected (i.e. identifying patients with false negative initial histology).

In order to ensure efficiency in the study design, whilst all patients with positive tests for methylation will undergo the reference standard colonoscopy, only a sample of those with negative tests will be selected for reference standard colonoscopy. The rationale for this is given in the sample size section below.

### 3.2. Study Schema



### 3.3. Timing of assessments

| | Prior to registration | 1st Colonoscopy | Post-colonoscopy | Prior to repeat colonoscopy | Repeat colonoscopy | Post repeat colonoscopy | End of trial)* |
|---|---|---|---|---|---|---|---|
| **Written Informed consent** | x | | | | | | |
| **Review inclusion / exclusion criteria** | x | | | x | | | |
| **Colonoscopy findings/ outcomes** | | x | | | x | | |
| **Histology outcomes** | | | x | | | x | |
| **Blood sample collection** | | | | | x | | |
| **Methylation outcomes** | | | x | | | x | |
| **Stool sample collection** | | | | | x | | |
| **Therapeutic endoscopy (if required)** | | | x | | | x | |
| **Surgery (if required)** | | | x | | | x | |
| **Adverse event evaluation** | | x | x | | x | x | x |

* After 4-12 months: Long term follow up of this cohort of patients will be undertaken to assess late conversion rate to neoplasia. This will be undertaken outside of the study.

# 4. ELIGIBILITY

## 4.1. Centre eligibility

ENDCaP-C will aim to recruit 1000 patients from up to 37 centres in the UK. Eligible centres will be those having active chronic IBD endoscopic surveillance programmes.

## 4.2. Patient Inclusion and Exclusion Criteria

### Inclusion criteria

- Diagnosis of either:
    - Chronic ulcerative colitis with symptoms for over 10 years duration
    - Primary Sclerosing Cholangitis
- On the surveillance programme and undergoing a routine colonoscopy during the study period
- Willing to accept the possibility of an additional colonoscopy between 4 and 12 months after registration.
- No previous history of colorectal cancer
- Aged 18 years or over
- Be able and willing to provide written informed consent for the study

### Exclusion criteria

- Bowel obstruction
- Patients in whom it is not possible to undergo complete colonoscopies
- Patients with proctitis only
- Unable to give written informed consent
- Less than 18 years of age
- For UC only patients:
    - Patients with fulminant colitis
    - Crohns colitis patients
    - Patients with unclassified IBD
    - Patients with microscopic colitis

# 5. CONSENT AND RECRUITMENT

## 5.1. Recruitment:

1000 patients will be recruited from up to 37 centres in the UK. Recruitment in the West Midlands will be facilitated by the West Midlands Inflammatory Bowel Disease (IBD) network, with 15000 IBD patients of whom 7,000 are on a centralised database. The Birmingham and Black Country IBD Network comprises; Queen Elizabeth, Heartlands and Solihull , Good Hope, City and Sandwell, New Cross, Russell's Hall, and Manor Hospital, Walsall. Other centres, undertaking protocol driven surveillance, based on national guidelines will also be invited to participate.

Eligible participants will be provided with study information, possibly with their appointment letter. Identification of those who have consented to participation will be through stickers on the patient notes. Team training meetings will be provided at each centre, and the information provided will be reinforced by investigator meetings and newsletters.

## 5.2. Informed consent

The study will be conducted in accordance with the Research Governance Framework for Health and Social Care and the International Conference on Harmonisation of Good Clinical Practice.

At the pre-assessment or colonoscopy appointment, the patient will meet with a consultant gastroenterologist or surgeon to discuss the study. Participants who potentially fulfil the inclusion criteria for this trial must have their eligibility confirmed by a medically qualified doctor with access to and a full understanding of the potential participant's medical history. The confirmation of eligibility must be documented in the patient's notes and on the registration form. Eligibility must be confirmed before registration.

The patient's written informed consent to participate in the study must be obtained before registration and after a full explanation of the study has been given. Written informed consent will be obtained by a trained member of the research team (with clinical training, GCP training, knowledge of the study protocol, and delegated authority from the local PI). Within the ENDCaP-C study, it is anticipated that consent will usually be obtained by a surgeon, gastroenterologist or research nurse at site.

Once written informed consent is obtained the original copy should be kept in the ENDCaP-C study site file, one given to the patient, one kept in the patient's notes and one sent to the ENDCaP-C Study Office. Patients will give their explicit consent for the movement of their consent form, giving permission for the study office to be sent a copy. This will be used to perform in-house monitoring of the consent process.

Informed consent must be obtained before any study-related procedures are undertaken.

If new information becomes available which may be relevant to the patient's consent, forms will be revised and informed consent sought again. The ENDCaP-C Study Office will advise when this is required.

## 5.3. Registration

Once eligibility has been confirmed and after written informed consent has been obtained, patients can be registered into the study.

Patients are registered into the study online at the ENDCaP-C website,
https://www.Trials.bham.ac.uk/ENDCaPC

Or by telephone call to the BCTU registration service (0800 953 0274)

Telephone registration is available Monday-Friday 09:00-17:00.

Online registration is available 24 hours a day, 7 days a week apart from short periods of scheduled maintenance and occasional network problems.

For the secure online registration website, each investigator will be provided with a unique username and password.

Registration notepads will be provided to investigators and should be used to collate the necessary information prior to registration. The person registering will need to answer all of the questions before registration and a study number is given.

## 5.4. Informing the participant's GP

With the patient's prior consent, their General Practitioner (GP) will also be informed that they are taking part in the study. A GP Letter is provided electronically for this purpose.

## 5.5. Screening logs and acceptance rate

A screening log will be kept of all eligible patients, including those who decline to take part with the reason for declining. Details recorded will include the reason for non-registration and the date this was

determined. This will establish the acceptance rate of the study by patients and the generalizability of the study participants.

