TRACC

Tracking mutations in cell free tumour DNA to predict **R**elapse in e**A**rly **C**olorectal **C**ancer

Clinical Protocol Version 12.0 dated 10.01.2024

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NRES No: 15/LO/1576

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1.1.1.1.1 PROTOCOL SIGNATURE PAGE

Study title: Tracking mutations in cell free tumour DNA to predict **R**elapse in eArly **C**olorectal **C**ancer**.**
Acronym: TRACC
Protocol version number: 12.0
Version date: 10.01.2024

Approved by Chief Investigator:

_____

Name: Professor David Cunningham

Date:

1.1.1.1.2 Investigator's Agreement

I have read the attached protocol v12.0 entitled Tracking mutations in cell free tumour DNA to predict Relapse in eArly Colorectal Cancer, dated 10.01.2024 and agree to abide by all provisions set forth therein.

I agree to comply with the principles of Good Clinical Practice (GCP), the EU and GCP Directives (2001/20/EC; 2005/28/EC) and The Medicines for Human Use (Clinical Trials) Regulations and Amendment Regulations 2006 (Statutory Instrument 2006 No. 1928).

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Gastrointestinal & Lymphoma Trials Unit of the Royal Marsden NHS Foundation Trust.

Signature	
Name of Principal Investigator	
Centre Name	
Date (DD/MM/YYYY)	

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Research Team:

Speciality	**Name & Location**
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Biological Specimen Co-ordinator Lead	Ruwaida Begum, RM

Protocol design and development: Dr Shelize Khakoo, Dr Gayathri Anandappa, Dr Susanna Slater, Mrs Clare Peckitt, Professor Ian Chau, Dr Naureen Starling & Professor David Cunningham

Note: All queries about this trial should be addressed to the GI & Lymphoma Trials Unit, including clinical queries. Clinical queries will then be referred to the Trial Physician or Chief Investigator.

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1.1.1.1.3 PROTOCOL SYNOPSIS

Study Title:	**T**racking mutations in cell free tumour DNA to predict **R**elapse in e**A**rly **C**olorectal **C**ancer
Short Study Title:	TRACC
Chief Investigator:	Professor David Cunningham
CCR number:	4344
Hypotheses:	1. In patients with stage I, II and III colorectal cancer (CRC), detection of mutations in circulating tumour DNA (ctDNA) in plasma can predict relapse. 2. ctDNA directed adjuvant chemotherapy administration will enable biomarker driven selection of patients who would benefit from adjuvant chemotherapy and thereby reduce the proportion of patients receiving adjuvant chemotherapy without compromising disease free survival.
Centres:	Multi-centre study across the UK. The TRACC study is open to any site within the United Kingdom treating patients with CRC. It is anticipated that up to 100 centres will participate in trial recruitment.
Study Population:	Part A (Feasibility Phase): This part of the study will include the first patients 48 with stage II or III CRC who have undergone curative surgery. Samples from these patients will allow assessment of ctDNA detection methods. Part B (Translational Study): The study population will include 1. Patients in Part A of the study 2. Patients with stage I CRC, low risk stage II CRC and any patient not willing or not eligible to take part in Part C of the study Part C (randomised study of ctDNA guided adjuvant chemotherapy versus standard of care adjuvant chemotherapy): This study population will include patients with high risk stage II or stage III colon or rectal cancer who have undergone curative surgery and are being recommended adjuvant chemotherapy. Adjuvant chemotherapy administration will be based on randomisation between standard of care arm and ctDNA guided arm. Patients with rectal cancer who have undergone neo-adjuvant radiotherapy or chemoradiotherapy followed by curative surgery will also be included. Participating centres may choose to enrol patients into Part B of the study alone or to Part B followed by Part C of the study, or Part C alone.

Objectives:	**Primary Objectives:** **For Part A (feasibility):** • To assess whether circulating cell free tumour derived DNA (ctDNA) is detectable in patients with stage II and III colorectal cancer (CRC) pre-operatively **For Part B (translational study):** • To assess whether detection of ctDNA predicts for relapse in patients with stage II and III CRC that have undergone surgery with curative intent **Part C (randomised study, ctDNA guided versus standard of care adjuvant chemotherapy study):** • To demonstrate a de-escalation strategy of ctDNA guided adjuvant chemotherapy is non-inferior to standard of care treatment as measured by 3-year disease free survival in patients with high risk stage II or stage III CRC with no evidence of minimal residual disease (ctDNA negative) **Secondary Objectives:** **For all patients within the study as relevant** • To assess whether mutations identified in formalin fixed paraffin embedded (FFPE) tumour tissue using targeted resequencing by a clinically validated method can be detected in circulating cell free DNA (cfDNA) using droplet digital PCR (ddPCR). Patients in Part C of the study will have ctDNA tested using next generation sequencing (NGS) based assays at pre-specified timepoints • To quantify levels of mutations in cfDNA and assess change from baseline, post-operatively and during chemotherapy • In patients with rectal cancer having neo-adjuvant radiotherapy/chemoradiotherapy, to quantify levels of mutations in ctDNA prior to radiotherapy/chemoradiotherapy and assess change in level prior to surgery and post-operatively • To quantify levels of mutations in cfDNA and assess change 3 monthly from the first post-operative visit for year 1, 6 monthly until year 3 and annually in years 4 and 5 or until relapse, if this occurs first in patients with stage II and III CRC • To assess whether serial quantification of ctDNA has the potential to predict loco-regional and/or distant relapse after treatment of stage II and III CRC • To correlate change in quantity of mutations in ctDNA with carcinoembryonic antigen (CEA), clinical and radiological parameters

	• To develop a threshold for the detection of ctDNA that is likely to lead to relapse, by using the first 500 patients recruited as a training set and the next 500 patients recruited as a validation set **For Part C only** • Assess proportion of patients who are ctDNA negative on post-operative ctDNA and receiving de-escalated adjuvant chemotherapy in interventional arm compared to standard arm • Assess proportion of patients who are ctDNA negative post-operatively who become ctDNA positive during follow-up and receive chemotherapy as an escalation of care • To compare overall survival between ctDNA directed adjuvant chemotherapy and standard of care adjuvant chemotherapy arms • To compare neurotoxicity in and quality of life in patients between arms • Health economic analysis to assess the cost-effectiveness of ctDNA directed therapy arm compared to the standard of care arm **Exploratory Objectives** **For all patients in the study (Part A + B + C)** • To evaluate whether the presence of a specific mutation or a pattern of mutations identified in both FFPE tumour tissue and cfDNA predict for relapse • To use targeted next generation sequencing (NGS) in plasma to identify mutations in cfDNA • To develop digital pathology as a complementary tool to predict relapse in stage II and III CRC • To analyse blood and tumour tissue for other potential predictive and prognostic biomarkers which show promise in emerging literature • To molecularly sub-classify tumour tissue according to the consensus classification reached by the colorectal cancer subtyping consortium • To assess whether particular molecular sub-types of CRC are more likely to have detectable ctDNA at the first post-operative visit • To assess whether particular molecular sub-types of CRC are more likely to relapse • To assess blood and tumour tissue of patients with synchronous colorectal primaries to evaluate mutational patterns in these patients • To explore if ctDNA is detectable in patients with stage I colorectal cancer

	• To assess whether detection of ctDNA predicts for relapse in patients with stage I CRC who have undergone surgery with curative intent • Patients with rectal adenocarcinoma receiving chemoradiotherapy (CRT) at The Royal Marsden Hospital will have mrTRG (Magnetic resonance imaging tumour regression grade) assessed, which will be correlated with ctDNA **For Part C only** • Analysis to assess the economic impact of ctDNA directed therapy on patients, their families, and the wider economy, compared to the standard of care arm.
Endpoints:	All of the endpoints will be analysed in all patients and by disease stage **Part A (Feasibility Study)** **Primary Endpoint:** • The percentage of patients with stage II and III CRC that have detectable ctDNA pre-operatively **Secondary Endpoints:** • The concordance rate between mutations detected by targeted resequencing in tumour tissue and mutations detected by digital PCR in ctDNA • The correlation between the change in detectable mutations in plasma ctDNA at the first post-operative visit and the change in CEA • The percentage of patients who have detectable mutations in ctDNA post-operatively, out of the patients with detectable mutations in tumour tissue and cfDNA pre-operatively • The percentage of patients that have detectable mutations in ctDNA post-operatively that did not have mutations in ctDNA pre-operatively but had mutation(s) in the tumour tissue alone **Part B (Translational Study)** **Primary Endpoint:** • The association between detectable ctDNA at the first post-operative visit & recurrence free survival (RFS). • Detectable ctDNA is defined as the presence of at least one tumour-derived mutation above the limit of detection (LOD) threshold for that particular mutation assay. **Secondary Endpoints:**

	• The association between detectable ctDNA with RFS, loco-regional relapse free survival, distant relapse free survival and Overall Survival (OS) at the following time-points: pre-operative, the first post-operative visit, during chemotherapy and post-chemotherapy • The association between the level of ctDNA with RFS, loco-regional relapse free survival, distant relapse free survival and OS at the following time-points: pre-operative, the first post-operative visit, during chemotherapy and post-chemotherapy • The association between the time of rise in the level of ctDNA and RFS, where rise is defined as an increase in the ctDNA level in two consecutive samples. • The association between the change in quantity of ctDNA from the pre-operative and the first post-operative sample with RFS • In patients having adjuvant chemotherapy, the percentage of patients who have detectable ctDNA post-operatively, that no longer have detectable ctDNA on completion of adjuvant chemotherapy • In patients receiving adjuvant chemotherapy, the association between the change in quantity of ctDNA from the pre and post chemotherapy samples with RFS • In rectal patients receiving neo-adjuvant radiotherapy/chemoradiotherapy, the association between the change in quantity of ctDNA from the pre and post radiotherapy/chemoradiotherapy sample with RFS • The association between the change in quantity of ctDNA from the end of all treatment and subsequent surveillance visits with RFS • To assess the presence or absence of measurable disease radiologically with the presence or absence of detectable mutations in plasma cfDNA post-operatively • The lead time between rise in ctDNA and rise in CEA from the post-operative values • The lead time between rise in ctDNA from the post-operative values and radiological relapse • The association between ctDNA and prognostic factors will be investigated • Multi-variate analysis of RFS will investigate the addition of ctDNA variables in significant univariate analysis to standard prognostic factors • To estimate a threshold for detection of ctDNA (actual level and rise) from that predicts relapse in a training set of patients and to be validated in the validation set. **Part C only** **Primary Endpoint**:

	Difference in 3 year disease-free survival from time of surgery to progression, between standard of care arm and ctDNA guided adjuvant chemotherapy arm. **Secondary Endpoints:** • Proportion of patients in the ctDNA guided arm who are ctDNA negative and therefore have de-escalation of adjuvant chemotherapy • Proportion of patients who are ctDNA negative post-operatively who become ctDNA positive during follow-up and receive chemotherapy as an escalation • Overall survival between both arms, defined as time from randomisation to death of any cause. • Sub-group analyses performed on 3-year DFS and OS including but not limited to the following will be performed: a) high risk stage II versus stage III b) site of primary tumour (right colon versus left colon versus rectum). • Neurotoxicity data between both arms (FACT/GOG-Ntx4 & CTCAE V5) • Quality of life data (EORTC QLQ-C30 and CR29 and EQ-5D-3L) • Health resource utilisation data between both arms (RUtInE™ questionnaire) • Descriptive analysis (frequencies and proportion) will be used to describe clinico-pathological characteristics in patients recruited in the MMRd cohort, according to study arm (see p16)
Study design:	This is a multi-centre, prospective, translational research study involving the collection and analysis of tumour tissue, serial blood samples and clinical data in patients with newly diagnosed stage I, II and III CRC. The study will be divided into 3 parts. Part A will serve as a feasibility study prior to proceeding to Part B, which will include patients with stage I, stage II or III CRC treated with curative surgery and undergoing standard of care treatments and follow-up. The third part of the study, Part C, will include those patients willing to participate in a ctDNA guided adjuvant chemotherapy approach. In Part A the proportion of patients with stage II and III CRC who have detectable ctDNA in plasma pre-operatively will be determined. Part B of the study will aim to determine whether detection of ctDNA in the first post-operative blood sample can be used to predict relapse in patients with stage I, II and III CRC. In addition, levels of ctDNA at other time points such as: pre-operative, during chemotherapy and post-chemotherapy will be evaluated. All patients are followed up every 3 months during first year, 6 months during 2nd

	and 3rd year and annually during years 4 and 5; bloods for ctDNA will be collected at all these time points. The association between the level of ctDNA at these time points with RFS and OS will be determined. Part C of the study will include patients with high risk stage II and stage III CRC as per histopathological assessment of the resection specimen who are willing to participate in the ctDNA guided interventional part of the study. Patients do not have to be enrolled in Part B in order to enrol in Part C of the study. Patients will be eligible to enrol regardless of whether their baseline ctDNA is positive or not. Patients will sign a separate consent form specific for Part C of the study. Once informed consent is obtained in the oncology clinic from patients, either face to face or over the telephone or video consultations, prior to randomisation, clinicians will decide the adjuvant chemotherapy of choice based on histopathological features of the tumour, patient's age, co-morbidities and patient's choice, as is current clinical practice. Following informed consent, patients will have ctDNA samples collected during 4-8 (+2) weeks post-operatively (month 0 time point). This blood sample will be tested for ctDNA, presence of ctDNA considered as 'positive' and absence of ctDNA will be considered as 'negative'. Patients will be randomised in 1:1 fashion between standard of care arm, where patients are offered standard of care adjuvant chemotherapy according to national guidelines, and the experimental arm, in which patients will be treated based on ctDNA results. **Standard of Care (SoC) arm** Patients will have blood collected post-operatively for ctDNA analysis 4-8 (+2) weeks after surgery. Blood samples for ctDNA will be banked for future analysis, hence patients will not receive ctDNA results if randomised to this arm. They will be offered standard of care capecitabine based adjuvant chemotherapy as per national guidelines (single agent capecitabine for 6 months or doublet CAPOX for 3 months). **ctDNA guided adjuvant chemotherapy arm** In patients assigned to the ctDNA guided adjuvant chemotherapy arm, results of ctDNA analysis will be made available within a 2 week turn-around period to allow for commencing adjuvant chemotherapy within 12 weeks after surgery. Based on the results, patients in this arm will be treated as follows: <u>Post-op (month 0) ctDNA positive patients (ctDNA detected)</u> Patients receive standard of care adjuvant chemotherapy

	Post-op (month 0) ctDNA negative patients (ctDNA not detected) If ctDNA is negative post-operatively, chemotherapy is de-escalated as follows (outlined in **Figures 5a** and **5b**): -if a doublet regimen (CAPOX) was recommended to the patient before randomisation, patient receives single agent chemotherapy (capecitabine) for 3 months. -if single agent chemotherapy (capecitabine) was recommended, then patient receives no chemotherapy. In patients in the ctDNA guided arm who are ctDNA negative at month 0, a further real time analysis of ctDNA with a 2 week turn-around time of ctDNA results will be performed at month 3. Based on the results, patients will be managed as follows: Post-op ctDNA (month 0) negative patients who become positive during follow-up at month 3 In those patients who are ctDNA negative during month 0 (post-op) but become positive at month 3, will undergo radiological imaging to assess for disease relapse (CT or MRI). If no evidence of macroscopic disease is noted, in this group of patients, systemic chemotherapy will be introduced, or escalated to doublet regimen with CAPOX for 3 months (capecitabine for 6 months is acceptable in patients not suitable to receive oxaliplatin). If there is evidence of macroscopic disease by radiological assessment, chemotherapy as per clinician's choice will be administered. Post-op ctDNA negative patients who remain negative at month 3 during follow-up Those patients who are ctDNA negative at month 0 and continue to remain negative at month 3 will have clinical follow-up only and no further adjuvant chemotherapy will be administered. They will be followed up at months 6, 9, 12, 18, 24, 30, 36, 48 and 60 as per protocol schedule. Patients participating in Part C, both standard of care arm and ctDNA guided arm, will have ctDNA levels measured during adjuvant chemotherapy at time points specified in the protocol, i.e., at these time points in total: 3 monthly blood samples during year 1, 6 monthly blood samples during years 2 and 3, annual blood samples during years 4 and 5.
Study design for patients with mismatch repair deficiency (MMRd)/ microsatellite	Patients with resected MMRd/MSI-H CRC being recommended standard of care adjuvant chemotherapy are also eligible to enrol in TRACC Part C. MMRd/MSI-H patients randomised to Arm A: standard of care arm will follow the same procedures as detailed above (see **Study Design**).

high (MSI-H) CRC in Part C	Patients randomised to Arm B: ctDNA guided ACT, patients who are ctDNA negative post-operatively (month 0) will be de-escalated to **no chemotherapy** as per the de-escalation/escalation schema in **Figure 5c.** Otherwise, patients who are ctDNA positive post-operatively (month 0) will follow the procedures as detailed above (see **Study Design**).
Interim Analysis:	<u>For feasibility and translational study (Parts A and B)</u> The study incorporates a feasibility part A study, and an interim analysis as follows: 1) Part A will incorporate the first 48 patients and be looking at the proportion of patients with detectable ctDNA pre-operatively. 2) The interim analyses will be performed once the first 150 patients have reached their one year post-operative visit and their plasma has been analysed. This analysis will be descriptive to see the proportion of patients that have detectable mutations in ctDNA in the first post-operative sample and if there is any evidence that detectable mutations can predict relapse. The interim analysis will also be used to check that all treatment modalities are being adequately represented. <u>For ctDNA guided interventional group of the study (For Part C)</u> Stopping for safety will be based on the recommendations from the Independent Data Monitoring Committee (IDMC) and endorsed by a Trial Steering Committee (TSC). Stopping based on lack of efficacy (futility) will be based on the combination of evidence from safety and the conditional power. If the conditional power is <20% after 25% or 50% of the DFS events, the study will be considered futile. The IDMC will offer the overall recommendation based on clinical and statistical data for stopping for futility. The IDMC may also meet at other times as required but as a minimum we will consider 2 interim/futility assessments planned using conditional power. • 25% events (n=125) would occur by year 4 (with 1280 patients recruited • 50% events (n=250) would occur by year 5 (with all patients recruited)
Number of patients and statistical considerations:	The first 48 patients will comprise the feasibility Part A of the study. These patients will be included in the overall analysis. This would be used as a guide as to whether to continue to the main study or not. Part B of the study will include at least 500 evaluable patients with stage II (low risk and high risk) CRC and 500 evaluable patients with stage III CRC. Patients with stage I CRC will also be included.

	For ctDNA guided interventional group of the study (Part C), a total of 1620 patients (810 patients in each arm) would need to be randomised, with 499 events required based on the following assumptions: 1) 5 year accrual 2) 3 year minimum follow up on all 3) 5% one-sided significance level 4) 80% power 5) 1, 2, 3, 4, 5, 6 year DFS estimated from SCOT study as 0.9, 0.8, 0.75, 0.725, 0.7, 0.68 6) Non inferiority margin = 1.25 (ruling out 69.8% 3 year DFS) A non-inferiority margin of 1.25 has been chosen to allow for a worsening of 3-year DFS from 75% up to 69.8% only as being clinically acceptable. The accrual target will be inflated by ~5% to account for drop-outs, therefore the overall total accrual target will be 1700 patients. It is anticipated that the overall study population will be at least 2700 (1700 more than initially planned). This is anticipated to take approximately 10 years in total from the start of study recruitment in 2016. Once confirmation that we have 1620 evaluable patients for Part C of the study and that Part B of the study has 500 evaluable stage II (low risk and high risk CRC) and 500 evaluable stage III evaluable patients, we will consider halting recruitment to that stage. We plan to over-recruit to account for drop-outs. In addition, we plan to recruit patients with high risk stage II and stage III resected MMRd CRC into the Part C sub-study.
"Eligibility Assessment:	**For Main Study (Part A and Part B) and ctDNA guided interventional group of the study (Part C)** All patients in Part B will have eligibility assessed in a 2-step process, prior to surgery and again post-operatively with the histopathology report from surgery using the criteria below. All patients meeting the eligibility criteria at the first assessment will be registered. Rectal cancer patients who undergo pre-operative radiotherapy or chemoradiotherapy will have an additional eligibility assessment after completion of this with the results of their response assessment imaging, after their management plan has been determined. This is most likely to be following multidisciplinary team discussion. For Part C of the study, patients may be initially registered as part of the translational study (Part B), although this is not mandatory. A further eligibility assessment will be undertaken specifically for Part C as per below criteria.

	All patients who were registered to Part C and subsequently excluded based on the eligibility criteria below will be replaced to ensure an adequate sample size is maintained for the statistical analysis.
Inclusion and Exclusion Criteria:	**Eligibility criteria to be used prior to registration (for all patients, Part A and B):** Inclusion Criteria: • New diagnosis of histologically confirmed CRC (colon and rectal) scheduled to undergo surgery with curative intent, with no radiological evidence of metastatic disease. • Histology consistent with adenocarcinoma or patients with high grade dysplasia whose imaging is suggestive of colorectal carcinoma (CRC). • Age≥18 • Ability to give informed consent • Able to adhere to follow up schedule NB: Synchronous CRC primaries are allowed in Part A and B. Exclusion Criteria: • Scheduled to have neo-adjuvant chemotherapy (neo-adjuvant radiotherapy or chemoradiotherapy for patients with rectal cancer is permitted) • Current or previous other malignancy within 5 years of study entry, except cured basal or squamous cell skin cancer, superficial bladder cancer, prostate intraepithelial neoplasm, carcinoma in situ of the cervix or other non-invasive malignancy **Additional eligibility criteria for rectal cancer patients following completion of pre-operative radiotherapy or chemoradiotherapy** Inclusion Criteria: • All patients proceeding to surgery Exclusion Criteria: • Patients scheduled to have further pre-operative treatment with chemotherapy • Patients that are no longer proceeding with surgery i.e., those in whom surgery is considered too high risk • Patients who are no longer proceeding with surgery as they are proceeding with a deferral of surgery approach

	Criteria to be used to confirm eligibility on the case report form (CRF) with the histopathology report at the first post-operative visit Inclusion Criteria: • Stage I, II or III CRC based on the post-operative histopathology report • Availability of FFPE tumour tissue (from either biopsy or surgery), for processing and analysis Exclusion criteria: • Patients with no confirmed tissue diagnosis or high-grade dysplasia included in the study based on imaging diagnosis but subsequent histopathology of surgical specimen confirms no carcinoma will be excluded • Scheduled to receive post-operative radiotherapy **Eligibility criteria Part C only** Inclusion Criteria: 1. Subject ≥ 18 years of age 2. Subjects with histologically proven high-risk stage II* or stage III (any T, and N1 or N2) colon or rectal cancer treated with curative intent with surgery alone with no evidence of metastatic disease. Subjects must be due to receive adjuvant chemotherapy following surgery. or Subjects with radiologically or histologically confirmed stage III (any T, N1 or N2) histologically proven locally advanced rectal cancer treated with neo-adjuvant radiotherapy or chemoradiotherapy with no evidence of metastatic disease are eligible. Subjects must be due to receive adjuvant chemotherapy following surgery. **High risk stage II is defined as having one or more of the following: T4 disease, tumour obstruction and/or perforation of the primary tumour during the pre-operative period, inadequate nodal harvest as indicated by <12 nodes examined, poorly differentiated grade on histology, peritoneal involvement or extramural perineural/venous/lymphatic invasion.* 3. Fully surgically resected tumour (R0) with clear resection margins (i.e., >1 mm) 4. Adequate organ function - Absolute neutrophil function ≥1.0 x 10^9/ L

	- Platelet Count ≥ 75 x 10^9 / L - Haemoglobin ≥80g/L (blood transfusion before randomisation is allowed) - Adequate renal function as calculated by Cockcroft and Gault equation (GFR ≥ 50ml/min if single agent capecitabine or CAPOX being administered) - Aspartate aminotransferase/ Alanine aminotransferase levels ≤ 2.5 upper limit of normal 5. Absence of major post-operative complications or other clinical conditions that, in the opinion of the investigator, would contraindicate adjuvant chemotherapy 6. Patients should be assessed by an Oncologist for suitability of adjuvant chemotherapy with 6 months (8 cycles) of capecitabine* or 3 months (4 cycles) of CAPOX, have a post-operative ctDNA blood sample collected and be randomised by week 4-8 (+2 weeks) after surgery, commencing adjuvant chemotherapy within 12 weeks after surgery (*6 cycles in patients already treated with 2 cycles of concomitant capecitabine as part of neo-adjuvant chemoradiotherapy) 7. ECOG performance status 0-2 8. Able to give informed consent Exclusion Criteria: 1. History of concurrent and previous malignancy within the last 5 years, including those on anti-cancer therapy (e.g., adjuvant endocrine therapy), except for curatively treated superficial malignancies, (e.g., non-melanomatous skin cancer and carcinoma in situ) 2. Any major post-operative complications or other clinical conditions that in the opinion of the investigator would contra-indicate adjuvant chemotherapy 3. Any subject not due to receive adjuvant chemotherapy will not be eligible for Part C of the study 4. Hypersensitivity or contraindication to the drug(s) associated with the planned choice of systemic chemotherapy (CAPOX or single agent capecitabine) as stated in the SmPC for each of the drugs 5. Subjects due to receive 5-flurouracil (5-FU) based adjuvant chemotherapy (either single agent 5-FU or in combination with oxaliplatin) will not be eligible for Part C of the study,

	these patients will continue to be followed up in the observation Part B of the study (if enrolled) 6. Patients with synchronous CRC primary tumours
Follow-up Schedule	All patients will be followed up for a minimum period of 5 years in all parts of the study. Following completion of adjuvant chemotherapy, follow up and assessments for clinical review, CEA and ctDNA samples will be as follows: • 3 monthly during the first year • 6 monthly during years 2 and 3 • annual follow-up during years 4 and 5 Surveillance CT chest/abdomen/pelvis performed as part of routine surveillance post-operatively, at end of year 1, 2 and 3. Further imaging will be done if clinically indicated or ctDNA becomes positive at month 3 in the ctDNA guided arm where the patient was initially ctDNA negative or CEA rises.
Health Economics	For Part C of the study only, a cost-effectiveness analysis at a later stage will help to assess the costs and health effects of the proposed intervention in comparison with the current standard of care. This will support future NHS implementation if appropriate. A pilot study on 40 patients who completed at least 6 months follow up will take place to help understand how well patients are responding to the RUtInE™ questionnaire. Objectives of the pilot will be to assess RUtInE™ questionnaire's response rate (RR) and completeness rate (CR),

Fig 1: Overall Study Design

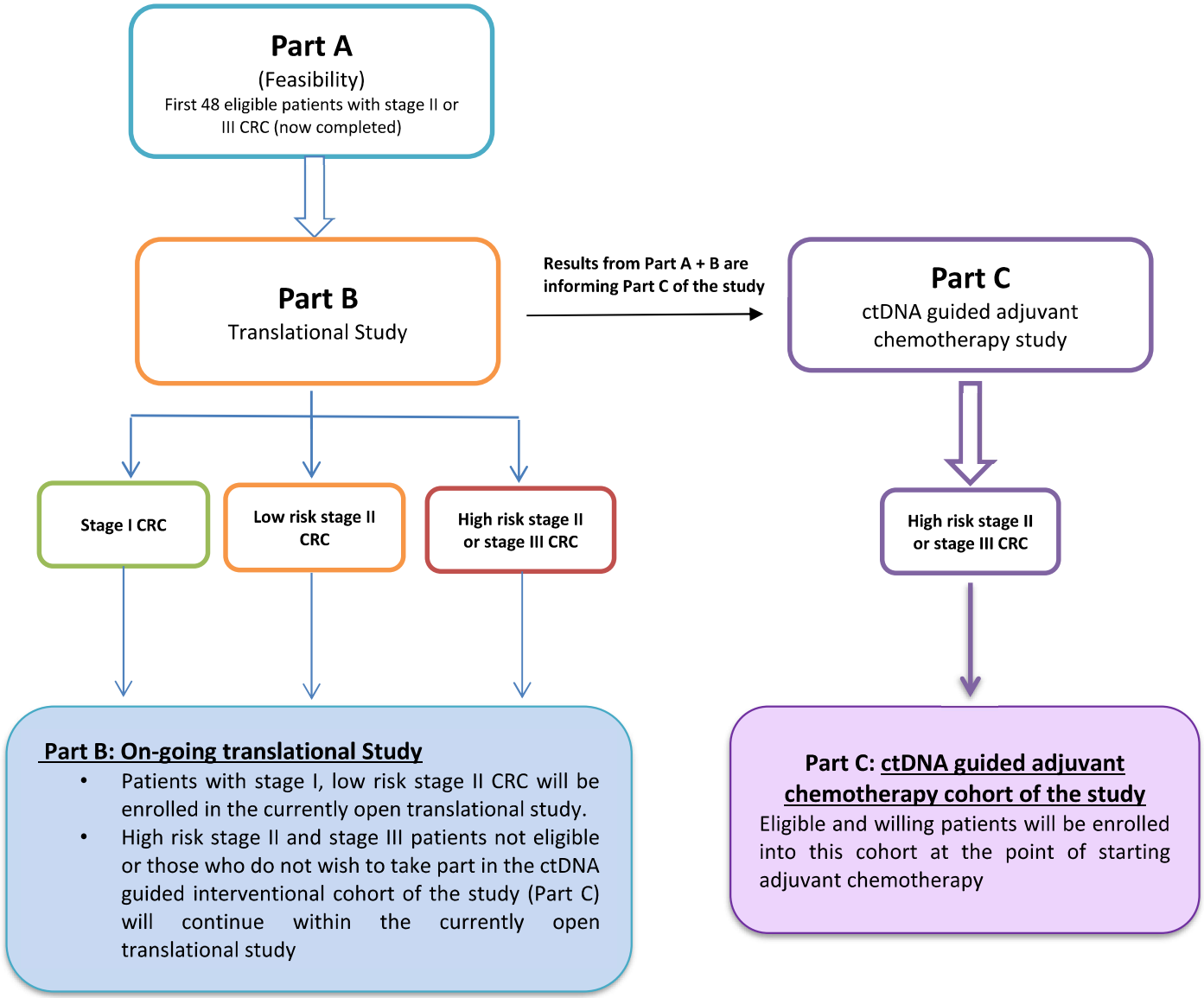


Figure 2: Time-points for blood samples and CT scans for stage II and III patients (for patients in Part B, translational study only)

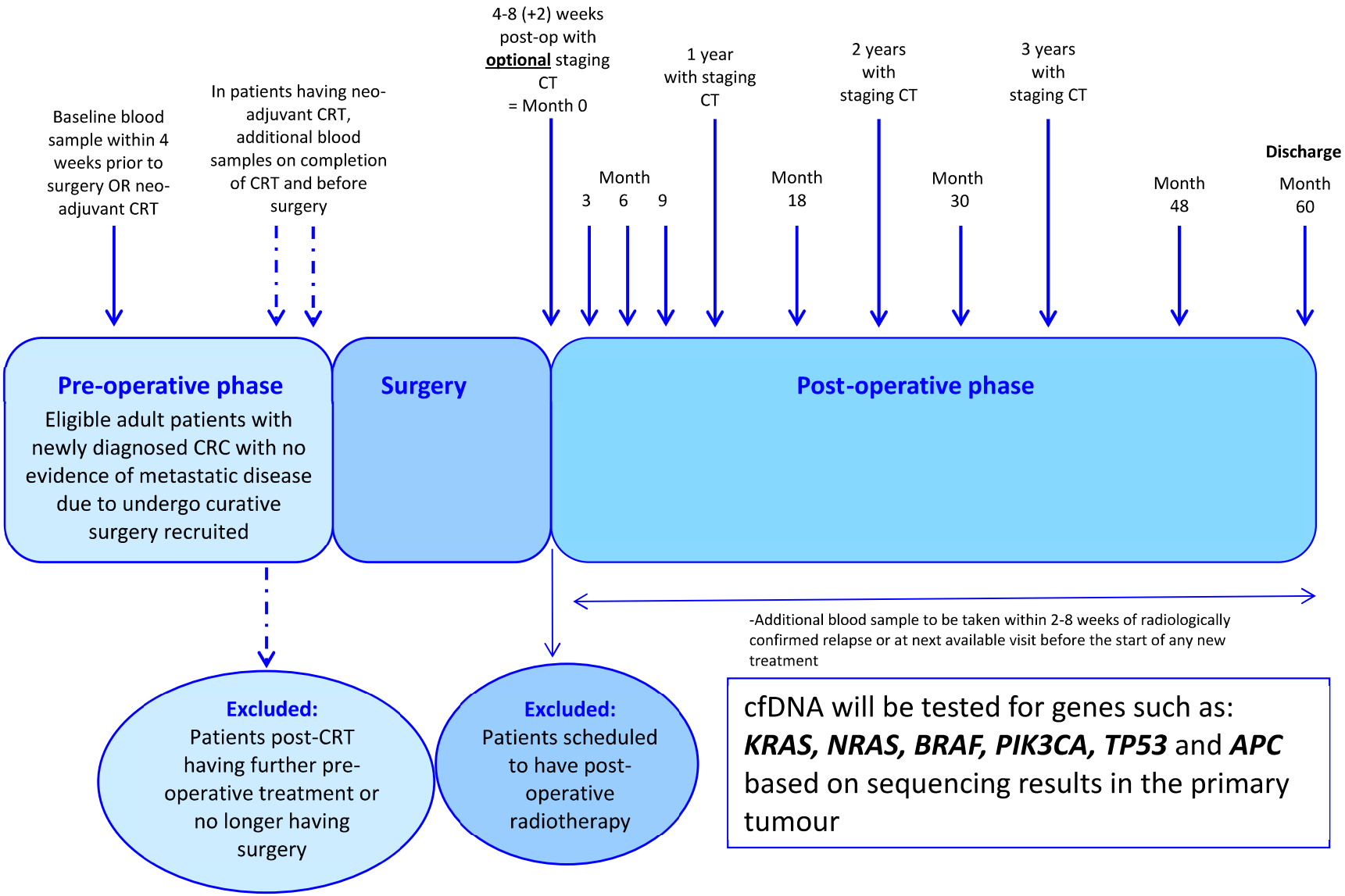
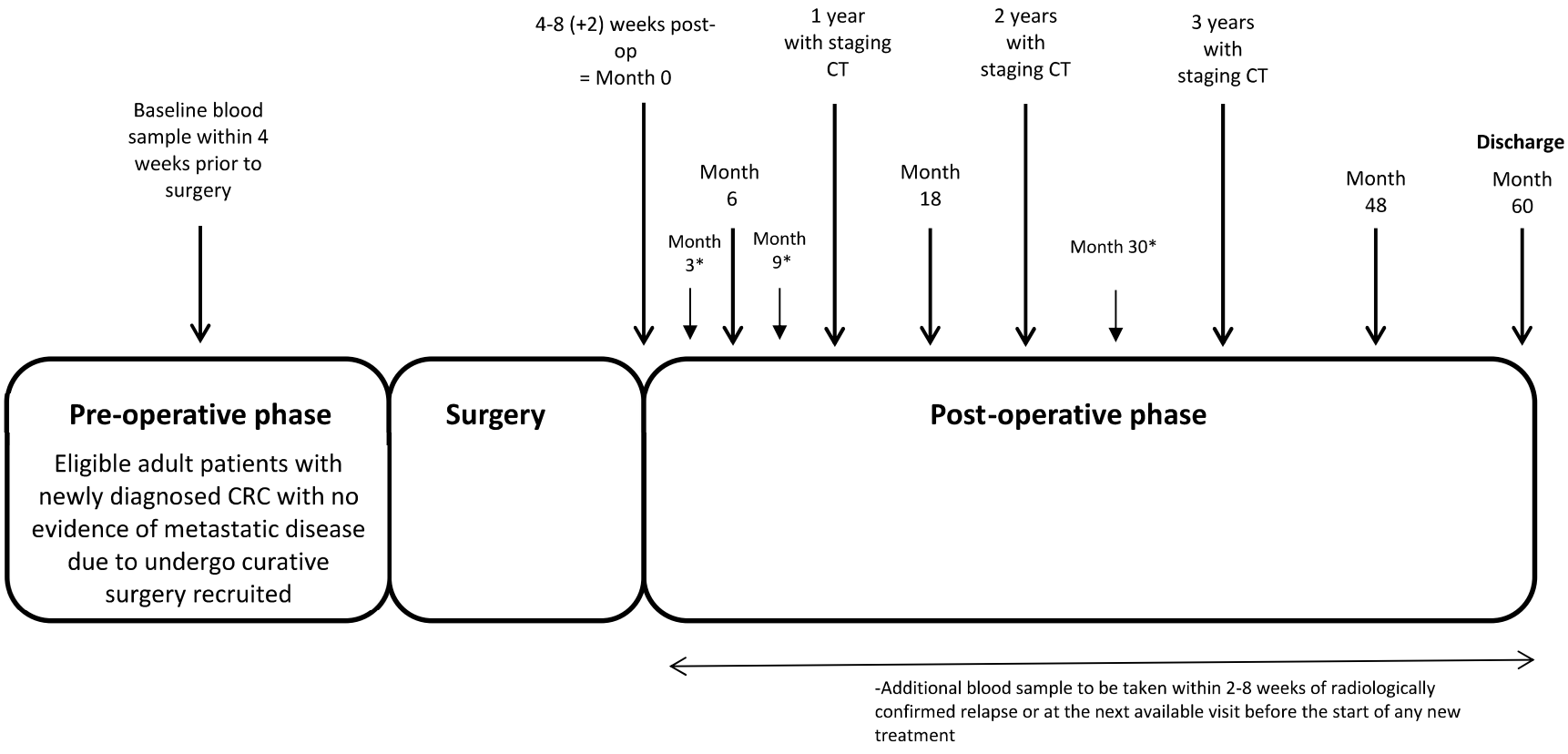


Figure 3: Time-points for blood samples and CT scans for stage I patients

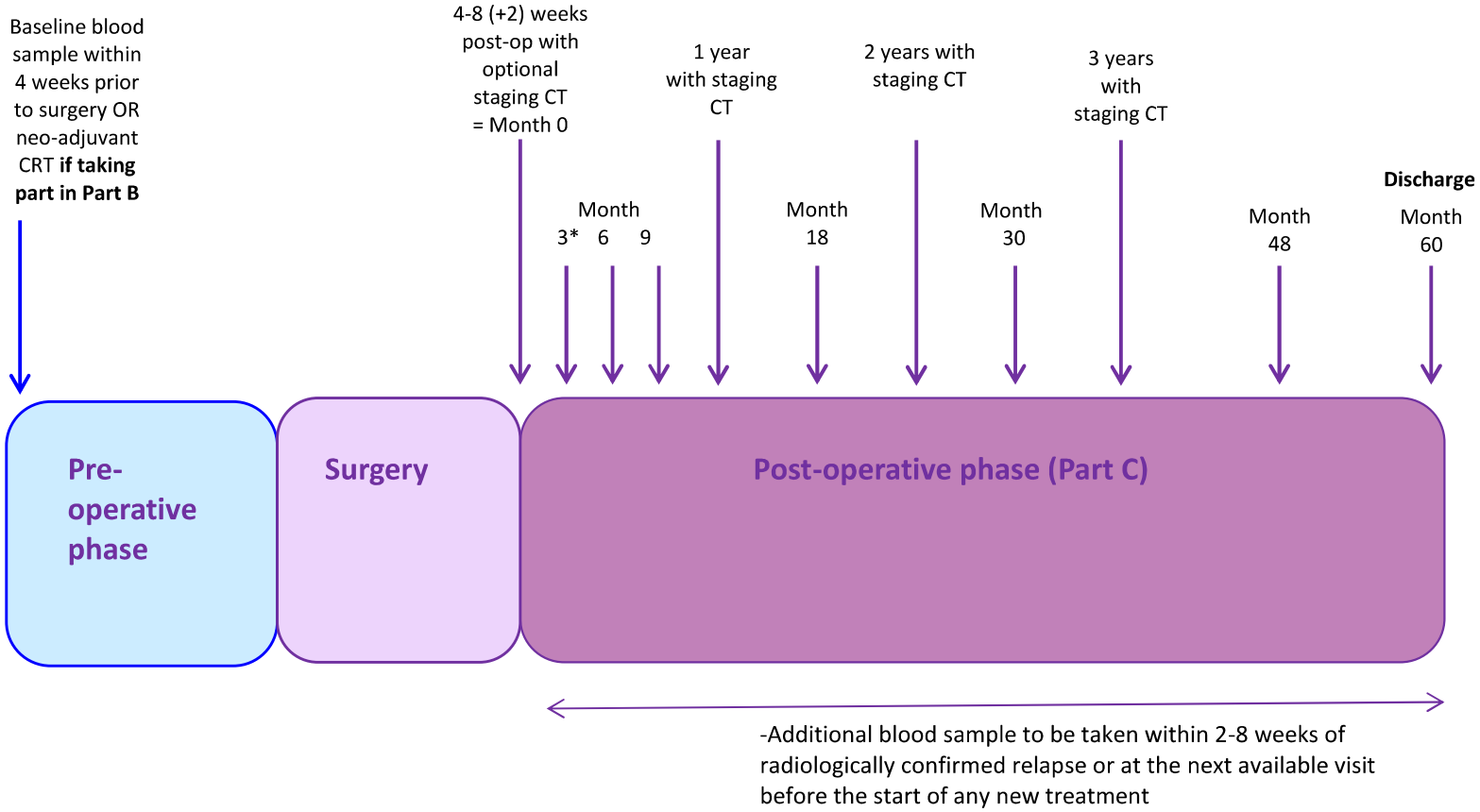
(Part B, Translational Study only)



*Month3, Month 9 & Month 30 follow up visits for Stage I patients are optional and can be collected if patients are coming on site at these timepoints.