# 6. TESTS AND PROCEDURES

## 6.1. Standard colonoscopy surveillance and histology

All patients will undergo a protocol-driven surveillance colonoscopy by a named colonoscopist as per usual NHS care.

During this procedure, routine biopsy samples will be taken. As a minimum, our requirements are for two samples from the left side of the colon, two from the right side of the colon and one from the rectum. We do not object to additional biopsies being taken and sent to us if this is preferred at a site. Each biopsy will involve two "bites" from the mucosa using a spiked endoscopy forceps as is current standard practice.

If it is not possible to collect all the required biopsy samples at the baseline colonoscopy due to, for instance, fulminant colitis or bowel obstruction not detected prior to colonoscopy, the patient may be invited approximately 6 weeks later for a repeat colonoscopy and this colonoscopy can be used as the baseline. These patients would remain in the study and would not be withdrawn.

Biopsy samples should be embedded in paraffin and processed and analysed as per local practice. It is important that biopsies from different sites (left, right and rectum) should not be embedded into the same block and so we expect a minimum of THREE blocks, one per site. If this is not possible or unavoidable, guidance on the how to identify the appropriate biopsies must be provided.

As these samples are those taken as part of routine practice (i.e. they are not additional blocks being taken specifically for the ENDCaP-C study), the blocks will be returned to site at the end of the trial and will not be exhausted. Blocks for an individual patient can be returned earlier if required for clinical reasons. Please contact the ENDCaP-C Study Office to discuss this if required.

The ENDCaP-C Study Office will send out instructions on sample requirements to all participating centres.

Analysis of FFPE sections from biopsy material will be coordinated by a named lead pathologist at each centre. If dysplasia is detected in any of the biopsies, patients will be offered endoscopic or surgical resection as decided by their MDT. Patients offered endoscopic or surgical resection will also have details of the type (endoscopy, laparotomy, and laparoscopy) and extent of resection recorded on the CRF along with updated pathological findings.

## 6.2. Index Test: Adjunctive methylation

The index test will be the DNA methylation panel of markers defined in Module 1 and refined as a quality-assured clinical assay in Module 2. These work packages will have defined the combination of genes and loci which result in a classification of hypermethylation.

After local histological analysis, FFPE blocks will be sent to the ENDCaP-C Trial Office. Blocks will be identified by study number and one other identifier. The blocks will then be transferred to the pathology department at the Queen Elizabeth Hospital Birmingham where all blocks will undergo a central histology review. DNA will be extracted and transferred to the Birmingham United Molecular Pathology (BUMP) laboratory at Birmingham Women's Hospital for methylation analysis. Methylation results will be held on the secure ENDCaP-C study database. This will ensure the reference standard is undertaken blinded to the methylation result. The methylation results will not be released to patients (in the trial) or clinicians during the study period.

## 6.3. Early Reference Standard (see 3.6, Study Schema)

The early reference standard will be the histological analysis obtained from the standard surveillance colonoscopy at trial entry as detailed above.

## 6.4. Late Reference Standard (see 3.6, Study Schema)

All histologically negative patients with positive methylation status (test positives) will be identified by the ENDCaP-C Study Office and will be invited to undergo early repeat colonoscopy. Twice as many histologically negative patients that have negative methylation status will be randomly selected without matching (test negatives) by the ENDCaP-C Study Office and also will be invited to undergo early repeat colonoscopy. The late reference standard colonoscopy will be undertaken 4-12 months after the standard colonoscopy, blinded to methylation status. This reference assessment will be standardised using dye spray and targeted biopsy to maximise dysplasia detection, and should be performed by nominated, experienced colonoscopists at each site.

During this procedure, biopsy samples will be taken. As with the standard colonoscopy at trial entry, our requirements are for two samples from the left side of the colon, two from the right side of the colon and one from the rectum. We do not object to additional biopsies being taken and sent to us if this is preferred at a site. Each biopsy will involve two “bites” from the mucosa using a spiked endoscopy forceps as is current standard practice.

Biopsy samples should be embedded in paraffin and processed and analysed as per local practice. It is important that biopsies from different sites (left, right and rectum) should not be embedded into the same block and so we expect a minimum of THREE blocks, one per site. If this is not possible or unavoidable, guidance on the how to identify the appropriate biopsies must be provided.

The ENDCaP-C Study Office will send out instructions on sample requirements to all participating centres.

After local histological analysis, FFPE blocks will be sent to the ENDCaP-C Trial Office. Blocks will be identified by study number and one other identifier. The blocks will then be transferred to the pathology department at the Queen Elizabeth Hospital Birmingham where all blocks will undergo a central histology review. DNA will be extracted and transferred to the Birmingham United Molecular Pathology (BUMP) laboratory at Birmingham Women’s Hospital for methylation analysis. Methylation results will be held on the secure ENDCaP-C study database. This will ensure the reference standard is undertaken blinded to the methylation result. The methylation results will not be released to patients (in the trial) or clinicians during the study period.

If dysplasia is detected following the repeat colonoscopy, patients will be offered endoscopic or surgical resection as decided by their MDT, in accordance with the protocol for usual care. Participants offered endoscopic or surgical resection will also have details of the type (endoscopy, laparotomy, and laparoscopy) and extent of resection recorded on their CRF along with updated pathological findings.

### 6.4.1 Stool sample collection

All patients scheduled to attend for the late reference standard colonoscopy and who have consented to sample collection will be sent a stool collection kit in the post with instructions to bring a stool sample (taken before bowel preparation) to the hospital on the day of the endoscopy. This will be used to test for Wnt antagonist methylation.