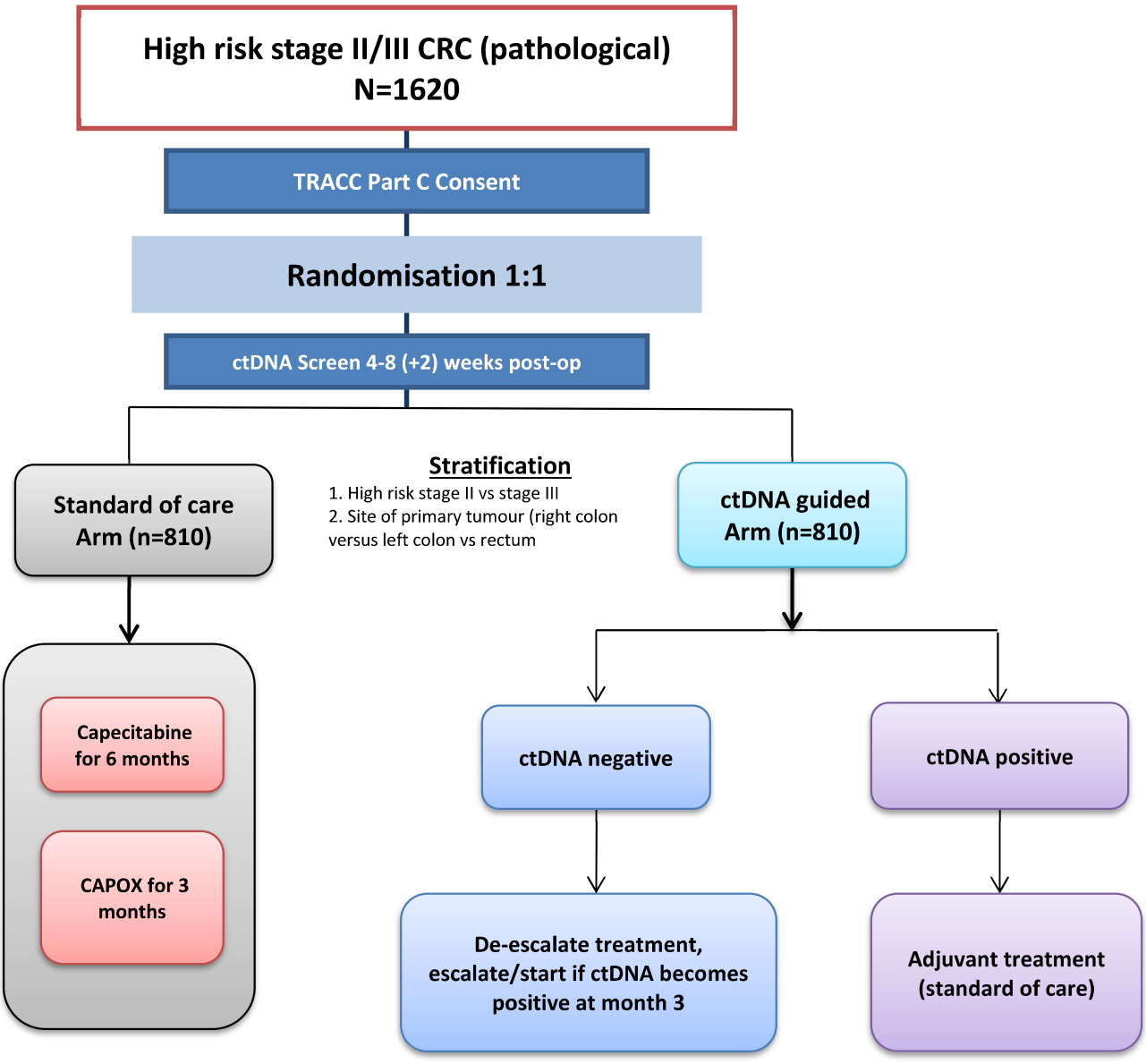
cfDNA will be tested for genes such as: ***KRAS, NRAS, BRAF, PIK3CA, TP53*** and ***APC*** based on sequencing results in the primary tumour

Fig 4: Time-points for blood samples and CT scans for high risk II and III patients in ctDNA guided adjuvant chemotherapy cohort of study (Part C)



* ctDNA samples at month 0 and month 3 will be analysed in real time for the CtDNA guided adjuvant chemotherapy arm and if ctDNA positive at month 3, patients will require CT scan at this time point (month 3)

Figure 5a: Study schema for Part C of the study



* See Fig 5b for treatment pathway for patients who are ctDNA negative post-op

Fig 5b: De-escalation/ Escalation strategy in ctDNA negative group in the ctDNA guided arm

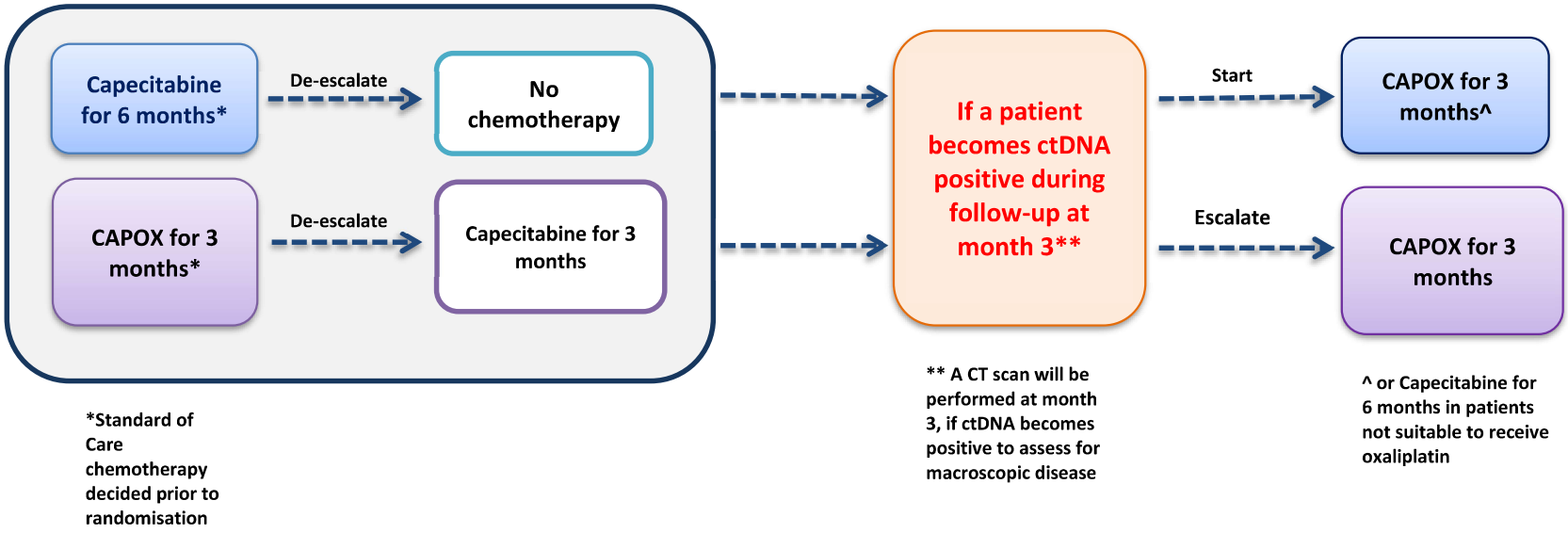


Fig 5c: De-escalation/escalation strategy for patients with MMRd/MSI-H CRC in ctDNA negative group in the ctDNA guided arm

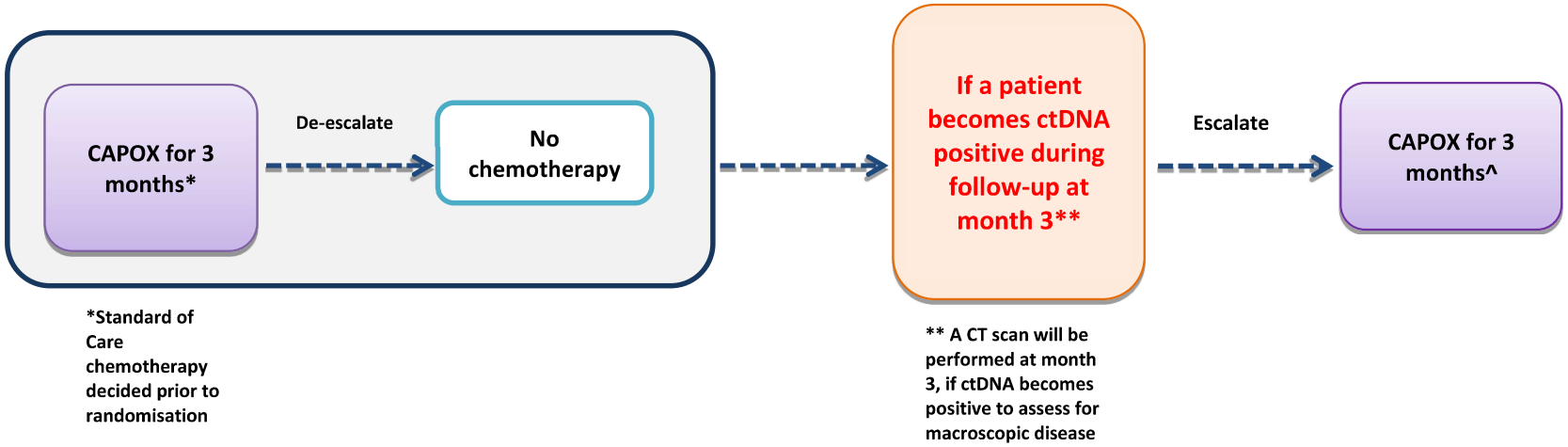
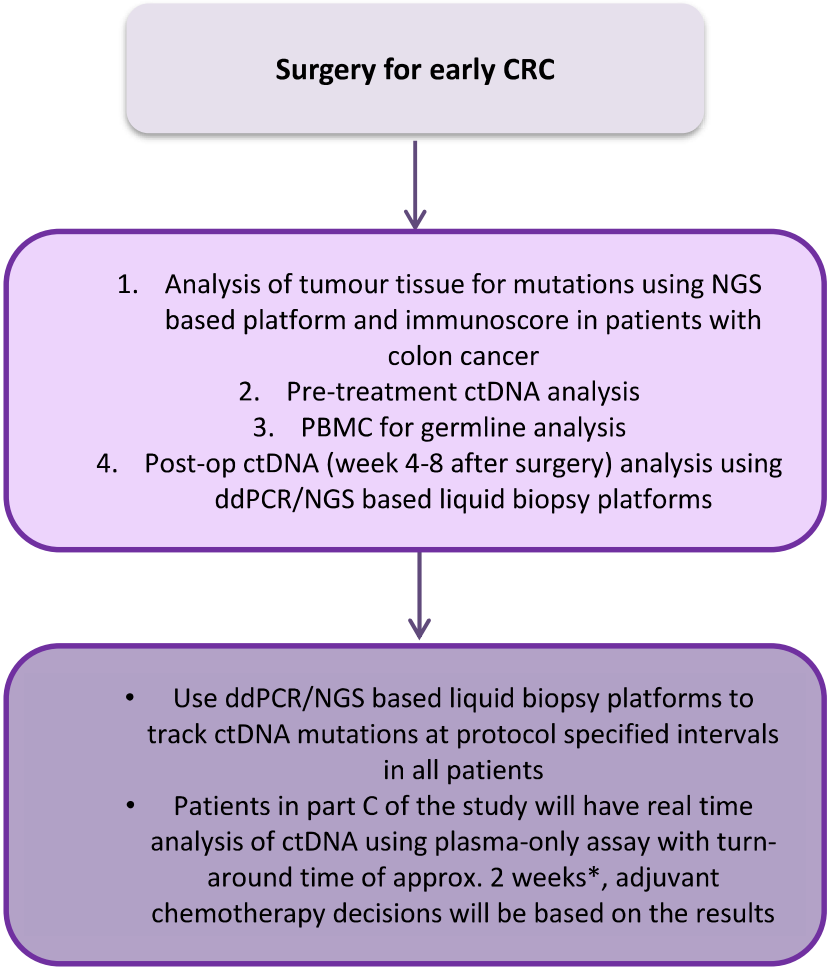


Figure 6: Analysis to be undertaken in tumour tissue and blood



*Patients in Part C of the study will have ctDNA analysis done post-operatively, if randomised to the ctDNA guided arm of the study and adjuvant chemotherapy will be de-escalated if ctDNA negative. Further real time analysis will be conducted at month 3, and decision to re-escalate will be undertaken based on ctDNA results.

(This has now been replaced by the Guardant Health Guardant Reveal ctDNA assay)

STUDY GLOSSARY

Abbreviation/Acronym	*Definition*
ASCO	American Society of Clinical Oncology
BRC	Biomedical Research Centre
CEA	Carcinoembryonic antigen
cfDNA	Circulating cell free DNA
CIMP	CpG island methylator phenotype
CIN	Chromosomal instability
CMP	Centre for Molecular Pathology
CMS	Colorectal cancer molecular sub-type
CRC	Colorectal cancer
CRCSC	Colorectal Cancer Subtyping Consortium
CRF	Case report form
CT	Computed tomography
ctDNA	Circulating cell free tumour derived DNA
DFS	Disease free survival
dMMR	Deficient mismatch repair status
DNA	Deoxyribonucleic acid
dPCR	Digital PCR
ddPCR	Droplet digital polymerase chain reaction
eCRF	Electronic case report form
EDTA	Ethylenediaminetetraacetic acid
FFPE	Formalin fixed paraffin embedded
H & E	Haematoxylin and eosin
ICR	Institute of Cancer Research
ITT	Intention to treat
KRAS	Kirsten-ras oncogene
LOD	Limit of detection
MMR	Mismatch repair
MRI	Magnetic resonance imaging
MSI	Microsatellite instability
MSI-H	Microsatellite instability high

NGS	Next generation sequencing
OS	Overall survival
PI	Principal Investigator
PIS	Patient information sheet
PP	Per protocol
pMMR	Proficient mismatch repair status
REC	Research Ethics Committee
RFS	Recurrence free survival
ROC	Receiver operating characteristic
RM	Royal Marsden NHS Foundation Trust
RNA	Ribonucleic acid
SOC	Standard of care
SOP	Standard operating procedure

Table of Contents

1. OBJECTIVES

Advances in sequencing technologies have enabled the characterisation of somatic genomic alterations in colorectal cancer (CRC). cfDNA refers to fragmented DNA in the cell free component of whole blood. A proportion of cfDNA carries the same tumour specific alterations and is termed ctDNA. We believe that ctDNA may be a surrogate for tumour burden and may reflect minimal residual disease in patients that have undergone curative surgery. This study aims to track ctDNA and assess whether detection ctDNA predicts for relapse in patients with stage II and III CRC that have undergone potentially curative surgery.

2. BACKGROUND

CRC is the fourth commonest cause of death from cancer worldwide, accounting for over 600 000 deaths per year.[1] The majority of patients presenting with stage I (Dukes' A) and low risk stage II (Dukes' B) can be cured by surgical resection alone. In patients with node positive stage III (Dukes' C) disease, adjuvant chemotherapy has been shown to reduce the risk of relapse and increase 5-year overall survival (OS) rates. In patients with stage II disease, any benefit from adjuvant chemotherapy is likely to be modest. In the case of rectal cancer, neo-adjuvant chemoradiation offers the benefit of reducing locoregional recurrence in appropriately selected patients.

2.0 Surveillance in CRC Following Surgery with Curative Intent

Despite multi-modality treatment, local or distant relapse remains a significant cause of mortality, thus highlighting the role for adequate surveillance to ensure the early detection of locoregional and oligometastatic disease, where curative treatment may still be possible. Four systematic reviews have demonstrated improved survival in patients undergoing intensive surveillance compared to minimal or no follow up with an estimated OS gain of 7-13%.[2][3][4][5]A recent trial compared minimal follow up to more intensive follow up with carcinoembryonic antigen (CEA) and/or computed tomography (CT) imaging.[6] Patients in the intensive follow up arm had a higher rate of surgical resection for recurrence with curative intent. The authors predict that observed absolute 6% increase in surgical resection rate would translate into a 2-3% OS advantage for intensive follow up.

In an analysis of 20 898 patients (66% with stage III and 33% with stage II disease), it was recognised that stage II patients tend to have later recurrences than stage III patients. During the 8 year follow up period, 74% of stage II patients who experienced a recurrence did so in the first 3 years compared to 82% of stage III patients. [7]

CEA is an oncofetal antigen that is widely accepted as a tool to monitor patients with CRC and assist in the early detection of recurrence. The most common sites of recurrence following resection include: liver (33%), lung (22%) and loco-regional (15% for colon, 35% for rectum and 14% for regional lymph nodes).[8] CEA monitoring can detect recurrent CRC with an average lead time of 5 months (range 4-10 months). [9]

CEA is elevated in approximately 75% of patients with recurrent CRC and will therefore miss a proportion of patients.[8] CEA is most sensitive for hepatic and retroperitoneal

metastases (approximately 70%) and least sensitive for local recurrences and peritoneal or pulmonary disease (<50%). Other limitations of CEA monitoring include the fact that CEA can be elevated with benign conditions and can rise with fluorouracil-based chemotherapy. The sensitivity and specificity of CEA for detecting recurrence is largely dependent on the cut off value used. With a cut off value of 5ng/ml, sensitivity is approximately 34% and specificity is approximately 84%.[10] In view of the limitations of CEA, new markers that would facilitate a more accurate and timely identification of recurrence are required.

There is limited data available regarding detection of cfDNA in patients with stage 1 colorectal cancer and we will include this group of patients as an exploratory cohort. The data generated will help decide if this technique is useful to detect early relapse in this group of patients.

2.1 Adjuvant Chemotherapy in Stage II and III disease

Adjuvant chemotherapy for patients with stage III colon cancer is currently the standard of care, as the risk of recurrence in such patients is estimated to be in the region of 15-50%[11] with an estimated 5 year OS of 30-60% with surgical resection alone.[12] Fluoropyrimidines alone lead to an absolute improvement of 10-15% in OS in stage III disease and the addition of oxaliplatin confers a further 4-5% benefit.[12] However, the survival benefit of oxaliplatin based chemotherapy may be limited to patients younger than 65 as demonstrated by the MOSAIC trial[13] or younger than 70 as reported by the ACCENT group database. [14]

The role of adjuvant chemotherapy for patients with stage II colon cancer, which represents 40% of resected cancers, [15] remains controversial. When compared to stage III disease, patients with stage II disease have a significantly better prognosis with a lower risk of recurrence and a better 5 year OS of 60-80% with surgical resection alone.[12] The QUASAR study which included both colon and rectal cancer patients, demonstrated a 3.6% improvement in OS with adjuvant fluorouracil and folinic acid chemotherapy compared to observation.[16] However, the improvement in survival could have been influenced by the fact that the trial encompassed both stage II and stage III patients, although patients with stage II disease represented the majority (91%). In this trial, patients with colorectal cancer aged >70 did not appear to benefit from adjuvant chemotherapy. In the MOSAIC study, there was no evidence of a 6-year OS benefit for the use of oxaliplatin based adjuvant chemotherapy compared to fluorouracil plus leucovorin in stage II patients. However, in an exploratory analysis of high-risk stage II patients, there was a non-significant trend towards increased 6 year OS in patients receiving oxaliplatin based chemotherapy (85% vs. 83.3%, p=0.648) but all patients receiving oxaliplatin experienced a much higher rate of grade 3 sensory peripheral neuropathy.

The small benefit of adjuvant chemotherapy in stage II patients must be weighed up against treatment toxicities, patient comorbidities and preferences. In addition, clinicopathological features such as: mismatch repair (MMR) status, T4 tumours, perforated tumour or bowel obstruction at the time of presentation, less than 12 lymph nodes removed during surgery, poorly differentiated histology and the presence of lymphovascular invasion should be taken into consideration. Although these clinico-pathological features have useful prognostic value, they do not necessarily correlate with

tumour biology and as a consequence of this, they fail to accurately predict response to adjuvant chemotherapy.

In stage II CRC patients, the QUASAR study[16] showed a statistically significant improvement in survival in patients receiving adjuvant chemotherapy (80.3% versus 77.4%) with a reduction in recurrence (22.2% versus 26.2%), and the improvement is more in patients with high risk disease (T4 disease, perforation, obstruction, < 10 nodes examined, poorly differentiated histology or extramural vascular or lymphatic invasion), although not statistically significant. In patients with high-risk disease, single agent capecitabine is used for a period of 6 months as 3- weekly cycles. Recurrence rates in stage II CRC is around 15% and SEER analysis calculated that 30% of stage II patients receive adjuvant chemotherapy.

Adjuvant chemotherapy is recommended in patients with stage III CRC. Until 2017, a total of six months of adjuvant chemotherapy was recommended. The SCOT study reported 3 months of adjuvant chemotherapy was non-inferior to 6 months of treatment in high risk stage II and stage III CRC patients.[17] In a study of 6088 patients were randomised in a 1:1 manner to receive adjuvant chemotherapy for 3 months or 6 months. The 3 year disease-free survival (DFS) in the 3-month group was 76·7% (95% CI 75·1–78·2) and in the 6-month group was 77·1% (75·6–78·6) for the 6 month group [HR: 1·006 (0·909–1·114, test for non-inferiority p=0·012)], significantly below the non-inferiority margin. The rate of peripheral neuropathy was 58% in the 6-month group versus 25% in the 3 month group. Based on these results, currently in the UK the practice has changed and patients with stage III CRC receive either 3 months of doublet chemotherapy with capecitabine and oxaliplatin (CAPOX) or 6 months of 5-flurouracil and oxaliplatin (FOLFOX) or 6 months of single agent capecitabine. Discussions regarding adjuvant chemotherapy are guided by age of the patient (<70 years), number of nodes involved and patient's wishes. Around 50% of patients with stage III disease in the UK receive doublet chemotherapy.

2.2 Molecular Biology of Colorectal Cancer

Understanding the heterogeneous molecular mechanisms underlying CRC would enable us to better correlate clinico-pathological features to the underlying molecular biology. This would in turn facilitate the development of better therapies and biomarkers of treatment response.

One of the early mechanisms proposed was the adenoma-carcinoma sequence driven by early *APC* gene mutations, activating *KRAS* mutations and inactivating *TP53* mutations in the presence of chromosomal instability (CIN). Later on it became apparent that about 15% of sporadic colorectal cancers develop through distinct molecular pathways. These cancers include those originating from serrated precursor lesions and are characterised by the CpG island methylator phenotype (CIMP) and activating BRAF oncogene mutations. Most cancers arising from sessile serrated adenomas display the high-level microsatellite instability (MSI-H) phenotype, as a consequence of MLH1 gene promoter methylation, and occur in the proximal colon of elderly patients, with a female predominance. [11][15]

Over recent years our understanding of CRC biology has substantially increased owing to improved high throughput genomic and proteomic technologies. In 2012, the Cancer Genome Atlas Network reported a comprehensive molecular characterisation of human colon and rectal cancer.[18] 16% of colorectal carcinomas were found to be hypermutated and the majority of this sub-group harboured the expected MSI-H phenotype. About a quarter of this hyper-mutated subgroup had somatic mismatch-repair gene and polymerase e (POLE) mutations. In addition, 46% of these tumours were found to have *BRAF* mutations. Excluding the hypermutated cancers, colon and rectum cancers were found to have considerably similar patterns of genomic alterations. In this non-hypermutated group, twenty-four genes were reported to be frequently mutated, including previously recognised genes such as: *APC* (81%), *TP53* (60%), *KRAS* (43%), *PIK3CA* (18%), *SMAD4* (10%), *NRAS* (9%) and other less recognised genes such as *ARID1A, SOX9 and FAM123B*.

More recently, a number of independent groups attempted to identify novel molecular signatures in CRC. These groups collaborated and formed the Colorectal Cancer Subtyping Consortium (CRCSC), in order to reach a consensus among the subtyping systems.[19] Despite the heterogeneity in cohorts and methods, subtype concordance analysis yielded a clear consensus on 4 CRC molecular subtypes (CMS1-4), enriched for key clinical, pathway and molecular traits. The 4 subtypes are depicted in the figure below. 16% of samples could not be assigned to the four distinct subtypes and the CRCSC speculated that this is likely to be due to an additional mixed subtype with variable epithelial-mesenchymal activation. The prognostic and predictive significance of these newly classified CRC subtypes is yet to be determined.

Table 1: 4 CRC molecular subtypes (extracted from the CRCSC)[20]

Molecular Subtype	Frequency	Characteristics
CMS1	14%	MSI, immune pathway activation/expression, right-side tumours, older age at diagnosis, females, hypermutation, *BRAF* mutant, intermediate survival
CMS2	41%	High CIN, MSS, strong WNT/MYC pathway activation, left-side tumours, *TP53*mutant, *EGFR* amplification/overexpression, better survival
CMS3	8%	Low CIN, moderate WNT/MYC pathway activation, *KRAS* mutant, *PIK3CA* mutant, IGFBP2 overexpression, intermediate survival
CMS4	20%	CIN/MSI heterogeneous, mesenchymal/TGF-beta activation, younger age at diagnosis, NOTCH3/VEGFR2 overexpression, worse survival

In the 2014 the American Society of Clinical Oncology (ASCO) conference, an informative analysis of the molecular characteristics of stage III colorectal cancer was presented. In particular, 3,018 stage III CRC samples were analysed and classified into 5 distinct sub-groups based on: the presence or absence of *BRAF*^{V600E}, *KRAS* (codons 12, 13) mutations, MMR deficiency (dMMR) *vs* proficiency MMR (pMMR) and the methylation of *MLH1*. [20]The five sub-groups identified were:

1. Traditional: (pMMR, wild-type BRAF and KRAS), 49% of all tumours
2. Alternate: (pMMR, wild-type BRAF, mutant KRAS), 35% of all tumours
3. Serrated:(pMMR, mutant BRAFV600E, wild-type KRAS),6.9% of all tumours
4. Serrated:(dMMR, mutant BRAFV600E, wild-type KRAS, hypermethylated MLH1), 6.8% of all tumours
5. Familial dMMR: (wild-type BRAF, any KRAS, unmethylated MLH1) 2.6% of all tumours

These 5 subtypes had distinct clinico-pathological features. For example, when comparing the traditional subtype to the alternate or serrated pMMR subtypes, the traditional pMMR tumours were more likely to arise in males (58% *vs* 51% or 41%; p<0.0001) and were more likely to be left sided tumours (67% *vs* 42% or 24%; p<0.0001). When comparing serrated and traditional pMMR CRCs, serrated pMMR tumours were more likely to be of high-grade histology (44% *vs* 19%, p <0.0001) and N2 stage (59% *vs* 41%, p<0.0001).

These subtypes of stage III CRC also appeared to be associated with distinct prognosis. Alternate or serrated pMMR tumours showed a statistically significant worse disease-free survival (DFS) when compared to the traditional subtype. Moreover, when compared to alternate CRCs, traditional [HR= 0.68 (0.58 -0.79), p<0.001], serrated dMMR [HR=0.73 (0.54-.99), p=0.042], and familial dMMR [HR=0.51 (0.30-.87), p=0.0130] CRCs showed favourable DFS. The DFS of dMMR and traditional subtypes were similar. The predictive role of these subtypes is yet to be determined.

2.3 Prognostic and Predictive Biomarkers in CRC

It is recognised that a proportion of patients with early colorectal cancer will be cured by surgical resection alone. Therefore, finding prognostic biomarkers that would identify patients with a better inherent outlook independent of treatment and predictive biomarkers that would determine groups of patients that are likely to benefit from specific chemotherapy regimens is of paramount importance, as this would spare a group of patients from the toxicities of chemotherapy. The identification and development of these biomarkers has been based on our increasing understanding of molecular biology. It is anticipated that the recent molecular classifications described above would lead to better biomarkers for the management of CRC cancers patients in the adjuvant setting.

To date the only marker of proven prognostic and predictive significance that is used in clinical practice for the management of patients with stage II CRC is the presence of microsatellite instability high (MSI-H) phenotype. This was based on the pooled results of a systematic review of 7642 patients with colorectal cancer which reported that patients with MSI-H had a better overall prognosis and did not benefit from fluorouracil adjuvant chemotherapy when compared to patients with microsatellite stability.[21] MMR genes encode for proteins which normally repair bases that are incorrectly added to, or deleted from, microsatellites (segments of nucleotide repeats) during DNA replication. In the presence of mutated MMR genes, base repair mechanisms are ineffective, leading to microsatellite instability (MSI). In clinical practice, immunohistochemistry is used to test for loss of the MMR gene products as a surrogate for MSI.

The prognostic role of KRAS and BRAF mutation status in stage II and III CRC has been investigated using the formalin fixed paraffin embedded (FFPE) samples from over 1000 patients.[22]KRAS and BRAF tumour mutation rates were 37.0% and 7.9%, respectively, and were not significantly different according to tumour stage. In conclusion, in stage II-III colon cancer, the KRAS mutation status did not have major prognostic value, whereas BRAF was of prognostic relevance for OS in patients with MSI-stable or MSI-low tumours. In the metastatic setting RAS mutation status is an established negative predictive biomarker for response to EGFR antibodies, however, in the adjuvant setting RAS status has not been proven to be of similar predictive value.

Multigene assay, Oncotype DX Colon Cancer Recurrence Score (Genomic Health, Redwood City, CA) has been shown in prospective studies to be a useful prognostic marker of recurrence in stage II and III CRC.[23][24]. This assay is able to calculate a recurrence score by measuring the expression of 12 genes (seven recurrence genes and five reference genes) in FFPE primary colon tumour tissue. This assay, which has been commercially licensed since 2010, has been shown to predict recurrence risk independently of pathologic T stage, tumour grade, number of nodes examined,

lymphovascular invasion and microsatellite status. However, assays such as this do not address the question of minimal residual disease following potentially curative resection.

Table 2: Common Mutations in CRC

Mutation	Frequency	Additional Information
KRAS[22][25] codon 12, 13, 61 and 146	34-37% in stage II and III CRC	85-90% of KRAS mutations in CRC occur in codons 12 or 13, the remainder mainly comprise codon 61 (5%) and 146 (5%)[25]
BRAF V600E mutation[22][25]	8-10% in stage II and III CRC	BRAF and KRAS mutually exclusive
NRAS[25] codons 12, 13 and 61 with mutations in codon 61 being the most common	3-5% in stage II and III CRC	NRAS and KRAS mutually exclusive
PIK3CA[25][26] exon 9 and exon 20 mutations	11-12% in stage II and III CRC	PIK3CA and KRAS/NRAS/BRAF mutations may co-exist >80% of PIK3CA mutations in CRC occur in exon 9 (60-65%) and exon 20 (20-25%)[27]
TP53[25]	44 % in stage II and III CRC	60% (non hyper-mutated) vs. 20%(hyper-mutated)[17]
APC[18]	81% (non hyper-mutated) vs. 51% (hyper-mutated)	

2.4 Circulating tumour DNA

Circulating cell free DNA (cfDNA) refers to fragmented DNA in the cell free component of whole blood. It was first discovered in 1948 by Mandel and Metais.[28] In 1977, Leon et al.[29] recognised that cfDNA is present in much higher concentrations in cancer patients than in healthy individuals and a few years later, Stroun et al.[30][31] found that a proportion of cfDNA is tumour derived (ctDNA) and therefore maintains the genomic alterations present in the original tumour. The concept that a proportion of cfDNA is tumour derived was proven in studies of pancreatic cancer patients where mutations in the *KRAS2* oncogene that were detected in cfDNA in the plasma of these patients, were found to be identical to those detected in their original pancreatic tumour. [32][33][34][35][36]

The exact mechanism accounting for the release of cfDNA into blood has yet to be elucidated but multiple theories have been proposed. In healthy individuals, lymphocytes and other nucleated cells are likely to contribute to a significant proportion of the cfDNA. In individuals with cancer, it has been suggested that cfDNA arises from deficient phagocytosis of apoptotic and necrotic cells due to the high cell turnover associated with tumour growth resulting in accumulation of cellular debris which is released into the circulation.[37][38] Other hypotheses include: the tumour may be actively releasing DNA fragments into blood or the cfDNA arises from lysis of circulating cancer cells or micro-metastases shed from the tumour. [39]

cfDNA has been actively researched as a prognostic tool in many areas of medicine[40][41][42] due to the recognition that levels can increase under certain conditions such as cellular injury or necrosis but the most success has been seen in foetal medicine, where foetal cfDNA has been detected in maternal plasma and analysed to detect germline anomalies.[43][44][45][46] In oncology, the presence of genetic aberrations such as somatic mutations, which are unique to ctDNA and absent in the remainder of the cfDNA provides many potential applications and opportunities to exploit. However, the challenge has largely been related to the fact that ctDNA often only represents a very small proportion of the total cfDNA (<1.0%) and therefore requires highly sensitive and reliable detection techniques.

The advent of digital genomic technologies and next generation sequencing has made the detection of rare mutant variants within normal DNA a reality. Potential clinical applications of ctDNA which require further investigation include screening for cancer, the early detection of relapse after potentially curative treatment, the detection of minimal residual disease, monitoring tumour dynamics in response to therapy, identification of targetable genetic alterations without the need for a tumour biopsy and the assessment of the emergence of resistant clones in response to treatment.

The role of ctDNA as a circulating biomarker has generated much interest over a spectrum of tumour types. In a recent study in metastatic breast cancer, Dawson et al.[47] utilised targeted deep sequencing to screen for point mutations in *PIK3CA* and *TP53,* followed by whole genome paired-end sequencing of tumour tissue and matched normal tissue and identified genomic alterations including point mutations and structural variants in 30 out of 52 patients. In these 30 patients, digital PCR or tagged amplicon deep sequencing of plasma was performed to track genomic alterations and demonstrated that ctDNA was detected in 97% of patients. In a subset of samples where both digital PCR and tagged amplicon deep sequencing were utilised, the technologies showed excellent concordance. The digital PCR assay allowed for the detection of a mutant allele fraction of at least 0.1% (one mutant molecule in a background of 1000 wild type molecules) and the sensitivity of tagged amplicon deep sequencing allowed for the detection of a mutant allele fraction of at least 0.14% with a confidence margin of 0.95. The median quantity of ctDNA across all samples was 150 amplifiable copies per millilitre of plasma and the median mutant allele fraction was 4% (interquartile range, 1 to 14). The study showed that ctDNA has superior sensitivity to other circulating biomarkers and often provides the earliest measure of treatment response. The role of ctDNA to predict relapse has also been investigated in early breast cancer in patients who underwent neo-adjuvant chemotherapy followed by surgery.[48] In this study, 60% of tumours had at least one mutation identified by targeted next generation sequencing and tumour specific mutations were detected in 75% of cfDNA samples at baseline. All patients that had mutations in cfDNA detectable post-surgery relapsed whereas none of the patients that had not relapsed had tumour specific mutations detectable in cfDNA post-surgery indicating clearance by the primary treatment. These findings suggest that tracking tumour specific mutations in cfDNA can predict early relapse however, the results are based on a cohort of only 20 patients and the length of follow-up was limited.

2.5 ctDNA as a biomarker in CRC

A number of studies have also investigated the role of ctDNA as a circulating biomarker in colorectal cancer and selected studies have been summarised in table 3 below. These studies have been very heterogeneous in that they used different methodologies, included patients with different stages of disease, assessed the detection of ctDNA at varying time-points and looked for different genomic alterations in cfDNA. Moreover, the studies included small sample sizes. Nevertheless, the results of studies to date have consistently shown that ctDNA may show promise as a measure of tumour burden. ctDNA levels have been shown to correlate with stage of disease and in a recent publication, ctDNA was shown to be detectable even in localised CRC where it was detected in 73% of patients compared to 100% of patients with metastatic disease, using next generation sequencing (NGS).[49]

The technology used to detect ctDNA impacts upon the likelihood of ctDNA detection. To date, digital polymerase chain reaction (dPCR) is considered the most sensitive technique for this purpose with some platforms being reported to be able to detect 1 mutant allele in a background of 100 000 wild type deoxyribonucleic acid (DNA) fragments.[50] Each technique has its own limitations and advantages. The main limitation of dPCR is that probes for the detection of each mutated allele are usually required and often the patient's tumour tissue needs to be tested in advance to determine the specific somatic mutations to track in plasma. On the other hand, whilst NGS can be used to analyse multiple genes at a time, it is limited by its lower sensitivity for detecting rare variants, the requirement for better quality DNA and the need for extensive data interpretation that often involves dedicated bioinformatics.

2.6 ctDNA pre-analytical considerations

It has been difficult to draw any definitive conclusions from ctDNA studies to date due to the variation in processing of samples. The matrix utilised has been serum in some studies whereas other studies have used plasma. Although increased levels of cfDNA have been detected in serum, evidence suggests that this is likely to be the result of contamination from genomic DNA released by white blood cells during the clotting process. Consequently, ctDNA is diluted by increased levels of non-specific genomic DNA. Plasma has therefore been recommended as the matrix of choice. [51]

The importance of appropriate blood collection tubes has also been established. The use of heparin as an anticoagulant should be avoided due to potential interference with downstream applications involving PCR. Ethylenediaminetetraacetic acid (EDTA) tubes are recommended for blood collection where samples can be processed soon after blood draw. Alternatively, cell free DNA blood collection tubes (Streck tubes) have been shown to maintain stability of ctDNA when blood processing is likely to be delayed.[52][53]

Table 3: Selected Studies evaluating the relationship between ctDNA in plasma and prognosis in Colorectal Cancer

Study	**Population**	**Genomic alterations investigated (and techniques utilised for ctDNA detection)**	**Blood Sampling time-points**	**Key Results**
Diehl 2008[54]	N=18 (total of 22 surgeries) Stage II=1 Stage III=1 Stage IV=16	*APC, KRAS, TP53, PIK3CA* (BEAMing)	Pre-surgery Post-op day 1 Post-op days 2-10 Post-op days 13-56	-Each tumour was found to have at least 1 mutation -ctDNA was detectable in all pre-surgery samples -17 out of 22 surgeries involved complete resections whereas 5 were incomplete resections -Of the 5 cases with incomplete resections, ctDNA levels increased 24 hours after surgery in 3 cases and ctDNA levels decreased only slightly in the other 2 cases -half-life of ctDNA estimated to be 114 min -Plasma samples were available from the first follow up visit (13-56 days post-op) in 20 instances -ctDNA was still detectable in 16 out of 20 instances and recurrences occurred in all but 1 of these. No recurrence occurred in cases where ctDNA was undetectable at the first follow up visit P=0.006
Frattini 2008[55]	N=70 Stage II=36 Stage III=28 Stage IV=6	*KRAS, p16$^{INK4\alpha}$* (mutant enriched PCR and fluorescent-methylation specific PCR)	Pre-surgery 4, 10 and 16months post-op	-18 samples were tested for genomic alterations in tumour and plasma -13 out of 18 (72%) had the same alterations present in tumour and plasma

				-11 patients remained disease free and none had detectable ctDNA post-op. 7 out of these 11 patients had ctDNA detectable pre-surgery -6 out of 7 patients (86%) with a recurrence had ctDNA detectable
Lecomte 2002[56]	N=58 Stage I=8 Stage II=21 Stage III=16 Stage IV=13	*KRAS, p16* (mutant allele specific amplification and methylation specific-PCR) Sensitivity of assay for *KRAS* was able to detect 1 mutant allele out of 1000 normal alleles For *p16*: 1 methylated genome in 500 unmethylated genomes could be detected	Pre-surgery	-39 out of 58 (67%) tumours had at least one genetic alteration - In 2 out of 39 patients, plasma DNA status was undetermined -26 out of 37 (70%) patients with a detectable genetic alteration in tumour, had detectable ctDNA - Mean length of follow up was 22 months -2 year OS was 48% in patients where ctDNA was detectable compared to 100% where ctDNA was undetectable (P<0.03) -25 out of these 37 patients had stage I, II or III disease and in this subgroup, the 2 year recurrence free survival rate for the 17 patients with detectable ctDNA was 66% compared to 100% for the 8 patients without detectable ctDNA (P=0.044)
Tie 2014[57]	N=250 All stage II patients with a subset of 175 patients having serial samples	*TP53, APC, KRAS, NRAS, BRAF, PIK3CA, CTNNB1, SMAD4*, and *FBXW7*	Post-operatively at 4-10 weeks and then 3 monthly for a subset of 175 patients	-In 178 patients who did not receive adjuvant chemotherapy, ctDNA was detected in 14 (7.9%) patients of whom 11 (79% of 14 patients) relapsed during median follow-up of 27 months

	230 patients with stage II colon cancer.	(massively parallel sequencing platform, Safe-SeqS)		-Of the 164 patients who were ctDNA negative only 16 (9.8%) had recurrence [hazard ratio (HR), 18; 95% confidence interval (CI), 7.9 to 40; P < 0.001]. ctDNA was positive in 6 of 52 patients (11%) who received at least 3 months of adjuvant chemotherapy -patients in whom ctDNA was positive immediately after completion of adjuvant chemotherapy had an increased risk of recurrence (HR, 11; 95% CI, 1.8 to 68; P = 0.001) with ctDNA preceding radiological recurrence -The sensitivity of ctDNA in predicting relapse at 36 months was 48% with a specificity of 97% -Patients with negative ctDNA post-operatively had low risk of radiological relapse, 3 year RFS of 90%
Tie 2018[58]	N= 159 patients, 462 samples Locally advanced rectal cancer (T3/4 and/or N+)	SafeSeq	ctDNA pre-treatment, post-chemoradiotherapy, 4-10 weeks after surgery	-ctDNA levels was detected in 77%, 8.3% and 12% of pre-treatment, post-chemoradiotherapy and postsurgical plasma samples -Recurrence-free survival was significantly worse in patients in whom ctDNA was detected after chemoradiotherapy (HR 6.6; p<0.001) or after surgery (HR 13.0; p<0.001), and postoperative ctDNA was predictive of recurrence irrespective of adjuvant chemotherapy use (chemotherapy: HR 10.0; p <0.001; without chemotherapy: HR 22.0; p<0.001)

Tie 2018[59]	Stage III colon cancer	SafeSeq	Serial plasma samples post-surgery, during chemotherapy and at treatment completion	-An inferior recurrence-free survival (RFS) in the 19 of 95 pts (20%) with positive ctDNA post-surgery (HR, 3.52; p = 0.004). -ctDNA status changed from negative to positive after CT in 6 of 71 pts (8%) and was associated with an inferior RFS (HR 5.30; p = 0.006).
Lindforss 2005[60]	N=25 Stage I=4 Stage II=7 Stage III=13 Stage IV=1	*KRAS* (temperature gradient gel electrophoresis)	Pre-surgery Day 3 post Surgery	-16 out of 25 patients (64%) had KRAS mutations in tumour tissue -9 out of the 16 patients (56%) had KRAS mutations in plasma pre-operatively - On day 3 post surgery, 8 out of the 16 patients (50%) still had KRAS mutations in plasma -There was no significant correlation between relapse of disease (38months, mean) during follow-up and presence of KRAS mutations in the tumour (p=0.40) or in plasma post-operatively on day 3 (p=0.99)
Bazan 2006[61]	N=66 Stage I=18 Stage II=18 Stage III=26 Stage IV=4	*KRAS, TP53, p16$^{INK4\alpha}$* (PCR-single strand conformational polymorphism and methylation specific PCR of bisulfite modified DNA)	Pre-surgery	-In 50 out 66 patients (76%), at least one genetic alteration was found in tumour tissue -18 out of the 50 patients (36%) had the same genetic alteration in plasma -Median follow-up of 26 months: the presence of *KRAS* mutations was related with a quicker relapse (P<0.01), a trend towards statistical significance was observed for *TP53* (P=0.083)

ctDNA detection using NGS based liquid biopsy panels

Commercial NGS based panels for ctDNA analysis are currently used as research and clinical tools in patients with metastatic cancers including CRC. The amount of tumour derived cell-free DNA (ctDNA) in smaller, early to mid-stage tumours can be very low and is usually at or below 0.1% VAF (variant allele frequency). With rapid technological advances, NGS ctDNA assays have a high degree of sensitivity and specificity to detect ctDNA in early stage CRC. Assays may be tumour informed (the tumour is sequenced to identify variants to track) or tumour naïve (blood ctDNA is sequenced alone).

Guardant Health (Redwood City, CA) has developed and validated its tumour-naive CLIA-certified LUNAR1 assay (recently commercialized under the name Guardant Reveal), an NGS-based assay for the qualitative detection of residual disease in individuals with CRC undergoing curative intent treatment. The assay evaluates the presence of ctDNA based on multiple analytic features, including detection of tumour-derived mutations and methylation signals and returns a result of “ctDNA detected” or “ctDNA not detected”. The integrated assay accurately reports genomic alterations down to allele frequencies of 0.01%, and effectively bioinformatically filters out biological noise sources such as mutations caused by clonal haematopoiesis. The addition of methylation analysis improved sensitivity by 36% over genomic assessment alone. In a study of 64 patients with colorectal cancer undergoing curative-intent treatment, the Guardant Reveal assay showed 100% positive predictive value (PPV) for a ctDNA detected result to predict recurrence, 56% sensitivity to detect participants who will ultimately recur using a single four week post-treatment “landmark” timepoint, and >90% sensitivity for recurrence using serial ctDNA assessment in the surveillance setting detection in CRC using a plasma-only integrated genomic and epigenomic ctDNA assay [62]. Since then, an improved version of the Guardant Reveal assay has been analytically validated, showing high concordance with the previous version (88% (44/50) positive predictive agreement and 98.8% (84/85) negative predictive agreement). The improved assay is anticipated to have similar clinical sensitivity and improved specificity (>99% vs. 95%).

2.7 ctDNA guided adjuvant chemotherapy

There is increasing evidence that ctDNA can reliably predict for minimal residual disease in patients with stage II or III CRC who have undergone curative surgery. In a study in 145 patients with stage II or stage III CRC who underwent R0 resection [63] Roche Avenio ctDNA kits were used to assess somatic mutations in 197 cancer-related genes in tissue and in plasma taken post-operatively (mean 10 days post-op). Patients were considered ctDNA positive or negative in post-operative plasma based on a single detection of single nucleotide variants (SNVs) in tumour tissue at an allele frequency of at least 5%. Of the 144 patients with tissue variants, 93 (65%) were colon and 51 (35%) were rectal. Of the 144 patients, 12 patients were ctDNA positive (8%) and 132 (92%) were ctDNA negative. Of the stage II patients, 4 out of 85 were ctDNA positive (5%) and in stage III patients 8 out of 59 patients (14%) were ctDNA positive. Median follow-up was 32 months. Time to recurrence, relapse free survival and OS was significantly shorter in patients who are ctDNA positive (n =12) compared to negative patients (n= 132). The sensitivity for predicting recurrence was 57.1% (12 out of 21 patients) with a specificity of 100% and

positive predictive value of 100%. Post-operative ctDNA was an independent predictor of recurrence in both stage II and III irrespective of adjuvant treatment.

In a study[59] in 95 patients with stage III colon cancer treated with adjuvant chemotherapy, an inferior disease free survival was noted in 20% patients who were ctDNA positive post-surgery (HR, 3.52; p = 0.004). In patients who were ctDNA positive post-op, adjuvant chemotherapy rendered 50% of them negative at time of completion of treatment. Patients who remained ctDNA positive at end of adjuvant chemotherapy had an inferior DFS (HR: 7.14; p < 0.001). In 5% of patients in this study ctDNA changed from negative to positive (HR: 5.30; p = 0.006) within 3 months.

There is lack of consensus around adjuvant chemotherapy in patients with locally advanced rectal cancer LARC who have undergone neo-adjuvant radiotherapy or chemoradiotherapy. The PETTAC6 data presented at ASCO previously in 2014 and in 2018 in patients with locally advanced rectal cancer who received neo-adjuvant chemoradiotherapy has shown a 3-year DFS of 72% with adjuvant capecitabine and no additional benefit with the addition of oxaliplatin.[64] A prospective study in patients with LARC (T3/T4 and/or N+) receiving chemoradiotherapy measuring ctDNA levels pre-treatment, post-chemoradiotherapy and 4 -10 weeks after surgery was conducted in 159 patients and 462 samples analysed.[58] ctDNA levels was detected in 77%, 8.3% and 12% of pre-treatment, post-chemoradiotherapy and postsurgical plasma samples respectively. Recurrence-free survival was significantly worse in patients in whom ctDNA was detected after chemoradiotherapy (HR 6.6; p<0.001) or after surgery (HR 13.0; p<0.001), and postoperative ctDNA was predictive of recurrence irrespective of adjuvant chemotherapy use (chemotherapy: HR 10.0; p<0.001; without chemotherapy: HR 22.0; p<0.001). Postoperative ctDNA levels could potentially be a prognostic biomarker, identifying patients with high or low risk of recurrence.

In a prospective study of 230 patients with resected stage II CC, 1046 plasma samples were analysed using Safe-Seq.[57] Post-op ctDNA was detected in 20 of 230 patients (9%). In 179 patients who did not receive adjuvant chemotherapy 14 patients (7.9%) were ctDNA positive of whom 11 (79%) had recurrence at median follow-up of 27 months; sixteen of 164 patients (9.8%) of patients who were ctDNA negative recurred [hazard ratio (HR), 18; 95% confidence interval (CI), 7.9 to 40; P < 0.001]. Patients with persistence of ctDNA after completion of chemotherapy has significantly reduced recurrence-free survival (HR, 11; 95% CI, 1.8 to 68; P = 0.001).

2.8 Immunoassays

Early detection of colorectal cancer can help improve morbidity and mortality. Due to low compliance with faecal-based screening and colonoscopy, various groups have designed non-invasive alternatives including blood-based multiplexed immunoassays for early detection. [65]

In a study of over 1005 patients with early stage, clinically detected cancers including CRC, a 61-amplicon panel PCR-based sequencing panel was positive in a median of 70% of the eight cancer types with sensitivities ranging from 69 to 98% for the detection of five

cancer types and specificity > 99% and anatomical localisation in a median of 83% of the patients.[66] The study will also potentially explore immunoassays in recruited patients that will help inform early relapse and develop methodologies for early detection of CRC helping the national health strategy for better screening procedures.

3. RATIONALE

Post-treatment follow up with CEA and CT scans for patients with resectable stage II and III CRC remains the current standard of care, as this is associated with higher rates of curative surgery for CRC recurrence and a subsequent improvement in OS. However, CT scans are not useful for identifying micro-metastatic disease and the number of scans that can be performed to detect recurrence is limited by the risks associated with frequent exposure to radiation and contrast media. CEA has other limitations including its potential to be elevated in benign conditions, as well as its low sensitivity for the detection of micro-metastatic disease.

ctDNA is a cancer specific marker because by definition somatic cancer mutations are identified by their presence in tumour DNA and absence in matched normal DNA. CtDNA has a short half-life and changes in its levels can potentially predate those seen on imaging studies or other biomarkers. CtDNA is detectable in over 70% of patients with localised CRC.[49] In addition, in CRC, primary tumours and metastases exhibit high genomic concordance.[67] Therefore, tracking and quantifying mutations in key genes, such as *APC, KRAS* and *TP53*, that are known to play a key role in early CRC and persist in metastatic disease in cfDNA is a promising strategy for the identification of minimal residual disease and recurrent disease.

This is further supported by previous CRC trials that utilised ctDNA and showed that by tracking ctDNA in the plasma of patients with CRC post-operatively, it was possible to predict disease recurrence, although these conclusions were admittedly based on a small sample size and included patients with all stages of disease. Diehl et al. monitored ctDNA after 22 CRC resections.[54] Plasma was available in 20 cases at the first follow up visit (range 13-56 days). CtDNA was detectable in 16 out of 20 instances and recurrences occurred in all but 1 of these. No recurrence occurred in cases where ctDNA was undetectable (p=0.006). More recently, an ongoing study provided preliminary results supporting the use of ctDNA 4-10 weeks post-operatively in stage II CRC patients that have undergone potentially curative surgery to predict relapse.[57]

Studies to date have provided promising evidence for the role of ctDNA in predicting relapse in CRC. However, due to the small sample sizes and heterogeneous nature of these studies which encompassed different methodologies, different disease stages, assessed the detection of ctDNA at varying time-points and looked for different genomic alterations in cfDNA, no definitive conclusions can be drawn.

We therefore propose an adequately powered study with standardized ctDNA analysis to establish whether there is an association between detectable ctDNA at the first post-operative visit and RFS. We will also attempt to elucidate the significance of ctDNA levels at other time-points. The study may help ultimately provide a platform for identifying a subset of patients that can be spared from adjuvant chemotherapy and its associated

toxicity safely on the basis of ctDNA analysis and the likelihood of relapse. Additionally, the study will allow us to assess whether in patients with detectable ctDNA post-operatively, completion of adjuvant chemotherapy renders ctDNA undetectable and consequently influences prognosis.

Improvements in technology allow some platforms to be able to detect rare variants at a level as low as 1 mutant allele in a background of 100 000 wild type DNA fragments. However, the clinical significance of such sensitive LOD remains to be determined. In an exploratory analysis we will also aim to evaluate what threshold of ctDNA is likely to be clinically meaningful in predicting relapse in early CRC patients.

The Bowel Cancer UK Critical Research Gaps in Colorectal Cancer Initiative has identified development of biomarkers that define the optimal curative therapeutic strategy preventing overtreatment and improving treatment selection as one of the major research gaps that requires to be addressed [68]. There is increasing evidence of the clinical utility of circulating tumour DNA (ctDNA) in detection of minimal residual disease in patients in patients with stage II and III colorectal cancer who undergo potentially curative surgery. Circulating DNA (ctDNA) guided adjuvant chemotherapy in this group of patients could potentially help individualise risk stratification, thereby reducing the use of unnecessary chemotherapy and tailor treatment decisions helping deliver personalised medicine.

Plasma samples from the ongoing TRACC study are currently being analysed as per protocol on behalf of the Royal Marsden Hospital. Different technological assays including ddPCR (digital droplet PCR) and NGS panels are being utilised to analyse samples, allowing for technical validation of ctDNA results in a systematic and centralised manner. The use of these highly sensitive assays allows for detection of even small amounts of DNA from early-stage tumours, which is at or below 0.1% variant allele frequency (VAF). With these technologies variant allele frequency of 0.05% to >1% can be assessed.

In light of accumulating scientific evidence and recent improvements in the sensitivity of the technological assays allowing for ctDNA in detecting residual microscopic disease, we amended the protocol to include a nested biomarker guided interventional study. This approach allows for rapid transition of research into the clinic that will ultimately benefit patients and also help NHS save costs on chemotherapy.

4. HYPOTHESES

Hypothesis 1: In patients with stage II and III CRC, detection of mutations in ctDNA in plasma can predict relapse.

Hypothesis 2: We hypothesise that ctDNA directed adjuvant chemotherapy administration will enable biomarker driven selection of patients who would benefit from adjuvant chemotherapy and thereby reduce proportion of patients receiving adjuvant chemotherapy without compromising disease free survival

5. STUDY AIMS AND OBJECTIVES

5.0 Study Aims

To assess whether ctDNA is detectable pre-operatively in stage I, II and III CRC and determine whether detection of ctDNA following surgery with curative intent is able to predict relapse and help identify the patients who are or are not likely to benefit from adjuvant chemotherapy.

5.1 Primary Objective

For Part A (Feasibility):

- To assess whether circulating cell free tumour derived DNA (ctDNA) is detectable in patients with stage II and III CRC pre-operatively

For Part B (Translational Study):

- To assess whether detection of ctDNA predicts for relapse in patients with stage II and III CRC that have undergone surgery with curative intent

For Part C (Randomised ctDNA guided adjuvant chemotherapy versus SoC study):

- To demonstrate de-escalation strategy of ctDNA guided adjuvant chemotherapy is non-inferior to standard of care treatment as measured by 3 year disease free survival in patients with high risk stage II or stage III CRC with no evidence of minimal residual disease (ctDNA negative)

5.2 Secondary Objectives

For all study patients (as applicable):

- To assess whether mutations identified in FFPE tumour tissue using targeted resequencing by a clinically validated method can be detected in circulating cell free DNA (cfDNA) using droplet digital PCR (ddPCR). Patients in Part C of the study will have ctDNA tested using next generation sequencing (NGS) based assays at pre-specified time-points
- To quantify levels of mutations in cfDNA and assess change from baseline, post-operatively and during chemotherapy
- In patients with rectal cancer having neo-adjuvant radiotherapy/chemoradiotherapy, to quantify levels of mutations in cfDNA prior to radiotherapy/chemoradiotherapy and assess change in level prior to surgery and post-operatively
- To quantify levels of mutations in cfDNA and assess change 3 monthly from the first post-operative visit for year 1, 6 monthly until year 3 and annually in years 4 and 5 or until relapse, if this occurs first
- To assess whether serial quantification of ctDNA has the potential to predict loco-regional and/or distant relapse after treatment of stage II and III CRC
- To correlate change in quantity of mutations in cfDNA with carcinoembryonic antigen (CEA), clinical and radiological parameters

- To develop a threshold for the detection of ctDNA that is likely to lead to relapse, by using the first 500 patients recruited as a training set and the next 500 patients recruited as a validation set

For patients in the randomised ctDNA guided adjuvant chemotherapy versus standard of care adjuvant chemotherapy study (Part C):

- Assess proportion of patients who are ctDNA negative on post-operative ctDNA and receiving de-escalated adjuvant chemotherapy in interventional arm compared to standard arm
- Assess proportion of patients who are ctDNA negative post-operatively who become ctDNA positive during follow-up and receive chemotherapy as an escalation of care
- To compare overall survival between ctDNA directed adjuvant chemotherapy and standard of care adjuvant chemotherapy arms
- To compare neurotoxicity and quality of life in patients between arms
- Health economic analysis to assess the cost-effectiveness of ctDNA directed therapy arm compared to the standard of care arm

5.3 Exploratory Objectives for all study patients (as relevant):

- To evaluate whether the presence of a specific mutation or a pattern of mutations identified in both FFPE tumour tissue and cfDNA predict for relapse
- To use targeted next generation sequencing (NGS) in plasma to identify mutations in cfDNA
- To develop digital pathology as a complementary tool to predict relapse in stage II and III CRC
- To analyse blood and tumour tissue for other potential predictive and prognostic biomarkers which show promise in emerging literature
- To molecularly sub-classify tumour tissue according to the consensus classification reached by the colorectal cancer subtyping consortium
- To assess whether particular molecular sub-types of CRC are more likely to have detectable ctDNA at the first post-operative visit
- To assess whether particular molecular sub-types of CRC are more likely to relapse
- To assess blood and tumour tissue of patients with synchronous primaries to evaluate mutational patterns in patients with synchronous colorectal primaries
- To explore if ctDNA is detectable in patients with stage 1 colorectal cancer
- To assess whether detection of ctDNA predicts for relapse in patients with stage I CRC that have undergone surgery with curative intent
- Patients with rectal adenocarcinoma receiving chemoradiotherapy at The Royal Marsden Hospital will have mrTRG (Magnetic resonance imaging tumour regression grade) assessed which will be correlated with cfDNA

For Part C only

- Analysis to assess the economic impact of ctDNA directed therapy on patients, their families, and the wider economy, compared to the standard of care arm.

6. STUDY DESIGN

The study will include the following:

1) Translational Study: All patients within the study will have ctDNA assessed at protocol-specified intervals (Part A and Part B)
2) Patients with high risk stage II and stage III colorectal cancer willing and eligible to take part in the randomised ctDNA guided adjuvant chemotherapy versus SoC chemotherapy study will be enrolled in Part C

6.0 Translational Study (Part A and Part B of the study)

This is a multi-centre, prospective, translational research study involving the collection and analysis of tumour tissue, serial blood samples and clinical data in patients with newly diagnosed stage I, II and III CRC. Blood samples will be collected at time-points specified in Figures 2 and 3 depending on the stage of the tumour. In patients with rectal cancer whose tumour is down-staged by radiotherapy or chemoradiotherapy (CRT), the staging prior to CRT will be considered as the stage for analysis. In 'straight to surgery' (STS) patients the surgical staging will be considered for analysis.

FFPE tumour tissue will be retrieved from pretreatment biopsy and surgery of patients with stage I, stage II and III CRC. FFPE blocks received will be assessed for tumour content and DNA and RNA will be extracted using standard kits. RNA will be extracted in order to enable the tumour tissue to be molecularly sub-classified according to the consensus classification reached by the colorectal cancer subtyping consortium and the assessment of other potential biomarkers such as micro-RNA. DNA will undergo quality control and if sufficient quantity and quality are present, will be sequenced using a clinically validated method. It is anticipated that the DNA extraction failure rate is likely to be <3%. Targeted resequencing of genes relevant to CRC such as *KRAS, NRAS, TP53, PIK3CA, BRAF* and *APC* will be performed. Instead we will only identify common mutations in the hotspots in the oncogenes and known mutations as well as frameshift/nonsense mutations in the tumour suppressor genes. We expect to identify 2-3 mutations per sample in these genes.

To identify these tumour mutations in the cfDNA we will design genotyping assays against the common hotspots for the above genes, based on COSMIC database mutation frequencies in CRC. Additional assays will be required for patient specific mutations (most likely in APC where nonsense mutations are not focal). These assays will be used in a digital droplet PCR system (QX200, Bio-Rad) for which there is extensive experience within the ICR and RM. Initially, assays will be tested on the tumour DNA and another DNA sample which is known not to have the mutations to ensure the specificity of the assay. DNA extracted from plasma will be run in triplicate for each mutation identified using ddPCR, plus required controls. Multiplexing of assays may be developed to reduce

running costs. Where apparent tumour specific mutations are unexpectantly abundant in the cfDNA, we will use DNA extracted from the buffy coat to re-test the assays to ensure the mutations are somatic. This approach will be more cost effective and time efficient than running the buffy coat DNA initially.

With rapid improvements in technology, we will adopt a tumour agnostic approach and test plasma directly for ctDNA detection using next generation sequencing targeted gene panels. We will also aim to compare this with tumour informed approaches including whole exome and whole genome sequencing approached on tumour informing variants in plasma ctDNA. Other biomarker approaches which will enhance sensitivity such as methylation assays will also be used. A subset of samples from Part B will be sent to Guardant Heath for testing with the Guardant Reveal assay.

In Part A the proportion of patients with stage II and III CRC who have detectable mutations in ctDNA in plasma pre-operatively will be determined as a feasibility step.

Part B of the study (translational study) will aim to determine whether detection of ctDNA in the first post-operative blood sample can be used to predict relapse in patients with stage II and III CRC. In addition, levels of ctDNA at other time-points such as: pre-treatment (baseline), post-CRT if applicable, post-adjuvant chemotherapy and during follow-up will be evaluated. The association between the level of ctDNA at these time points with RFS and OS will be determined.

Part B of the study will include:

1. Patients in Part A of the study
2. Patients with stage I CRC, low risk stage II CRC, recruited high-risk stage II and stage III patients not willing or not eligible to take part in Part C of the study

Participating centres may choose to enrol patients into Part B of the study alone or to Part B followed by Part C, or to Part C alone.

6.1 Part C of the study

Part C of the study will be a prospective, randomised, multi-centre study and will include high risk stage II and stage III CRC patients who have had resection of their primary tumour and are due to receive adjuvant chemotherapy. Patients will be randomised in a 1:1 manner to standard of care arm where patients are offered adjuvant chemotherapy according to national guidelines, or to the ctDNA guided arm, in which patients are treated based on post-operative (month 0) ctDNA results. Patients will be stratified according to:
1. High risk stage II versus stage III
2. Site of primary tumour: right colon versus left colon versus rectum

Screening and consent for Part C of the study: Patients will be screened within 4-8 (+2) weeks after surgery. Informed consent will be sought either face to face or over the telephone or video consultations. Patients will sign a written consent form specific for Part

C of the study. Following informed consent, clinicians assign standard of care adjuvant chemotherapy regimen prior to randomisation. Clinicians will decide the adjuvant chemotherapy of choice based on histopathological features of the tumour, patient's age, co-morbidities and patient's choice, as is current clinical practice. Following informed consent patients will have ctDNA samples collected during week 4-8 (+2 weeks) post-operatively and screening completed following which randomisation will be performed.

Randomisation: This will be performed by the Institute of Cancer Research Statistical Unit in a 1:1 manner between standard of care adjuvant chemotherapy or ctDNA guided adjuvant chemotherapy.

Standard of Care (SoC) arm

Patients will have blood collected post-operatively for ctDNA analysis 4-8 (+2) weeks after surgery. Blood samples for ctDNA will be banked for future analysis, hence patients will not receive ctDNA results if randomised to this arm. They will be offered standard of care capecitabine based adjuvant chemotherapy as per national guidelines (single agent capecitabine for 6 months or doublet CAPOX for 3 months).

ctDNA guided adjuvant chemotherapy arm

In patients assigned to the ctDNA guided adjuvant chemotherapy arm, results of post-operative (month 0) ctDNA analysis will be made available within a 2 week turn-around period and by week 12 to allow for commencing adjuvant chemotherapy within 12 weeks after the date of surgery. Based on the results, patients in this arm will be treated as follows:

Post-op (month 0) ctDNA positive patients (ctDNA detected)

Patients receive standard of care adjuvant chemotherapy if they have ctDNA detected.

Post-op (month 0) ctDNA negative patients (ctDNA not detected)

If ctDNA is negative post-operatively, patients are de-escalated as follows (outlined in **Figures 5a** and **5b**):
-if a doublet regimen (CAPOX) was assigned to the patient before randomisation, patient receives single agent chemotherapy (capecitabine) for 3 months.
-if single agent chemotherapy (capecitabine) has been assigned, then patient receives no chemotherapy.

Patients in the ctDNA guided arm of the study whose month 0 results were negative (ctDNA negative) will have month 3 (3-5 weeks after the last chemotherapy tablet/dose or 3 months after month 0 sample in those not receiving chemotherapy) ctDNA tested in real time and results made available to guide decisions as follows:

Post-op ctDNA (month 0) negative patients who become ctDNA positive at month 3 (at the end of ACT where applicable) during follow-up

Those patients who are ctDNA negative at month 0 but become positive at month 3 will undergo radiological imaging to assess for disease relapse. If no evidence of macroscopic disease is noted, systemic chemotherapy will be escalated to doublet regimen with CAPOX for 3 months (capecitabine for 6 months is acceptable in patients unsuitable to receive oxaliplatin). If there is evidence of macroscopic disease by radiological assessment, further management will be as per clinician's discretion within the advanced disease protocol as per local guidelines.

Post-op ctDNA (month 0) negative patients who remain ctDNA negative at month 3 (at the end of ACT where applicable) during follow-up

Those patients who are ctDNA negative at month 0 and continue to remain negative at month 3 will have clinical follow-up only and no further adjuvant chemotherapy will be administered. They will be followed up at months 6, 9, 12, 18, 24, 30, 36, 48 and 60 as per protocol schedule.

Study design for patients with mismatch repair deficient/microsatellite instability high CRC

Patients with resected MMRd/MSI-H CRC being recommended standard of care adjuvant chemotherapy are also eligible to enrol in TRACC Part C.

Patients will be randomised in a 1:1 manner to standard of care arm where patients are offered adjuvant chemotherapy according to national guidelines, or to the ctDNA guided arm, in which patients are treated based on post-operative (month 0) ctDNA results in the same way as the main Part C study.

MMRd/MSI-H patients randomised to Arm A: standard of care arm will follow the same procedures as detailed previously (see **Study Design**).

Patients randomised to Arm B: ctDNA guided ACT, who are ctDNA negative post-operatively (month 0) will be de-escalated to **no chemotherapy** as per the de-escalation/escalation schema in **Figure 5c.** Otherwise, patients who are ctDNA positive post-operatively (month 0) will follow the procedures as detailed above (see **Study Design**).

Patients participating in Part C, both the SoC and ctDNA guided arms, will have ctDNA levels collected during adjuvant chemotherapy (if receiving 6 months treatment), after completion of adjuvant chemotherapy (3-5 weeks after the last tablet/dose), and at following time points: every 3 months during the first year, every 6 months during the second and third years and annually during years 4 and 5. Patients in both arms will have ctDNA levels collected when disease recurrence is confirmed clinically and/or by radiology. Follow-up blood sample results will be banked for future analysis and not processed in real time.

ctDNA assay to be used for Part C of the study

Given recent improvements in technology and logistical challenges in coordinating tumour-informed ctDNA analyses across multiple centres, Guardant Health's Guardant Reveal ctDNA assay (UKCA marked) will be utilized for Part C of the study. Whole blood samples will be collected in four (4) 10mL Streck tubes in blood collection kits provided by Guardant Health and shipped to Guardant Health for processing and real time return of ctDNA test results will occur at the following time-points:

1. Post-operative (month 0) samples from patients participating in TRACC Part C who are randomised to ctDNA guided adjuvant chemotherapy arm
2. Post-ACT/month 3 samples from patients randomised to ctDNA guided adjuvant chemotherapy arm who were ctDNA negative (not detected) at month 0.

Please refer to the trial laboratory manual regarding shipping location and logistics for samples.

Post-operative samples collected from patients recruited to Part C who are randomised to the standard of care arm will be banked for future analysis by Guardant Health's Guardant Reveal assay. TRACC Part C participants recruited on prior protocol versions pre-dating implementation of the Reveal assay will have additional plasma samples banked for retrospective analysis by the Guardant Health Reveal assay (derivatives from at least 20mL whole blood preferred where possible).

If sufficient data accumulates real-time analysis will be considered for month 6, year 1 and year 2 samples. Advice will be sought from the TMG, IDMC and TSC with regards to this.

For all TRACC patients who co-enrol into the TRIGGER trial [a multi-centre, prospective, translational study sponsored by the Royal Marsden NHS Foundation Trust exploring the magnetic resonance tumour regression grade (mrTRG) as a novel biomarker to stratify management of good and poor responders to chemoradiotherapy] data generated from the molecular sub-classification of FFPE tumour tissue from pre-CRT biopsies and post-CRT resection specimens, described above, will securely share between the TRACC and TRIGGER research teams. The clinical and translational data relating to ctDNA and any re-sequencing results from FFPE tumour tissue for the co-enrolled patients will also be securely shared between the TRACC-TRIGGER research teams. For the co-enrolled patients randomised to the interventional arm of TRIGGER, the follow-up will be done as part of the TRIGGER trial and all clinical and translational bio-specimen data will be shared with the TRACC team. The data from this group of patients may be analysed as separate cohort and details specified in a statistical analysis plan. This is to avoid duplication of work by research teams from the same sponsor and to optimise resource utilisation.

6.2 Additional Research

Subject to funding, it is anticipated that surplus blood and tissue collected during this study in conjunction with clinical data, may also be used for future research projects, where patients have provided consent. Access to these samples for future research will only be available following agreement amongst members of the trial management group.

Additional research into protein biomarkers will be undertaken as part of this study to help explore other biomarkers of early relapse. The biomarkers will be studied in high risk stage II and stage III patients initially and if appropriate, stage I and low risk stage II patients will be included. Additional research may involve (but is not limited to) epigenomics, metabolomics, tumour biology and genomics (including whole genome sequencing) as well as epidemiological studies.

7. NUMBER OF CENTRES

This trial is open to any qualifying site within the United Kingdom. The TRACC study is open to any site within the United Kingdom treating patients with CRC. It is anticipated that up to 100 centres will participate in trial recruitment.

8. NUMBER OF SUBJECTS

The first 48 patients recruited will comprise the pilot phase or the feasibility part of the study (Part A).

Part B of the study will include at least 500 evaluable patients with stage II (low risk and high risk) CRC and 500 evaluable patients with stage III CRC. Patients with stage I CRC will also be included.

Part C of the study will include the ctDNA guided versus SoC adjuvant chemotherapy group, a total of 1620 patients (810 patients in each arm) would need to be randomised for the primary analysis, and 499 events required. The accrual target will be inflated by ~5% to account for drop-outs, therefore the overall total accrual target will be ~ 1700 patients.

It is anticipated that the overall study population will be at least 2700 (1700 more than initially planned). This is anticipated to take approximately 10 years in total from the start of study recruitment in 2016. There will be a proportion of patients who begin the study within Part B and proceed to Part C following surgery. Once it is confirmed that we have recruited 1620 evaluable patients for Part C of the study and that Part B of the study has 500 stage II (high and low risk not in Part C) and 500 stage III evaluable patients, we will consider halting recruitment to that stage. We plan to over-recruit to account for drop-outs. In addition, we plan to recruit patients with high risk stage II and stage III resected MMRd CRC into the Part C sub-study.

9. ESTIMATED STUDY DURATION

The study is planned to continue until a minimum of 2,700 patients have been enrolled. This is anticipated to take approximately 10 years in total from start of study recruitment in 2016.

10. STUDY DURATION FOR PARTICIPANTS

Follow up will continue until death, discharge from routine follow up, or withdrawal from the study. All patients will be followed up for a minimum of 5 years.

11. END OF STUDY

The 'end of study' will be defined as the date that the last patient has had their last visit, or when adequate follow up has occurred for all the study end points to be assessed and reported, whichever is sooner. At this point, the 'end of trial notification' will be submitted to the relevant regulatory authorities and ethics committees.

12. SUBJECT ELIGIBILITY

Investigators will be expected to maintain a screening log of all potential study candidates that includes limited information about the potential candidate, date, and outcome of the screening process (e.g., enrolled into study, reason for ineligibility, or refused to participate).

All patients in Part B will have eligibility assessed in a 2-step process, prior to surgery and again post-operatively with the histopathology report from surgery using the criteria below. All patients meeting the eligibility criteria at the first assessment will be registered.

Rectal cancer patients who undergo pre-operative radiotherapy or chemoradiotherapy will have an additional eligibility assessment after completion of this with the results of their response assessment imaging, after their management plan has been determined. This is most likely to be following multidisciplinary team discussion.

The Clinical Trials Unit at the Royal Marsden Hospital will check and confirm eligibility at relevant timepoints throughout the study with histopathology reports, multidisciplinary team outcomes and clinical documentation. The data collection system has been updated in this regard.

All patients that were registered and subsequently excluded based on the eligibility criteria below, will be replaced to ensure an adequate sample size is maintained for the statistical analysis.

12.0 Eligibility criteria to be used prior to registration (for all patients, Part A and B)

Inclusion Criteria:

- New diagnosis of histologically confirmed CRC (colon and rectal) scheduled to undergo surgery with curative intent, with no radiological evidence of metastatic disease.
- Histology consistent with adenocarcinoma or patients with high grade dysplasia whose imaging is suggestive of colorectal carcinoma (CRC)
- Age ≥18
- Ability to give informed consent
- Able to adhere to follow up schedule

NB: Synchronous CRC primaries are allowed in Part A and B.

Exclusion Criteria:

- Scheduled to have neo-adjuvant chemotherapy (neo-adjuvant radiotherapy or chemoradiotherapy for patients with rectal cancer is permitted).
- Current or previous other malignancy within 5 years of study entry, except cured basal or squamous cell skin cancer, superficial bladder cancer, prostate intraepithelial neoplasm, carcinoma in situ of the cervix or other non-invasive malignancy

12.1 Additional eligibility criteria for rectal cancer patients following completion of pre-operative radiotherapy or chemoradiotherapy

Inclusion Criteria:

- All patients proceeding to surgery

Exclusion Criteria:

- Patients scheduled to have further pre-operative treatment with chemotherapy
- Patients who are no longer proceeding with surgery i.e., those in whom surgery is considered too high risk
- Patients that are no longer proceeding with surgery as they are proceeding with a deferral of surgery approach

12.2 Criteria to be used to confirm eligibility on the case report form (CRF) with the histopathology report at the first post-operative visit

Inclusion Criteria:

- Stage I, II or III CRC based on the post-operative histopathology report
- Availability of FFPE tumour tissue (from either biopsy or surgery), for processing and analysis

Exclusion criteria:

- Patients with no confirmed tissue diagnosis or high grade dysplasia included in the study based on imaging diagnosis but subsequent histopathology of surgical specimen confirms no carcinoma will be excluded
- Scheduled to receive post-operative radiotherapy

12.3 Eligibility criteria Part C only

Inclusion Criteria:

1. Subject ≥ 18 years of age
2. Subjects with histologically proven high-risk stage II* or stage III (any T, and N1 or N2) colon or rectal cancer treated with curative intent with surgery alone with no evidence of metastatic disease. Subjects must be due to receive adjuvant chemotherapy following surgery.
   or
   Subjects with radiologically or histologically confirmed stage III (any T, N1 or N2) histologically proven locally advanced rectal cancer treated with neo-adjuvant radiotherapy or chemoradiotherapy with no evidence of metastatic disease are eligible. Subjects must be due to receive adjuvant chemotherapy following surgery.

   **High risk stage II is defined as having one or more of the following: T4 disease, tumour obstruction and/or perforation of the primary tumour during the pre-operative period, inadequate nodal harvest as indicated by <12 nodes examined, poorly differentiated grade on histology, peritoneal involvement or extramural perineural/venous/lymphatic invasion.*
3. Fully surgically resected tumour (R0) with clear resection margins (i.e., >1 mm)
4. Adequate organ function

- Absolute neutrophil function ≥1.0 x 10^9/ L
- Platelet Count ≥ 75 x 10^9 / L
- Haemoglobin ≥80g/L (blood transfusion before randomisation is allowed)
- Adequate renal function as calculated by Cockcroft and Gault equation (GFR ≥ 50ml/min if single agent capecitabine or CAPOX being administered)

- Aspartate aminotransferase/ Alanine aminotransferase levels ≤ 2.5 upper limit of normal

5. Absence of major post-operative complications or other clinical conditions that, in the opinion of the investigator, would contraindicate adjuvant chemotherapy
6. Patients should be assessed by an Oncologist for suitability of adjuvant chemotherapy with 6 months (8 cycles) of capecitabine* or 3 months (4 cycles) of CAPOX, have a post-operative ctDNA blood sample collected and be randomised by week 4-8 (+2 weeks) after surgery, commencing adjuvant chemotherapy within 12 weeks after surgery
   (*6 cycles in patients already treated with 2 cycles of concomitant capecitabine as part of neo-adjuvant chemoradiotherapy)
7. ECOG performance status 0-2
8. Able to give informed consent

Exclusion Criteria:

1. History of concurrent and previous malignancy within the last 5 years, including those on anti-cancer therapy (e.g., adjuvant endocrine therapy), except for curatively treated superficial malignancies (e.g., non-melanomatous skin cancer and carcinoma in situ
2. Any major post-operative complications or other clinical conditions that in the opinion of the investigator would contra-indicate adjuvant chemotherapy
3. Any subject not due to receive adjuvant chemotherapy will not be eligible for Part C of the study
4. Hypersensitivity or contraindication to the drug(s) associated with the planned choice of systemic chemotherapy (CAPOX or single agent capecitabine) as stated in the SmPC for each of the drugs
5. Subjects due to receive 5-Flurouracil (5-FU) based adjuvant chemotherapy (either single agent 5-FU or in combination with oxaliplatin) will not be eligible for Part C of the study, these patients will continue to be followed up in the observation Part B of the study (if enrolled).
6. Patients with synchronous CRC primary tumours

13. SUBJECT ENROLLMENT

Patients will be considered as enrolled in the trial after written informed consent and registration has occurred. Any health care professional with GCP training who has been delegated by the principal investigator can obtain consent from eligible patients. At registration, each patient will be assigned a study number which will be used to identify the patient.

Before patients may be enrolled into the trial, the sponsor must receive proof that all required regulatory approval (both local and, if relevant, national) has been obtained for the site.