The ENDCaP-C Study Office will send out instructions on sample requirements to all participating centres.

### 6.4.2 Blood sample collection

At the time of the late reference standard colonoscopy, an addition 10ml venous blood sample will be taken patients who have consented to sample collection. This will be used to test for Wnt antagonist methylation in serum.

The ENDCaP-C Study Office will send out instructions on sample requirements to all participating centres.

## 6.5. Avoidance of Study Biases*:*

The generalisability of the study will be assured by aiming to recruit a consecutive sample of patients attending for routine surveillance, who fall within the high risk category. Information will be collected on risk factors in all study patients and investigate whether the diagnostic value of methylation varies according to patient characteristics.

Misclassification bias can occur in test accuracy studies where the reference standard test is inaccurate. The initial colonoscopy will be a routine surveillance procedure, making the study a pragmatic one which assesses the incremental value of methylation over and above standard current practice. The reference standard colonoscopy will be of the highest quality possible, utilising dye spray to increase dysplasia detection and will be restricted to experienced, nominated colonoscopists at sites. In addition, this colonoscopy will occur 4-12 months after the initial colonoscopy, which will increase the chances of finding dysplasia that was initially not detected (i.e. identifying patients with false negative initial histology). Whilst there is a risk that new dysplasia may develop during this period, this time period has been chosen to minimise the risk. This should not introduce bias as it should be equal in both methylation test positives and negatives. Long term follow up of this cohort of patients will be undertaken, to assess late conversion rate to neoplasia. This will be undertaken outside of this study.

Reference standard colonoscopies will only be undertaken in a sample of methylation test negatives. As this sample will be chosen randomly without matching it should be representative of all test negatives, and the results not suspect to partial verification bias.

It is acknowledged that verification bias cannot be completely eliminated due to the fact that the histologist will be aware that any repeat colonoscopy will consist of both test positives and test negatives. Hence to minimise this bias so that histologists cannot base their decision criteria for histological classification by knowledge of the allocation ratio, we will match the numbers of controls recruited at the study level and not within each hospital.

## 6.6. Withdrawal of treatment or protocol violation

Patients may withdraw at any time during the study if they choose not to continue, or if their clinical team feel that continued participation in the study is inappropriate.

There are different types of withdrawal:

- The patient would like to withdraw from the study specific tests and procedures, but is willing to be followed-up according to the study protocol (i.e. has agreed to attend study specific follow-up visits and that follow-up data can be collected)
- The patient does not want to attend study specific follow-up visits, but has agreed to be followed-up according to standard practice (i.e. has agreed that follow-up data can be collected at standard clinic visits)
- The patient is not willing to be followed up for study purposes at any further visits (i.e. has agreed that any data collected prior to the withdrawal of consent can be used in the study final analysis)

Full details of the reason(s) for withdrawal should be recorded on the Case Report Forms (CRFs) if healthcare professional-initiated, otherwise a simple statement reflecting patient preference, the date of the decision and any reasons given will suffice. Patients who withdraw from study treatment, but continue with on-going follow-up and data collection should be followed-up in accordance with the protocol.

### 6.7. Compatibility with other studies

Patients can be in both ENDCaP-C and other non-interventional studies.

If the patient is part of another interventional study, they may still be able to be recruited to ENDCaP-C. Please contact the ENDCaP-C Study Office to discuss these patients' eligibility prior to entry into other studies.

## 7. SAFETY MONITORING PROCEDURES

The collection and reporting of data on adverse events and serious adverse events will be in accordance with ICH GCP and the Research Governance Framework 2005. It is imperative that all investigators have a thorough understanding of anticipated adverse events and the reporting process of these events.

There are no (serious) adverse events which would be anticipated as a unique consequence of participation in the study. Any study-related serious adverse events (SAEs) which require immediate reporting will be reported on a study-specific SAE form and will follow the procedure/timeframes outlined in this section of the protocol.

### 7.1. General Definitions

#### Serious Adverse Events (SAEs)

An SAE is an untoward event which:

- Results in death
- Immediately threatens the life of participant*
- Results in hospitalisation or a longer than anticipated stay in hospital
- Results in a persistent or significant disability
- Results in any congenital anomaly or birth defect in any pregnancy

*Life-threatening in the definition of a serious adverse event or serious adverse reaction refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe. Important adverse events/reactions that are not immediately life-threatening or do not result in death or hospitalisation, but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Serious adverse events specific to the ENDCaP-C study include, but are not limited to:

- Bowel injury/perforation
- Post-colonoscopy bleed requiring admission to hospital
- Inpatient admission for exacerbation of chronic colitis

#### Expected SAEs

The following are SAEs that could be reasonably expected for this group of patients during the course of the study:

- Hospitalisations for routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
- Hospitalisations for treatment, which was elective or pre-planned, for a pre-existing condition that is unrelated to the indication under study, and did not worsen
- Admission to a hospital or other institution for general care, not associated with any deterioration in condition
- Treatment on an emergency, outpatient basis for an event not fulfilling any of the definitions of serious given above and not resulting in hospital admission
- Hospitalisations for planned surgery following non-response to UC treatment

For the purposes of this study these expected SAEs do **NOT** require reporting on an SAE form. These events should continue to be recorded in the source data according to local practice and be included on the routine follow-up CRFs.

Disease related morbidity and routine treatment or monitoring of a pre-existing condition that has not worsened will **NOT** be considered as SAEs and should **NOT** be reported to the Study Office.

### 7.2. Serious Adverse Events for expedited reporting

SAEs occurring within 1 week from the date of the colonoscopy (and not listed as 'expected' as defined above) will always be reportable to the ENDCaP-C Study Office on an SAE form. The assessment of relatedness to the study intervention is a clinical decision and will be based on all available information at the time. This applies for both the baseline and follow-up colonoscopy.