Before registration, each potential patient must be given a patient information sheet (colon PIS for patients with colon cancer; rectal and recto-sigmoid cancer patients who are due to receive radiotherapy or chemoradiotherapy will be given a separate rectal PIS). Informed consent will be obtained according to the requirements of GCP. Consent must be taken by the principal investigator (PI) or a qualified doctor, nurse or health care professional who is authorised by the PI on the site delegation log to take consent.

For patients with high risk stage II or stage III CRC eligible and wishing to take part in the ctDNA guided adjuvant chemotherapy study (Part C), a PIS for Part C will be given. A separate consent form will need to be signed by the patient. Patients can sign consent to Part C of the study only after they have had at least 24 hours to consider the study. Consent for Part C can only be taken by a qualified doctor or specialist health care professional with the required qualifications of their Trust who is authorised to prescribe, consent and make decisions regarding adjuvant chemotherapy.

The registration and baseline assessment forms must also be completed. Only patients fulfilling all pre-registration inclusion criteria and exclusion criteria should be registered. Any queries about eligibility should be addressed directly to the lead centre.

14. REGISTRATION

Completed registration pages from the trial Case Report Form (CRF) and baseline assessment reports should be emailed to the GI Trials Unit at the Royal Marsden Hospital. The original documents should be retained by the site. All documents that need to be e-mailed are listed on the CRF.

E-mail	**TRACCstudy@rmh.nhs.uk**

Once eligibility has been reviewed and all information has been received in the sponsor CTU the registration and study number assigned to the patient will be entered onto the relevant CRF page and emailed back to the study personnel at the participating site responsible for recruitment of the patient.

15. STUDY PROCEDURES

15.0 Study Recruitment and consent

Suitable patients will be identified in colorectal multidisciplinary team meetings and outpatient clinics. Patients will only be approached once the appropriate official notification has been received by the chief investigator that the trial is open to recruitment. Eligibility screening will be conducted by the study team after reviewing the medical records. Patients will be provided with an up to date copy of the patient information sheet (PIS);

colon PIS for patients with colon cancer; rectal and recto-sigmoid cancer patients who are due to receive neo-adjuvant radiotherapy or chemoradiotherapy will be given a separate rectal PIS which specifically outlines study procedures related to radiotherapy, and patient consent will be sought.

Consenting procedures will conform to GCP and local and national regulations and consent can be taken by an appropriately trained member of the clinical multidisciplinary team, including medical and nursing staff. Please note that for Part C only a qualified doctor or specialist health care professional with the required qualifications of their Trust who is authorised to prescribe, consent and make decisions regarding adjuvant chemotherapy can obtain trial consent. Patients will have the opportunity to discuss the study in detail prior to giving consent.

Patients may consent to Part B on the same working day as receipt of the PIS provided they are willing and able to decide that they wish to participate. For Part C, patients will need at least 24 hours to read through the Part C specific patient information sheet and consider the study before signing an informed consent form. Informed consent can be obtained for Part B and C either during a face to face consultation or over the telephone or video consultations.

Patients will not be pressurised into making a decision and will be informed that their care will not be compromised if they choose not to participate. Patients will also be asked for permission to inform their GP if they consent to participate in the study. Additionally, patients will be asked to consent for their research tissue and blood samples to be stored and used in future research projects.

Patients recruited to Part B (translational study) will have standard of care chemotherapy as per local policy. They will have tumour tissue and bloods collected as per protocol. The following guidelines are not applicable to patients in Part B and are only applicable for patients in Part C of the study.

15.1 Study Treatments (only applicable for patients in Part C of the study)

All chemotherapeutic agents used for patients recruited in Part C of the study are standard of care treatments administered as per local hospital policy including standard doses for the Trust. This includes dose reductions before and during treatment, for example those used for patients with DPYD variants and toxicity. Patients in the ctDNA guided arm receive de-escalated chemotherapy if ctDNA is negative on the post-operative (month 0) blood sample. In those patients in whom ctDNA is negative in the post-operative sample but become positive at month 3/post-ACT, chemotherapy is started or escalated.

Single agent capecitabine regimen

Capecitabine: dose as per local hospital policy, twice a day oral administration from days 1-14 in 3 weekly cycles

Total number of cycles will be as follows:

Total of 6 months of treatment (8 cycles) in standard of care and ctDNA guided arms for those patients who need their chemotherapy escalated at the month 3 timepoint but are not suitable to receive oxaliplatin.

Total of 3 months of treatment (4 cycles) in ctDNA guided arm

In patients with locally advanced rectal cancer who have undergone neo-adjuvant chemoradiotherapy with 2 cycles of concomitant capecitabine (6 weeks), the following number of cycles will be administered in the adjuvant period:

Total of 18 weeks of treatment (6 cycles) in standard of care arm

Total of 12 weeks of treatment (4 cycles) in ctDNA guided arm in case of de-escalation from CAPOX to capecitabine.

Capecitabine and Oxaliplatin doublet regimen

Oxaliplatin: dose as per local hospital policy administered intravenously once every 3 weeks in 3 weekly cycles

Capecitabine: dose as per local hospital policy, twice a day oral administration from days 1-14 in 3 weekly cycles

Total number of cycles will be as follows:

Total of 3 months of treatment (4 cycles) in standard of care and ctDNA guided arms for those patients who need their chemotherapy escalated at the month 3 timepoint.

15.1.1 Chemotherapy Drugs used in the trial for patients in Part C

The following chemotherapy drugs are used in the clinical trial: capecitabine with or without oxaliplatin.

All chemotherapy agents must be sourced from local hospital stock and prepared as per local practice. All chemotherapy agents used must be stored according to Summary of Product Characteristics and labelled as per local practice.

Drug Accountability

As per risk adapted approach, sites may use standard documents as drug accountability e.g., in-house preparation worksheets, orders from external partners. This should completed in accordance with local Trust policies.

15.1.2 Duration of treatment for patients in Part C

Eligible patients will have treatment as per protocol unless there is disease progression, unacceptable toxicity or withdrawal of consent for any reason or at clinician's discretion within that time period.

15.1.3 Management of Infusion Reactions for patients in Part C

In case of hypersensitivity reactions, the drug infusion should be interrupted and the acute management should occur as per local clinical practice. Decision on re-challenge with the same drug on subsequent cycles will be made according to the severity of the hypersensitivity reaction at the discretion of the clinical investigator.

15.1.4 Dose Modifications for Toxicity for patients in Part C

Expected toxicities as detailed in SmPC will form the reference safety information (RSI) for this study. The links to SmPC for individual drugs are as follows:

Capecitabine: Capecitabine 150 mg film-coated tablets - Summary of Product Characteristics (SmPC) - (emc) (medicines.org.uk)

Oxaliplatin: Oxaliplatin 5 mg/ml concentrate for solution for infusion - Summary of Product Characteristics (SmPC) - (emc) (medicines.org.uk)

For toxicities or combinations of toxicities not specifically covered in detail in the SmPC, doses of chemotherapy can be reduced at the discretion of the investigator as per local practice. All dose modifications documented in the CRF including reasons. Dose modifications due to neurotoxicity should be documented separately.

Dose banding of chemotherapy agents as per local practice based on National Guidelines are to be followed. Local guidelines for DPYD (dihydroprymidine dehydrogenase) testing and dose modifications should be followed. Crossover from capecitabine to infusional 5-FU is not allowed but if clinically indicated (e.g., for toxicity), please discuss with the Chief Investigator or Trial Physician. Use of alternative chemotherapeutic agent in case of toxicity (e.g., raltitrexed in case of cardiac side effects with capecitabine) is allowed and should be recorded in the database. The total duration of treatment should remain the same. Please do not hesitate to contact your coordinating trial office for advice.

15.1.5 Neurosensory Toxicity (applicable for patients in Part C)

Neurosensory toxicity due to oxaliplatin may require dose reduction and dose adjustments according to local protocol may be followed as long as the dose given is carefully annotated in the CRF.

Wherever possible, oxaliplatin should be dose-reduced (as per Investigator discretion) rather than discontinued and can be given over a longer period of time if it is the hyperacute neurotoxicity which is particularly a problem. In the situation where oxaliplatin is discontinued due to toxicity, adjuvant treatment can continue with capecitabine alone if deemed appropriate by the local Investigator. In this case, the dose per m^2 of the single agent capecitabine can be increased as per local practice at the discretion of the Investigator and documented in the CRF.

CTCAE version 5.0 should be used to grade the neurotoxicity as follows:

Nervous System Disorders					
CTCAE Term	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Peripheral Sensory Neuropathy	Asymptomatic	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self-care ADL	Life-threatening consequences; urgent intervention required	
Definition: A disorder characterised by damage or dysfunction of the peripheral sensory nerves					

15. 2 Blood sample collection for all patients in the study

Blood samples should only be collected after the patient has provided informed consent for study participation. A total of up to 30-50 mL (about 6-10 teaspoons of blood will be collected). Every effort will be made to ensure that blood samples are taken at the same time as for routine clinical purposes in order to avoid the need for additional venepuncture. Blood will be collected at time-points as specified in Figures 2, 3, and 4. However, for visits that have been delayed as part of clinical care or for other circumstances such as but not limited to COVID-19 restrictions, blood samples should be taken at the next available visit and assigned to the nearest or the most appropriate follow up timepoint.
For all patients where repeat samples for a time-point have been submitted these samples will be retained and analysed.

For stage I patients, collection of Month 3, Month 9 and Month 30 samples are optional and can be collected by sites if these visits are part of their local practice or if patients are visiting the site at these time-points. All samples that have been previously received for stage I patients at Month 3, Month 9 & Month 30 visits will be retained and analysed.

Around 30-50mL of blood will be collected in cell free DNA blood collection tubes™ (Streck) or equivalent at each time-point and will be shipped and processed as outlined in the TRACC study laboratory manual. Amendments to the laboratory manual may be required in the future to allow for developments in technologies and laboratory techniques.

For Part C of the study only, real-time analysis of ctDNA with a 2-week turn-around time of ctDNA results will be performed in patients randomised to the ctDNA guided adjuvant chemotherapy arm at the following time points:

- Post-operative ctDNA at month 0 (4-8 (+2) weeks following surgery, before randomisation)
- Post-operative ctDNA at month 3 (3-5 weeks after the last chemotherapy tablet/dose or 3 months after month 0 sample in those not receiving chemotherapy) in patients who are ctDNA negative (not detected) at month 0 and have chemotherapy de-escalated

If patients have a radiological relapse, a final blood sample will be collected 2-8 weeks following clinical or radiological confirmation of relapse (and prior to new treatment

starting). Following this, no further blood samples will be collected. These patients should be contacted annually by telephone to confirm their clinical status.

All blood samples and derivatives will be shipped, stored and analysed on behalf of the Royal Marsden.

Please see the Laboratory Manual for full details on the collection, processing, storage and shipment of the blood samples.

15.3 Histological specimen collection for all patients in the study

FFPE tumour blocks from surgical resection and diagnostic biopsy specimens will be requested from relevant hospitals and will be shipped, stored and analysed on behalf of the Royal Marsden provided patients have provided informed consent for this, which is a prerequisite for study entry. Tissue will be removed from representative tumour blocks and sections will subsequently be prepared for the purposes of nucleic acid extraction and further immunohistochemical testing (e.g., MMR testing in stage II patients where this has not been performed as part of standard clinical practice at local sites).

Please see the Laboratory Manual for full details on the collection, processing, storage and shipment of the blood samples.

15.4 Biological specimen labelling and storage for all patients in the study

Biological samples retained for the study will be encoded with a unique study identifier and other patient identifiers (e.g., name, date of birth and NHS number) will be removed prior to storage, in order to maintain patient confidentiality. Instead samples should be labelled with the study name TRACC, the patient's study number (assigned at registration) and initials. The date of collection and collection time-point as outlined in Figures 1 and 2 should also be recorded on blood samples. Centres should also keep a record of blood and tissue samples collected that includes the same information. In addition, the date of transfer of samples to the lead centre should be recorded in the CRF.

For research material and analyses, certain key individuals within the study, within the Royal Marsden Hospital GI Clinical Trials Unit, will be able to link the unique identifier with the patient's identification details. This is to allow the collection of retrospective and prospective demographic, and retrospective clinico-pathological data, and also to ensure that on receipt, samples are encoded correctly. All repeat samples received for any visits, will be retained and analysed.

Prior to transfer to the Royal Marsden GI & Lymphoma Trials Unit, centres should ensure that blood and tumour samples are appropriately stored. Blood samples should be sent ambient on the day of collection, whereas FFPE tissue blocks may be stored at room temperature away from light.

Blood and tissue samples will be stored indefinitely. However, the patient retains the right to have the sample material returned to their hospital pathology department or destroyed at any time by contacting the principal investigator at the site at which they were registered

for the study. The site principal investigator will then be responsible for contacting the sponsor via the chief investigator to arrange for the return or destruction of the samples.

All blood samples and derivatives will be shipped, stored, and analysed on behalf of the Royal Marsden.

Blood samples from Part C participants that are due to be analysed by the Guardant Reveal assay will be shipped to Guardant Health at 505 Penobscot Dr.; Redwood City, CA 94063, U.S.A. Please refer to the trial laboratory manual regarding shipping location and logistics for samples. Results for all samples that pass quality control measures will be sent directly to the Principal Investigator, site study team and TRACC trials unit.

The sponsor will be the exclusive owner of any data, discoveries, or derivative materials from the sample materials and is responsible, via the chief investigator, for the destruction of the sample(s) at the request of the research patient through the site principal investigator or at the end of the storage period. The site principal investigator will provide the chief investigator with the required patient study numbers so that any unused blood and tissue samples can be located and destroyed. If a commercial product is developed from this research project, the sponsor will own the commercial product with the exception that Guardant Health will retain ownership of its existing commercial products and any product improvements made by Guardant Health to its existing Guardant Reveal assay. The patient will have no commercial rights to such product and will have no commercial rights to the data, information, discoveries, or derivative materials gained or produced from the sample. See Section 18.3 for patient confidentiality.

15.5 Additional blood and tumour analyses that may be conducted

MMR status is both a prognostic and predictive biomarker in stage II CRC patients. As such, analysis for loss of expression of MMR gene products by immunohistochemistry may be performed in this group of patients, only where this has not been performed by immunohistochemistry as standard of care in other sites. Analysis for loss of expression of MMR gene products will not be performed in real time as delays may be experienced in retrieving tumour blocks from participating sites. We expect that in majority of the participating centres, assessment of MMR status will be conducted as standard of care in stage II CRC patients.

Ribonucleic acid (RNA) may also be extracted from FFPE tumour tissue in order to enable the tumour tissue to be molecularly sub-classified according to the consensus classification reached by the CCSC as an exploratory objective.

Haematoxylin and Eosin (H&E) slides may be used to develop digital pathology as a complementary tool to predict relapse in stage II and III CRC as another exploratory objective.

In an exploratory objective, other blood based potential biomarkers including (but not limited to) microRNAs may also be analysed. Similarly, tumour tissue may also be analysed for other potential predictive and prognostic biomarkers which show promise in

emerging literature. Detailed description of the procedures for both sample collection and processing for additional research will be outlined in the TRACC laboratory manual.

With the exception of MMR status, where results may be provided to local investigators as and when they become available, if they are of significance, individual patients will not be informed of their results as they are unlikely to influence management.

15.6 Collection of clinical data

This study will use study specific electronic CRFs to collect data. Data collected may include but will not be limited to demographic characteristics, clinico-pathological parameters and treatment received including type of surgery and complications related to surgery.

15.7 Quality of Life and Neurotoxicity Questionnaires and Health Economics Questionnaires

At the outset of the trial sites opted to participate in the collection of quality of life and neurotoxicity questionnaires (EORTC QLQ-C30 & CR29, GOG-NTX 4 and EQ-5D) and health economics questionnaire (RUtInE^{TM}) at clinic visits as follows:

1. At randomisation
2. Months 3, 6, 9 and 12 during first year
3. 6 monthly during years 2 and 3
4. End of year 4
5. End of year 5

The sites enter patient reported quality of life data and neurotoxicity data directly into CRFs for each patient.

15.8 Pregnancy

Patients should agree to use reliable birth control during the time they are receiving chemotherapy and for a year after stopping chemotherapy. If the patient or their partner becomes pregnant either whilst receiving trial chemotherapy or in the first year after stopping trial chemotherapy it must be stressed that they are requested to inform their Investigator immediately. Acceptable forms of effective contraception

Acceptable forms of effective contraception include:
1. Established use of oral, injected or implanted hormonal methods of contraception.
[The decision to allow use of hormonal contraceptives should be based on the Investigational Medicinal Product's (IMP's) metabolism and potential for interactions, pharmacology and the adverse event profile (e.g., vomiting)].

2. Placement of an intrauterine device (IUD) or intrauterine system (IUS).
[Consideration should be given to the type of device or system being used, as there are higher failure rates quoted for certain types, e.g., steel or copper wire].

3. Barrier methods of contraception: Condom or Occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository.
The use of barrier contraceptives should always be supplemented with the use of a spermicide. The following should be noted:

- Failure rates indicate that, when used alone, the diaphragm and condom are not highly effective forms of contraception. Therefore, the use of additional spermicides does confer additional theoretical contraceptive protection.
- However, spermicides alone are inefficient at preventing pregnancy when the whole ejaculate is spilled. Therefore, spermicides are not a barrier method of contraception and should not be used alone.

4. Male sterilisation (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate).
[For female subjects on the study, the vasectomised male partner should be the sole partner for that subject].

5. True abstinence: When this is in line with the preferred and usual lifestyle of the subject.
[Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception].

Once informed of a pregnancy, sites must immediately complete and email a Pregnancy Notification Form to their coordinating trial office. The Pregnancy Notification Form must be updated and emailed again as soon as anything relating to the pregnancy changes such as miscarriage, termination or delivery of the baby.

Table 1: Summary of Study assessments for Part A and Part B (Translational Study)

	Baseline[A] Pre-operative phase	Post CRT if applicable	Post – operative [C] Month 0	If patient enrolling in Part C, please refer to Table 2	Month 3/end of ACT where applicable[D]	Month 6/end of ACT where applicable[D]	Month 9	Month 12	Month 18	Month 24	Month 30	Month 36	Month 48	Month 60	Relapse[E]
Informed Consent	X														
Eligibility Assessment	X														
Eligibility assessment after completion of pre-operative (chemo)radiotherapy		X													
Confirmation of eligibility with histopathology report			X												
Blood collection for ctDNA analysis for stage II and III patients	X	(X)[B] X	X		X	X	X	X	X	X	X	X	X	X	X
Blood collection for ctDNA analysis for patients with stage I CRC	X		X		X_F	X	X_F	X	X	X	X_F	X	X	X	X
CEA	X	X	X		X^H	X	X^H	X	X	X	X^H	X	X	X	X
CT for patients with stage II and III CRC	X	X	X^G					X		X		X			X
CT for patients with stage I CRC	X							X		X		X			
Clinical Data Collection	X	X	X		X	X	X	X	X	X	X	X	X	X	X

Tissue (biopsy/resection sample)	X[I]		X												

CRT=Chemoradiotherapy

All time-points should be counted from Month 0.

A: Baseline blood samples within 4 weeks prior to surgery or start of neo-adjuvant radiotherapy or chemoradiotherapy in CRT cohort.
B: In patients having CRT, the ctDNA blood sample taken on completion of CRT is optional. A ctDNA blood sample should be taken within 4 weeks prior to surgery in these patients (see laboratory manual).
C: 4-8 (+2) weeks post-surgery
D: In both arms, Month 3 ctDNA blood sample should be taken at the end of ACT (3-5 weeks after the last chemotherapy tablet/dose) where applicable, otherwise take sample 3 months after Month 0 time point. Likewise, Month 6 ctDNA blood sample should be taken at the end of ACT (3-5 weeks after the last chemotherapy tablet/dose) where applicable, otherwise take sample 3 months after Month 3 time point.
E: Patients should be contacted annually by telephone from relapse to confirm their clinical status until they have reached at least 5 years from their surgery.
F: For Stage 1 patients Month 3, Month 9 and Month 30 samples for ctDNA are optional and can be collected if the patient is attending the site at these timepoints.
G: Post-op CT scan at Month 0 (4-8 weeks after surgery) is optional
H: For Stage 1 patients Month 3, Month 9 and Month 30 visits and samples for CEA are optional and can be collected if the patient is attending the site at these timepoints.
I: Diagnostic biopsy tissue only required for patients receiving neo-adjuvant radiotherapy or chemoradiotherapy (CRT), and in those with stage I CRC fully resected at biopsy.

Table 2: Summary of study assessments for Part C (ctDNA guided adjuvant chemotherapy interventional study)

	Post-operative[B] Month 0	Month 3/end of ACT where applicable[G]	Month 6/end of ACT where applicable[G]	Month 9	Month 12	Month 18	Month 24	Month 30	Month 36	Month 48	Month 60	Relapse[J]
Eligibility Assessment[A]	X											
Informed consent for Part C of the study	X											
Blood collection for ctDNA analysis for high risk stage II and III patients	X[C]	X[C]	X	X	X	X	X	X	X	X	X	X
CEA	X	X	X	X	X	X	X	X	X	X	X	X
CT for patients with high risk stage II and III CRC	X[D]	X[E]			X[F]		X[F]		X[F]			X[F]
Clinical Data Collection for neurotoxicity	X	X	X	X	X	X	X	X	X	X	X	X
Clinical Data Collection of QoL and RUtInE™ data	X	X	X	X	X	X	X	X	X	X	X	X
Routine haematology and biochemistry blood tests (including eGFR)	X[H]											
Tissue (biopsy/resection sample)	X[I]											

ACT=Adjuvant chemotherapy

All time-points should be counted from Month 0.

A: Sites should submit the following as a minimum to the sponsor for confirmation of eligibility and registration of patient to Part C: post-operative histopathology report (and biopsy report where applicable), CTTAP (and MRI rectum/pelvis where applicable), blood results including full blood count, biochemistry, , and renal function as calculated by Cockcroft and Gault equation.
B: 4-8 (+2) weeks post-surgery.
C: Real time analyses of ctDNA samples will be performed at Month 0 and Month 3/end of ACT (for patients randomised to Arm B/ctDNA guided adjuvant chemotherapy arm).
D: Post-op CT scan at Month 0 is optional before randomisation for patients in Part C of the study.
E: Additional CT scan at Month 3 will be performed for patients randomised to Arm B/ctDNA guided adjuvant chemotherapy arm of the study if they were ctDNA negative at baseline but become ctDNA positive during follow-up. This is classified as a research scan.
F: CT scanning at end of years 1, 2 and 3 and at suspicion of relapse are done as part of routine clinical follow-up.
G: In both arms, Month 3 ctDNA blood sample should be taken 3-5 weeks after the last chemotherapy tablet/dose where applicable. For patients having 6 months of ACT, Month 6 ctDNA blood sample should be taken 3-5 weeks after the last chemotherapy tablet/dose. For further clarification of timing of Month 3 and Month 6 samples, please see 'Scenarios' box below.
H: Routine blood tests should be taken after surgery and before registration/randomisation and ACT is commenced.
I: Diagnostic biopsy tissue also required for patients receiving neo-adjuvant radiotherapy or chemoradiotherapy (CRT)
J: Patients should be contacted annually by telephone from relapse to confirm their clinical status until they have reached a minimum of 5 years from their surgery.

Scenarios:

For patients on **standard of care adjuvant chemotherapy**:

1. If patient receiving **6 months of Capecitabine monotherapy**, please take Month 3 ctDNA blood sample halfway through ACT and Month 6 ctDNA blood sample 3-5 weeks after the last chemotherapy tablet/dose.
2. If patient receiving **3 months of CAPOX chemotherapy**, please take Month 3 ctDNA blood sample 3-5 weeks after the last chemotherapy tablet/dose.

For patients receiving **ctDNA-guided adjuvant chemotherapy**:

1. ctDNA positive for standard of care chemotherapy
    a. If patient receiving **6 months of Capecitabine monotherapy**, please take Month 3 ctDNA blood sample halfway through ACT and Month 6 ctDNA blood sample 3-5 weeks after the last chemotherapy tablet/dose.
    b. If patient receiving **3 months of CAPOX chemotherapy**, please take Month 3 ctDNA blood sample 3-5 weeks after the last chemotherapy tablet/dose.
2. ctDNA negative for de-escalation of chemotherapy
    a. If patient de-escalated to no chemotherapy, please take Month 3 ctDNA blood sample 3 months after Month 0 sample.
    b. If patient receiving **3 months of Capecitabine monotherapy**, please take Month 3 ctDNA blood sample 3-5 weeks after the last chemotherapy tablet/dose.

For ctDNA blood tests beyond Month 6, timepoints should be measured from Month 0

16. WITHDRAWAL OF SUBJECTS

Patients have the right to withdraw fully or partially from the study at any time and for any reason without prejudice to his or her future medical care by the physician at the institution. Patients who are withdrawn from Part B of the study will not be replaced. Patients who are withdrawn from Part C of the study may need replacing, depending on the reason for withdrawal.

Withdrawal of full consent for a study means that the patient does not wish to receive further investigational treatment/procedures and does not wish to or is unable to continue further study participation. Any patient may withdraw full consent to participate in the study at any time during the study. The investigator will discuss with the patient the most appropriate way to withdraw to maintain the patient's care. In this case, the patient's data and samples already collected up to the point of withdrawal will still be included in the analysis of data, and the patient censored from that point onwards, unless the patient has explicitly requested that none of the data collected should be used for analysis.

Withdrawal of partial consent means that the patient does not wish to undergo any further procedures (e.g., blood tests) but is still willing to collaborate in providing further data by continuing on study (e.g., permit further data collection from hospital records including survival data).

Reasons for withdrawal or removal of patients from the study may include:

- withdrawal of consent/patient choice (see above)
- administrative decision by the investigator, chief investigator or sponsor
- ineligibility
- significant protocol deviation
- patient non-compliance
- clinician choice
- blood sample problems/ctDNA failure

Patients who relapse will be partially withdrawn from the study following confirmation of relapse, no further blood samples will be taken, and the patient will be followed up annually for survival for at least 5 years from surgery. Additionally, patients who develop a new primary malignancy during study participation will be withdrawn at time of new primary diagnosis.

REMINDER

- Full withdrawal = no further trial procedures (including bloods), no study follow-up including survival follow-up
- Partial withdrawal = no further trial procedures (e.g., blood tests), no study follow-up, but continues annual survival follow-up (regardless of reason for partial withdrawal)

17. STATISTICAL CONSIDERATIONS

17.0 Study Design

This is a multi-centre, prospective, translational research study involving the collection and analysis of tumour tissue, serial blood samples and clinical data in patients with stage I, II and III CRC.

Part C (ctDNA guided adjuvant chemotherapy versus standard of care adjuvant chemotherapy) is a nested biomarker guided group which will include patients with high risk stage II or stage III colon or rectal cancer who have undergone curative surgery and are willing to take part in ctDNA guided adjuvant chemotherapy cohort of the study. Patients with rectal cancer who have undergone neo-adjuvant radiotherapy or chemoradiotherapy followed by curative surgery will also be included.

17.1 Analysis populations

For the Translational study (Part B)
All data from all eligible patients will comprise the main analysis set and will be used for all analysis unless otherwise specified.

For our a-priori hypotheses (section 4 and as below), we will include the following study population.

Hypothesis 1: In patients with stage II and III CRC, detection of mutations in ctDNA in plasma can predict relapse.

Hypothesis 2: We hypothesise that ctDNA directed adjuvant chemotherapy administration will enable biomarker driven selection of patients who would benefit from adjuvant chemotherapy and thereby reduce proportion of patients receiving adjuvant chemotherapy without compromising disease free survival

For testing hypothesis 1:
All analyses will be presented including all patients and then split by disease stage II & III. This will include the following patients:

1. Patients in Part A of the study
2. Patients in Part B of the study

For testing hypothesis 2:
This will include all patients treated in Part C (randomised study of ctDNA guided adjuvant chemotherapy versus standard of care adjuvant chemotherapy). The primary population for analysis will be the intention to treat (ITT) population, defined as all eligible randomised patients. A sensitivity per protocol (PP) analysis will also be performed defined as all those receiving treatment as planned per randomisation on Part C excluding major protocol deviations.

17.2 Study Endpoints

Endpoints will be analysed as outlined below. In patients with rectal cancer whose tumour is down-staged by neo-adjuvant radiotherapy or chemoradiotherapy (CRT), the baseline radiological staging prior to CRT will be considered as the stage for analysis. In straight to surgery patients , the surgical staging will be considered for analysis.

Primary end point for Part A (Feasibility Study):

- The percentage of patients with stage II and III CRC that have detectable ctDNA pre-operatively

  Detectable ctDNA is defined as the presence of at least one tumour-derived mutation above the limit of detection (LOD) threshold for that particular mutation assay in a duplicate investigation.

Secondary Endpoints for Part A:

- The concordance rate between mutations detected by targeted resequencing in tumour tissue and mutations detected by digital PCR in cfDNA
- The correlation between the change in detectable mutations in plasma cfDNA at the first post-operative visit and the change in CEA
- The percentage of patients who have detectable mutations in cfDNA post-operatively, out of the patients with detectable mutations in tumour tissue and cfDNA pre-operatively
- The percentage of patients that have detectable mutations in cfDNA post-operatively that did not have mutations in cfDNA pre-operatively but had mutation(s) in the tumour tissue alone

Primary Endpoint for patients in Part B of the study

- The association between detectable ctDNA at the first post-operative visit & RFS.

Secondary Endpoints for all patients (Part A+B, as relevant):

- The association between detectable ctDNA with RFS, loco-regional relapse free survival, distant relapse free survival and OS at the following time-points: pre-operative, the first post-operative visit, during chemotherapy and post-chemotherapy
- The association between the level of ctDNA with RFS, loco-regional relapse free survival, distant relapse free survival and OS at the following time-points: pre-operative, the first post-operative visit, during chemotherapy and post-chemotherapy
- The association between the time of rise in the level of ctDNA and RFS, where rise is defined as an increase in the ctDNA level in two consecutive samples.
- The association between the change in quantity of ctDNA from the pre-operative and the first post-operative sample with RFS

- In patients having adjuvant chemotherapy, the percentage of patients who have detectable ctDNA post-operatively, that no longer have detectable ctDNA on completion of adjuvant chemotherapy
- In patients receiving adjuvant chemotherapy, the association between the change in quantity of ctDNA from the pre and post chemotherapy samples with RFS
- In rectal patients receiving neo-adjuvant radiotherapy and chemoradiotherapy, the association between the change in quantity of ctDNA from the pre and post radiotherapy/chemoradiotherapy sample with RFS
- The association between the change in quantity of ctDNA from the end of all treatment and subsequent surveillance visits with RFS
- To assess the presence or absence of measurable disease radiologically with the presence or absence of detectable mutations in plasma cfDNA post-operatively
- The lead time between rise in ctDNA and rise in CEA from the post-operative values
- The lead time between rise in ctDNA from the post-operative values and radiological relapse
- The association between ctDNA and prognostic factors will be investigated
- Multi-variate analysis of RFS will investigate the addition of ctDNA variables in significant univariate analysis to standard prognostic factors
- To estimate a threshold for detection of ctDNA (actual level and rise) from that predicts relapse in a training set of patients and to be validated in the validation set.

Primary Endpoint for Part C of the study
Difference in 3 year disease-free survival from time of surgery to progression, between standard of care arm and ctDNA guided adjuvant chemotherapy arm

Secondary Endpoints for Part C of the study

1. Proportion of patients in the ctDNA guided arm receiving standard of care chemotherapy in the ctDNA negative group
2. Proportion of patients who are ctDNA negative post-operatively who become ctDNA positive during follow-up and receive chemotherapy as an escalation
3. Overall survival between both arms, defined as time from randomisation to death of any cause.
4. Sub-group analyses performed on 3-year DFS and OS in including but not limited to the following will be performed:
   (a) high risk stage II versus stage III
   (b) site of primary tumour (right colon versus left colon versus rectum).
5. Neurotoxicity data between both arms (FACT/GOG-Ntx4 & CTCAE V5)
6. Quality of life data (EORTC QLQ-C30 and CR29 and EQ-5D-3L)
7. Health resource utilisation data between both arms (RUtInE™ questionnaire)
8. Descriptive analysis (frequencies and proportion) will be used to describe clinico-pathological characteristics in patients recruited in the MMRd cohort, according to study arm

17.3 Sample Size Considerations

For Feasibility and Main Study (Part A and B)
In total it is anticipated that a minimum of 1000 patients will be recruited, of which a minimum of 500 evaluable will have stage II and 500 evaluable stage III disease. We plan to over-recruit to account for drop-outs.

The first 48 patients will comprise the pilot study (Part A). These patients will be included in the overall planned recruitment target of >1000 patients. The statistical analysis for the pilot study will be conducted after the first 24 patients with confirmed stage II disease and the first 24 patients with stage III disease have been recruited. If 12 or more of the 48 patients have trackable mutations detected in the cfDNA pre-operatively then this would rule out a 15% detection rate in favour of a 30% rate, with 80% power and 5% significance level. This would be used as a guide as to whether to continue to the main study or not.

In the stage II patients, it is assumed that 16% of patients will have detectable ctDNA at the first post-operative visit and that overall in stage II patients, the 5 year DFS will be 80%. With 90% power and 5% 2-sided significance, a difference of 10% in 5 year DFS can be detected from 75% (in 79 subjects with detectable ctDNA) to 85% (in 395 subjects with no-detectable ctDNA). Therefore in total, 474 subjects will be needed and 78 events. If the actual detectable rate is 10% or 25% rather than 16% then still less than 500 subjects would be required (n=460 and 492 respectively).

In the stage III patients, it is assumed that 40% of patients will have detectable ctDNA at the first post-operative visit and that overall in stage III patients, the 5 year DFS will be 65%. With 90% power and 5% 2-sided significance, a difference of 15% in 5 year DFS can be detected from 57.5% (in 144 subjects with detectable ctDNA) to 72.5% (in 216 subjects with no detectable ctDNA). Therefore in total, 360 subjects will be needed and 119 events. If the actual detectable rate is 30% or 50% rather than 40% then still less than 500 subjects would be required (n=330 and 410 respectively).

One of the secondary endpoints is to estimate a threshold for detection of ctDNA that predicts relapse by using a training set in half of the patients and a validation set in the second half of the patients. As this is a secondary endpoint as opposed to the primary endpoint, the study does not necessarily need to be adequately powered for this purpose but if 500 patients are recruited into each cohort then each cohort will have 250 in the training set and 250 in the validation set, which should give sufficient numbers for estimation under ROC analysis.

It is anticipated that we would also recruit at least 60 stage I patients per year (assuming 10-15% rate) for 3 years, giving a total of 180. With these numbers we can estimate the ctDNA detection rate in stage I patients pre-op sample with $\pm$ 7.3%. Descriptive analysis will be used to describe data from patients with stage I colorectal cancer.

For randomised ctDNA guided versus standard of care adjuvant chemotherapy, Part C of the study
Based on a standard 3-year DFS of 75% to demonstrate non-inferiority in survival with 80% power, one-sided $\alpha = 0.05$ with non-inferiority hazards ratio of 1.25, a sample size of 810 subjects in each arm is estimated for a total number of 499 events.

A total of 1620 subjects (810 patients in each arm) would need to be randomised, with 499 events required based on the following assumptions:

1) 5 year accrual
2) 3 year minimum follow up on all
3) 5% one-sided significance level
4) 80% power
5) 1, 2, 3, 4, 5, 6 year DFS estimated from SCOT study as 0.9, 0.8, 0.75, 0.725, 0.7, 0.68
6) Non inferiority margin = 1.25 (ruling out 69.8% 3 year DFS)

For Part C of the study, a non-inferiority margin of 1.25 was chosen as this will allow for a worsening of 3-year DFS from 75% to 69.8% as being clinically acceptable. The accrual target will be inflated by ~5% to account for drop-outs, therefore the overall total accrual target will be around 1700 patients.

For randomised MMRd/MSI-H patients
The analysis of this exploratory cohort will be descriptive, therefore a sample size calculation has not been performed. 10-15% of patients with early stage resectable CRC have MMRd/MSI-H disease. The accrual number for this sub-study will be limited by the number of patients with MMRd/MSI-H disease who enrol whilst Part C is open to recruitment. It is anticipated up to 100 patients will be recruited into this cohort.

Overall study population
It is anticipated that the overall study population will be at least 2700 (1,700 more than initially planned) including drop-outs. This is anticipated to take approximately 10 years in total from the start of study recruitment in 2016. The protocol requires 1,620 contemporaneously evaluable randomised patients for Part C.
It is considered that to test the first hypothesis of ctDNA as a biomarker of relapse, 500 evaluable stage II and 500 evaluable stage III patients will be derived from the recruited patients.

Once confirmation that we have recruited 1620 evaluable patients for Part C of the study and that the non-interventional study has 500 evaluable stage II (and 500 evaluable stage III, we will consider halting recruitment to that stage. We plan to over-recruit to account for drop-outs.

17.4 Interim Analyses and Futility Analyses

The study incorporates a feasibility part A study and an interim analysis for Part B as follows:

1) Part A will incorporate the first 48 patients and be looking at the proportion of patients with trackable mutations in cfDNA pre-operatively.
2) The interim analyses will be performed once the first 150 patients have reached their one year post-operative visit and their plasma has been analysed. This analysis will be descriptive to see the proportion of patients that have detectable

mutations in cfDNA in the first post-operative sample and if there is any evidence that detectable mutations can predict relapse. The interim analysis will also be used to check that all treatment modalities are being adequately represented.

Interim/ Futility Analysis for ctDNA guided adjuvant chemotherapy arm of the study (Part C)

Stopping for safety will be based on the recommendations from the IDMC and endorsed by a TSC. Stopping based on lack of efficacy (futility) will be based on the combination of evidence from safety and the conditional power. If the conditional power is <20% after 25% or 50% of the DFS events, the study will be considered futile. The IDMC will offer the overall recommendation based on clinical and statistical data for stopping for futility.

The IDMC may also meet at other times as required but as a minimum we will consider 2 interim/futility assessments planned using conditional power.

- 25% events (n=125) would occur by year 4 (with 1280 patients recruited)
- 50% events (n=250) would occur by year 5 (with all patients recruited)

17.5 Planned Methods of Analysis

A detailed statistical analysis plan will be created by the study statistician and approved by the Chief Investigator prior to study reporting as per the Trust's Standard Operational Procedures.

Analysis population - Interventional study (Part C)

The primary analysis population will be the intention to treat (ITT) population. The ITT population will be defined as all eligible randomised patients.

A sensitivity analysis will be performed on the per (pp) protocol population. The PP population will be defined as all patients randomised to study treatments, standard of care or ctDNA guided adjuvant chemotherapy, who received the treatment assigned at randomisation without major protocol deviations.

Survival endpoints are defined as follows:

Recurrence Free Survival (RFS) is defined as the time from date of surgery to first radiologically confirmed relapse or death from CRC. All patients who are alive, disease free, or who have died from any other cause will be censored at last follow-up or at time of death.

Disease Free Survival (DFS) as time from date of surgery to first radiologically confirmed relapse or death of any cause. All patients alive and disease free will be censored at last follow-up.

Overall Survival (OS) as time from date of surgery to death of any cause. All patients alive will be censored at last follow-up.

Loco-regional relapse free survival as date of surgery to radiological confirmation of first loco-regional relapse. All patients alive and event free will be censored at last follow-up or at death if death prior to loco-regional relapse.

Distant relapse free survival as time from date of surgery to first distant relapse. All patients alive and event free will be censored at last follow-up or at death if death prior to distant relapse.

Feasibility Study (Part A)

The analysis for the feasibility study is going to be mainly descriptive. Continuous data will be summarised as n, mean, standard deviation, median, minimum value and maximum value. Categorical data will be presented as frequencies and percentages.

The primary endpoint, percentage of patients with stage II and III CRC that have detectable ctDNA pre-operatively, will be presented with 95% confidence intervals. This will be calculated in all patients and secondly by disease stage (II & III)

Translational Study (Part B)

A CONSORT flow diagram will be produced to describe the flow of patients through the study.

Continuous data will be summarised as n, mean, standard deviation, median, minimum value and maximum value. Categorical data will be presented as frequencies and percentages.

The primary endpoint, RFS by detectable ctDNA or not at the first post-operative visit will be presented as median RFS and 1, 2, 3 year RFS using Kaplan Meier methods. Differences in RFS between those with detectable ctDNA or not post-operatively will be compared using a log rank test. Hazard ratios and 95% confidence intervals will be presented from Cox regression model.

The association between detectable ctDNA and RFS, loco-regional relapse free survival, distant relapse free survival and OS at the following time-points: pre-operative, the first post-operative visit, during chemotherapy and post-chemotherapy, will be performed as per the primary endpoint,

Sensitivity is defined as the proportion of participants who develop recurrence that have ctDNA detected at or before the time of clinical detection of recurrence.

Specificity is defined as the proportion of participants who do not develop recurrence that have ctDNA not detected at or before the time of their last clinical follow-up.

RFS will be assessed as the primary endpoint but by the following factors:

- Time of rise in ctDNA level grouped into 6 months.
- Changes in ctDNA levels from the pre-operative to first post-operative sample
- Changes in ctDNA levels from the post-operative to the first post-chemotherapy samples (in those receiving adjuvant chemotherapy)
- Changes in ctDNA levels from the pre-and post-radiotherapy/chemoradiotherapy samples (in those rectal patients receiving neo-adjuvant radiotherapy or chemoradiotherapy)
- Changes in ctDNA levels from the pre- operative to subsequent surveillance visit samples

If any ctDNA variables in the above analyses are found significant univariately (p-value<0.1) then they will be added to a multivariate cox regression model (including but not limited to TNM stage, tumour location, extramural, vascular and perineural invasion status, grade of tumour differentiation, treatment received and performance status) to see if the association remains after adjustment for standard prognostic factors.

The association between presence / absence of measurable disease and presence / absence of detectable ctDNA post-operatively will be performed using a chi-squared test or exact test as appropriate.

The difference in time to rise in CEA and rise in ctDNA in post-operative values will be summarised descriptively.

The difference in time to rise in CEA in post-operative values and radiological relapse will be summarised descriptively.

Logistic regression will be used to investigate if any prognostic factors are associated with detectable ctDNA or level of ctDNA (above and below median). At least the following prognostic factors will be assessed: TNM stage, tumour location, extramural, vascular and perineural invasion status, grade of tumour differentiation, treatment received and performance status. Odds ratios and 95% confidence intervals will be presented.

Receiver operating characteristic (ROC) analysis, including sensitivity and specificity will be used to define a cut off for ctDNA (actual value and rise) defining response in the test set (first 50% patients). This will then be tested in the validation set (second 50% patients). Descriptive analysis will be used to describe data from patients with stage I colorectal cancer.

For Part C of the study

Primary Endpoint
The primary endpoint, disease-free survival, will be calculated by arm using Kaplan Meier method and 3 year DFS will be presented with 95% confidence intervals. Hazard ratio (HR) and 95% confidence intervals (CI) for difference between groups will be produced from a cox regression model. If the HR CI includes the non-inferiority margin then non-inferiority will not have been demonstrated. If the HR CI excludes the non-inferiority margin then non-inferiority will have been demonstrated.

Secondary Endpoints

1. Proportion of patients in the ctDNA guided arm receiving standard of care chemotherapy will be presented as a proportion with 95% confidence intervals and proportion of patients in whom standard of care chemotherapy is de-escalated is calculated.
2. Proportion of patients in the ctDNA guided arm who are ctDNA negative but become ctDNA positive will be calculated and 3-year DFS and OS assessed in this group of patients.
3. Overall survival between both arms will be reported as per DFS.
   Sub-group analyses performed on 3-year DFS and OS by arms, including but not limited to the following will be performed:
   (a) high risk stage II versus stage III
   (b) site of primary tumour (right colon versus left colon versus rectum).
   Hazard ratio and 95% confidence intervals for difference between sub-groups will be produced from a cox regression model.
4. Neurotoxicity data between both arms will be presented as proportion of patients, with 95% confidence interval, reporting neurotoxicities as per CTCAE V5 and will be compared using chi-squared or exact test as appropriate. The FACT/GOG-Ntx4 neurotoxicity scores will be presented as per the standard guidelines.

MMRd/ MSI-H Cohort

Descriptive analysis only will be used to present data from patients included in the MMRd-MSI-H cohort. Descriptive statistics including n, mean, SD, median, minimum, and maximum for numeric variables and frequency and percentage for categorical variables will be presented.

17.6. Quality of Life and Cost-effectiveness Analysis

Quality of life data (EORTC QLQ-C30 & CR29 and EQ-5D-3L) will be presented as per standard guidelines and compared between two study arms. The derived QOL scores will be described as medians or means (depending on distribution) and plotted overtime.

Economic evaluation will assess the cost-effectiveness of ctDNA directed therapy in comparison with standard care over the projected lifetime of individuals in the two groups. Furthermore, we will also investigate whether the proposed intervention is cost-saving. The analysis will be carried out within the UK setting, from the perspective of the National Health System (NHS) and Personal Social Services (PSS), in line with the National Institute for Health and Care Excellence (NICE) guidelines on economic evaluation (National Institute for Health and Clinical Excellence. Guide to the methods of technology appraisal. NICE: London, 2013). This will enable us to provide the NHS with timely evidence on the potential cost-effectiveness of the proposed intervention.

Healthcare costs for the control and intervention groups will be estimated using the latest NHS Reference Costs combined with health resource utilisation data. The latter will be captured through trial records and self-report questionnaires (RUtInE^{TM}). Quality of life data (EQ-5D-3L) will inform the analysis along with data on the observed disease free interval and overall survival between the treatment arms. Results of the cost-effectiveness analysis will be reported as cost per quality-adjusted life year (QALY) gained.

Cost-effectiveness analysis assumptions will be tested through univariate, multivariate and probabilistic sensitivity analysis, with the latter used to characterise uncertainty in parameter estimates.

Health resource utilisation questionnaires are usually developed ad hoc and are not standardised nor assessed on their quality. Piloting such questionnaires is not typical, while response rates are not usually reported in health economics literature, thus no guidelines exist on appropriate standards.

A pilot study on 40 patients will be carried out to help us understand how well patients are responding to the RUtInE^{TM} questionnaire (Appendix 11). We will assess the RUtInE^{TM} questionnaire's response rate (RR), defined as the percentage of patients returning a questionnaire at each time point out of those expected (i.e., not withdrawn or died). The response rate will be assessed in the first 40 evaluable patients at the following timepoints: randomisation, months 3 and month 6. Patients will be included in the pilot sample only if they were provided with a copy of the RUtInE^{TM} questionnaire during their clinic visit at all time points included in the analysis.

Questionnaires will be considered returned if they have been returned to the clinic or by post by their next planned visit. If the mean response rate across all time points is less than 60%, consideration will be given to adopting alternative data collection strategies to increase the response rate. A mean response rate of 60% or higher will be considered satisfactory and the data collection strategy will not be changed.

Alternative strategy could take the form of i) reducing the RUtInE^{TM} length to only include data necessary to the cost-effectiveness analysis (questions 1 to 9) and thus removing

questions relevant to the patient-perspective on costs (questions 10 to 21); ii) reducing number of timepoints the RUtInE^{TM} is given to the patients.

We will also analyse the questionnaire's completeness rate (CR) defined as the percentage of patient responses to questionnaire items out of the total number of items included in the questionnaire. Pilot participants who returned their questionnaires at all three time points (randomisation and months 3 and 6) will be included in the CR analysis.

Data included in the pilot study will also be part of the final analysis, while the completion of RUtInE^{TM} will continue while the pilot study's data are analysed. During this pilot we will also monitor the percentage of patients who have returned questionnaires at all time points, as well as data completeness.

17.7 Randomisation Procedure (PART C only and MMRd/MSI-H study)

Completed randomisation and anonymised baseline assessment forms should be e-mailed to the sponsor GI and Lymphoma CTU. The original documents should be retained by the site. The documents which should be e-mailed at the same time are listed on the randomisation case report form (CRF).

Randomisation E-mail	**TRACCstudy@rmh.nhs.uk**

Randomisation will be performed at the Institute for Cancer Research – Clinical Trials and Statistical Unit (ICR-CTSU), by random permuted blocks.

The randomisation will be stratified by the following factors:

TRACC Part C (patients with MMRp tumours)

1. High risk stage II versus stage III
2. Site of primary tumour: right colon versus left colon versus rectum

MMRd/MSI-H cohort

1. High risk stage II versus stage III
2. Site of primary tumour: right colon versus left colon versus rectum

The result of the randomisation and the study number assigned to the patient will be emailed to the study personnel at the participating site responsible for the randomisation.

Statistical software
Stata version 18 will be used for all analyses.

18. REGULATORY OBLIGATIONS

18.0 Informed Consent

Prior to participation in the clinical study, the principal investigator (or a member of the study team named in the site signature log and authorised by the site principal investigator – for Part C this individual must be a qualified doctor or specialist health care professional with the required qualification of their Trust who is authorised to prescribe, consent and make decisions regarding adjuvant chemotherapy) is responsible for obtaining written informed consent from the patient after adequate explanation of the aims, methods, anticipated benefits, and potential risks of the study. This must take place before protocol-specific screening procedures or treatment is administered. Where patients are being seen in person, face-to-face consent should proceed as usual.
Due to the COVID-19 pandemic the patient pathway has changed and routine care now involves telephone or video consultations. For patients with early colorectal cancers, remote consultations may take place prior to curative surgery, and/or prior to commencing adjuvant chemotherapy.

Remote consent for parts B and C of the TRACC trial can be taken by telephone or video consultation. A member of the study team will contact the patient to discuss the trial. Should the patient wish to participate and agree to remote consent, this should be documented in their notes. The remote consent will follow a structured process, details of which can be found in the guidance note on Remote Consent.

For Part B, informed consent can be obtained on the same day that the PIS is issued. 24 hours must be allowed for the patient to read the PIS/ICF prior to consenting for Part C of the study.

The acquisition of informed consent should be documented in the patient's medical records, and the ICF should be signed and personally dated by the patient and by the authorised person who conducted the informed consent discussion. The original signed informed consent form should be retained in accordance with institutional policy and GCP, and a copy of the signed consent form should be provided to the patient or legally acceptable representative.

If a patient is illiterate or visually impaired and does not have a legally acceptable representative, the investigator must provide an impartial witness to read the informed consent form to the patient and must allow for questions. Thereafter, both the patient and the witness must sign the informed consent form to attest that informed consent was freely given and understood. If a patient requires help with language, an interpreter can be present on the call for remote consent to translate. A witness must be present and this must be documented in the patient's notes.

18.1 Independent Ethics Committee/Institutional Review Board

A copy of the protocol, proposed informed consent form, other written patient information, and any proposed advertising material must be submitted to an independent ethics committee and any other relevant regulatory authorities, subject to the regulations of the country of each participating site, for written approval. A copy of the written approval of

the protocol and informed consent form must be received by the sponsor before recruitment of patients into the study and shipment of investigational product.

The investigator must submit and, where necessary, obtain approval from the independent ethics committee concerned and any other relevant regulatory authorities for all subsequent protocol amendments and changes to the informed consent document. The investigator should notify the same of deviations from the protocol.

18.2 Pre-study Documentation Requirements

The investigator is responsible for forwarding the following documents to the study sponsor for review before study initiation from the sponsor can occur:

- Signed and dated protocol signature page (see Investigator's Agreement on page 3)
- Copy of local research and development office and site specific assessment approvals, or equivalents thereof.
- Copy of approved patient information sheet and informed consent form on headed paper which will be used at the site
- Up-to-date curricula vitae of the principal investigator and lead pharmacist
- GCP training certificate (which must be within 3 years)
- Contact details for key study personnel and completed, signed site delegation log
- Signed study contract
- Any relevant pharmacy forms (drug supply and accountability).

18.3 Patient Confidentiality

The investigator must ensure that the patient's confidentiality is maintained in compliance with the UK Data Protection Act of 2018. On the case report forms or other documents submitted to the sponsor, patients should be identified by their initials and a patient study number only. Documents that are not for submission to the sponsor (e.g., signed informed consent forms) should be kept in strict confidence by the investigator.

In compliance with GCP guidelines, it is required that the investigator and institution permit authorised representatives of the sponsor and of the regulatory agency(s) direct access to review the patient's original medical records for verification of study-related procedures and data. Direct access includes examining, analysing, verifying, and reproducing any records and reports that are important to the evaluation of the study. The investigator is obligated to inform and obtain the consent of the patient to permit named representatives to have access to his/her study-related records without violating the confidentiality of the patient.

18.4 Investigator Signatory Obligations

Each clinical study report or case report form should be signed by the investigator or a member of the study team named in the site signature log and authorised by the site principal investigator.

Original copies of all completed case report forms should be retained by the site once they have been emailed to the GI & Lymphoma Trials Unit.

19. SAFETY DATA COLLECTION, RECORDING AND REPORTING

Adverse Events Reporting

Data on neurotoxicity related to oxaliplatin treatments as per CTCAE v5.0 will be collected and reported in the CRFs. Only those toxicities listed in the SmPC leading to dose reductions in chemotherapy need to be recorded in CRFs. All other toxicities listed in the SmPC for these drugs do not need to be reported or recorded.

Serious Adverse Events Reporting

The study will use chemotherapy drugs that are routinely used as standard of care, adverse events and serious adverse events will not need to be reported for any known adverse reaction to Oxaliplatin and/or Capecitabine as listed in the Summary of Product Characteristics (SmPC) for each drug does not need to be reported.

Exemptions from SAE reporting will also include the following:

- SAEs that occur after consent and registration/randomisation but prior to any trial treatment do not require to be reported.

- SAEs that are unrelated to the trial treatment including the following:

  - Elective hospitalisation and surgery for treatment of locally advanced rectal carcinoma or its complications e.g., bowel obstruction

  - Elective hospitalisation to planned procedures e.g., central venous access device insertion

  - Elective hospitalisation for any treatment including administration of adjuvant chemotherapy in standard of care arm or de-escalation/ treatment/ re-escalation of chemotherapy in ctDNA guided arm

  - Elective hospitalisation for palliative care

  - Disease progression leading to hospitalization, or prolongation of hospitalization, or death as a result of disease progression

- Pregnancy itself is not regarded as an AE unless there is a suspicion that the trial treatment under investigation may have interfered with the effectiveness of a contraceptive medication. Patients must be withdrawn from the trial if they become pregnant. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented. All reports of congenital abnormalities or birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs.

If any unexpected serious adverse reactions deemed related to either of the chemotherapy agents used in the study are identified, they should be reported to the MHRA using the Yellow card system.

20. ADMINISTRATIVE AND LEGAL OBLIGATIONS

20.0 Study Documentation and Archive

The investigator should maintain a list of appropriately qualified persons to whom he/she has delegated study duties. All persons authorised to make entries and/or corrections on case report forms will be included on the site signature log.

Source documents are original documents, data, and records from which the patient's case report form data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence. Case report form entries may be considered source data if the case report form is the site of the original recording (i.e., there is no other written or electronic record of data). In this study, case report form may be used as source documents.

The investigator and study staff are responsible for maintaining a comprehensive and centralised filing system of all study-related (essential) documentation, suitable for inspection at any time by representatives from the sponsor and/or applicable regulatory authorities. Elements should include:

- Patient files containing completed case report forms, informed consent forms, and patient identification list
- Study files containing the protocol with all amendments, investigator's brochure, copies of pre-study documentation (see Section 18.2), and all correspondence to and from the sponsor and institutional review board.
- If kept, proof of receipt, Investigational Product Accountability Record, Return of Investigational Product for Destruction, Final Investigational Product Reconciliation Statement, and all drug-related correspondence

In addition, all original source documents supporting entries in the case report forms must be maintained and be readily available.

No study document should be destroyed without prior written agreement between the sponsor and the investigator. Should the investigator wish to assign the study records to another party or move them to another location, he/she must notify the sponsor in writing of the new responsible person and/or the new location.

20.1 Study Monitoring

The study will be centrally monitored using the following procedures.

Central Eligibility checking at registration
Registration eligibility criteria will be checked against source documentation emailed to the trials office. Registration will only take place once central checking of documents is complete and any queries addressed.

Statistical Monitoring
The trial statistician will regularly examine the data for anomalies and outliers, such as too few or too many events. Queries will be raised by the trial coordinators in such situations and communication with the clinical teams will take place. In addition statistical monitoring of unusual dates and inconsistent data will take place. Again these will raise queries via the trial coordinators.

Sponsor GI Unit Research Meetings
Recruitment will be discussed regularly at the weekly GI Unit Research meetings.

Trial Management Group (TMG) meetings
There will be regular trial meetings to review data accrual and completeness. Problems with individual centres will be raised and addressed at the TMG meetings. The TMG will meet at minimum frequency of every 6 months.

Independent Data Monitoring Committee (IDMC)
The IDMC will perform a monitoring role in examining the emerging data in the study. They will be privy to all the results as necessary to perform this role. They will meet annually during the life of the study to review safety, scientific validity and the conduct of the trial. This will be conducted according to the IDMC charter and will meet annually.

Trial Steering Committee (TSC)
The role of the TSC is to provide oversight for the trial on behalf of the sponsor. It should also provide advice through its independent Chairman to the Trial Management Group (TMG) on all aspects of the trial. All proceedings will be conducted as per the TSC charter and they will meet annually.

Database validation checks
This study will use eCRFs and the MACRO database. The investigator will ensure that data are recorded on the eCRFs as specified in the study protocol and in accordance with the instructions provided. The investigator ensures the accuracy, completeness, legibility

and timeliness of the data recorded and of the provision of answers to data queries according to the Clinical Study Agreement.

20.2 Publication Policy

Authorship of any publications resulting from this study will be determined on the basis of the Uniform Requirement for Manuscripts Submitted to Biomedical Journals (International Committee of Medical Journal Editors, 2005), which states:

- Authorship credit should be based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.
- When a large, multicentre group has conducted the work, the group should identify the individuals who accept direct responsibility for the manuscript. These individuals should fully meet the criteria for authorship defined above.
- Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship.
- All persons designated as authors should qualify for authorship, and all those who qualify should be listed.
- Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.

20.3 Compensation

No specific compensation arrangement exists for harmful events which might arise from participation in the study. However, the study is covered for negligent claims occurring with the NHS by Crown indemnity. There is no pre-existing arrangement for non-negligent claims arising from the conduct of the study.

20.4 Trial sponsorship and financing

The study sponsor is the Royal Marsden NHS Foundation Trust, Downs Road, Sutton, Surrey. SM2 5PT, United Kingdom. There is support from the National Institute of Health Research and the Royal Marsden Cancer Charity for this study.

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Appendix Ia: AJCC staging of colon and rectal cancer (7th edition)

Definitions

Primary Tumour (T)	
Tx	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ: intraepithelial or invasion of lamina propria
T1	Tumour invades sub-mucosa
T2	Tumour invades muscularis propria
T3	Tumour invades through the muscularis propria into pericolorectal tissues
T4a	Tumour penetrates to the surface of the visceral peritoneum
T4b	Tumour directly invades or is adherent to other organs or structures
Regional Lymph Nodes (N)	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in 1-3 regional lymph nodes
N1a	Metastasis in one regional lymph nodes
N1b	Metastasis in 2-3 regional lymph nodes
N1c	Tumour deposit(s) in the subserosa, mesentery, or non-retroperitonealized pericolic or perirectal tissues without regional nodal metastasis
N2	Metastasis in 4 or more regional lymph nodes
N2a	Metastasis in 4-6 regional lymph nodes
N2b	Metastasis in 7 or more regional lymph nodes
Distant Metastasis	
M0	No distant metastasis
M1	Distant metastasis
M1a	Metastasis confined to one organ or site (for example, liver, lung, ovary, nonregional node)
M1b	Metastases in more than one organ/site or the peritoneum

ANATOMIC STAGE/ PROGNOSTIC GROUPS				
Stage	**T**	**N**	**M**	**Dukes'**
0	Tis	N0	M0	-
I	T1	N0	M0	A
	T2	N0	M0	A
IIA	T3	N0	M0	B
IIB	T4a	N0	M0	B
IIC	T4b	N0	M0	B
IIIA	T1-T2	N1/N1C	M0	C
	T1	N2a	M0	C
IIIB	T3-T4a	N1/N1c	M0	C
	T2-T3	N2a	M0	C
	T1-T2	N2b	M0	C
IIIC	T4a	N2a	M0	C
	T3-T4a	N2b	M0	C
	T4b	N1-N2	M0	C
IVA	Any T	Any N	M1a	-
IVB	Any T	Any N	M1b	-

cTNM is the clinical classification, pTNM is the pathological classification. The y prefix is used for those cancers that are classified after neo-adjuvant pre-treatment (for example, ypTNM). Patients who have a complete pathological response are ypT0N0cM0 that may be similar to stage Group 0 or I.

Appendix Ib: AJCC Colon and Rectum Cancer Staging (8th Edition)

Primary tumour (pT)

TX: primary tumour cannot be assessed
T0: no evidence of primary tumour
Tis: carcinoma in situ, intramucosal carcinoma (involvement of lamina propria with no extension through muscularis mucosae)
T1: tumour invades submucosa (through the muscularis mucosa but not into the muscularis propria)
T2: tumour invades muscularis propria
T3: tumour invades through the muscularis propria into the pericolorectal tissues
T4:
- **T4a:** tumour invades through the visceral peritoneum (including gross perforation of the bowel through tumour and continuous invasion of tumour through areas of inflammation to the surface of the visceral peritoneum)
- **T4b:** tumour directly invades or adheres to other adjacent organs or structures

Regional lymph nodes (pN)

NX: regional lymph nodes cannot be assessed
N0: no regional lymph node metastasis
N1: metastasis in 1 - 3 regional lymph nodes
- **N1a:** metastasis in 1 regional lymph node
- **N1b:** metastasis in 2 - 3 regional lymph nodes
- **N1c:** no regional lymph nodes are positive but there are tumour deposits in the subserosa, mesentery or nonperitonealized pericolic or perirectal / mesorectal tissues

N2: metastasis in 4 or more regional lymph nodes
- **N2a:** metastasis in 4 - 6 regional lymph nodes
- **N2b:** metastasis in 7 or more regional lymph nodes

Distant metastasis (pM)

M0: no distant metastasis by imaging; no evidence of tumour in other sites or organs
M1: distant metastasis
- **M1a:** metastasis confined to 1 organ or site without peritoneal metastasis
- **M1b:** metastasis to 2 or more sites or organs is identified without peritoneal metastasis
- **M1c:** metastasis to the peritoneal surface is identified alone or with other site or organ metastases

Stage grouping

Stage 0:	Tis	N0	M0
Stage I:	T1 - T2	N0	M0
Stage IIA:	T3	N0	M0
Stage IIB:	T4a	N0	M0
Stage IIC:	T4b	N0	M0
Stage IIIA:	T1 - T2	N1 / N1c	M0
	T1	N2a	M0
Stage IIIB:	T3 - T4a	N1 / N1c	M0
	T2 - T3	N2a	M0
	T1 - T2	N2b	M0
Stage IIIC:	T4a	N2a	M0
	T3 - T4a	N2b	M0
	T4b	N1 - N2	M0
Stage IVA:	any T	any N	M1a
Stage IVB:	any T	any N	M1b
Stage IVC:	any T	any N	M1c

Appendix (II) RUtInE™ questionnaire (health resource utilisation questionnaire for health economic analysis)

RUtInE™ – Bowel Cancer

A questionnaire on health-related costs

This questionnaire will help us estimate the costs related to your health and explore the costs of different approaches to treatment.

Some of the questions below will help with estimating the costs to you and your family, while others will help us understand the costs to the health care system and to the wider economy.

Please try to answer the questions as accurately as possible.

1. Please provide information for any tests/investigations you had in the **last three months** because of your **bowel cancer or its treatment**.

Test/investigation	Have you had any?	How many?
Blood test	Yes ☐ No ☐	.....
Magnetic Resonance Imaging (MRI)	Yes ☐ No ☐	.....
CT /CAT scan	Yes ☐ No ☐	.....
PET-CT scan	Yes ☐ No ☐	.....
Sigmoidoscopy	Yes ☐ No ☐	.....
Colonoscopy	Yes ☐ No ☐	.....
Other (please specify)		.....
Other (please specify)		.....

2. In the **last three months**, have you used any of the following health or care services because of your **bowel cancer or its treatment**? Please exclude any appointments already listed under question 1.

Service	Have you used this service?	How many times?
Doctor at GP practice	Yes ☐ No ☐	.....surgery visits
	Yes ☐ No ☐	.....home visits
	Yes ☐ No ☐	.....phone calls
Nurse at GP practice	Yes ☐ No ☐	.....surgery visits
Hospital doctor (not GP)	Yes ☐ No ☐	.....hospital visits
Hospital nurse (doctor not present)	Yes ☐ No ☐	.....hospital visits
Nurse home visits	Yes ☐ No ☐	.....home visits
Hospital stay	Yes ☐ No ☐	.....days
Intensive care unit	Yes ☐ No ☐	.....days
999 calls (emergencies)	Yes ☐ No ☐	.....phone calls
111 calls (non-emergencies)	Yes ☐ No ☐	.....phone calls
Other hospital phone calls for treatment or follow-up (made or received)	Yes ☐ No ☐	.....phone calls
Accident & emergency (A&E)	Yes ☐ No ☐	.....attendances (arrived by ambulance)
	Yes ☐ No ☐	.....attendances (no ambulance used)
Physiotherapist	Yes ☐ No ☐	.....visits
Social worker	Yes ☐ No ☐	.....visits
Occupational therapist	Yes ☐ No ☐	.....visits
Home care worker	Yes ☐ No ☐	.....visits
Dietitian	Yes ☐ No ☐	.....visits
Individual counselling	Yes ☐ No ☐	.....visits
Other (please specify)		.....
Other (please specify)		.....
Other (please specify)		.....

3. In the **last three months**, have you used the services of any of the following institutions because of your **bowel cancer or its treatment**? Please provide the number of days you have received each service.

Service	Have you used this service?	How many days?
Residential home	Yes ☐ No ☐	..... days
Nursing home	Yes ☐ No ☐	..... days
Hospice	Yes ☐ No ☐	..... days
Respite care	Yes ☐ No ☐	..... days
Other (please specify)		.....

4. Do you pay for your prescriptions? Yes ☐ No ☐

5. Please provide information about any medication you have taken in the **last three months** because of your **bowel cancer or its treatment** in the table below. If you have received chemotherapy, please do not include the chemotherapy drugs here. Any other items you may have purchased or received on prescription will be covered under questions 8 and 9.

   The following two examples provide more information on how to complete the 'Dosage' and 'How often?' columns.

   Example 1:
   One tablet of 30mg, twice a day for one week:

Dosage	**How often?**
30 mg	2 times daily over a period of 7 days

   Example 2:
   Two tablets of 15mg in the morning and one tablet of 15mg in the evening, every day for one week:

Dosage	**How often?**
15 mg	3 times daily over a period of 7 days

Medications		**Dosage**	**How often?**	**On prescription?**
Anti-diarrhoea medication (please specify brand name if known)	Have you taken this? Yes ☐ No ☐	.....mg	.....times daily over a period of .....days	Yes ☐ No ☐
Anti-spasmodics (to slow down bowel movements) (please specify brand name if known)	Have you taken this? Yes ☐ No ☐	.....mg	.....times daily over a period of .....days	Yes ☐ No ☐
Laxatives (to relieve constipation) (please specify brand name if known)	Have you taken this? Yes ☐ No ☐	.....mg	.....times daily over a period of .....days	Yes ☐ No ☐

(Question 5 continued)				
Medications		**Dosage**	**How often?**	**On prescription?**
Anti-sickness medication (please specify brand name if known)	Have you taken this? Yes ☐ No ☐	.....mg	.....times daily over a period of .....days	Yes ☐ No ☐
Pain killers (please specify brand name if known)	Have you taken this? Yes ☐ No ☐	.....mg	.....times daily over a period of .....days	Yes ☐ No ☐
Anti-depressants (to help with chemotherapy side effects) (please specify brand name if known)	Have you taken this? Yes ☐ No ☐	.....mg	.....times daily over a period of .....days	Yes ☐ No ☐
Anti-epileptic drugs (to help with chemotherapy side effects) (please specify brand name if known)	Have you taken this? Yes ☐ No ☐	.....mg	.....times daily over a period of .....days	Yes ☐ No ☐
Other (please specify brand name if known)		.....mg	.....times daily over a period of .....days	Yes ☐ No ☐
Other (please specify brand name if known)		.....mg	.....times daily over a period of .....days	Yes ☐ No ☐

(Question 5 continued)			
Medications	**Dosage**	**How often?**	**On prescription?**
Other (please specify brand name if known)	.....mg	.....times daily over a period of .....days	Yes ☐ No ☐
Other (please specify brand name if known)	.....mg	.....times daily over a period of .....days	Yes ☐ No ☐
Other (please specify brand name if known)	.....mg	.....times daily over a period of .....days	Yes ☐ No ☐

6. Have you had a stoma? Yes ☐ No ☐

If you have had a stoma, please continue with question 7. If not, please go to question 9.

7. What type of stoma have you had? Ileostomy ☐ Colostomy ☐

8. Please provide information about any stoma products, appliances and accessories you have purchased or received on prescription in the **last three months** in the table below.

   If you have purchased a pack of items, please provide the number of individual items you have purchased under the 'How many?' column.
   (e.g. Two packs of 30 stoma bags = 60,
   One pack of 20 adhesive remover wipes = 20)

Stoma products/ appliances/ accessories	Have you purchased any or received any on prescription?	How many?	On prescription?
Stoma bags	Yes ☐ No ☐	.....	Yes ☐ No ☐
Flanges	Yes ☐ No ☐	.....	Yes ☐ No ☐
Flange extenders	Yes ☐ No ☐	.....	Yes ☐ No ☐
Adhesive removers	Yes ☐ No ☐	.....spray cans	Yes ☐ No ☐
		.....wipes	Yes ☐ No ☐
Belts	Yes ☐ No ☐	.....	Yes ☐ No ☐
Lubricating deodorant gel	Yes ☐ No ☐	.....bottles	Yes ☐ No ☐
		.....sachets	Yes ☐ No ☐
Bottles of odour neutralising liquid	Yes ☐ No ☐	.....	Yes ☐ No ☐
Other (please specify)		.....	Yes ☐ No ☐

9. Please provide your best estimates about any other items you have purchased or received on prescription in the **last three months** in the tables below. Please only include items you have purchased or received on prescription because of your **bowel cancer or its treatment.**

   If you have purchased a pack of items, please provide the number of individual items you have purchased under the 'How many?' column.
   (e.g. Two packs of 15 pads for faecal incontinence = 30,
   One pack of 25 bed pads = 25)

Prescription items	Have you purchased any or received any on prescription?	How many?	On prescription?
Pads for faecal incontinence	Yes ☐ No ☐	.....	Yes ☐ No ☐
Waterproof mattress cover/ Absorbent bed pads	Yes ☐ No ☐	.....	Yes ☐ No ☐
Emollient/ Barrier/ Soothing cream	Yes ☐ No ☐	.....	Yes ☐ No ☐
Special soaps	Yes ☐ No ☐	.....	Yes ☐ No ☐
Vaginal dilator	Yes ☐ No ☐	.....	Yes ☐ No ☐
Other (please specify)		.....	Yes ☐ No ☐

Non-prescription items	Have you purchased any?	Cost to you?
New underwear	Yes ☐ No ☐	£ .....
New clothes	Yes ☐ No ☐	£ .....
New bed linen	Yes ☐ No ☐	£ .....
Extra laundry items	Yes ☐ No ☐	£ .....
Extra toilet paper	Yes ☐ No ☐	£ .....
Other (please specify)		£ .....
Other (please specify)		£ .....
Other (please specify)		£ .....

10. Do you have private health insurance covering you for bowel cancer diagnosis, treatment or aftercare? Yes ☐ No ☐

11. Tick the box next to the description best matching your current employment status:

Full time, self-/paid employment (30 hours/week or more)	☐
Part time, self-/paid employment (less than 30 hours/week)	☐
Employed (self-/paid) but on sick leave	☐
Training/education (not in self-/paid employment)	☐
Retired (not in self-/paid employment)	☐
Not in self-/paid employment for other reasons	☐

If you are in employment (even if on sick leave), please continue with question 12. If not, please go to question 15.

12. In the **last three months**, have you been away from work because of your **bowel cancer or its treatment?** Yes ☐ No ☐

13. If yes, for how many days? ☐ .....

14. Tick the box next to the description best matching your current gross annual employment income (before any deductions):

No employment income	☐
Below £20,000	☐
£20,000-£50,000	☐
Over £50,000	☐
Prefer not to say	☐

15. In the **last three months**, have you received help from family or friends (informal carers) because of your **bowel cancer or its treatment?** Yes ☐ No ☐

If you answered yes to question 15, please continue with question 16. If you answered no, please go to question 21.

16. On average, how many hours per week has your informal carer spent helping you in the **last three months**? (If you have more than one informal carer, provide information for the one you consider to be your **main** informal carer.)

0-9 hours per week	☐
10-19 hours per week	☐
20-34 hours per week	☐
35-49 hours per week	☐
50-99 hours per week	☐
100 or more hours per week (or 24/7)	☐

17. Tick the box next to the description best matching your informal carer's current employment status:

Full time, self-/paid employment (30 hours/week or more)	☐
Part time, self-/paid employment (less than 30 hours/week)	☐
Employed (self-/paid) but on sick leave	☐
Training/education (not in self-/paid employment)	☐
Retired (not in self-/paid employment)	☐
Not in self-/paid employment for other reasons	☐

If your informal carer is currently in employment (even if on sick leave), please continue with question 18. If not, then please go to question 21.

18. In the **last three months**, has your informal carer been away from work to help you? Yes ☐ No ☐

19. If yes, for how many days? ☐ .....

20. Tick the box next to the description best matching your informal carer's current gross annual employment income (before any deductions):

No employment income	☐
Below £20,000	☐
£20,000-£50,000	☐
Over £50,000	☐
Prefer not to say	☐

21. Please fill the tables below providing your best estimates on travel to all your health care appointments of the **last three months**, related to your **bowel cancer or its treatment.**

    If a family member or friend **(companion)** has accompanied you to any of these appointments, please also provide estimates for them.

    (One way journey = 1 journey, Return journey = 2 journeys)

		You		Companion	
Travel mode	**Have you used this?**	**Number of journeys**	**Average Cost per journey**	**Number of journeys**	**Average Cost per journey**
Tube / Tram	Yes ☐ No ☐	.....	£	.....	£ .....
Bus	Yes ☐ No ☐	.....	£	.....	£ .....
Train	Yes ☐ No ☐	.....	£	.....	£ .....
Other (please specify)		.....	£	.....	£ .....

Do you have a Freedom Pass or an older person's bus pass? Yes ☐ No ☐

		You		Companion
Travel mode	**Have you used this?**	**Number of journeys**	**Average mileage per journey**	**Number of journeys**
Car	Yes ☐ No ☐	.....	.....miles	.....

Did you or your companion pay any parking costs? Yes ☐ No ☐

If yes, how many times did you or your companion pay?	.....
How much on average did you pay for parking each time?	£ .....

		You	Companion	You/ Companion
Travel mode	**Have you used this?**	**Number of journeys**	**Number of journeys**	**Average Cost per journey**
Taxi	Yes ☐ No ☐	.....	.....	£ .....

Appendix (III) (Sub study protocol)

Refer to a separate document, TRACC Process Evaluation Protocol Version 1.1 dated 10.01.2024.

Appendix (IV) Summary of Protocol Amendments

Refer to a separate document, Summary of Protocol Amendments Version 12 dated 10.01.2024.

The ROYAL MARSDEN
NHS Foundation Trust

Short Title: TRACC: Process evaluation and implementation study

Full Title: A mixed methods process evaluation and implementation study of the TRACC intervention

(Tracking mutations in cell free tumour DNA to predict Relapse in eArly Colorectal Cancer)

Protocol version 1.1

Chief Investigator: Professor David Cunningham
Work stream Lead for the Process Evaluation: Professor Susanne Cruickshank

Sponsor: The Royal Marsden NHS Foundation Trust, Fulham Road, SW3 6JJ

Funder: This project (NIHR128529) is funded by the Efficacy and Mechanism Evaluation (EME) Programme, an MRC and NIHR partnership. The views expressed in this publication are those of the author(s) and not necessarily those of the MRC, NIHR or the Department of Health and Social Care

Coordinating trials unit: Applied Health Research Department & The Royal Marsden Clinical Trials Unit

REC number: 15/LO/1576
CCR number: 4344

IRAS Project ID: This project evaluates the ctDNA guided chemotherapy intervention within the TRACC protocol (RMH CCR no:4344 NRES No: 15/LO/1576) and is a work stream being conducted within the wider NIHR funded programme

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TRACC Process Evaluation Advisory Group	
Monica Jefford	Patient and Public Involvement (PPI) Representative Group Chair, Royal Marsden NHS Foundation Trust

Version / Amendment History	
Version No. / Amendment No.	SUMMARY OF CHANGES
1.0	First submission to CCR
1.1/Amendment 1	• Updated personnel including Work Stream Lead change from Prof Theresa Wiseman to Prof Susanne Cruickshank • Change throughout of focus group to **focus groups or individual interviews** • Sample changed from 15 sites for patient focus groups/interviews and HCP focus groups/interviews to **10** sites • Change in terminology around timepoints for data collection: Timepoint 2 (T^2) 36-42 months changed to **'mid Part C trial recruitment'** Timepoint 3 (T^3) 54-60 months changed to **'6 months post Part C recruitment end'**

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2 STUDY SUMMARY

Title	**A mixed methods process evaluation and implementation study of the TRACC intervention**
Study Design	Mixed-methods, longitudinal study collecting data from the clinical teams and patients participating in the TRACC trial (CCR4344)
Clinical Indication	High-risk stage II or stage III colon or rectal cancer who have undergone curative surgery and need adjuvant chemotherapy
Patient population	Patient participants entered into Part C of the TRACC protocol will be invited to attend patient focus groups or individual interviews High-risk stage II or stage III colon or rectal cancer who have undergone curative surgery and need adjuvant chemotherapy
Trial Type	Non-randomised sampling
Type of control	None
Study Groups	• Healthcare professionals from TRACC Part C sites completing NoMAD instrument survey (NoMAD HCP) • Focus group/ interviews with patients TRACC Part C participants from selected sites (FGIPatients) • Focus group/ interviews with healthcare professionals TRACC Part C MDT meetings from selected sites (FGIHCP)
Number of trial patients /Sites	Up to 40 sites **NoMAD HCP**: Between 80 - 100 healthcare professionals from all TRACC Part C sites to complete NoMAD instrument survey at 3 time-points **FGIPatients:** Purposive sample of 10 sites to conduct patient focus groups or individual interviews (up to 10 participants in each) at 3 time-points **FGIHCP**: Purposive sample of sites to conduct 10 healthcare professional focus groups or individual interviews from staff at 10 sites at 3 time-points

Estimated duration of trial & completion of Activities	Data Collection maps the timeline of the main TRACC trial and occurs at the following time-points (measured from the first recruitment in TRACC Part C) • T^1 12-18 months • T^2 mid Part C trial recruitment • T^3 6 months post Part C recruitment end
Duration of Active Participation	**NoMADHCP**: 5-10 minutes completed at each of the 3 time-points **FGIPatients:** duration up to 60 minutes **FGIHCP**: duration up to 40 minutes
Trial Objectives	Primary Objective • To identify, characterise and explain mechanisms that promote or inhibit the implementation of the TRACC programme for the management for early stage CRC Secondary Objectives • To describe both anticipated and actual barriers and facilitators to implementation of the TRACC programme from the perspective of healthcare professionals using the NoMAD survey instrument at 3 time-points • To assess change in survey responses between T^1 (12 – 18 months), T^2 (mid Part C trial recruitment) and T^3 (6 months post Part C recruitment end) to depict any shift from anticipated to experienced barriers and facilitators to implementation of TRACC • To elicit healthcare professionals' interpretations of NoMAD instrument survey results and their explanations of the processes that led to the results within focus group interviews conducted at different sites • To describe differences in processes and their outcomes between different settings • To describe the patient experience of those participating in the TRACC programme including factors which facilitate

	and inhibit implementation, adherence and impact on everyday activities
Trial Endpoints	Primary Endpoint • Information to develop an implementation strategy for TRACC Secondary Endpoints: • Knowledge and understanding of the anticipated and actual barriers and facilitators to implementation of the TRACC programme in the clinical pathway from the healthcare professionals' perspective • Change in NoMAD survey instrument from T1 to T2 and T3 time-points • Knowledge and understanding of healthcare professionals' explanations regarding the findings of the NoMAD and the process of implementation in different settings • Knowledge and understanding of the patient experience of TRACC and the impact on everyday activities • Knowledge and understanding of the barriers and facilitators to implementation and adherence from patients' perspectives
Summary of Inclusion and Exclusion Criteria in the Three Study Groups	
Inclusion Criteria: 1.NoMADHCP	Healthcare Professionals • Healthcare professionals working within the colorectal cancer services of sites taking part in TRACC Part C (ctDNA guided adjuvant chemotherapy). These could include Surgeons, Medical Oncologists, Clinical Oncologists, Colorectal Clinical Nurse Specialists, Research Nurses
Exclusion Criteria 1.NoMADHCP	• HCPs who have worked less than 3 months in the study site.