SAEs outside of this timeframe can also be reported if it is the opinion of the Investigator that there is a possible causal relationship to another aspect of the study. An assessment of relatedness and expectedness will be undertaken by the Chief Investigator (or delegated Clinical Coordinator). All SAEs will be followed-up until the event has resolved or a decision has been taken for no further follow-up.

### 7.3. Reporting SAEs

All SAEs must be recorded on the SAE Form and faxed to the ENDCaP-C Study Office on 0121 415 8871 within 24 hours of the research staff becoming aware of the event.

The Principal Investigator (or other nominated clinician) has to assign seriousness and causality to the SAE as part of the reporting process.

For each SAE, the following information will be collected:

- full details in medical terms with a diagnosis, if possible
- its duration (start and end dates; times, if applicable)
- action taken
- outcome
- causality, in the opinion of the investigator*
- whether the event would be considered expected or unexpected*

*Assessment of causality must be made by a doctor. If a doctor is unavailable, initial reports without causality assessment should be submitted to the BCTU by a healthcare professional within 24 hours, but

must be followed up by medical assessment as soon as possible thereafter, ideally within the following 24 hours.

The local investigator and others responsible for patient care should institute any supplementary investigations of SAEs based on their clinical judgement of the likely causative factors and provide further follow-up information as soon as available. If a participant dies, any post-mortem findings must be provided to the BCTU. The BCTU will report all deaths to the DMEC for continuous safety review.

### 7.4. Notification of deaths

All deaths will be reported to the BCTU on the SAE Form irrespective of whether the death is related to disease progression or an unrelated event. If a participant dies, any post-mortem findings must be provided to the BCTU with the SAE form. The BCTU will report all deaths to the DMEC for continuous safety review.

### 7.5. Pharmacovigilance responsibilities

**Local Principal Investigator (or nominated individual in PI's absence):**

- Medical judgement in assigning seriousness and causality to SAEs.
- To fax SAE forms to BCTU within 24 hours of becoming aware, and to provide further follow-up information as soon as available.
- To report SAEs to local committees if required, in line with local arrangements.
- To sign an Investigator's Agreement accepting these responsibilities.

**Chief Investigator (or nominated individual in CI's absence):**

- To assign causality and expected nature of SAEs where it has not been possible to obtain local assessment
- To review all events assessed as SAEs in the opinion of the local investigator

**Birmingham Clinical Trials Unit:**

- To prepare annual safety reports to main REC and TSC.
- To prepare SAE safety reports for the DMEC at a minimum of 12-monthly intervals.
- To report all fatal SAEs to the DMEC for continuous safety review.

**Study Steering Committee (TSC):**

- To provide independent supervision of the scientific and ethical conduct of the study on behalf of the Study Sponsor and funding bodies.
- To review data, patient compliance, completion rates, adverse events (during treatment and up to end of follow-up).
- To receive and consider any recommendations from the DMEC on protocol modifications.

**Data Monitoring & Ethics Committee (DMEC):**

- To review overall safety and morbidity data to identify safety issues which may not be apparent on an individual case basis
- To recommend to the TSC whether the study should continue unchanged, continue with protocol modifications, or stop.

### 7.6. Notification of Serious Breaches of GCP and/or the protocol

A “serious breach” is a breach which is likely to effect to a significant degree:

(a) the safety or physical or mental integrity of the participants of the study; or

(b) the scientific value of the study.

The BCTU on behalf of the Sponsor shall notify the REC in writing of any serious breach of:

(a) the conditions and principles of GCP in connection with the study; or

(b) the protocol relating to the study, as amended from time to time, within 7 days of becoming aware of that breach.

The Sponsor will be notified immediately of any case where the above definition applies during the study conduct phase.

## 8. DATA HANDLING AND RECORD KEEPING

It will be the responsibility of the Principal investigator to ensure the accuracy of all data entered in the CRFs. The ENDCaP-C Delegation & Signature Log will identify all those personnel with responsibilities for data collection.

### 8.1. Data management and validation

Data should be collated directly from the patient (for Quality of Life questionnaires) or from the patient hospital notes using the ENDCaP-C case report forms. Within the ENDCaP-c study, source data is the participants’ medical notes generated and maintained at site.

Data will be collected via paper CRFs; paper forms should be sent to the ENDCaP-C Study Office for central input. Data validation is built into the database, so that range, date and logic checks are performed at the point of data entry.

Paper CRFs must be completed, signed/dated and returned to the ENDCaP-C Study Office by the Investigator or an authorised member of the site research team (as delegated on the ENDCaP-C Study Signature & Delegation Log) as soon as possible. Data reported on each CRF should be consistent with the source data or the discrepancies should be explained. If information is not known, this must be clearly indicated on the CRF. All missing and ambiguous data will be queried. All sections on the CRFs are to be completed.

The ENDCaP-C Study office will request any CRF not received within 1 week of the due date.

Email and letter reminders will be sent to the investigator and research coordinator for missing CRFs; Data Clarification Forms (DCFs) will be sent to request missing data or to resolve data inconsistencies.

Once completed, original CRFs and DCFs will be sent to the ENDCaP-C Study Office and copies retained at site to be filed in the Investigator Site File.

#### 8.1.1 Confidentiality of personal data

All data will be handled in accordance with the UK Data Protection Act 1998.

Participants will give their explicit permission for a copy of their consent form, which contains their full name, to be sent to the ENDCaP-C Study Office to be sent a copy. This will be used to perform in-house monitoring of the consent process.