Inclusion Criteria: 2.FGIPatients	• Age ≥ 18 years at the time of consideration • Patients participating in TRACC Part C (ctDNA guided adjuvant chemotherapy) • Life-expectancy > 3 months • Able to give informed consent, participate in focus group discussions in English or able to attend with local translator and comply with study procedures
Exclusion Criteria 2.FGIPatients	• Age <18 years at the time of consideration • In the opinion of the investigator the patient is not suitable to approach for participation
Inclusion Criteria: 3.FGIHCP	• Members of MDT meetings at selected sites taking part in TRACC Part C (ctDNA guided adjuvant chemotherapy) and open at Time-point 1 • Willing to consent to participate in focus group discussion
Exclusion Criteria 3.FGIHCP	• HCPs who have worked less than 3 months in the study site.
Treatment / Main Trial Procedures and Follow-up	**a) NoMAD HCP:** Survey of healthcare professionals at all TRACC Part C sites using the NoMAD instrument at T^1 T^2 T^3 and delivered electronically by online survey software b) **FGIPatients**: Focus Group or Individual Interviews for patients (conducted by Applied Health Researchers at 10 purposively selected TRACC Part C sites: T^1 T^2 T^3) **c) FGIHCP:** Focus Group or Individual Interviews for healthcare professionals (conducted by Applied Health Researchers at 10 purposively selected TRACC Part C sites: T^1 T^2 T^3)

3 PRINCIPAL INVESTIGATOR SIGNATURE PAGE

Study Title: A mixed methods process evaluation and implementation study of the TRACC intervention (Tracking mutations in cell free tumour DNA to predict Relapse in eArly Colorectal Cancer)

Sponsor protocol number:

I have read and agree to the protocol, as detailed in this document. I am aware of my responsibilities as an Investigator under the UK Clinical Trials Regulations, the guidelines of Good Clinical Practice (GCP) the Declaration of Helsinki and the applicable regulations of the relevant NHS Trusts and the study protocol. I agree to conduct the study according to these regulations and guidelines and to appropriately direct and assist the staff under my control who will be involved in the study, and ensure that all staff members are aware of their clinical study responsibilities.

Site Address: _____

_____

_____

Name of Investigator: Professor David Cunningham

Title: Chief Investigator

Signed: _____

Date: _____

List of Abbreviations	
Abbreviation	Definition
CCR	Joint RM / ICR Committee for Clinical Research
CRF	Case Report Form
CRC	Colorectal Cancer
CNS	Clinical Nurse Specialist
ctDNA	Circulating cell free tumour derived DNA
DNA	Deoxyribonucleic acid
EME	Efficacy and Mechanism Evaluation
FGIPatients	Focus Group Interviews for Patients
FGIHCP	Focus Group Interviews for Healthcare Professionals
GCP	Good Clinical Practice
GP	General Practitioner
HRA	Health Research Authority
ICF	Informed Consent Form
IDMC	Independent Data Monitoring Committee
MDT	Multidisciplinary team
MRC	Medical Research Council
NIHR	National Institute of Health Research
NoMAD HCP	Healthcare professionals completing NoMAD Instrument survey
NPT	Normalisation Process Theory
PI	Principal Investigator
PIS	Patient Information Sheet
REC	Research Ethics Committee
RM CTU	Royal Marsden Clinical Trials Unit
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOC	Standard of Care
SOP	Standard Operating Procedure
TMG	Trial Management Group

4 BACKGROUND

TRACC is a multi-centre, prospective observational longitudinal study of ctDNA after surgery for stage I to III CRC. Part C adds randomisation and ctDNA guided chemotherapy components for patients with high risk stage II or stage III CRC (CCR 4344). Part C of TRACC, and the process evaluation described in this protocol are funded by the Efficacy and Mechanism Evaluation Programme (EME) (NIHR 128529).

This study is a Mixed Methods Process Evaluation informed by Normalisation Process Theory (NPT) and conducted by the RM Applied Health Research Group. The process evaluation will inform an implementation strategy for a future TRACC clinical pathway.

NPT is a socio-behavioural theory which provides a rigorous and robust means of conceptualising the dynamics of implementation processes in healthcare settings and has been widely used as a basis for process evaluations in studies of complex interventions in hospital settings[1,2]. The theory consists of four main constructs: coherence (or sense-making); cognitive participation (or engagement); collective action (work done to enable the intervention to happen); and reflexive monitoring (formal and informal appraisal of the benefits and costs of the intervention).[3] This study includes a survey of healthcare professionals using the NoMAD instrument which is a structured assessment tool based on these four theoretical constructs and which will be completed at each of the 3 study time-points. This tool is a self-report measure of individuals' perceptions of the process of the implementation and is an appropriate way to compare implementation progress across multiple sites and activity over time.[4]

In order to provide a fuller understanding of the embedding of practice, the study also incorporates qualitative methods in the form of focus group discussions or individual interviews at each of the 3 time-points. These focus group discussions or individual interviews will be conducted with patients taking part in the TRACC Part C clinical trial and with healthcare professionals who form the MDT at selected trial sites.

Both the results of the survey and the findings from the focus group interviews will be used to develop guidance in an iterative process across the 3 time-points and inform the development of an implementation strategy for the TRACC intervention.

5 STUDY OBJECTIVES & ENDPOINTS

5.1 Aim

- To conduct a mixed-methods, longitudinal process evaluation of the TRACC intervention of ctDNA guided chemotherapy, informed by Normalisation Process Theory.

5.2 Primary Objectives

- To identify, characterise and explain mechanisms that promote and inhibit the implementation of ctDNA guided chemotherapy for the management for early stage CRC

5.3 Secondary Objectives

- To describe both anticipated and actual barriers and facilitators to implementation of the TRACC programme from the perspective of healthcare professionals using the NoMAD survey instrument at 3 time-points
- To assess change in survey responses between T^1 (12 – 18 months), T^2 (mid Part C trial recruitment) and T^3 (6 months post Part C trial recruitment) to depict any shift from anticipated to experienced barriers and facilitators to implementation of TRACC
- To elicit healthcare professionals' interpretations of NoMAD instrument survey results and their explanations of the processes that led to the results within focus group interviews conducted at different sites
- To describe differences in processes and their outcomes between different settings
- To describe the patient experience of those participating in the TRACC programme including factors which facilitate and inhibit implementation, adherence and impact on everyday activities

5.4 Primary Endpoint

Information to develop an implementation strategy for TRACC

5.5 Secondary Endpoints

- Knowledge and understanding of the anticipated and actual barriers and facilitators to implementation of the TRACC programme in the clinical pathway from healthcare professionals' perspective
- Change in NoMAD survey instrument from T1 to T2 and T3 time-points
- Knowledge and understanding of healthcare professionals' explanations regarding the findings of the NoMAD survey and the process of implementation in different settings
- Knowledge and understanding of the patient experience of TRACC and the impact on everyday activities
- Knowledge and understanding of the barriers and facilitators to implementation and adherence from patients' perspectives

6 STUDY DESIGN

6.1 Summary

This mixed-methods process evaluation is closely linked to the timelines of the Part C TRACC protocol. It begins by performing a survey of clinical staff and by conducting a set of focus groups for patients and healthcare professionals at the first time-point which takes place when Part C of the TRACC trial has been open and recruiting for one year. These interventions are repeated at a further two time-points as described in the data collection section below.

6.2 Data collection

Data collection will take place at 3 time points (measured from the first TRACC Part C patient recruitment):

T^1 12-18 months; T^2 mid Part C trial recruitment; T^3 6 months post Part C trial recruitment

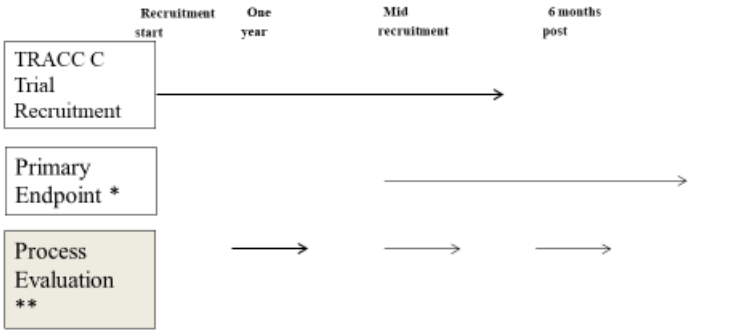
It will consist of:

a) Survey of clinical staff at all TRACC Part C sites using the NoMAD instrument at T^1 T^2 T^3 and delivered electronically by online survey software

b) Focus Group discussion or individual interviews for patients (conducted by Applied Health Researchers at 10 purposively selected TRACC Part C sites: T^1 T^2 T^3)

c) Focus Group discussion or individual interviews for healthcare professionals (conducted by Applied Health Researchers at 10 purposively selected TRACC Part C sites: T^1 T^2 T^3)

Study Flowchart



* Difference in 3 year disease-free survival from time of surgery to progression, between standard of care arm and ctDNA guided adjuvant chemotherapy arm

** Description of factors that promote or inhibit implementation of TRACC approach, including the patient experience.
Each of the 3 time-points include Surveys of Clinical Staff, Patient Focus Groups/Interviews & Healthcare Professional Focus Groups/Interviews

6.3 Identification and recruitment of participants

The Applied Health Research team will work closely with the TRACC Trial Management team to inform trial sites about the purpose and scope of the process evaluation and to ensure appropriate introductions and consent to contact is established. By processing the TRACC trial amendment which adds this work stream, sites are agreeing to participate in the process evaluation. They will be asked to complete a registration form containing some key aspects about the participating site (Appendix 14.1)

A unique site identification number will be assigned once they have processed the amendment and joined the study.

Survey (NoMAD instrument)

An initial small pilot of the survey will be conducted before the study starts to establish how well the NoMAD (an already validated tool) has been adapted for use within TRACC and to test its function and usability. Two sites will be identified with the help of the TRACC trial manager, and six healthcare professionals from those sites will be invited to complete and test the survey. They will be asked to comment upon the accuracy of the information provided regarding how

long it takes to complete; the wording clarity and comprehensibility of the adapted survey questions and the functionality of the survey platform. Members of the Applied Health Research Team will review the feedback and make amendments to the survey accordingly before it is circulated more widely as part of the first time point of data collection.

Once finalised following the pilot, the survey will be circulated electronically by the Applied Health Research Team to all study PIs for completion. Research nurses at site may be approached to forward the survey link to their clinical nurse specialist colleagues and other relevant clinical staff. Survey participants will be asked to provide their email address. Some of the same participants may continue to complete the survey at each time point and their email address will help identify them and provide information on how individuals' perceptions change over time. Staff email addresses will be collected within the survey solely for the purposes of tracking responses over time. Repeated completion is not likely to be possible in all cases given the length of the study. We will also look at change in responses over time according to site and more generally across all participants.

Focus Group Discussions or Individual Interviews for patients

The Applied Health Research team will liaise with PI and research nurse contacts at purposively selected sites in order to identify patients taking part in Part C of the TRACC trial who may be willing to take part in a focus group discussion. Patient focus group discussions or individual interviews will be conducted at each time point from a selection of 10 sites The study sites from which the patients will be recruited will be selected to represent a range of experiences in adopting the TRACC trial. They will include sites who have successfully adopted the trial and those who have experienced difficulties. They will also include a range of high, low and moderately recruiting sites. Research nurses will be asked to hand out Patient Information sheets to patients they identify, which will contain contact details of the Applied Health Researcher who can be contacted to provide further information as required and answer any questions. The Research Nurse will obtain verbal consent from identified patients for the researcher to contact them by phone. Initial consent to participate in the focus group/interview will be obtained over the phone in a discussion with the researcher who will then continue to liaise with the patient about the date and venue.. If the focus groups/interviews proceed as face to face this will be followed up by written informed consent taken by the Applied Health Researcher in person on the day.. If according to circumstances, the focus groups/interviews take the form of an online meeting, then consent will be provided via phone or video call as reflected within TRACC guidelines.

Focus Group Interviews for healthcare professionals

The Applied Health Research team will liaise with PI and research nurse contacts at 10 purposively selected sites in order to schedule the focus group/interviews with clinical staff. The 10 study sites will be selected to represent a range of experiences in adopting the TRACC trial. They will include sites who have successfully adopted the trial and those who have experienced difficulties. They will also include a range of high, low and moderately recruiting sites. The focus group discussions or interviews may most conveniently be held as part of an MDT meeting and will be facilitated in person or online, according to circumstances, by an Applied Health Researcher for a duration of up to 40 minutes. Selected results of the survey will be presented and the focus group discussion will aim to capture the participants' interpretations and explanations for the survey results.

6.4 Data Analysis and Feeding Back to the TRACC TMG

Survey Data: Quantitative data will be analysed using descriptive statistics to provide a picture of anticipated and actual barriers and facilitators to the TRACC intervention. Analysis will be performed at each time-point and change will be assessed between time-points. No formal statistical testing is required for this study. Results will be presented to the main stakeholder group at each time-point to inform the iterative development of an implementation strategy. Selected results from the survey will be presented at the Focus Group Discussions or Individual Interviews for healthcare professionals to facilitate discussion.

Focus Group Discussions/Individual Interviews Patients: The patient focus group interviews will be exploratory, eliciting participants' experience of TRACC, including factors which facilitate and inhibit implementation, adherence and impact on everyday activities. Data will be analysed using thematic analysis.[5] The findings will be presented to the main stakeholder group at each time-point to inform the iterative development of an implementation strategy

Focus Group Discussions/Individual Interviews Healthcare Professionals: Selected results of the NoMAD survey will be presented to elicit healthcare professionals' interpretations of the data and their explanations of the processes that have led to the results. This data will be analysed using process tracing analyses to explore:

a) Participants' implicit causal models of factors that shape the adoption, implementation and integration of TRACC

b) Comparative analyses of differences in processes and their outcomes between different settings

Results will be presented to the main stakeholder group at each time-point to inform the iterative development of an implementation strategy

6.5 Follow up and end of study definitions

Participants will be recruited until

- Surveys are completed by clinical staff from all study sites across each of the three time-points
- Focus group discussions/individual interviews of patient participants from 10 sites are conducted at each of the three time-points
- Focus group discussions/individual interviews of clinical staff in the MDT are conducted at 10 sites at each of the three time-points

Participants will not be followed up after the end of the study which will be defined as the last survey completed and last focus group/interview conducted in both study groups at time-point three

7 INCLUSION/EXCLUSION CRITERIA

Study Group	Inclusion Criteria	Exclusion Criteria
NoMAD HCP Healthcare professionals	• Healthcare professionals working within the colorectal cancer services of sites taking part in TRACC Part C (ctDNA guided adjuvant chemotherapy). These could include Surgeons, Medical Oncologists, Clinical Oncologists, Colorectal Clinical Nurse Specialists, Research Nurses	• HCPs who have worked less than 3 months in the study site
FGIPatients	• Age ≥ 18 years at the time of consideration	• Life expectancy <3 months • In the opinion of the local PI the patient is

Focus Group Discussion/ Individual Interviews Patients	• Patients participating in TRACC Part C (ctDNA guided adjuvant chemotherapy) • Able to give informed consent, participate in focus group discussions in English or able to attend with local translator and comply with study procedures	not suitable to approach for participation
FGI HCP Focus Group Discussion/Individual Interviews Healthcare Professionals	• Members of MDT meetings at selected sites taking part in TRACC Part C (ctDNA guided adjuvant chemotherapy) • Willing to consent to participate in focus group discussion	• HCPs who have worked less than 3 months in the study site

8 STUDY PROCEDURES

Patient participation in this study will not affect any decisions regarding patients' ongoing care or participation in TRACC part C in any way. Informed consent will be as per GCP and the TRACC trial guidelines.

8.1 Study entry

For the NoMAD survey consent will be implied should the healthcare professional choose to complete it using the online link.

For the patient focus group interviews the Principal Investigator, or delegated Sub-Investigator, should ensure that each patient, prior to inclusion in the study, is given full and adequate written information regarding the objectives and procedures of the study and the possible risks involved (the PIS) and will ensure that the patient consents to be contacted by the research team by phone. The Applied Health Research Team will follow up by telephone and answer any questions that the patient may have about their participation, they will then obtain verbal consent if appropriate and discuss the practicalities of attending the focus group discussion. At least 24 hours should be allowed for the patient to decide on study entry. Patients must be informed about their right to withdraw from the study at any time without affecting their treatment. If the patient focus group interviews proceed as face to face, consent forms will be signed by the patient and the researcher on the day of the focus group discussion and securely retained. If, according to circumstances, the focus group interviews are conducted online then consent will be obtained by phone or video call according to TRACC guidelines. A copy of the consent form will be given to the patient for their records. Patients must sign and date an informed consent form (ICF) before engaging in any study related procedure.

For the healthcare professional focus group discussions, these may be conducted conveniently as part of the MDT meeting. Written notice of the Applied Health Research Team's intention to present selected survey findings will be given in advance to the PI's at sites and will be circulated along with participant information sheets and consent forms to the relevant MDT members by the research team at site. Consent will be obtained virtually if the discussion is conducted online or in person if conducted face to face and will be in keeping with TRACC guidelines.

8.2 NoMAD instrument survey and Focus Group Interviews

The study objectives are to assess trends in changes in scores for the NoMAD instrument delivered at T^1, T^2 and T^3. This validated instrument is a self-report measure of individuals'

perceptions of the process of implementation. It is based around four constructs; coherence, cognitive participation, collective action and reflexive monitoring. Showing a trend towards higher scores in how these constructs are assessed over the study period will provide evidence for the positive impact of an implementation strategy.

Qualitative focus group discussions or individual interviews will provide a fuller understanding of the embedding of practice. The patient FGIs will be exploratory, eliciting participants' experience of TRACC including factors which facilitate and inhibit implementation, adherence and impact on everyday activities. The data will be analysed using thematic analysis in order to understand the meaning of the experience for patients. The findings will be fed back to the TRACC TMG, along with the survey results. The healthcare professional FGIs will be aimed at eliciting participants' interpretations of the survey data and their explanations of the processes that have led to the results. We will analyse these data using process tracing analyses[6,7.] to explore a) participants' implicit causal models of factors that shape the adoption, implementation and integration of TRACC and b) comparative analyses of differences in processes and their outcomes between different settings. Again the findings will be presented to the stakeholder group to inform the guidance development. Integrating the qualitative and quantitative data will further our understanding of the results.

8.3 Study Documentation and CRF completion

A TRACC Process Evaluation excel spread sheet recruitment log will be developed for each of the three study groups: clinical staff completing the survey; patients participating in focus group interviews and members of the MDT participating in focus group interviews.

Focus group interviews will be audio recorded by the Applied Health Researchers and then transcribed into word documents. Data from the interviews will be stored and organised in the NVIVO software package to enable thematic analysis which will be performed by members of the Applied Health Research Team.

8.4 Schedule of events

	T[1] 12 – 18 months	T[2]mid Part C trial recruitment	T[3] 6 months post Part C recruitment
Informed Consent[a]	X	X	X
NoMAD Survey Completion	X	X	X
Focus Group/Interviews Patients	X	X	X
Focus Group/ Interviews Healthcare Professionals	X	X	X

[a] Written informed consent/or telephone/video consent will be taken according to circumstances ; from patients approached to take part in the focus group interviews. For healthcare professionals who are members of the MDT, information sheets will be circulated in advance and consent to take part in focus group interviews will be implied if staff stay on within the MDT meeting to participate in the discussion.. For the NoMAD survey consent will be implied should the healthcare professional choose to complete it using the online link

9 STUDY APPROVALS & MANAGEMENT

This trial will be conducted in accordance with the conditions and principles of GCP as defined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and any subsequent amendments of the clinical trial regulations and GCP guidelines. It will also adhere to the Sponsor's SOPs and local centres' SOPs as appropriate and other regulatory requirements as amended.

9.1 Study Approvals

9.1.1 Sponsor and Regulatory Approvals

Before starting the study, the protocol, patient information sheet, consent form, and any other written information that will be provided to the patients must be approved by the Royal Marsden/Institute of Cancer Research joint Committee for Clinical Research (CCR), who will, on approval accept sponsorship of the study. Once sponsorship approval is obtained, the study will then be submitted to the local REC and HRA as an amendment to the TRACC clinical trial for review and approval. The study will not begin until favourable opinion from REC and HRA is received and local R&D Capacity and Capability has been given for the amendment

9.1.2 Protocol Amendments

Any protocol amendment should be agreed with the TMG (see below), classified as substantive or non-substantive, and be approved by the sponsor prior to submission and review by REC and HRA. Once a favourable opinion has been received, the amendment can be distributed to sites for each to complete their local capacity review. Prior to implementation at the site, a local approval from R&D should be obtained.

It is the responsibility of the RM CTU to submit amendments to the Sponsor for approval and to distribute them to all participating centres for capacity review and implementation. Amendments requiring REC approval may be implemented only after a copy of the REC/HRA's approval letter has been obtained. Amendments that are intended to eliminate an apparent immediate hazard to patients may be implemented prior to receiving Sponsor or REC/HRA approval. However, in this case, approval must be obtained as soon as possible after implementation.

9.1.3 Insurance Arrangements

Indemnity for participating hospitals is provided by the usual NHS indemnity arrangements for clinical negligence.

9.1.4 Provision of Information to the Participant's GP

It is the Investigator's responsibility to inform the patient's GP by letter that the patient is taking part in the study provided the patient agrees to this, and information to this effect is included in the PIS and ICF. A copy of the letter should be filed in the Investigator Site File. A template letter approved by the REC/HRA will be provided by the Sponsor to all participating sites.

9.2 Trial Management

9.2.1 Site Agreements

The TRACC Process Evaluation and Implementation Study is a project that evaluates the ctDNA guided chemotherapy intervention within the TRACC protocol (RMH CCR no:4344 NRES No: 15/LO/1576) and is a work stream being conducted within the wider NIHR funded programme. TRACC is a non-commercial trial sponsored by the Royal Marsden NHS Foundation Trust.

Agreements between Sponsor and NHS participating site will be described within the Organisation Information Document – Non Commercial.

9.2.2 RM-CTU Trial Management Responsibilities

RM-CTU is responsible for ensuring appropriate Sponsor oversight for the trial and for obtaining the required Sponsor and regulatory approvals including co-ordinating the production and incorporation of protocol amendments. In addition, RM-CTU is responsible for the processing of trial safety data including expedited reporting to the relevant authorities.

9.2.3 Trial Management Group

The TRACC Trial Management Group (TMG) will be set up with membership and remit as defined in the TMG Charter. Membership will include Chief Investigator, study Statistician, Co-Investigators and the Trial Manager. Principal Investigators and other key study personnel will be invited to join the TMG as appropriate. The TMG has operational responsibility for the conduct of the trial and for overseeing its timely completion including achievement of recruitment targets. The TMG will meet with a frequency as determined by the Chief Investigator in discussion with the trial manager. The frequency of meetings and / or the need for ad-hoc meetings will reflect emerging needs. For this work stream the process evaluation and implementation findings will be shared with the TMG following each time-point of data collection and appropriate guidelines will be developed and shared with sites as a result of this consultation.

9.3.4 Data quality management

During the study, the Applied Health Research team will be responsible for the quality of the data captured in the trial database. Data completeness reports will be prepared for the TMG.

9.3.5 Licenses and Agreements

Confirmation will be obtained to ensure that that the NoMAD instrument included in this study can be used free of charge as it is being employed for academic research purposes only. Where licence fees are required, these will be discussed and reviewed by the Research Team and an assessment made to ensure that appropriate funds are available to cover their use. No data from completed questionnaires will be transferred to third parties.

9.3.6 Registration & NIHR Portfolio Listing

The TRACC trial is registered on ClinicalTrials.Gov database and adopted onto the national (NIHR) trial portfolio. A summary of the Process Evaluation and Implementation work stream will be added to existing TRACC information

10 DATA MANAGEMENT

10.1 Patient Confidentiality

The Chief Investigator is responsible for ensuring that the patient's confidentiality is maintained in compliance with the UK General Data Protection Regulations 2018. Focus Group Interview transcripts will identify the participant only by their trial ID, initials and year of birth. The ICF should state that confidentiality will be preserved. The PIS will include the required GDPR statements and inform the patient that their GP will be informed of their participation in the TRACC Process Evaluation and Implementation trial.

10.2 Study Data Management

The Applied Health Research Team Lead is responsible for ensuring that appropriate trial data management procedures are adhered to at their site including:

- Ensuring that all study related documentation is accurate, complete, maintained and accessible.
- Ensuring that original consent forms for the patient focus groups/interviews are dated and signed by both patient and researcher and are kept together in a central file.
- Ensuring that all essential documents are retained after the study ends to comply with current legislation (to include amendment paperwork indicating sites agreement to participate in the process evaluation study; the accompanying registration form completed with demographic information about the site; recruitment lists of patients participating in the focus groups/interviews)

10.3 Study Database

Only the personnel listed on the Delegation Log as having data collection and entry responsibilities should enter or change data.

10.4 End of Trial Report

Findings from the data will be presented at the end of the trial based on the statistical and framework analysis plan. The CI/designee will prepare a brief clinical study report / publication based on the final study report, produced by the study team. A summary of the report must be provided to the Research Ethics Committee within 1 year from the submission of the end of trial notification.

10.5 Record retention

Essential documents are documents that individually and collectively permit evaluation of the conduct of the study and substantiate the quality of the data collected. During the clinical study and after study closure the Investigator must maintain adequate and accurate records to enable both the conduct of a clinical study and the quality of the data produced to be evaluated and verified in accordance with current legislation.

The study team will maintain essential documents to facilitate the management of the study, audit and inspection in accordance with local SOPs and in compliance with the clinical study regulatory requirements. Records will be retained for a period of at least 5 years in accordance with the required regulations.

11 STATISTICAL CONSIDERATIONS

11.1 Sample Size

The analysis for this study will be purely descriptive and as such will not be powered. The link to the survey will be sent to all participating sites (up to 40 sites and between 80 and 100 HCPs).

11.2 Analysis Plan

A statistical analysis plan (SAP) will be approved by the Statistician and Chief Investigator (or delegate) prior to any analyses being undertaken. Participant characteristics will be described and numbers with percentages for categorical variables plus means and standard deviations, or medians along with lower and upper quartiles for continuous variables will be presented.

Specifically:

- Questionnaire data will be analysed descriptively. Mean and standard deviation of scale scores and change in scale scores will be presented for each scale of each measure;
- Mean and standard deviation of scale scores and change in scores will be stratified by potential covariates
- Qualitative interview data will undergo thematic analysis.

13. REFERENCES

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3. Murray, E., Treweek, S., Pope, C, MacFarlane, A., Ballini, L., Dowrick et al. Normalisation process theory: a framework for developing, evaluating and implementing complex interventions. BMC Medicine. 2010, 8:63
4. Finch TL, Girling M, May CR, Mair FS, Murray E, Treweek S, et al. Improving the normalization of complex interventions: part 2 - validation of the NoMAD instrument for assessing implementation work based on normalization process theory (NPT). Bmc Med Res Methodol. 2018;18(1):135.
5. C G Qualitative Data Analysis: An Introduction. London. Sage Publications Limited. 2007

6. Beach D PR. Process-Tracing Methods: Foundations and Guidelines. Ann Arbbor: University of Michigan Press. 2013

7. Schneider CQ, Rohfling I. Combining QCA and Process Tracing in Set-Theoretic Multi-Method Research. *Sociol Methods Res*.2013;42:559-97

14. APPENDICES

14.1 Registration Form / Demographics

To include information on the size of site and oncology team; whether the site is a specialist cancer centre or an oncology service within a general hospital; approximate number of colorectal patients treated per year; whether the site has a separate R&D department; does the site give chemotherapy on site or transfer to specialist centre; is the PI a surgeon or oncologist

14.2 NoMAD Instrument amended for TRACC

Survey Instructions

This survey is designed to help get a better understanding of how to apply and integrate new technologies and complex interventions in health care

This survey asks questions about the implementation of ctDNA guided chemotherapy as part of the TRACC clinical trial (Part C). We understand that people involved in the care of patients receiving ctDNA guided chemotherapy as part of TRACC have different roles, and that people may have more than one role

From the statements below please choose an option that best describes ***your main role*** in relation to **the care of patients receiving ctDNA guided chemotherapy (Part C of the TRACC trial)**

 I am part of the research team who are running Part C of the TRACC trial at site

 I am not part of the research team but am involved in the care of patients who are participating in Part C of the TRACC trial

For this survey, please answer all the statements from the perspective of this role. Depending on your role or responsibilities, some statements may be more relevant than others.

The survey is in 3 parts. Part A asks some brief questions about yourself and your role. Part B includes three general questions about the care of patients receiving ctDNA guided chemotherapy as part of the TRACC clinical trial. Part C contains a set of more detailed questions about how you view this new practice. For each statement in Part C, there is the option to agree or disagree with what is being asked (OPTION A). However, if you feel that the statement is not relevant to you, there are also options to tell us why (OPTION B).

Please take the time to decide which answer <u>best suits your experience for each statement and tick the appropriate circle</u>

Part A: About yourself

1. **How many years have you worked for this organization?** ***(If your Trust has merged with another or changed its name, please include in your answer all the time you have worked with this Trust and its predecessors)***

    - Less than one year
    - 1-2 years
    - 3-5 years
    - 6-10 years
    - 11-15 years
    - More than 15 years

2. **How would you describe your professional job category?** (Principal Investigator, Co-Investigator, Research Fellow, Clinical Nurse Specialist, Research Nurse, Other, please specify)

3. **How long have you been qualified?**

    - Less than one year
    - 1-2 years
    - 3-5 years
    - 6-10 years
    - 11-15 years
    - More than 15 years

4. **Please provide the name of your site and your email address**

Part B: General questions about the intervention

When you use ctDNA guided chemotherapy, <u>how familiar</u> does it feel?

Still feels very new Feels completely familiar

0 1 2 3 4 5 6 7 8 9 10

Do you feel that ctDNA guided chemotherapy <u>is currently</u> a normal part of your work?

Not at all Somewhat Completely

0 1 2 3 4 5 6 7 8 9 10

Do you feel that ctDNA guided chemotherapy <u>will become</u> a normal part of your work?

Not at all Somewhat Completely

0 1 2 3 4 5 6 7 8 9 10

Part C: Detailed questions about the intervention

For each statement please select an answer that best suits your experience using Option A. If the statement is not relevant to you please select an answer from Option B.

Section C1

1. **I can see how ctDNA guided chemotherapy within TRACC differs from usual ways of working**
2. **Staff in this organization have a shared understanding of the purpose of ctDNA guided chemotherapy within TRACC**
3. **I understand how ctDNA guided chemotherapy within TRACC affects the nature of my work**

4. **I can see the potential value of ctDNA guided chemotherapy for my work**

For all choose either

Option A: Strongly Agree, Agree, Neither agree nor disagree, Disagree, Strongly disagree

Option B: Not relevant to my role, Not relevant at this stage, Not relevant to the intervention

Section C2

1. **There are key people who drive ctDNA guided chemotherapy within TRACC forward and get others involved**
2. **I believe that supporting the delivery of ctDNA guided chemotherapy within TRACC is a legitimate part of my role**
3. **I'm open to working with colleagues in new ways to support the delivery of ctDNA guided chemotherapy within TRACC**
4. **I will continue to support the delivery of ctDNA guided chemotherapy within TRACC**

For all choose either

Option A: Strongly Agree, Agree, Neither agree nor disagree, Disagree, Strongly disagree

Option B: Not relevant to my role, Not relevant at this stage, Not relevant to the intervention

Section C3

1. **I can easily integrate work required to support ctDNA guided chemotherapy within TRACC into my existing work**
2. **Supporting the delivery of ctDNA guided chemotherapy within TRACC disrupts working relationships**

3. **I have confidence in other people's ability to support the delivery of ctDNA guided chemotherapy within TRACC**

4. **Work is assigned to those with skills appropriate to implement ctDNA guided chemotherapy within TRACC**

5. **Sufficient training is provided to enable staff to implement ctDNA guided chemotherapy within TRACC**

6. **Sufficient resources are available to support the delivery of ctDNA guided chemotherapy within TRACC**

7. **Management adequately supports the delivery of ctDNA guided chemotherapy within TRACC**

For all choose either:

Option A: Strongly Agree, Agree, Neither agree nor disagree, Disagree, Strongly disagree

Option B: Not relevant to my role, Not relevant at this stage, Not relevant to the intervention

Section C4

1. **I am aware of reports about the effects of ctDNA guided chemotherapy within the TRACC trial**

2. **The staff agree that ctDNA guided chemotherapy within the TRACC trial is worthwhile**

3. **I value the effects that ctDNA guided chemotherapy within the TRACC trial has had on my work**

4. **Feedback about ctDNA guided chemotherapy within the TRACC trial can be used to improve practice in the future**

5. **I can modify how I work on ctDNA guided chemotherapy within the TRACC trial**

For all choose either:

Option A: Strongly Agree, Agree, Neither agree nor disagree, Disagree, Strongly disagree

Option B: Not relevant to my role, Not relevant at this stage, Not relevant to the intervention

SURVEY CONCLUSION

Thank you for completing our survey

Summary of Protocol Amendments

Table of Contents

Protocol changes from V3.0 to V4.0

Page No	Version 3.0	Version 4.0	Explanation
Throughout	V3.0 dated 14.08.15	V4.0 dated 13.10.15	
Page 1 and header		Rec information included	
Page 2		Addition of Claire Saffery as Senior Trial Manager	
Page 6 & 34	Multi-Centre study, encompassing selected centres from to the London Cancer Alliance catchment area	Multi-Centre study, encompassing selected centres from but not limited to the London Cancer Alliance catchment area	Addition of text “but not limited to” added in line with the rest of the protocol.
Page 41	Physical examination was included in the study schedule		Deletion of physical examination from schedule. The decision was made to remove this as not all sites perform this in clinic.

Protocol changes from V4.0 to V5.0

Page No	Version 4.0	Version 5.0	Explanation
Throughout	V4.0 dated 13.10.15	V5.0 dated 01 Jun 2017	
2		Inclusion of NRES number, 15/LO/1576	Inclusion of NRES number
2		Inclusion of Dr. Gayathri Anandappa as trial physician	Study personnel update
2		Inclusion of Annette Bryant as senior trial manager	Study personnel update
2		Inclusion of Ethics Committee under Confidentiality Notice	Confidentiality Notice update
5		Inclusion of Prof. Gina Brown, Dr. Irene Chong, Dr. Mike Hubank as co-investigators and removal of Dr. David Gonzalez Castro and Dr. Brian Walker from the list of co-investigators	Study personnel update
6		Inclusion of Dr. Mike Hubank in the research team Inclusion of Aleruchi Okachi as biological specimen co-ordinator	Study personnel update
6		Protocol design and development includes Dr. Ian Chau and Dr. Gayathri Anandappa	
7	London Cancer Alliance	RM partners Cancer Vanguard (previously London Cancer Alliance)	Change in name update
11		The study will also include patients with stage I disease and assess if ctDNA is detectable in this subset of patients, and if ctDNA predicts for relapse.	Inclusion of patients with stage I colorectal cancer (CRC)

Page No	Version 4.0	Version 5.0	Explanation
7	To quantify levels of mutations in cfDNA and assess change 3 monthly from the first post-operative visit for year 1, 6 monthly until year 3 and annually in years 4 and 5 or until relapse, if this occurs first	To quantify levels of mutations in cfDNA and assess change 3 monthly from the first post-operative visit for year 1, 6 monthly until year 3 and annually in years 4 and 5 or until relapse, if this occurs first in patients with stage II and III CRC	Clarification that this specific secondary objective will only be performed in stage II and III CRC, to reflect the follow-up pathway in this group of patients.
8	To determine whether ctDNA analysis is useful for predicting relapse in patients with rectal cancer who have a favourable response to long course (chemo)radiotherapy and proceed with a deferral of surgery approach		Patients in whom deferral of surgery approach is used will be withdrawn from the study
22		There is limited data available regarding detection of cfDNA in patients with stage 1 colorectal cancer and we will include this group of patients as an exploratory cohort. The data generated will help decide if this technique is useful to detect early relapse in this group of patients.	Rationale for including patients with stage 1 CRC
8		Inclusion of following exploratory end-points To assess blood and tumour tissue of patients with synchronous colorectal primaries to evaluate mutational patterns in these patients To explore if ctDNA is detectable in patients	Inclusion of exploratory end-points

Page No	Version 4.0	Version 5.0	Explanation
		with stage I colorectal cancer To assess whether detection of ctDNA predicts for relapse in patients with stage I CRC who have undergone surgery with curative intent Patients with rectal adenocarcinoma receiving chemoradiotherapy at The Royal Marsden Hospital will have mrTRG (Magnetic resonance imaging tumour regression grade) assessed which will be correlated with cfDNA	
10		The study will also include collection and analysis of tissue, serial blood samples and clinical data of patients with newly diagnosed stage I CRC.	Study design being updated to include patients with stage I CRC
10	London Cancer Alliance (LCA)	RM Partners Cancer Vanguard (previously London cancer alliance (LCA))	Update of name change
11		The study will also include patients with stage I disease and assess if ctDNA is detectable in this subset of patients, and if ctDNA predicts for relapse.	Study design updated to include patients with stage I CRC
12		Descriptive analysis will be used to describe data from patients with stage I colorectal cancer.	Statistical analysis plan for patients with stage I CRC
13	Stage II and III	Stage I, II and III	Extending the Eligibility inclusion criteria at the first

Page No	Version 4.0	Version 5.0	Explanation
			post-operative visit to include stage 1 patients
13	Patients that are no longer proceeding with surgery as they are proceeding with a deferral of surgery approach (NB these patients will remain in the study as an exploratory cohort and will therefore continue to have bloods taken)	Patients that are no longer proceeding with surgery as they are proceeding with a deferral of surgery approach	This group of patients will no longer be followed up as part of the TRACC study.
13	Stage I patients based on the post-operative histopathology report should be excluded with the exception of rectal cancer patients that had pre-operative (chemo)radiotherapy. In such patients, the pre-chemoradiotherapy-magnetic resonance imaging (MRI) staging should eb used. Patients with stage II or III disease on this initial MRI scan with stage I disease on the post-operative histopathology reporet due to response to (chemo) radiotherapy can still be included.		This exclusion criterion has been removed.
13		Time points for blood samples and CT scans for stage II and III patients	Update of study schema for stage II and III patients in Figure 1. The legend has been updated to now clarify that patients in the deferral arm of surgery will no longer continue on the study.
13	4-8 weeks post- op with staging CT	4-12 weeks post- op with staging CT	Extension of time period for staging CT scans
13	Stage 1 patients		This exclusion criterion has been removed

Page No	Version 4.0	Version 5.0	Explanation
13	Additional blood samples to be taken within 2 weeks of radiologically confirmed relapse	Additional blood samples to be taken within 2-8 weeks of radiologically confirmed relapse	Time period for blood collection increased
13		It is anticipated that we would also recruit 60 stage 1 patients per year (assuming 10-15% rate) for 3 years, giving a total of 180. With these numbers we can estimate the ctDNA detection rate in stage 1 patients pre-op sample with $\pm$ 7.3%. Descriptive analysis will be used to describe data from patients with stage I colorectal cancer	The statistical explanation of the expected number of stage 1 patients to be recruited
14		New trial schema for stage I patients included in Figure 2	Stage I patients will have different trial study visits
15		Patients with high grade dysplasia included in the study based on imaging diagnosis but subsequent histopathology confirms no carcinoma will be excluded	This exclusion criterion has been added
22		There is limited data available regarding detection of cfDNA in patients with stage 1 colorectal cancer and we will include this group of patients as an exploratory cohort. The data generated will help decide if this technique is useful to detect early relapse in this group of patients.	Rationale for including patients with stage I CRC in this amendment
34	To determine whether ctDNA analysis is useful for predicting relapse in patients with		Patients in whom deferral of surgery approach is used will

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	rectal cancer who have a favourable response to long course (chemo)radiotherapy and proceed with a deferral of surgery approach		be withdrawn from the study
34		To assess blood and tumour tissue of patients with synchronous primaries to evaluate mutational patterns in patients with synchronous colorectal primaries To explore if ctDNA is detectable in patients with stage 1 colorectal cancer To assess whether detection of ctDNA predicts for relapse in patients with stage I CRC that have undergone surgery with curative intent	Exploratory endpoints updated to reflect inclusion of patients with stage I CRC
34	This is a multi-centre, prospective, translational research study involving the collection and analysis of tumour tissue, serial blood samples and clinical data in patients with newly diagnosed stage II and III CRC. Blood samples will be collected at time-points as specified in the trial schema.	This is a multi-centre, prospective, translational research study involving the collection and analysis of tumour tissue, serial blood samples and clinical data in patients with newly diagnosed stage I, II and III CRC. Blood samples will be collected at time-points specified in Figures 1 and 2 depending on the stage of the tumour. In patients with rectal cancer whose tumour is down-staged by chemoradiotherapy (CRT), the staging prior to CRT will be considered as the stage for analysis. In patients in whom surgery upstages or	Study design and timing of blood samples specified

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		down-stages the tumour, the surgical staging will be considered for analysis.	
35	FFPE tumour tissue will be retrieved from surgery of patients with stage II and III CRC.	FFPE tumour tissue will be retrieved from surgery of patients with stage I, stage II and III CRC.	Inclusion of stage I patients
36		For patients at the Royal Marsden Hospital who co-enrol into the TRIGGER trial [a multi-centre, prospective, translational study sponsored by the Royal Marsden NHS Foundation Trust exploring the magnetic resonance tumour regression grade (mrTRG) as a novel biomarker to stratify management of good and poor responders to chemo-radiotherapy] data generated from the molecular sub-classification of FFPE tumour tissue from pre-CRT biopsies and post-CRT resection specimens, described above, will be securely shared with the TRACC research team. In addition translational data relating to ctDNA and re-sequencing results from FFPE tumour tissue, acquired in the TRACC trial for co-enrolled patients will be securely shared with the TRIGGER research team. This is to avoid duplication of work by	Collection and sharing of study data for patients on TRACC and TRIGGER study clarified in protocol

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		research teams from the same sponsor and to optimise resource utilisation.	
37		The Clinical Trials Unit at the Royal Marsden Hospital will check and confirm eligibility at relevant time-points through-out the study with histopathology reports, multidisciplinary team outcomes and clinical documentation. The data collection system has been updated in this regard.	Eligibility checks for patients to continue with the study.
37		Patients with high grade dysplasia whose imaging is suggestive of colorectal carcinoma (CRC) will be included but will be excluded post-surgery if carcinoma diagnosis is not confirmed	Add to inclusion criteria
38	Patients that are no longer proceeding with surgery as they are proceeding with a deferral of surgery approach (NB these patients will remain in the study as an exploratory cohort and will therefore continue to have bloods taken)	Patients that are no longer proceeding with surgery as they are proceeding with a deferral of surgery approach	This group of patients will no longer be followed up as part of the TRACC study.
38	Rectal cancer patients who have a favourable response to chemo-radiotherapy or radiotherapy and proceed with a deferral of surgery approach will be analysed as a separate cohort if		Patients who proceed to have a deferral of surgery approach will now be excluded from the study analysis

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	archival tumour specimen from their diagnostic biopsy is available for re-sequencing. Radiological staging will be used for this subset of patients who will not be included in the main statistical analysis of this study but will form the basis of an exploratory analysis. These patients will have a blood sample taken 6-12 weeks following completion of chemo-radiotherapy/radiotherapy (instead of the blood sample that is being collected within 4 weeks prior to surgery in all other patients proceeding to surgery). The month 0 blood sample on the trial schema will then be collected 4-8 weeks from this time-point or prior to starting adjuvant chemotherapy if this is sooner. All subsequent blood collection time-points will remain the same.		
38	Stage II or III CRC based on the post-operative histopathology report	Stage I, II or III CRC based on the post-operative histopathology report	Inclusion of stage I patients
38	Stage I patients based on the post-operative histopathology report should be excluded, with the exception of rectal cancer patients that had pre-operative chemoradiotherapy. In such patients, the pre-chemoradiotherapy magnetic resonance imaging (MRI) staging		Exclusion criterion updated

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	should be used. Patients with stage II or III disease on initial MRI scan with stage I disease on the post-operative histopathology report due to response to (chemo) radiotherapy can still be included.		
37		Patients with high grade dysplasia included in the study based on imaging diagnosis but subsequent histopathology confirms no carcinoma will be excluded	Inclusion of patients with high grade dysplasia only if subsequently carcinoma is proven
38		Any health care professional with GCP training who has been delegated by the principal investigator can obtain consent from eligible patients.	Update of study personnel able to take consent
39	Patients will be provided with an up to date copy of the patient information sheet (PIS)	Before registration, each potential patient must be given a patient information sheet (colon PIS for patients with colon cancer; rectal and recto-sigmoid cancer patients who are due to receive chemoradiotherapy will be given a separate rectal PIS), and informed consent obtained according to the requirements of GCP.	Clarification of study PIS which will be given to patients.
39	Patients will be provided with an up to date copy of the patient information sheet (PIS)	Patients will be provided with an up to date copy of the patient information sheet (PIS); colon PIS for patients with colon cancer; rectal and recto-sigmoid	Clarification of study PIS which will be given to patients.

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		cancer patients who are due to receive chemoradiotherapy will be given a separate rectal PIS which specifically outlines study procedures related to radiotherapy, and patient consent will be sought.	
40	Blood will be collected at time-points as specified in trial schema.	Blood will be collected at time-points as specified on Figures 1 and 2.	Figure reference updated
40	In patients in whom no mutation is detected on resequencing of tumour tissue, blood samples will continue to be collected in accordance with the trial schema for future research projects, where patients have provided consent.	In patients in whom no mutation is detected on resequencing of tumour tissue, blood samples will continue to be collected in accordance with the Figure 3 for future research projects, where patients have provided consent.	Figure reference updated
40		Please see the Laboratory Manual for full details on the collection, processing, storage and shipment of the blood samples.	Update regarding laboratory manual.
41		Please see the Laboratory Manual for full details on the collection, processing, storage and shipment of the blood samples.	Update regarding laboratory manual.
41	Instead samples should be labelled with the study name TRACC, the patient's study number (assigned at registration) and initials. The date of collection and collection time-point as outlined in the trial schema should also be recorded on blood samples.	Instead samples should be labelled with the study name TRACC, the patient's study number (assigned at registration) and initials. The date of collection and collection time-point as outlined in Figures 1 and 2 should also	Figure reference updated

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		be recorded on blood samples.	
43		Table of study assessments including the legend updated	Study assessments updated to include patients with stage I CRC
43	This is a multi-centre, prospective, translational research study involving the collection and analysis of tumour tissue, serial blood samples and clinical data in patients with stage II and III CRC	This is a multi-centre, prospective, translational research study involving the collection and analysis of tumour tissue, serial blood samples and clinical data in patients with stage I, II and III CRC	Inclusion of patients with stage I CRC
44		All of the endpoints will be analysed in all patients and by disease stage. In patients with rectal cancer whose tumour is down-staged by chemoradiotherapy (CRT), the staging prior to CRT will be considered as the stage for analysis. In patients in whom surgery upstages or down-stages the tumour, the surgical staging will be considered for analysis.	Staging that will be used for statistical analysis clarified.
48	DFS as time from date of surgery to first relapse or death. All patients alive and disease free will be censored at last follow-up.	DFS as time from date of surgery to first radiologically confirmed relapse or death. All patients alive and disease free will be censored at last follow-up.	Clarification of radiological confirmation of relapse for estimation of DFS.
49		Descriptive analysis will be used to describe data from patients with stage I colorectal cancer.	Statistical analysis plan for patients with stage I CRC.
51		It is anticipated that we would also recruit 60 stage 1 patients per year (assuming	Statistical justification of the number of stage 1 patients expected over 3 years

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		10-15% rate) for 3 years, giving a total of 180. With these numbers we can estimate the ctDNA detection rate in stage 1 patients pre-op sample with ± 7.3%. Descriptive analysis will be used to describe data from patients with stage I colorectal cancer.	

Protocol changes from V5.0 to V6.0

Page No	Version 5.0	Version 6.0	Explanation
Throughout	V5.0 dated 01.08.17	V6.0 dated 01.02 2018	
15	4-12 weeks post-op with staging CT	4-12 weeks post-op with optional staging CT	Protocol being amended from mandatory to optional CT scan and figure 1 changed accordingly
44		E: optional CT scan 4-12 weeks after surgery	Protocol being amended from mandatory to optional CT scan after surgery, so summary of study assessments changed accordingly

Protocol changes from V6.0 to V7.0 (draft)

Page No	Version 6.0	Version 7.0	Explanation
Throughout	**V6.0 dated 01.02.2018**	**V7.0 dated 21.09 2018(draft)**	
2		**Inclusion of Health Economist,Dr. Kyriaki Giorgakoudi**	**Study personnel update**
6		**Inclusion of Dr. Naureen Starling and Dr. Ian Chau in the Trial Management Group**	**Study personnel update**
6		**Dr. Larissa Sena removed from histopathology team and Dr. Katharina von Loga added instead**	**Study personnel update**
6		**Aleruchi Okachi removed as Biological Specimen Co-ordinator**	**Study personnel update**
6		**LCA research changed to RM partners Vanguard**	**Change in name update**
6		**Dr. Naureen Starling and Mrs. Clare Peckitt added in list of authors in protocol development**	**Study personnel update**
8	**In patients with stage I, II and III CRC, detection of mutations in ctDNA in plasma can predict relapse**	**In patients with stage I, II and III colorectal cancer (CRC), detection of mutations in circulating tumour DNA (ctDNA) in plasma can predict relapse** ctDNA directed adjuvant chemotherapy administration will enable biomarker driven selection of patients who would benefit from adjuvant chemotherapy and thereby reduce the proportion of patients receiving adjuvant chemotherapy without compromising disease free survival.	**Hypothesis row moved above, abbreviations expanded and hypothesis for Part C of the study explained**
8	**Multi-Centre study, encompassing selected centres from, but not limited to the RM partners Cancer Vanguard (previously**	**Multi-Centre study across the UK. The TRACC study is open to any site within the United Kingdom treating patients with CRC. It is anticipated that up to 100**	**Update on anticipated sites**

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	London Cancer Alliance) catchment area	**centres will participate in trial recruitment.**	
8		Part A (Feasibility Phase): This part of the study will include the first patients 48 with stage II or III colorectal cancer who have undergone curative surgery. Samples from these patients will allow assessment of ctDNA detection methods. Part B (Main Study): The overall study population will include Patients in Part A of the study Patients with stage I CRC, low risk stage II CRC and any patient not willing or not eligible to take part in Part C of the study *(current on-going study)* Patients with high risk stage II or stage III CRC who are willing and are randomised in Part C of the study as below. Part C (ctDNA guided adjuvant chemotherapy group): This biomarker guided group will include patients with high risk stage II or stage III colon or rectal cancer who have undergone curative surgery and need adjuvant chemotherapy. Adjuvant chemotherapy will be based on randomization between standard of care arm and ctDNA guided arm. Patients with rectal cancer who have undergone neo-adjuvant chemo-radiotherapy followed by curative surgery will also be included.	**Study population described**

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9	**Patients will be recruited over 3 years and will be followed up for up to 5 years.**	Study patients will be recruited over 5 years and followed up for 5 years.	**Change in study period updated**
9	**Primary Objective:** **For Part A (feasibility):** To assess whether circulating cell free tumour derived DNA (ctDNA) is detectable in patients with stage II and III colorectal cancer (CRC) pre-operatively **For Part B:** To assess whether detection of ctDNA predicts for relapse in patients with stage II and III CRC that have undergone surgery with curative intent	**Primary Objectives:** **For Part A (feasibility):** To assess whether circulating cell free tumour derived DNA (ctDNA) is detectable in patients with stage II and III colorectal cancer (CRC) pre-operatively **For Part B (includes all patients):** To assess whether detection of ctDNA predicts for relapse in patients with stage II and III CRC that have undergone surgery with curative intent **Part C: For ctDNA guided interventional group of the study** To demonstrate de-escalation strategy of ctDNA guided adjuvant chemotherapy is non-inferior to standard of care treatment as measured by 3 year disease free survival in patients with high risk stage II or stage III colorectal cancer in patients with no evidence of minimal residual disease (ctDNA negative)	**Primary objective updated**
9-10	**Secondary Objectives:** To assess whether mutations identified in formalin fixed paraffin embedded (FFPE) tumour tissue using targeted resequencing by a clinically validated method can be detected in	**Secondary Objectives:** **For all patients within the study (Part B)** To assess whether mutations identified in formalin fixed paraffin embedded (FFPE) tumour tissue using targeted resequencing by a clinically validated method can be	**Secondary objectives amended**

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	circulating cell free DNA (cfDNA) using droplet digital PCR (ddPCR) To quantify levels of mutations in cfDNA and assess change from baseline, post-operatively and during chemotherapy In patients with rectal cancer having neoadjuvant chemoradiation, to quantify levels of mutations in cfDNA prior to chemoradiation and assess change in level prior to surgery and post-operatively To quantify levels of mutations in cfDNA and assess change 3 monthly from the first post-operative visit for year 1, 6 monthly until year 3 and annually in years 4 and 5 or until relapse, if this occurs first in patients with stage II and III CRC To assess whether serial quantification of ctDNA has the potential to predict loco-regional and/or distant relapse after treatment of stage II and III CRC To correlate change in quantity of mutations in cfDNA with carcinoembryonic antigen (CEA), clinical and radiological parameters To develop a threshold for the detection of ctDNA that is likely to lead to relapse, by using the first 500 patients recruited as a training set and the next 500 patients recruited as a validation set	detected in circulating cell free DNA (cfDNA) using droplet digital PCR (ddPCR) To quantify levels of mutations in cfDNA and assess change from baseline, post-operatively and during chemotherapy In patients with rectal cancer having neoadjuvant chemoradiation, to quantify levels of mutations in ctDNA prior to chemoradiation and assess change in level prior to surgery and post-operatively To quantify levels of mutations in cfDNA and assess change 3 monthly from the first post-operative visit for year 1, 6 monthly until year 3 and annually in years 4 and 5 or until relapse, if this occurs first in patients with stage II and III CRC To assess whether serial quantification of ctDNA has the potential to predict loco-regional and/or distant relapse after treatment of stage II and III CRC To correlate change in quantity of mutations in ctDNA with carcinoembryonic antigen (CEA), clinical and radiological parameters To develop a threshold for the detection of ctDNA that is likely to lead to relapse, by using the first 500 patients recruited as a training set and the next 500 patients recruited as a validation set **For Part C only (ctDNA guided interventional group of the study)** Assess proportion of patients who are ctDNA negative on post-operative ctDNA and not receiving adjuvant	

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		chemotherapy in interventional arm compared to standard arm Assess proportion of patients who are ctDNA negative post-operatively who become ctDNA positive during follow-up and receive chemotherapy as an escalation of care To compare overall survival between ctDNA directed adjuvant chemotherapy and standard of care adjuvant chemotherapy arms To compare neurotoxicity in and quality of life in patients between arms Economic analysis to assess the cost-effectiveness of ctDNA directed therapy arm compared to the standard of care arm	
10	**Exploratory Objectives**	**Exploratory Objectives For all patients in the study (Part B)**	**Clarified that exploratory objectives apply to all patients within the study**
11	**Part B**	**Part B (for all patients in the study**	**Clarified that these end-points apply to all patients**
12-13		**Part C only (ctDNA guided interventional group of the study:** **Primary Endpoint**: Difference in 3 year disease-free survival from time surgery to progression, between standard of care arm and ctDNA guided adjuvant chemotherapy arm. **Secondary Endpoints:**	End-points specific to Part C of the study added

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		Proportion of patients in the ctDNA guided arm receiving standard of care chemotherapy in the ctDNA negative group Proportion of patients who are ctDNA negative post-operatively who become ctDNA positive during follow-up and receive chemotherapy as an escalation Overall survival between both arms, defined as time from randomisation to death of any cause. Sub group analyses performed on 3-year DFS and OS in including but not limited to the following will be performed: high risk stage II versus stage III site of primary tumour (right colon versus left colon versus rectum). Neurotoxicity data between both arms (FACT/GOG-Ntx4 & CTCAE V5) Quality of life data (EORTC QLQ-C30 and CR29 and EQ-5D-3L) Health economics	
13-15	This is a multi-centre, prospective, translational research study involving the collection and analysis of tumour tissue, serial blood samples and clinical data in patients with newly diagnosed stage II and III CRC. The study will also include collection and analysis of tissue, serial blood samples and clinical data of patients with newly diagnosed stage I CRC. Resources from three biomedical research centres (BRCs) will be	This is a multi-centre, prospective, translational research study involving the collection and analysis of tumour tissue, serial blood samples and clinical data in patients with newly diagnosed stage I, II and III CRC. The study will be divided into 3 parts. Part A will serve as a feasibility study prior to proceeding to Part B, which will include patients with stage I, stage II or III colorectal cancer treated with curative surgery and undergo standard of care	Study design amended

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	pooled to acquire blood samples and tumour tissue from centres encompassed within but not limited to the RM Partners Cancer Vanguard (previously London cancer alliance (LCA)) catchment area. These will be analysed centrally at the centre for molecular pathology (CMP) in Sutton, Surrey. The three BRCs involved will be: Royal Marsden Hospital (RMH) and its academic partner the Institute of Cancer Research (ICR), Imperial and King's College London. FFPE tumour tissue will be retrieved from surgery of patients with stage I, II and III CRC and targeted resequencing by a clinically validated method for a panel of pre-specified genes such as *KRAS, NRAS, BRAF, PIK3CA, TP53* and *APC* will be performed. The study will also collect plasma from these patients and extract cfDNA. ddPCR assays will be used to detect and track tumour specific mutations in cfDNA. The study will be divided into 2 parts. Part A will serve as a feasibility study prior to proceeding to Part B. In part A the proportion of patients with stage II and III CRC who have detectable ctDNA in plasma pre-operatively will be determined.	treatments and follow-up. The third part of the study, Part C, will include those patients willing to participate in a ctDNA guided adjuvant chemotherapy approach. In part A the proportion of patients with stage II and III CRC who have detectable ctDNA in plasma pre-operatively will be determined. Part B of the study will aim to determine whether detection of ctDNA in the first post-operative blood sample can be used to predict relapse in patients with stage I, II and III CRC. In addition, levels of ctDNA at other time points such as: pre-operative, during chemotherapy and post-chemotherapy will be evaluated. All patients are followed up every 3 months during first year, 6 months during 2nd and 3rd year and annually during years 4 and 5; bloods for ctDNA will collected at all these time points. The association between the level of ctDNA at these time points with DFS and OS will be determined. Part C of the study will include patients willing to participate in the ctDNA guided interventional group. Patients will sign a separate consent form specific for Part C of the study. Once informed consent is obtained in the oncology clinic from patients willing to take part in part C of the study, prior to randomization, clinicians will decide the adjuvant chemotherapy of choice based on histo-pathological features of the tumour, patient's age, co-	

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	Part B of the study will aim to determine whether detection of ctDNA in the first post-operative blood sample can be used to predict relapse in patients with stage II and III CRC. In addition, levels of ctDNA at other time points such as: pre-operative, during chemotherapy and post-chemotherapy will be evaluated. The association between the level of ctDNA at these time points with DFS and OS will be determined. The study will also include patients with stage I disease and assess if ctDNA is detectable in this subset of patients, and if ctDNA predicts for relapse.	morbidities and patient's choice, as is current clinical practice. Following consent and before randomization, patients will have ctDNA samples collected during week 4-8 post-operatively. Patients will then be randomised to 1:1 fashion between standard of care arm where patients are offered standard of care adjuvant chemotherapy according to national guidelines, and the experimental arm, in which patients will be treated based on ctDNA results. Results of ctDNA analysis will be made available within a 2 week turn-around period by week 6-10 following surgery in patients assigned to the ctDNA- guided adjuvant chemotherapy arm (Arm B) as follows:. <u>Post-op ctDNA positive patients</u> Patients receive standard of care adjuvant chemotherapy <u>Post-op ctDNA negative patients</u> If ctDNA is negative post-operatively, patients are de-escalated as follows: -if a doublet regimen was assigned to the patient before randomisation, patient receives either single agent chemotherapy or no chemotherapy as per clinician's decision prior to randomisation. -if single agent chemotherapy has been assigned, then patient receives no chemotherapy. Real time analysis of ctdNA with a two –week turn-around time of ctDNA results will be	

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		performed at following time points: Post-operative ctDNA at month 0 (before randomisation) Post-operative ctDNA at month 3 <u>Post-op ctDNA negative patients who become positive during follow-up</u> In those patients who are ctDNA negative during month 0 but become positive at month 3, will undergo radiological imaging to assess for disease relapse (CT or MRI). If no evidence of macroscopic disease is noted, in this group of patients, systemic chemotherapy will be introduced with single agent capecitabine, or chemotherapy escalated to doublet regimen with CAPOX if they had capecitabine during the adjuvant setting. If there is evidence of macroscopic disease by radiological assessment, chemotherapy as per clinician's choice will be administered. Patients in part C, both standard of care arm and experimental arm will have ctDNA levels measured during adjuvant chemotherapy at all-time points as specified in the main part B of the study.	
15- 16		<u>For feasibility and Main study (Parts A and B)</u> <u>For ctDNA guided interventional group of the study (For Part C)</u> Stopping for safety will be based on the recommendations from the IDMC and endorsed by a Trial Steering Committee. Stopping	Interim analysis for Parts A, B and C clarified

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		based on lack of efficacy (futility) will be based on the combination of evidence from safety and the conditional power. If the conditional power is <20% after 25% or 50% of the DFS events, the study will be considered futile. The IDMC will offer the overall recommendation based on clinical and statistical data for stopping for futility. The IDMC may also meet at other times as required but as a minimum we will consider 2 interim/futility assessments planned using conditional power. 25% events (n=125) would occur by year 4 (with 1280 patients recruited 50% events (n=250) would occur by year 5 (with all patients recruited)	
16-17	In total it is anticipated that 1000 patients will be recruited over 3 years, of which 500 will have stage II and 500 stage III disease. Once we have confirmation that we have recruited 500 patients of a particular stage, we will halt recruitment to that stage. We plan to over-recruit to account for drop-outs. The first 48 patients will comprise the pilot study. These patients will be included in the overall planned recruitment target of 1000 patients. The statistical analysis for the pilot study will be conducted after the first 24 patients with confirmed	The first 48 patients will comprise the feasibility part A of the study. These patients will be included in the overall analysis. This would be used as a guide as to whether to continue to the main study or not. For ctDNA guided interventional group of the study (Part C), A total of 1621 subjects (810 patients in each arm) would need to be randomised, with 499 events required based on the following assumptions: 5 year accrual 3 year minimum follow up on all 10% 2 sided significance level 80% power	**Number of patients and statistical considerations changed to reflect amendment**

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	stage II disease and the first 24 patients with stage III disease have been recruited. If 12 or more of the 48 patients have trackable mutations detected in the cfDNA pre-operatively then this would rule out a 15% detection rate in favour of a 30% rate, with 80% power and 5% significance level. This would be used as a guide as to whether to continue to the main study or not. In the stage II patients, it is assumed that 16% of patients will have detectable ctDNA at the first post-operative visit and that overall in stage II patients, the 5 year DFS will be 80%. With 90% power and 5% 2-sided significance, a difference of 10% in 5 year DFS can be detected from 75% (in 79 subjects with detectable ctDNA) to 85% (in 395 subjects with no-detectable ctDNA). Therefore in total, 474 subjects will be needed and 78 events. In the stage III patients, it is assumed that 40% of patients will have detectable ctDNA at the first post-operative visit and that overall in stage III patients, the 5 year DFS will be 65%. With 90% power and 5% 2-sided significance, a difference of 15% in 5 year DFS can be detected from 57.5% (in 144 subjects with	1, 2, 3, 4, 5, 6 year DFS estimated from SCOT study as 0.9, 0.8, 0.75, 0.725, 0.7, 0.68 Non-inferiority margin = 1.25 (ruling out 69.8% 3 year DFS) A non-inferiority margin of 1.25 has been chosen to allow for a worsening of 3-year DFS from 72-75% up to 69.3% only as being clinically acceptable. The overall study population (Part B) will include Patients in Part A of the study Patients with high risk stage II or stage III CRC who are willing and randomised in Part C of the study Patients with stage I CRC, low risk stage II CRC and any patients not willing or not eligible to take part in Part C of the study It is anticipated that the overall study population will be around 1800 (800 more than initially planned) and will be recruited over 5 years. Once confirmation that we have recruited 1621 patients for part C of the study and that the main study has 500 stage II (low risk and high risk CRC not in part C) and 500 stage III (not in part C), we will halt recruitment to that stage. We plan to over-recruit to account for drop-outs.	

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	detectable ctDNA) to 72.5% (in 216 subjects with no detectable ctDNA). Therefore in total, 360 subjects will be needed and 119 events. It is anticipated that we would also recruit 60 stage 1 patients per year (assuming 10-15% rate) for 3 years, giving a total of 180. With these numbers we can estimate the ctDNA detection rate in stage 1 patients pre-op sample with ± 7.3%. Descriptive analysis will be used to describe data from patients with stage I colorectal cancer.		
18-19	Patients with high grade dysplasia whose imaging is suggestive of colorectal carcinoma (CRC) will be included but will be excluded post-surgery if carcinoma diagnosis is not confirmed	Patients with lesions with high degree of suspicion on histology but not confirmed to be an adenocarcainoma andwhose imaging is strongly suggestive of colorectal carcinoma (CRC) will be included. These patients will be excluded post-surgery if carcinoma diagnosis is not confirmed **For Main Study (Part A and Part B) and ctDNA guided interventional group of the study (Part C)** For part C of the study, patients are initially registered as part of the main study. If post-operative histopathology confirms high-risk stage II or stage III colorectal cancer, and patient is willing to take part in the ctDNA guided adjuvant chemotherapy management, then eligibility assessment will	**Eligibility assessments for whole study and for part C clarified**

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		be undertaken as per below criteria. Note for those patients who were not enrolled into the TRACC study before surgery and wish to specifically take part in Part C of the study, can take part in the study as long as they meet all eligibility criteria for part C of the study.. All patients who were registered and subsequently excluded based on the eligibility criteria below will be replaced to ensure an adequate sample size is maintained for the statistical analysis.	
18, 20-21		**Eligibility criteria to be used prior to registration (for all patients, Part A and B):** **For patients in the ctDNA guided interventional arm of the study only (Part C)** Inclusion Criteria: Subject ≥ 18 years of age Subjects with histologically proven high risk stage II or stage III colon cancer treated with curative intent with surgery alone (any T, N1 or N2) with no evidence of metastatic disease. Subjects must be due to receive adjuvant chemotherapy following surgery. Subjects with histologically proven stage III rectal cancer are eligible, including patients treated with neoadjuvant chemoradiotherapy (any T, N1 or N2, M0) with no evidence of metastatic disease. Subjects must be due to receive adjuvant chemotherapy following surgery.	**Inclusion and exclusion criteria for part C of the study clarified**

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		Fully surgically resected tumour with clear resection margins (i.e., >1 mm) Adequate organ function Absolute neutrophil function ≥1.0 x 10^9/ L Platelet Count ≥ 75 x 10^9 / L Haemoglobin ≥80g/L (blood transfusion before randomisation is allowed) Adequate renal function as calculated by Cockcroft and Gault equation (GFR ≥ 30ml/min if FOLFOX chemotherapy chosen and GFR ≥ 50ml/min if single agent capecitabine or CAPOX chosen) Aspartate aminotransferase/ Alanine aminotransferase levels ≤ 2.5 upper limit of normal Patients should be assessed by Oncology team for suitability and assessment for adjuvant chemotherapy, be able to have post-operative ctDNA sample collected and be randomised by week 4-8 after surgery. ECOG performance status 0- 2 Able to give informed consent Patients who were not recruited pre-surgery for the main study can take part as long as they fulfil all eligibility criteria for part C of the study Exclusion Criteria: History of concurrent and previous malignancy within the last 3 years, with the exception of non- melanomatous skin cancer and carcinoma in situ Any major post-operative complications or other clinical conditions that in the opinion of the investigator would contra-	

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		indicate adjuvant chemotherapy Any subject not due to receive adjuvant chemotherapy will not be eligible for Part C of the study Hypersensitivity or contraindication to the drug(s) associated with the planned choice of systemic chemotherapy (CAPOX, FOLFOX or single agent 5-FU or capecitabine) as stated in the SmPC for each of the drugs	
22		All patients will be followed up for a minimum period of 5 years in all parts of the study. Following completion of adjuvant chemotherapy, follow up and assessments for clinical review, CEA and ctDNA samples will be as follows: 3 monthly during the first year 6 monthly during years 2 and 3 annual follow-up during years 4 and 5 Surveillance CT chest/ abdomen/ pelvis performed as part of routine surveillance post-operatively, at end of year 1, 2 and 3. Further imaging will be done if clinically indicated or ctDNA status or CEA show rise.	**Follow-up schedule added**
22		For Part C of the study only, a cost-effectiveness analysis at a later stage would help us assess the costs and health effects of the proposed intervention in comparison with the current standard of care. This will support future NHS implementation if appropriate.	**Health economics section added**
23-33		**Figures updated**	**Update to include part**

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			C of the study
36-40		**Table of Contents updated**	
43		In stage 2 CRC patients, the QUASAR study[16] showed a statistically significant improvement in survival in patients receiving adjuvant chemotherapy (80.3% versus 77.4%) with a reduction in recurrence (22.2% versus 26.2%), and the improvement is more in patients with high risk disease (T4 disease, perforation, obstruction, < 10 nodes examined, poorly differentiated histology or extramural vascular or lymphatic invasion), although not statistically significant. In patients with high risk disease, single agent capecitabine is used for a period of 6 months as 3- weekly cycles. Recurrence rates in stage 2 CRC is around 15% and SEER analysis calculated that 30% of stage 2 patients receive adjuvant chemotherapy. Adjuvant chemotherapy is recommended in patients with stage 3 CRC. Until 2017, a total of six months of adjuvant chemotherapy was recommended. The SCOT study reported 3 months of adjuvant chemotherapy was non-inferior to 6 months of treatment in high risk stage 2 and stage 3 CRC patients.[17] In a study of 6088 patients were randomised in a 1:1 manner to receive adjuvant chemotherapy for 3 months or 6 months. The 3 year disease-free survival (DFS) in the 3-month group was 76·7% (95% CI 75·1–78·2) and in the 6-	**Background for addition of Part C of the study added**

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		month group was 77·1% (75·6–78·6) for the 6 month group [HR: 1·006 (0·909–1·114, test for non-inferiority p=0·012)], significantly below the non-inferiority margin. The rate of peripheral neuropathy was 58% in the 6 month group versus 25% in the 3 month group. Based on these results, currently in the UK the practice has changed and patients with stage 3 CRC receive either 3 months of doublet chemotherapy with capecitabine and oxaliplatin (CAPOX) or 6 months of 5-flurouracil and oxaliplatin (FOLFOX) or 6 months of single agent capecitabine. Discussions regarding adjuvant chemotherapy are guided by age of the patient (<70 years), number of nodes involved and patient's wishes. Around 50% of patients with stage 3 disease in the UK receive doublet chemotherapy.	
51-53		Table of studies amended to reflect changing landscapes of treatment of patients	
54-55		**ctDNA detection using NGS based liquid biopsy panels** NGS based panels including Guardant Health, Roche Avenio panels are currently used as research and clinical tools in patients with metastatic disease. The amount of tumour derived cell-free DNA (ctDNA) in smaller, early to mid-stage tumors can be very low and is usually at or below 0.1% VAF (variant allele frequency).	**Validity of technological assay added to background**

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		The study will use Roche Avenio panel for detection of ctDNA in patients following surgery. The panel is highly specific with a variant -level specificity of 99.99% for the expanded panel, with each sample having a variant level specificity greater than 99.4%. Even with as low as 5ng of cfDNA the sensitivity was with expanded Kit was 93% sensitivity at a VAF of 0.5% with 50 million reads, and for early stage tumours, with lowest level of residual disease, with the higher reads, more sequencing allowed for higher sensitivity — with 200 million reads (3-4 samples per NextSeq High Output run) and 50ng input, the Targeted Kit showed >99% sensitivity at a VAF of 0.05% and the Expanded Kit gives near 90% sensitivity at a VAF of 0.05% (https://sequencing.roche.com/content/dam/rochesequence/worldwide/resources/SEQ100108_AVENIO%20ctDNA_Performance_White_Paper.pdf). 2.7 ctDNA guided adjuvant chemotherapy There is increasing evidence that ctDNA can reliably predict for minimal residual disease in patients with stage II or III CRC who have undergone curative surgery. In a study in 145 patients with stage II or stage III CRC who underwent R0 resection (Diehn et al., ASCO 2017), Roche Avenio ctDNA kits were used to assess somatic mutations in 197 cancer-related genes in	

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		tissue and in plasma taken post-operatively (mean 10 days post-op). Patients were considered ctDNA positive or negative in post-operative plasma based on a single detection of single nucleotide variants (SNVs) in tumour tissue at an allele frequency of at least 5%. Of the 144 patients with tissue variants, 93 (65%) were colon and 51 (35%) were rectal. Of the 144 patients, 12 patients were ctDNA positive (8%) and 132 (92%) were ctDNA negative. Of the stage 2 patients, 4 out of 85 were ctDNA positive (5%) and in stage 3 patients 8 out of 59 patients (14%) were ctDNA positive. Median follow-up was 32 months. Time to recurrence, relapse free survival and OS was significantly shorter in patients who are ctDNA positive (n =12) compared to negative patients (n= 132). The sensitivity for predicting recurrence was 57.1% (12 out of 21 patients) with a specificity of 100% and positive predictive value of 100%. Post-operative ctDNA was an independent predictor of recurrence in both stage 2 and 3 irrespective of adjuvant treatment. In a further study (Tie et al., ASCO 2018) in 95 patients with stage 3 colon cancer treated with adjuvant chemotherapy, an inferior disease free survival was noted in 20% patients who were ctDNA positive post-surgery (HR, 3.52; p = 0.004). In patients who were ctDNA	

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		positive post-op, adjuvant chemotherapy rendered 50% of them negative at time of completion of treatment. Patients who remained ctDNA positive at end of adjuvant chemotherapy had an inferior DFS (HR: 7.14; p < 0.001). In 5% of patients in this study ctDNA changed from negative to positive (HR: 5.30; p = 0.006) within 3 months. There is lack of consensus around adjuvant chemotherapy in patients with locally advanced rectal cancer LARC who have undergone neo-adjuvant chemo-radiotherapy. The PETTAC6 data presented at ASCO previously in 2014 and in 2018 in patients with locally advanced rectal cancer who received neo-adjuvant chemo-radiotherapy has shown a 3-year DFS of 72% with adjuvant capecitabine and no additional benefit with the addition of oxaliplatin. A prospective study (Tie et al., Gut Feb 2018) in patients with LARC (T3/T4 and/or N+) receiving chemo-radiotherapy measuring ctDNA levels pre-treatment, post-chemo-radiotherapy and 4 -10 weeks after surgery was conducted in 159 patients and 462 samples analysed (Tie et al., Gut 2018). ctDNA levels was detected in 77%, 8.3% and 12% of pre-treatment, post-chemo-radiotherapy and postsurgical plasma samples respectively. Recurrence-free survival was significantly worse in patients in whom ctDNA was detected after chemo-radiotherapy (HR 6.6; p<0.001) or after surgery	

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		(HR 13.0; p<0.001), and postoperative ctDNA was predictive of recurrence irrespective of adjuvant chemotherapy use (chemotherapy: HR 10.0; p<0.001; without chemotherapy: HR 22.0; p<0.001). Postoperative ctDNA levels could potentially be a prognostic biomarker, identifying patients with high or low risk of recurrence. In a prospective study of 230 patients with resected stage 2 CRC, 1046 plasma samples were analysed using Safe-Seq (Tie et al., Science Translational Medicine, 2016). Post-op ctDNA was detected in 20 of 230 patients (9%). In 179 patients who did not receive adjuvant chemotherapy 14 patients (7.9%) were ctDNA positive of whom 11 (79%) had recurrence at median follow-up of 27 months; sixteen of 164 patients (9.8%) of patients who were ctDNA negative recurred [hazard ratio (HR), 18; 95% confidence interval (CI), 7.9 to 40; P < 0.001]. Patients with persistence of ctDNA after completion of chemotherapy has significantly reduced recurrence-free survival (HR, 11; 95% CI, 1.8 to 68; P = 0.001). 2.8 Immunoassays Early detection of colorectal cancer can help improve morbidity and mortality. Due to low compliance with fecal-based screening and colonoscopy, various groups have designed non-invasive	

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		alternatives including blood-based multiplexed immunoassays for early detection. In a study of over 1005 patients with early stage, clinically detected cancers including CRC, a 61-amplicon panel PCR-based sequencing panel was positive in a median of 70% of the eight cancer types with sensitivities ranging from 69 to 98% for the detection of five cancer types and specificity > 99% and anatomical localisation in a median of 83% of the patients. The study will explore immunoassays in stage I, II and III CRC patients that will help inform early relapse and develop methodologies for early detection of CRC helping the national health strategy for better screening procedures.	
57		The Bowel Cancer UK Critical Research Gaps in Colorectal Cancer Initiative has identified development of biomarkers that define the optimal curative therapeutic strategy preventing overtreatment and improving treatment selection as one of the major research gaps that requires to be addressed. There is increasing evidence of the clinical utility of circulating tumour DNA (ctDNA) in detection of minimal residual disease in patients in patients with stage II and III colorectal cancer who undergo potentially curative surgery. Circulating DNA (ctDNA) guided adjuvant chemotherapy in this group of patients could potentially help individualise risk stratification, thereby	**Rationale for amending the current study to include Part C of added**

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		reducing the use of unnecessary chemotherapy and tailor treatment decisions helping deliver personalised medicine. Plasma samples from the on-going TRACC study are currently being analysed as per protocol at the GCLP accredited laboratory in the Centre for Molecular Research at The Royal Marsden Hospital. Two different technological asssays including ddPCR (digital droplet PCR) and Roche Avenio panel are being used to analyse samples, allowing for technical validation of ctDNA results in a systematic and centralised manner. The use of these highly sensitive assays allows for detection of even small amounts of DNA from early stage tumours, which is at or below 0.1% variant allele frequency (VAF). With these technologies variant allele frequency of 0.05% to >1% can be assessed. Furthermore, the sensitivity of the Roche Avenio panel is 90% at 0.05% VAF, with a specificity of 99.9%. In light of accumulating scientific evidence and recent improvements in the sensitivity of the technological assays allowing for ctDNA in detecting residual microscopic disease, we propose amending the current protocol to include a nested biomarker guided interventional study. This approach allows for rapid transition of research into the clinic that will ultimately benefit	

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		patients and also help NHS save costs on chemotherapy.	
57	In patients with stage II and III CRC, detection of mutations in cfDNA in plasma can predict relapse.	Hypothesis 1: In patients with stage II and III CRC, detection of mutations in cfDNA in plasma can predict relapse. Hypothesis 2: We hypothesise that ctDNA directed adjuvant chemotherapy administration will enable biomarker driven selection of patients who would benefit from adjuvant chemotherapy and thereby reduce proportion of patients receiving adjuvant chemotherapy without compromising disease free survival	**Hypotheses of the study amended to reflect addition of Part C to the study**
58-59	**For Part B**	**For Part B (For all study patients):** **For Part C (patients in the ctDNA guided adjuvant chemotherapy arm):** To demonstrate de-escalation strategy of ctDNA guided adjuvant chemotherapy is non-inferior to standard of care treatment as measured by 3 year disease free survival in patients with high risk stage 2 or stage 3 colorectal cancer in patients with no evidence of minimal residual disease (ctDNA negative) **For patients in the ctDNA guided adjuvant chemotherapy arm (Part C):** Assess proportion of patients who are ctDNA negative on post-operative ctDNA and not receiving adjuvant	**Primary, secondary and exploratory Objectives for Part B and Part C of the study clarified**