Participants' names will not be written on any CRF. Participants' month and year of birth, last four digits of their hospital number and study identification number will be used for identification. The exception will be the registration form where participants' name, date of birth and hospital number will be recorded in full.

### 8.2. Definition of the End of Study

The end of the study for regulatory purposes is defined as the date of the last visit of the last patient undergoing protocol based treatment and collection of associated data. Within ENDCaP-C, this is once the last participant has undergone the late reference procedure at4-12 months. This will allow sufficient time for the completion of protocol procedures, data collection and data input.

Follow-up at 4-12 months post-study entry will constitute the end of the non-interventional phase of the study. It is possible that the patient will be eligible to continue with further follow up in another study and consent for this possibility will be obtained along with consent to participate in the ENDCaP-C study.

## 9. DATA ACCESS AND QUALITY ASSURANCE

### 9.1. Confidentiality of personal data

Personal data and sensitive information required for the ENDCaP-C Study will be collected directly from study participants and hospital notes. Participants will be informed about the transfer of this information to the ENDCaP-C Study Office at the BCTU and asked for their consent. The data will be entered onto a secure computer database indirectly from paper forms by BCTU staff.

All personal information received in paper format for the study will be held securely and treated as strictly confidential according to BCTU policies. All staff involved in the ENDCaP-C Study (clinical, academic, BCTU) share the same duty of care to prevent unauthorised disclosure of personal information. No data that could be used to identify an individual will be published. Data will be stored on a secure server at Birmingham Clinical Trials Unit (BCTU) under the provisions of the Data Protection Act and/or applicable laws and regulations.

### 9.2. In-house Data Quality Assurance

#### 9.2.1 Monitoring and Audit

Investigators and their host Trusts will be required to permit study-related monitoring and audits to take place by the ENDCaP-C Study Office, providing direct access to source data and documents as requested. Trusts may also be subject to inspection by the Research and Development Manager of their own Trust and should do everything requested by the Chief Investigator in order to prepare and contribute to any inspection or audit. Study participants will be made aware of the possibility of external audit of data they provide in the participant information sheet.

#### 9.2.2 Statistical monitoring throughout the study

Real-time reports will be available to research staff indicating missing test data for all participants at that centre. This will be supplemented by regular reminders from the Study Office for incomplete data. The study statistician will regularly report on recruitment, compliance and completeness of verification to the Steering Committee.

### 9.3. Independent Trial Steering Committee

The TSC provides independent supervision for the study, providing advice to the Chief and Co- Investigators and the Sponsor on all aspects of the study and affording protection for patients by ensuring the study is conducted according to the MRC Guidelines for Good Clinical Practice in Clinical Trials.

If the Chief and Co-Investigators are unable to resolve any concern satisfactorily, Principal Investigators, and all others associated with the study, may write through the Study Office to the chairperson of the TSC, drawing attention to any concerns they may have about the possibility of particular side-effects, or of particular categories of patient requiring special study, or about any other matters thought relevant.

### 9.4. Data Monitoring and Ethics Committee

During the study, interim analyses of safety and outcome data will be supplied, in strict confidence, to an independent Data Monitoring and Ethics Committee (DMEC) along with any other analyses that the committee may request. Further details of DMEC functioning are presented in the DMEC Charter.

### 9.5. Long-term storage of data

All records created by following trial procedures and all documents listed in guidance relating to the conduct of the trial must be retained and archived.

Archiving will be authorised by the BCTU on behalf of the Sponsor following submission of the end of study report.

Principal Investigators are responsible for the secure archiving of essential study documents and source documents (e.g. signed Informed Consent Forms, Investigator Site Files, participants' hospital notes, copies of CRFs etc.) (for their site) as per their NHS Trust policy. All essential documents will be archived for a minimum of 15 years after completion of study.

Destruction of these documents will require authorisation from the BCTU on behalf of the Sponsor.

## 10. OUTCOME MEASURES

### 10.1. Protection from bias

The generalisability of the study will be assured by aiming to recruit a consecutive sample of patients attending for routine surveillance who fall within the high risk category. We will collect information on risk factors in all study patients and investigate whether the diagnostic value of methylation varies according to patient characteristics.

Misclassification bias can occur in test accuracy studies where the reference standard test is inaccurate. Whilst we are ensuring the initial colonoscopy is a routine surveillance procedure to ensure the study is pragmatic and will address the incremental value of methylation over and above standard current practice, the reference standard colonoscopy will be of the highest quality possible, utilising dye spray to increase dysplasia detection and restricting to experienced, nominated colonoscopists at each site. In addition this colonoscopy will occur 4-12 months after the initial colonoscopy, which will increase the chances of finding dysplasia that was initially not detected (i.e. identifying patients with false negative initial histology). Whilst there is a risk that new dysplasia may develop during this period, we have chosen a time period to minimise this risk, which should not introduce bias as it should be equal in both methylation test positives and negatives. Long term follow up of this cohort of patients will be undertaken, to assess late conversion rate to neoplasia. This will be undertaken outside of this study.

We have elected only to undertake reference standard colonoscopies in a sample of methylation test negatives. As this sample will be chosen randomly without matching it should be representative of all test negatives, and the results not suspect to partial verification bias.

### 10.2. Outcome Measures

#### 10.2.1 Primary Outcome Measures

The primary outcome measures are:

- The occurrence of dysplasia in mucosal biopsies taken at follow up colonoscopy at 4-12 months in patients demonstrating hypermethylation (the positive predictive value)
- The ability of hypermethylation to discriminate between patients with and without dysplasia in mucosal biopsies taken at follow up colonoscopy at 4-12 months (the diagnostic odds ratio)

### 10.2.2 Decision tool for a positive methylation

The decision for a patient to be classified whether they are a test positive will be based on the model equation developed from Module 1. Due to some biomarkers tendency to fail amplification, it was decided by the Study Management Group that for the accuracy of the prediction of classifying a patient to be test positive based on their methylation data, at least 3 out of 5 biomarkers were required to be successfully amplified.

Hence all possible combinations of model equations consisting of at least 3 or more biomarkers were produced from the Module 1 data. Altogether 16 different models were conducted to be used as a decision tool for a positive methylation.

### 10.2.3 Secondary Outcome Measures

The secondary outcome measures for the study are:

1. Complications from colonoscopy

## 10.3. Assessment and Follow up

Primary assessment will be undertaken by both histology and methylation analysis on the biopsy specimens (>/= 5 per patient). Follow up within the study will be by colonoscopy at 4-12 months after initial assessment, with repeat biopsy, and repeat methylation assessment. Long term follow up of this cohort of patients will be undertaken, to assess late conversion rate to neoplasia. This will be undertaken outside of this study. Consent will be taken to allow future linkage to patient data available in NHS routine clinical datasets, including primary care data (e.g. CPRD, THIN, QResearch), secondary care data (Hospital Episode Statistics; HES) and mortality data from the Office of National Statistics (ONS) through The Health and Social Care Information Centre and other central UK NHS bodies. The consent will also allow access to other new central UK NHS databases that will appear in the future. This will allow us to extend the follow-up of patients in the trial and collect long-term outcome and health resource usage data without needing further contact with the study participants. This is important as it will link a trial that may become a clinical standard of care to long-term outcomes that are routinely collected in clinical data, but which will not be collected during the follow-up period of the trial.

## 10.4. Assessment of efficacy

This study is designed as an accuracy study and thus there will be no direct measurement of the efficacy or effectiveness of the inclusion of methylation testing in a surveillance programme. As the results of the methylation test will not be routinely provided to clinicians or patients during the study, the outcomes of patients in this study will not be affected by the test. However, from the results of this study it will be possible to model the potential benefit of changing the surveillance programme. Primarily we expect that the benefits of the test will be in advancing the time point at which CAN is detected in a patient, increasing the chances of preventing cancer and curative therapy being possible.

We would propose that should methylation be found to advance the time of detection of CAN in this study, its full impact on health outcomes could be assessed as it is included in surveillance programmes using a stepped-wedge study design, as high quality baseline data is routinely collected in this well-defined patient group. This is outside the remit of the current protocol.

### 10.5. Assessment of safety

The key safety issue is the additional colonoscopy, performed at 4-12 months. This is up toupto 8 months earlier than the planned surveillance examination and is associated with a small but significant risk of bowel injury/perforation (0.1-0.3%). This risk must be balanced by the benefit of early detection of CAN and effective cancer prevention. The additional risk is only marginally above that incurred by each patient within the surveillance programme, who will undergo perhaps 10-20 examinations through their lifetime. All colonoscopies and incurred morbidity will be collected in a standardised fashion, and provided in IDMC reports. All colonoscopies will be undertaken by experienced colonoscopists, and follow up colonoscopies will be performed by named specialists at each site.

## 11. STATISTICAL CONSIDERATIONS

### 11.1. Sample size

Positive and negative predictive values of methylation for neoplasm will be computed from test positives and negatives respectively, sensitivities and specificities will be estimated adjusting for the sampling proportion in the test negatives (see 11.3).

Computation of the sample size is based on (1) records indicating that 4% are detected with dysplasia by histology from a high risk cohort; (2) an assumption that a further 4% are missed (assuming a detection rate of 50% for routine colonoscopy) of which (3) 50% will be detected by methylation testing (i.e. sensitivity=50%) which will (4) give false positive results in 5% free of dysplasia (i.e. specificity=95%). The sensitivity and specificity of methylation testing have been estimated to be 90% and 100% in retrospective samples, thus these figures are conservative.

The study is powered to have adequate power to show that the test is discriminatory (measured by having an odds ratio different from 1) and that the positive predictive value is high enough to be useful for identification of the high risk patients, being at least 15%. The clinical consequence of a positive test result would be a further colonoscopy, and we consider that detecting neoplasia in 1 in 7 selected for further investigation would be regarded a clinically useful yield. It is not feasible to power the study to show that the negative predictive value is low enough to identify a low risk group.

In our cohort of 1000 we estimate that there will be 80 with underlying dysplasia: 40 of these would be detected by histology from the initial biopsies, and 20 of the remaining 40 will be identified by the methylation test. Following the assumptions about test performance, the methylation test will thus give 46 false positive results (5% of the 920 without dysplasia) giving an expected positive predictive value of 30% (20 of 66). With the assumed test performance, a sample size of 66 test positives will have 87% power to show (in a one sample test) that the positive predictive value is over 15% with $P<0.05$.

We will additionally verify the status of 132 (twice as many) test negatives to obtain estimates of the sensitivity and specificity of the test (computed adjusting for the sampling fraction of test negatives). We would expect that these would include 3 found positive for dysplasia and 129 without dysplasia, which will provide over 90% power to show the diagnostic odds ratio is significantly ($P<0.05$) different from one. A specificity of 95% will be estimated with a confidence interval of less than 4% points.

### 11.2. Projected accrual and attrition rates

Active recruitment to the study will take place over approximately seventeen months. Over this time we intend to recruit a total of 1000 patients from up to 37 UK sites. An audit of UC patient activity at University Hospital Birmingham has already been performed in late 2012 to estimate throughput rates of potentially eligible patients for this study. This showed that around 5-6 patients per month are successfully medically treated for a UC relapse, who would thus be eligible for this study. The rate of two patients per month (33-

40% successfully recruited) seems a realistic target. A final point to note is that patients are eligible for up to a year after a disease relapse, as such they can be approached any time in this period when they routinely attend follow-up outpatient clinic, further increasing the achievability of these recruitment targets.

### 11.3. Statistical Analysis for Test Accuracy

Analysis for the primary outcome will estimate positive and negative predictive values of methylation as the proportion of those methylation positive at the initial colonoscopy that are detected with CAN at the reference colonoscopy, and the proportion methylation negative that are free of CAN at the reference colonoscopy, respectively. The diagnostic value of the test will also be summarised as a diagnostic odds ratio, and values of sensitivity and specificity will be computed from predictive values utilising knowledge of the prevalence of CAN and the sampling fraction in those methylation negative. All estimates will be presented with 95% confidence intervals.

The baseline characteristics of the patients enrolled in the study will be examined and planned subgroup analyses will be undertaken using generalised estimating equation logistic regression models. Subgroup analyses are limited by statistical power and will be interpreted with appropriate caution.

Analyses for the primary outcome comparing results of the reference colonoscopy with methylation testing will only be made at a patient level. Although multiple samples are available from each, they cannot be matched at a lesion level as they are based on separate colonoscopic examinations. A patient will be defined as methylation positive if they are positive for any sample, likewise they will be defined as CAN positive if they are histology positive for any sample.

Analysis of the secondary objective of the diagnostic value of methylation will compare results from the same examination, and both lesion and patient level analyses will be possible. Lesion based analyses will take into account repeated samples from individuals using robust standard errors and hierarchical model structures.

We will also investigate the correlation of methylation in biopsies with methylation of proteins detected in serum and of proteins detected in faecal samples.

## 12. ORGANISATION AND RESPONSIBILITIES

To ensure the smooth running of the study and to minimise the overall procedural workload, it is proposed that each participating centre should designate individuals who would be chiefly responsible for local co-ordination of clinical and administrative aspects of the study.

All investigators are responsible for ensuring that any research they undertake follows the agreed protocol, for helping care professionals to ensure that participants receive appropriate care while involved in research, for protecting the integrity and confidentiality of clinical and other records and data generated by the research, and for reporting any failures in these respects, adverse reactions and other events or suspected misconduct through the appropriate systems.

### 12.1. Principal Investigator at each centre

Each Centre should nominate one person to act as the Local Principal Investigator. This person should be a **Consultant Gastroenterologist**.

The local PI shall bear responsibility for the conduct of research at their centre. The responsibilities of the local PI will be to ensure that all medical and nursing staff involved in the care of patients are well informed about the study and trained in study procedures, including obtaining informed consent. The local PI should

liaise with the Study Coordinator on logistic and administrative matters connected with the study. Updates and newsletters would be sent to the local PI, and they would be invited to training and progress meetings.

### 12.2. Research Co-ordinator at each centre

Each participating centre should also designate a researcher as local Research Coordinator; this is usually a research nurse. This person would be responsible for ensuring that all eligible patients are considered for the study, that patients are provided with study information sheets, and have an opportunity to discuss the study if required. The coordinator may be responsible for collecting the baseline patient and follow-up data. Again, this person would be sent updates and newsletters, and would be invited to training and progress meetings.

### 12.3. The ENDCaP-C Study Office

The ENDCaP-C Study Office will assist local PIs in obtaining relevant Trust approvals.

The ENDCaP-C Study Office at the University of Birmingham Clinical Trials Unit (BCTU) is responsible for providing collaborating centres with the following study materials:

- The Investigator Site File, containing all documentation required under ICH GCP to define the involvement of the centre in the study along with printed materials, such as participant information sheets, consent forms and study schema.
- An online registration system, including individual log-ins and passwords and guidance.

All of the above, will be supplied to each collaborating centre, after relevant Trust approval has been obtained. Additional supplies of any printed material can be obtained on request. The Study Office also provides the central registration service and is responsible for collection and checking of data (including reports of serious adverse events), for reporting of serious adverse events to the sponsor and/ or regulatory authorities and for analyses. The Study Office will help resolve any local problems that may be encountered in study participation.

## 13. RESEARCH GOVERNANCE

The study will be conducted in compliance with the approved protocol, the principles of Good Clinical Practice (GCP), the UK Data Protection Act and the National Health Service (NHS) Research Governance Framework for Health and Social Care (RGF).

All centres will be required to sign an Investigator's Agreement, detailing their commitment to accrual, compliance, Good Clinical Practice, confidentiality and publication. Deviations from the agreement will be monitored and the TSC will decide whether any action needs to be taken, e.g. withdrawal of funding, suspension of centre.

The Study Office will ensure researchers not employed by an NHS organisation hold an NHS honorary contract for that organisation.

### 13.1. Regulatory and Ethical Approval

#### 13.1.1 Ethical and Trust Management Approval

Prior to the recruitment of any participants, the Sponsor, will ensure that the appropriate regulatory bodies have approved the trial protocol, PIS and consent form and supporting documentation.

The Study has a favourable ethical opinion from South East Coast - Surrey Research Ethics Committee (REC) approval, determining that the study design respects the rights, safety and wellbeing of the participants.

With the assistance of the Study Office, the local Principal Investigator at each site is required to obtain local approvals prior to the start of recruitment at site. It is the responsibility of the PI to ensure that all subsequent amendments gain the necessary local approval. This does not affect the individual clinicians' responsibility to take immediate action if thought necessary to protect the health and interest of individual participants.

Sites will not be permitted to enrol participants until written confirmation from the Sponsor of all necessary approvals is received by the PI.

### 13.2. Funding and Cost implications

The research costs of the study are funded by a grant from the Efficacy and Mechanism Evaluation Programme (EME) programme of NIHR awarded to the University of Birmingham.

### 13.3. Sponsor

Sponsorship will be provided by the University of Birmingham upon signing of the Clinical Study Site Agreement with each study site.

### 13.4. Indemnity

ENDCaP-C was developed by the Inflammatory Bowel Disease Network and Birmingham Clinical Trials Unit.

The University of Birmingham is the study 'sponsor.' The Sponsor (University of Birmingham) holds Public Liability (negligent harm) and Clinical Study (negligent harm) insurance policies, which apply to this study. Participants may be able to claim compensation, if they can prove that the University of Birmingham has been negligent. However, as this clinical study is being carried out in a hospital setting, NHS Trust and Non-Trust Hospitals have a duty of care to patients treated, whether or not the patient is taking part in a clinical study. Compensation is only available via NHS indemnity in the event of clinical negligence being proven. University of Birmingham does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees.

Participants *may* also be able to claim compensation for injury caused by participation in this clinical study without the need to prove negligence on the part of University of Birmingham or another party. Participants who sustain injury and wish to make a claim for compensation should do so in writing in the first instance to the Chief Investigator, who will pass the claim to the Sponsor's Insurers, via the Sponsor's office.

There are no specific arrangements for compensation made in respect of any serious adverse events occurring though participation in the study, whether from the side effects listed, or others yet unforeseen.

Hospitals selected to participate in this study shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary should be provided to University of Birmingham, upon request.

### 13.5. Clinical Trials Unit

Data from this study will be handled by the BCTU at the University of Birmingham. BCTU is a full-time research facility dedicated to, and with substantial experience in, the design and conduct of randomised clinical Trials. The BCTU recognises the responsibilities of a data management centre with respect to the ethical practice of research and the adequate protection of human subjects.

### 13.6. Confidentiality of Personal Data

The study will collect personal data about participants, and medical records will be reviewed for all patients and routine physical examinations will be performed. Participants will be informed that their study data and information will be securely stored at the study office at the BCTU, and will be asked to consent to this.

The BCTU abide by the UK law Data Protection Act 1998. The data will be stored on a secure computer database, and all personal information obtained for the study will be held securely and treated as strictly confidential. Any data processed outside of the BCTU will be anonymised.

### 13.7. Publication

A meeting will be held after the end of the study to allow discussion of the main results among the collaborators prior to publication. The success of the study depends entirely on the wholehearted collaboration of a large number of doctors, nurses and others. For this reason, chief credit for the main results will be given not to the committees or central organisers but to all those who have collaborated in the study. Centres will be permitted to publish data obtained from participants in the ENDCaP-C Study that use study outcome measures but do not relate to the study randomised evaluation and hypothesis.

# REFERENCES


**Azarschab** P, et al Epigenetic control of E-cadherin (CDH1) by CpG methylation in metastasising laryngeal cancer. Oncol Rep. 2003 10:501-3.

**Cairns** S and Scholefield JH. Guidelines for colorectal cancer screening in high risk groups. Gut. 2002 51 Suppl 5:V1-2.

**Cairns** SR, et al Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). Gut. 2010 59:666-89.

**Caldwell** GM, et al Reorganisation of Wnt-response pathways in colorectal tumorigenesis. Br J Cancer. 2008 98:1437-42.

**Caldwell** GM, et al The Wnt antagonist sFRP1 in colorectal tumorigenesis. Cancer Res. 2004 64:883-8.

**Caldwell** GM, et al The Wnt antagonist sFRP1 is downregulated in premalignant large bowel adenomas. Br J Cancer. 2006 94:922-7.

**Caldwell** GM, et al Wnt signalling in adenomas of familial adenomatous polyposis patients. Br J Cancer.2010 103:910-7.

**Dhir** M, et al Epigenetic regulation of WNT signaling pathway genes in inflammatory bowel disease (IBD) associated neoplasia. J Gastrointest Surg. 2008 12:1745-53.

2000 95: 2710-9.

**Garrity-Park** MM, et al Methylation status of genes in non-neoplastic mucosa from patients with ulcerative colitis-associated colorectal cancer. Am J Gastroenterol. 2010 105:1610-9.

**Kukitsu** T, et al Aberrant crypt foci as precursors of the dysplasia-carcinoma sequence in patients withulcerative colitis. Clin Cancer Res. 2008 14:48-54.

**Moriyama** T, et al Hypermethylation of p14 (ARF) may be predictive of colitic cancer in patients with ulcerative colitis. Dis Colon Rectum. 2007 50:1384-92.

**NICE** Guidance Document CG118: Colonoscopic surveillance for prevention of colorectal cancer in people with ulcerative colitis, Crohn's disease or adenomas: guidance. Available from http://guidance.nice.org.uk/CG118/Guidance

**Osborn** NK, et al Aberrant methylation of the eyes absent 4 gene in ulcerative colitis-associated dysplasia. Clin Gastroenterol Hepatol. 2006 4:212-8.

**Sato** F, et al Aberrant methylation of the HPP1 gene in ulcerative colitis-associated colorectal carcinoma.Cancer Res. 2002 62:6820-2.

**Shu** X, et al Survival in cancer patients hospitalized for inflammatory bowel disease in Sweden. Inflamm.Bowel Dis. 2011 17:816-22

**Tominaga** K, et al Prediction of colorectal neoplasia by quantitative methylation analysis of estrogen receptor gene in nonneoplastic epithelium from patients with ulcerative colitis. Clin Cancer Res.2005 ;11:8880-5.

**Wildt**, et al A randomised double-blind placebo-controlled trial with Lactobacillus acidophilus La-5 and Bifidobacterium animalis subsp. lactis BB-12 for maintenance of remission in ulcerative colitis. J Crohns Colitis. 2011 5:115-21.