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		chemotherapy in interventional arm compared to standard arm Assess proportion of patients who are ctDNA negative post-operatively who become ctDNA positive during follow-up and receive chemotherapy as an escalation of care To compare overall survival between ctDNA directed adjuvant chemotherapy and standard of care adjuvant chemotherapy arms To compare neurotoxicity in and quality of life in patients between arms Health economic analysis of cost-effectiveness of ctDNA directed therapy arm compared to the standard of care arm 5.3 Exploratory Objectives for all study patients:	
60	Resources from three biomedical research centres (BRCs) will be pooled to acquire blood samples and tumour tissue from centres encompassed within but not limited to the London cancer alliance (LCA) catchment area. These will be analysed centrally at the centre for molecular pathology (CMP) in Sutton, Surrey. The three BRCs involved will be: Royal Marsden Hospital (RMH) and its academic partner the Institute of Cancer Research (ICR), Imperial and King's College London.		**This has now been removed**

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61	The study will be divided into 2 parts. Part A will serve as a feasibility study prior to proceeding to Part B.	The study will include the following: All patients within the study will have ctDNA assessed at protocol- specified intervals (Part A and Part B) Patients with high risk stage II and stage III colorectal cancer willing to take part in the ctDNA guided adjuvant chemotherapy part of the study will be studied in part C of the study	**Protocol amended to include part C**
61-63	In part A the proportion of patients with stage II and III CRC who have detectable mutations in ctDNA in plasma pre-operatively will be determined. Part B of the study will aim to determine whether detection of ctDNA in the first post-operative blood sample can be used to predict relapse in patients with stage II and III CRC. In addition levels of ctDNA at other time points such as: pre-operative, during chemotherapy and post-chemotherapy will be evaluated. The association between the level of ctDNA at these time points with DFS and OS will be determined.	6.0 ctDNA analysis in stage I, II and III CRC (Part A and Part B of the study) In part A the proportion of patients with stage II and III CRC who have detectable mutations in ctDNA in plasma pre-operatively will be determined as a feasibility step. Part B of the study will aim to determine whether detection of ctDNA in the first post-operative blood sample can be used to predict relapse in patients with stage II and III CRC. In addition levels of ctDNA at other time points such as: pre-operative, during chemotherapy and post-chemotherapy will be evaluated. The association between the level of ctDNA at these time points with DFS and OS will be determined. <u>All patients in the study (Part B of the study)</u> Patients in Part A of the study Patients with stage I CRC, low risk stage II CRC and any patients not willing or not eligible to take part in Part C of the study *(current on-going study)*	**Study design elaborated to include Part C of the study**

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		Patients with high risk stage II or stage III CRC who are willing and are randomised in Part C of the study as below. 6.1 ctDNA guided adjuvant chemotherapy group (Part C of the study) Part C of the study will be a prospective, multi-centre study where patients will be randomised in a 1:1 manner to receive standard of care arm where patients are offered adjuvant chemotherapy according to national guidelines, or to the experimental arm, in which patients are treated based on ctDNA results. Patients will be stratified according to 1. High risk stage II versus stage III 2. Site of primary tumour: right colon versus left colon versus rectum <u>Screening and consent for Part C of the study</u>: Patients will be screened within 4-8 weeks (±after surgery. Patients will sign a consent form specific for part C of the surgery. Following informed consent, clinicians assign standard of care adjuvant chemotherapy regimen at this stage prior to randomisation. Clinicians will decide the adjuvant chemotherapy of choice based on histo-pathological features of the tumour, patient's age, co-morbidities and patient's choice, as is current clinical practice. At this stage the clinician will also decide upfront on the de-escalation regimen if ctDNA is negative post-operatively. Following	

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		informed consent patients will have ctDNA samples collected during week 4-8 post-operatively and randomisation will then be performed. <u>Randomisation</u>: This will be performed by the Institute of Cancer Research Statistical Unit in a1:1 manner between standard of care adjuvant chemotherapy or ctDNA guided adjuvant chemotherapy. Results of ctDNA analysis will be made available by week 8-10 following surgery in patients assigned to the ctDNA- guided adjuvant chemotherapy arm (Arm B) as follows: <u>*Post-op ctDNA positive patients*</u> Patients receive adjuvant chemotherapy if they have ctDNA detected and deemed positive for detection. <u>*Post-op ctDNA negative patients*</u> If ctDNA is negative post-operatively, patients are de-escalated as follows: -if a doublet regimen was assigned to the patient before randomisation, patient receives either single agent chemotherapy or no chemotherapy as per clinician's discretion decided previously. -if single agent chemotherapy has been assigned, then patient receives no chemotherapy. Patients in both arms will have ctDNA levels measured during	

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		adjuvant chemotherapy at following time points. All patients are followed up every 3 months during first year, 6 months during 2nd and 3rd year and annually during years 4 and 5. Patients in both arms will have ctDNA levels collected during adjuvant chemotherapy at all the above time points or when disease recurrence is suspected clinically and confirmed by radiology. Real time analysis of ctdNA with a two-week turn-around time will be performed at following time points: Post-operative ctDNA at month 0 (week 4-8 after surgery, before randomisation) Post-operative ctDNA at month 3 The ctDNA results at month 0 will guide decision of de-escalation of adjuvant chemotherapy in the ctDNA guided arm. Results of ctDNA at month 3 will guide decisions regarding escalation or starting systemic chemotherapy for microscopic disease in those patients who were initially negative but become positive at month 3 as follows. *Post-op ctDNA negative patients who become positive during follow-up* In those patients who are ctDNA negative during month 0 but become positive at month 3, will undergo radiological imaging to assess for disease relapse. If no evidence of macroscopic disease is noted, in this group	

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		of patients, systemic chemotherapy will be introduced with single agent capecitabine, or chemotherapy escalated to doublet regimen with CAPOX if they had capecitabine during the adjuvant setting. If there is evidence of macroscopic disease by radiological assessment, chemotherapy as per clinician's choice will be administered. If sufficient data accumulates real time analysis will be considered for month 6, year 1 year 2 samples. Advice will be sought from the TMG, IDMA and TSC with regards to this.	
63		Additional research into protein biomarkers will be undertaken as part of this study to help explore other biomarkers of early relapse.	**Protocol updated to reflect that patients will have blood samples collected for protein biomarkers**
63	This trial is open to any qualifying site within but not limited to the London Cancer Alliance catchment area.	This trial is open to any qualifying site within the United Kingdom. The TRACC study is open to any site within the United Kingdom treating patients with CRC. It is anticipated that up to 100 centres will participate in trial recruitment.	**Number of Centres amended**
64	Total of 1000 patients of which 500 will have stage II and 500 will have stage III disease. The first 48 patients recruited will comprise the pilot study.	The first 48 patients recruited will comprise the pilot phase or the feasibility part of the study (Part A). Part B of the study will include all patients in the study including part A and Part C of the study.	**Number of subjects amended**

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		Part C of the study will include the ctDNA guided adjuvant chemotherapy group, a total of 1621 subjects (810 patients in each arm) would need to be randomized and 499 events required. It is anticipated that the overall study population (Part B) will be around 1800 (800 more than initially planned) and will be recruited over 5 years. Once confirmation that we have recruited 1621 patients for part C of the study and that the main study has 500 stage II (low risk and high risk CRC not in part C) and 500 stage III (not in part C), we will halt recruitment to that stage. We plan to over-recruit to account for drop-outs.	
64	The study is planned to continue until 1000 patients have been enrolled. This is anticipated to take up to 3 years.	The study is planned to continue until a total of 2,000 patients have been enrolled. This is anticipated to take up to 7 years in total from start of study recruitment in 2016.	**Estimated study duration amended**
65	Patients with high grade dysplasia whose imaging is suggestive of colorectal carcinoma (CRC) will be included but will be excluded post-surgery if carcinoma diagnosis is not confirmed	Patients with lesions with high degree of suspicion on histology but not confirmed to be an adenocarcainoma and whose imaging is strongly suggestive of colorectal carcinoma (CRC) will be included. This patients will be excluded post-surgery if carcinoma diagnosis is not confirmed	**Eligibility criteria for main study amended**
66-67		12.3 Eligibility criteria for ctDNA guided adjuvant chemotherapy study (Part C) only Inclusion Criteria:	**Eligibility criteria for Part C added to protocol**

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		Subject ≥ 18 years of age Subjects with histologically proven high risk stage II or stage III colon cancer treated with curative intent with surgery alone (any T, N1 or N2) with no evidence of metastatic disease. Subjects with histologically proven stage III rectal cancer are eligible, including patients treated with neoadjuvant chemoradiotherapy (any T, N1 or N2, M0) with no evidence of metastatic disease. Fully surgically resected tumour with clear resection margins (i.e., >1 mm). Adequate organ function Absolute neutrophil function ≥1.0 x 10^9/ L Platelet Count ≥ 75 x 10^9 / L Haemoglobin ≥80g/L (blood transfusion before randomisation is allowed) Adequate renal function (GFR ≥ 30ml/min if FOLFOX chemotherapy chosen and GFR ≥ 50ml/min if single agent capecitabine or CAPOX chosen) as calculated by Cockcroft and Gault equation Aspartate aminotransferase/ Alanine aminotransferase levels ≤ 2.5 upper limit of normal Absence of major post-operative complications or other clinical conditions that, in the opinion of the investigator, would contraindicate adjuvant chemotherapy Patients should be assessed by Oncology team for suitability and assessment for adjuvant chemotherapy, be able to have post-operative ctDNA sample collected and	

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		be randomised by week 8 after surgery. ECOG performance status 0- 2 Able to give informed consent Patients who were not recruited pre-surgery for the main study can take part as long as they fulfil all eligibility criteria for part C of the study. Exclusion Criteria: History of concurrent and previous malignancy within the last 3 years, with the exception of non- melanomatous skin cancer and carcinoma in situ Hypersensitivity or contraindication to the drug(s) associated with the planned choice of systemic chemotherapy (CAPOX, FOLFOX or single agent 5-FU or capecitabine) as stated in the SmPC for each of the drugs.	
68	Before registration, each potential patient must be given a patient information sheet (colon PIS for patients with colon cancer; rectal and recto-sigmoid cancer patients who are due to receive chemoradiotherapy will be given a separate rectal PIS), and informed consent obtained according to the requirements of GCP. Consent must be taken by the principal investigator (PI) or a qualified doctor or nurse who is authorised by the PI on the site delegation log to take	Before registration, each potential patient must be given a patient information sheet (colon PIS for patients with colon cancer, rectal and recto-sigmoid cancer patients who are due to receive chemoradiotherapy will be given a separate rectal PIS. informed consent obtained according to the requirements of GCP. Consent must be taken by the principal investigator (PI) or a qualified doctor or nurse who is authorised by the PI on the site delegation log to take consent. For patients with high risk stage II or III CRC eligible and wishing to take part in the	**Subject enrollment clarified for part C of the study**

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	consent. The registration and baseline assessment forms must also be completed. Only patients fulfilling all pre-registration inclusion criteria and without any exclusion criteria should be registered. Any queries about eligibility should be addressed directly to the lead centre.	ctDNA guided adjuvant chemotherapy study (Part C), a PIS for part C will be given. A separate consent form will need to be signed by the patient. Patients can sign consent Part C of the study only after they have had at least 24 hours to consider the study. Consent for Part C can only be taken by a qualified doctor who is authorised to make decisions regarding adjuvant chemotherapy.	
68	**Fax number**	**E-mail added**	**TRACC e-mail added**
69		Please note that for Part C only a qualified doctor who is authorised to make decisions regarding adjuvant chemotherapy can obtain consent.	**Consenting personnel clarified for Part C of the study**
69- 71		15.1 Study Treatments All chemotherapeutic agents used in the study are standard of care treatments. Patients in the experimental arm are de-escalated if ctDNA is negative on the post-operative blood sample. In those patients in whom ctDNA is negative in the post-operative sample, but become positive during surveillance, chemotherapy is started or escalated. *Single agent capecitabine regimen* Capecitabine: 2000mg/m2 per day in two divided doses, administered orally from days 1-14 of a 3 week cycle Total number of cycles will be as follows: Total of 6 months of treatment (8 cycles) in standard of care arm Total of 3 months of treatment (4 cycles) in experimental arm	**Study Procedures for Part C clarified**

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		Capecitabine and Oxaliplatin doublet regimen A total of 4 cycles will be administered at following doses Oxalipatin: 130mg/m2 administered intravenously every 3 weeks of a 3 weekly cycles Capecitabine: 1700mg/m2 per day in two divided doses, administered orally from days 1-14 of a 3 weekly cycle Total number of cycles will be as follows: Total of 3 months of treatment (4 cycles) in standard of care and experimental arms 5-Flurouracil and Oxaliplatin doublet regimen A total of 12 cycles will be administered at following doses as per standard Oxaliplatin: 85mg/m2 administered intravenously every two weeks of a 2-weekly cycle 5-Fluro-uracil (5-FU): a bolus of 400mg/m2 administered on day 1 of chemotherapy and 5-FU at dose of 2400mg/m2 administered over two days (1200mg/m2 per day) of a 2-weekly cycle Total number of cycles will be as follows: Total of 6 months of treatment (12 cycles) in standard of care arm 15.1.1 Chemotherapy Drugs used in the trial for patients in Part C	

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		The following chemotherapy drugs are used in the clinical trial: Capecitabine, Oxaliplatin and 5-flurouracil. All chemotherapy agents must be sourced from local hospital stock and prepared as per local practice. All chemotherapy agents used must be stored according to Summary of Product Characteristics and labelled as per local practice. Drug Accountability As per risk adapted approach, sites may use standard documents as drug accountability e.g. in-house preparation worksheets, orders from external partners. This should include batch numbers and expiries. 15.1.2 Duration of treatment Eligible patients will have treatment as per protocol unless there is disease progression, unacceptable toxicity or withdrawal of consent for any reason or at clinician's discretion within that time period. 15.1.3 Management of Infusion Reactions In case of hypersensitivity reactions, the drug infusion should be interrupted and the acute management should occur as per local practice. Decision on re-challenge with the same drug on subsequent cycles will be made according to the severity of the hypersensitivity reaction at the discretion of the clinical investigator.	

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		15.1.2 Dose Modifications for Toxicity Expected toxicities are detailed in Appendix___. SmPCs will form the reference safety information (RSI) for this study. There will be an annual update of the SmPCs which will be submitted to the regulatory authorities. For toxicities or combinations of toxicities not specifically covered in detail in the SmPC (see protocol appendix), doses of chemotherapy can be reduced at the discretion of the Investigator as per local practice. All dose modifications documented in the CRF including reasons. Dose modifications due to neurotoxicity should be documented separately. Crossover from capecitabine to infusional 5-FU and vice versa is allowed if this is required to control toxicity. At all times all endeavours should be made to keep the total number of weeks of treatment as determined by randomisation. Please do not hesitate to contact your coordinating trial office for advice. 15.1.3 Neurosensory Toxicity Neurosensory toxicity due to oxaliplatin may require dose reduction and dose adjustments according to local protocol may be followed as long as the dose given is carefully annotated in the CRF. Wherever possible, oxaliplatin should be dose-reduced (as	

<table>
<tr><th>Page No</th><th>Version 6.0</th><th>Version 7.0</th><th>Explanation</th></tr>
<tr><td></td><td></td><td>per Investigator discretion) rather than discontinued and can be given over a longer period of time if it is the hyperacute neurotoxicity which is particularly a problem. In the situation where oxaliplatin is discontinued due to toxicity, adjuvant treatment can continue with 5-FU or capecitabine alone if deemed appropriate by the local Investigator. In this case, the dose per m^2 of the single agent 5-FU/capecitabine can be increased as per local practice at the discretion of the Investigator and documented in the CRF.

CTCAE version 5.0 should be used to grade the neurotoxicity as follows:

<table>
<tr><th colspan="3">Nervous System Disorders</th></tr>
<tr><th>CTCAE Term</th><th>Grade 1</th><th>G</th></tr>
<tr><td>Peripheral Sensory Neuropathy</td><td>Asymptomatic</td><td>M
s
li
in
A</td></tr>
<tr><td colspan="3">Definition: A disorder characte
nerves</td></tr>
</table>
</td><td></td></tr>
<tr><td>72- 73</td><td></td><td>For part C of the study only, real time analysis of ctDNA with a two –week turn-around time of ctDNA results will be performed at following time points:
Post-operative ctDNA at month 0 (week 4-8 following surgery, before randomisation)
Post-operative ctDNA at month 3</td><td>Part C ctDNA blood sample analysis clarified along with extra blood sample collection</td></tr>
</table>

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		Where appropriate, centres will be informed of germline mutations that are inherited if previously not already known. Further referral of patients to genetics team will be as per local policies at individual centres. At the Royal Marsden, for example, patients will be referred to the Genetics team and Tumour Board. Blood samples will also be collected and centrifuged for plasma and serum protein biomarkers in centres where resources are available for processing of blood. Known plasma and serum biomarkers including tumour markers such as CEA and CA19-9, and new exploratory protein biomarkers will be studied.	
75		15.7 Quality of Life and Neurotoxicity Questionnaires At the outset of the trial sites opted to participate in the collection of quality of life and neurotoxicity questionnaires (EORTC QLQ-C30 & CR29, GOG-NTX 4 and EQ-5D) at clinic visits as follows At randomisation Months 3, 6, 9 and 12 during first year 6 monthly during years 2 and 3 End of year 4 End of year 5	**QoL and neurotoxicity questionnaires will be collected in Part C of the study**
75-76		15.8 Pregnancy Patients should agree to use reliable birth control during the time they are receiving chemotherapy and for a year after stopping chemotherapy. If the patient or their partner becomes pregnant either whilst receiving trial	**Section of pregnancy added**

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		chemotherapy or in the first year after stopping trial chemotherapy it must be stressed that they are requested to inform their Investigator immediately. Acceptable forms of effective contraception Acceptable forms of effective contraception include: 1. Established use of oral, injected or implanted hormonal methods of contraception. [The decision to allow use of hormonal contraceptives should be based on the Investigational Medicinal Product's (IMP's) metabolism and potential for interactions, pharmacology and the adverse event profile (e.g. vomiting)]. 2. Placement of an intrauterine device (IUD) or intrauterine system (IUS). [Consideration should be given to the type of device or system being used, as there are higher failure rates quoted for certain types, e.g. steel or copper wire]. 3. Barrier methods of contraception: Condom or Occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository. The use of barrier contraceptives should always be supplemented with the use of a spermicide. The following should be noted: Failure rates indicate that, when used alone, the diaphragm and condom are	

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		not highly effective forms of contraception. Therefore the use of additional spermicides does confer additional theoretical contraceptive protection. However, spermicides alone are inefficient at preventing pregnancy when the whole ejaculate is spilled. Therefore, spermicides are not a barrier method of contraception and should not be used alone. 4. Male sterilisation (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate). [For female subjects on the study, the vasectomised male partner should be the sole partner for that subject]. 5. True abstinence: When this is in line with the preferred and usual lifestyle of the subject. [Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception]. Once informed of a pregnancy, sites must immediately complete and fax a Pregnancy Notification Form to their coordinating trial office. The Pregnancy Notification Form must be updated and faxed again as soon as anything relating to the pregnancy changes such as miscarriage, termination or delivery of the baby	
77-79		Table updated to reflect addition of Part C of the study	

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		Part C (ctDNA guided adjuvant chemotherapy group) is a nested biomarker guided group which will include patients with high risk stage II or stage III colon or rectal cancer who have undergone curative surgery and are willing to take part in ctDNA guided adjuvant chemotherapy cohort of the study. Patients with rectal cancer who have undergone neo-adjuvant chemo-radiotherapy followed by curative surgery will also be included. **For the Main Study (Part B)** All data from all eligible patients with a trackable mutation identified in tissue sample will comprise the main analysis set and will be used for all analysis unless otherwise specified. **For testing hypothesis 1:** All analyses will be presented including all patients and then split by disease stage II & III. This will include the following patients: Patients in Part A of the study All patients in part B of the study apart from the experimental arm of Part C of the study **For testing hypothesis 2:** This will include all patient treated in Part C (ctDNA guided adjuvant chemotherapy group). The primary population for analysis will be the Intent to Treat (ITT) population, defined as all patients randomised to study treatments, standard of care or ctDNA guided adjuvant chemotherapy; a sensitivity per protocol analysis will also be	

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		performed defined as all those receiving treatment as planned per randomisation on Part C.	
80		Part C (ctDNA guided adjuvant chemotherapy group) is a nested biomarker guided group which will include patients with high risk stage II or stage III colon or rectal cancer who have undergone curative surgery and are willing to take part in ctDNA guided adjuvant chemotherapy cohort of the study. Patients with rectal cancer who have undergone neo-adjuvant chemo-radiotherapy followed by curative surgery will also be included.	**Study design updated**
81		**For testing hypothesis 1:** All analyses will be presented including all patients and then split by disease stage II & III. This will include the following patients: Patients in Part A of the study All patients in part B of the study apart from the experimental arm of Part C of the study **For testing hypothesis 2:** This will include all patient treated in Part C (ctDNA guided adjuvant chemotherapy group). The primary population for analysis will be the Intent to Treat (ITT) population, defined as all patients randomised to study treatments, standard of care or ctDNA guided adjuvant chemotherapy; a sensitivity per protocol analysis will also be	**Hypothesis clarified for Part B and Part C**

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		performed defined as all those receiving treatment as planned per randomisation on Part C.	
83		**Primary Endpoint for ctDNA guided adjuvant chemotherapy arm of the study (Part C)** Difference in 3 year disease-free survival from time to randomisation to progression, between standard of care arm and ctDNA guided adjuvant chemotherapy arm **Secondary Endpoints for ctDNA guided adjuvant chemotherapy arm of the study (Part C)** Proportion of patients in the ctDNA guided arm receiving standard of care chemotherapy in the ctDNA negative group Proportion of patients who are ctDNA negative post-operatively who become ctDNA positive during follow-up and receive chemotherapy as an escalation Overall survival between both arms, defined as time from randomisation to death of any cause. Sub group analyses performed on 3-year DFS and OS in including but not limited to the following will be performed: (a) high risk stage 2 versus stage 3 (b) site of primary tumour (right colon versus left colon versus rectum). Neurotoxicity data between both arms (FACT/GOG-Ntx4 & CTCAE V5)	**Primary Endpoint for ctDNA guided adjuvant chemotherapy specified**

Page No	Version 6.0	Version 7.0	Explanation
		Quality of life data (EORTC QLQ-C30 and CR29 and EQ-5D-3L) Health economics	
84-84		**For ctDNA guided adjuvant chemotherapy Study (Part C)** Based on a standard 3- year DFS of 75% to demonstrate non-inferiority in survival with 80% power at α = 0.1 with non-inferiority hazards ratio of 1.25, a sample size of 810 subjects in each arm is estimated for a total number of 499 events. A total of 1621 subjects (810 patients in each arm) would need to be randomised, with 499 events required based on the following assumptions 5 year accrual 3 year minimum follow up on all 10% 2 sided significance level 80% power 1, 2, 3, 4, 5, 6 year DFS estimated from SCOT study as 0.9, 0.8, 0.75, 0.725, 0.7, 0.68 Non inferiority margin = 1.25 (ruling out 69.8% 3 year DFS) For part C of the study, a non-inferiority margin of 1.25 was chosen as this will allow for a worsening of 3-year DFS from 75% to 69.8% as being clinically acceptable. It is anticipated that the overall study population will be around 2000 (1,000 more than initially planned) and will be recruited over 5 years. We have currently recruited 250 patients and the protocol requires 1,621 contemporaneously randomised patients for Part C. It is considered that to test	**Study population clarified for Part C of the study**

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		the our first hypothesis of ctDNA as a biomarker of relapse, 500 stage II and 500 stage III patients will be derived from the recruited patients, the patients who will be in the non-interventional arm of the study and the standard of care arm of part C of the study. Once confirmation that we have recruited 1621 patients for part C of the study and that the main study has 500 stage II (low risk and high risk CRC not in part C) and 500 stage III (not in part C), we will halt recruitment to that stage. We plan to over-recruit to account for drop-outs.	
85		*Interim/ Futility Analysis for ctDNA guided adjuvant chemotherapy arm of the study (Part C)* Stopping for safety will be based on the recommendations from the IDMC and endorsed by a Trial Steering Committee. Stopping based on lack of efficacy (futility) will be based on the combination of evidence from safety and the conditional power. If the conditional power is <20% after 25% or 50% of the DFS events, the study will be considered futile. The IDMC will offer the overall recommendation based on clinical and statistical data for stopping for futility. The IDMC may also meet at other times as required but as a minimum we will consider 2 interim/futility assessments	**Interim analysis for Part C specified**

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		planned using conditional power. 25% events (n=125) would occur by year 4 (with 1280 patients recruited 50% events (n=250) would occur by year 5 (with all patients recruited)	
85-86		**Survival endpoints are defined as follows:** Disease Free Survival (DFS) as time from date of surgery to first radiologically confirmed relapse or death of any cause. All patients alive and disease free will be censored at last follow-up. Overall Survival (OS) as time from date of surgery to death of any cause. All patients alive will be censored at last follow-up. Loco-regional relapse free survival as date of surgery to first loco-regional relapse. All patients alive and event free will be censored at last follow-up or at death if death prior to loco-regional relapse. Distant relapse free survival as time from date of surgery to first distant relapse. All patients alive and event free will be censored at last follow-up or at death if death prior to distant relapse.	**Survival end-points for the study clarified**
88		**ctDNA guided adjuvant chemotherapy (Part C)** Primary Endpoint The primary endpoint, disease-free survival, will be calculated	**End-points for Part C specified**

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		by arm using Kaplan Meier method and 3 year DFS will be presented with 95% confidence intervals. Hazard ratio (HR) and 95% confidence intervals (CI) for difference between groups will be produced from a cox regression model. If the HR CI includes the non-inferiority margin then non-inferiority will not have been demonstrated. If the HR CI excludes the non-inferiority margin then non-inferiority will have been demonstrated. Secondary Endpoints Proportion of patients in the ctDNA guided arm receiving standard of care chemotherapy will be presented as a proportion with 95% confidence intervals. Proportion of patients in the ctDNA guided arm who are ctDNA negative but become ctDNA positive will be calculated and 3-year DFS and OS assessed in this group of patients. Overall survival between both arms will be reported as per DFS. Sub group analyses performed on 3-year DFS and OS by arms, including but not limited to the following will be performed: (a) high risk stage 2 versus stage 3 (b) site of primary tumour (right colon versus left colon versus rectum). Hazard ratio and 95% confidence intervals for difference between sub-groups will be produced from a cox regression model.	

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		Neurotoxicity data between both arms will be presented as proportion of patients, with 95% confidence interval, reporting neurotoxicities as per CTCAE V5 and will be compared using chi-squared or exact test as appropriate. The FACT/GOG-Ntx4 neurotoxicity scores will be presented as per the standard guidelines. The derived QOL scores will be described as medians or means (depending on distribution) and plotted overtime.	
89		17.6. Quality of Life and Cost-effectiveness Analysis Quality of life data (EORTC QLQ-C30 & CR29 and EQ-5D-3L) will be presented as per standard guidelines and compared between two study arms. The derived QOL scores will be described as medians or means (depending on distribution) and plotted overtime. Economic evaluation will assess the cost-effectiveness of ctDNA directed therapy in comparison with standard care over the projected lifetime of individuals in the two groups. Furthermore, we will also investigate whether the proposed intervention is cost-saving. The analysis will be carried out within the UK setting, from the perspective of the National Health System (NHS) and Personal Social Services (PSS), in line with the National Institute for Health and Care Excellence (NICE) guidelines on economic	**QoL and cost-effectiveness analysis specified**

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		evaluation (National Institute for Health and Clinical Excellence. Guide to the methods of technology appraisal. NICE: London, 2013). This will enable us to provide the NHS with timely evidence on the potential cost-effectiveness of the proposed intervention. Healthcare costs for the control and intervention groups will be estimated using the latest NHS Reference Costs combined with health resource utilisation data. The latter will be captured through trial records and self-report questionnaires. Quality of life data (EQ-5D-3L) will inform the analysis along with data on the observed disease free interval and overall survival between the treatment arms. Results of the cost-effectiveness analysis will be reported as cost per quality-adjusted life year (QALY) gained. Sensitivity analysis will be employed to test the reliability of results to changes in model assumptions (e.g. sensitivity and specificity of ctDNA detection). Cost-effectiveness analysis assumptions will be tested through univariate, multivariate and probabilistic sensitivity analysis, with the latter used to characterise uncertainty in parameter estimates.	
89-90		**Randomisation Procedure (PART C only)**	**Randomisation procedure**

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		Completed randomisation and anonymised baseline assessment forms should be e-mailed to the RM GI and Lymphoma CTU. The original documents should be retained by the site. The documents which should be e-mailed at the same time are listed on the randomisation case report form (CRF). **Randomisation E-mail** Randomisation will be performed at the Institute for Cancer Research – Clinical Trials and Statistical Unit (ICR-CTSU), by random permuted blocks. The randomisation will be stratified by the following factors: 1. High risk stage II versus stage III 2. Site of primary tumour: right colon versus left colon versus rectum The result of the randomisation and the study number assigned to the patient will be faxed to the study personnel at the participating site responsible for the randomisation.	**for part C of the study clarified**
92-93		19. <u>SAFETY DATA COLLECTION, RECORDING AND REPORTING</u> **<u>Adverse Events Reporting</u>** Data on neurotoxicity related to oxaliplatin treatments as per CTCAE v5.0 will be collected and reported in the CRFs. Only those toxicities listed in the SmPC leading to dose	**Safety reporting specified for Part C of the study**

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		reductions in chemotherapy need to be recorded in CRFs. All other toxicities listed in the SmPC for these drugs do not need to be reported or recorded. **Serious Adverse Events Reporting** The study will use chemotherapy drugs that are routinely used as standard of care, adverse events and serious adverse events will not need to be reported for any known adverse reaction to Oxaliplatin and/or Capecitabine/5FU as listed in the Summary of Product Characteristics (SmPC) for each drug does not need to be reported. **Exemptions from SAE reporting will also include the following:** SAEs that occur after consent and registration/randomisation but prior to any trial treatment do not require to be reported. SAEs that are unrelated to the trial treatment including the following: Elective hospitalisation and surgery for treatment of locally advanced rectal carcinoma or its complications e.g. bowel obstruction Elective hospitalisation to planned procedures e.g. central venous access device insertion Elective hospitalisation for any treatment including administration of adjuvant chemotherapy in standard of	

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		care arm or de-escalation/ treatment/ re-escalation of chemotherapy in experimental arm Elective hospitalisation for palliative care Disease progression leading to hospitalization, or prolongation of hospitalization, or death as a result of disease progression Pregnancy itself is not regarded as an AE unless there is a suspicion that the trial treatment under investigation may have interfered with the effectiveness of a contraceptive medication. Patients must be withdrawn from the trial if they become pregnant. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented. All reports of congenital abnormalities or birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. Any toxicity not listed in the SmPC or not covered by the above exemptions will be need to be reported as a serious unexpected serious adverse adverse reaction (SUSAR). <u>Suspected Unexpected Serious Adverse Reaction</u>	

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		(SUSAR): A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question as outlined in the SmPC of the medication qualifies for a SUSAR and will need to be reported to the RMH Clinical Trial Units team.	
94		**Independent Data Monitoring Committee (IDMC)** The IDMC will perform a monitoring role in examining the emerging data in the study. They will be privy to all the results as necessary to perform this role. They will meet annually during the life of the study to review safety, scientific validity and the conduct of the trial. This will be conducted according to the IDMC charter and will meet annually. **Trial Steering Committee (TSC)** The role of the TSC is to provide oversight for the trial on behalf of the sponsor. It should also provide advice through its independent Chairman to the Trial Management Group (TMG) on all aspects of the trial. All proceedings will be conducted as per the TSC charter and they will meet annually.	**IDMC and TSC added to monitor conduct of the study**
95	**The study sponsor is the Royal Marsden NHS Foundation Trust, Downs Road, Sutton, Surrey. SM2 5PT, United Kingdom.**	The study sponsor is the Royal Marsden NHS Foundation Trust, Downs Road, Sutton, Surrey. SM2 5PT, United Kingdom. There is support from the National Institute of Health Research for this study.	**Funding from Roche – Hoffman added to the protocol**

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		Roche Hoffmann is funding the Avenio sequencing kits for ctDNA analysis and providing sequencing support. Roche are also analysing immunoassays in plasma and serum.	

Protocol changes V7: clarifications made to reflect recommendations from RMH- Committee for Clinical Research

Page No	Version 7.0	Version 7.0 dated 15.01.2019	Explanation
Throughout	**V7.0 dated 01.02.2018**	**V7.0 dated 15.01.2019 (final)**	**Version change**
8	Part B (Main Study): The overall study population will include Patients in Part A of the study Patients with stage I CRC, low risk stage II CRC and any patient not willing or not eligible to take part in Part C of the study *(current on-going study)* Patients with high risk stage II or stage III CRC who are willing and are randomised in Part C of the study as below	Part B (Translational Study): The overall study population will include Patients in Part A of the study Patients with stage I CRC, low risk stage II CRC and any patient not willing or not eligible to take part in Part C of the study	**Part B and Part C clarified**
Through-out	**Part B (all patients)**	**Part B(Translational study)**	**Clarified that current ongoing Part B study is translational study**
12-13	Proportion of patients in the ctDNA guided arm receiving standard of care chemotherapy in the ctDNA negative group	Proportion of patients in the ctDNA guided arm not receiving standard of care chemotherapy (i.e., proportion of patients who are ctDNA negative and therefore having de-escalation of adjuvant chemotherapy)	**Secondary end-point for Part C clarified**
21, 24, 27		**Figures clarified to reflect changes to Part B and Part C of the study**	
53	<u>All patients in the study (Part B of the study)</u> Patients in Part A of the study Patients with stage I CRC, low risk stage II CRC and any patients not willing or not eligible to take part in	<u>Part B of the study includes</u> Patients in Part A of the study Patients with stage I CRC, low risk stage II CRC and any patients not willing or not eligible to take part in Part C of the study	**Part B of the study clarified**

Page No	Version 7.0	Version 7.0 dated 15.01.2019	Explanation
	Part C of the study *(current on-going study)* Patients with high risk stage II or stage III CRC who are willing and are randomised in Part C of the study as below.		
61	15.1 Study Treatments All chemotherapeutic agents used for patients recruited are standard of care treatments.	15.1 Study Treatments (only for Part C of the study) All chemotherapeutic agents used for patients recruited in Part C of the study are standard of care treatments used as per local policy.	**Study treatment for Part C of the study clarified**
61-63		Section 15.1 applicable to patients in Part C of the study only	**Clarification**
67		Link to Quality of Life Questionnaires included	**Clarification**
80	Secondary Endpoints Proportion of patients in the ctDNA guided arm receiving standard of care chemotherapy will be presented as a proportion with 95% confidence intervals.	Secondary Endpoints Proportion of patients in the ctDNA guided arm receiving standard of care chemotherapy will be presented as a proportion with 95% confidence intervals and proportion of patients where chemotherapy is de-escalated is calculated.	**Secondary end-point clarified**

Protocol changes from V7.0 to V8.0

Page No	Version 7.0	Version 8.0 dated 17.09.2019	Explanation
Throughout	**V7.0 dated 15.01.2019**	**V8.0 dated 17.09.2019**	**Version change**
5, 6		Addition of Mr. Pete Wheatstone, Mr. Nicholas West and Prof. Theresa Wiseman as Co-investigators Deletion of Dr. Andrew Wotherspoon, Dr. Anguraj Sadanandam, Dr. Nicola Valeri And Dr. Yinyin Yuan.	**Study team updated**
9		Participating centres may choose to enroll patients into Part B of the study alone or to Part B followed by Part C of the study.	**Clarification that participation in Part C is optinal for centres.**
9	Study patients will be recruited over 5 years and followed up for 5 years.	Study patients will be recruited over 4 years and followed up for 5 years.	**Clarification of recruitment period**
13	Part C only (ctDNA guided interventional group of the study)	Part C only	**Clarification**
14-15	Part C of the study will include patients willing to participate in the ctDNA guided interventional group. Patients will sign a separate consent form specific for Part C of the study. Once informed consent is obtained in the oncology clinic from patients willing to take part in part C of the study, prior to randomization, clinicians will decide the adjuvant chemotherapy of choice based on histo-pathological features of the tumour, patient's age, co-morbidities and patient's choice, as is current clinical practice.	Part C of the study will include patients with high risk stage II and stage III CRC as per histopathological assessment of the resection specimen willing to participate in the ctDNA guided interventional part of the study. Only patients who were enrolled within Part B of the study and have had a baseline blood test taken before surgery or before chemo-radiotherapy will be eligible to be screened for Part C of the study. Only those patients whose baseline blood test shows presence of ctDNA will be allowed to continue within the Part C of the study. Patients will sign a separate consent form specific for Part C of the study. Once informed	Clarification that only those patients whose baseline blood test shows evidence of ctDNA will be included in part C of the study

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	Following consent and before randomization, patients will have ctDNA samples collected during week 4-8 post-operatively. Patients will then be randomised to 1:1 fashion between standard of care arm where patients are offered standard of care adjuvant chemotherapy according to national guidelines, and the experimental arm, in which patients will be treated based on ctDNA results. Results of ctDNA analysis will be made available within a 2 week turn-around period by week 6-10 following surgery in patients assigned to the ctDNA- guided adjuvant chemotherapy arm (Arm B) as follows:. <u>Post-op ctDNA positive patients</u> Patients receive standard of care adjuvant chemotherapy	consent is obtained in the oncology clinic from patients, prior to randomization, clinicians will decide the adjuvant chemotherapy of choice based on histo-pathological features of the tumour, patient's age, co-morbidities and patient's choice, as is current clinical practice.	
15	Following consent and before randomization, patients will have ctDNA samples collected during week 4-8 post-operatively. Patients will then be randomised to 1:1 fashion between standard of care arm where patients are offered standard of care adjuvant chemotherapy according to national guidelines, and the experimental arm, in which patients will be treated based on ctDNA results. Results of ctDNA analysis will be made available	Following consent, patients will have ctDNA samples collected during week 4-8 post-operatively (month 0 time point). This blood sample will be tested for ctDNA levels, presence of ctDNA considered as 'positive' and absence of ctDNA will be considered as 'negative'. Patients will be randomised in 1:1 fashion between standard of care arm where patients are offered standard of care adjuvant chemotherapy according to national guidelines, and the experimental arm, in which patients will be treated based	**Clarification that only those patients whose baseline blood test shows evidence of ctDNA will be included in part C of the study**

Page No	Version 7.0	Version 8.0 dated 17.09.2019	Explanation
	within a 2 week turn-around period by week 6-10 following surgery in patients assigned to the ctDNA- guided adjuvant chemotherapy arm (Arm B) as follows:. <u>Post-op ctDNA positive patients</u> Patients receive standard of care adjuvant chemotherapy <u>Post-op ctDNA negative patients</u> If ctDNA is negative post-operatively, patients are de-escalated as follows:	on ctDNA results. Results of ctDNA analysis will be made available within a 2 week turn-around period by week 6-10 following surgery in patients assigned to the ctDNA- guided adjuvant chemotherapy arm (Arm B) as follows:. <u>Post-op ctDNA positive patients</u> Patients receive standard of care adjuvant chemotherapy <u>Post-op ctDNA negative patients</u> If ctDNA is negative post-operatively, chemotherapy is de-escalated as follows:	
17	For ctDNA guided interventional group of the study (Part C), A total of 1621 subjects (810 patients in each arm) would need to be randomised, with 499 events required based on the following assumptions: 5 year accrual A non-inferiority margin of 1.25 has been chosen to allow for a worsening of 3-year DFS from 72-75% up to 69.3% only as being clinically acceptable.	For ctDNA guided interventional group of the study (Part C), A total of 1621 subjects (810 patients in each arm) would need to be randomised, with 530 events required based on the following assumptions: 4 year accrual A non-inferiority margin of 1.25 has been chosen to allow for a worsening of 3-year DFS from 75% up to 69.8% only as being clinically acceptable.	**Modification of event rate and accrual assumption.** Typo in non-inferiority margin in synopsis corrected to be consistent with what is in the body of the protocol
18	For part C of the study, patients can be initially registered as part of the translational study. If post-operative histopathology confirms high-risk stage II or stage III colorectal cancer, and patient is willing to take part in the ctDNA guided adjuvant chemotherapy	For part C of the study, patients will be initially registered as part of the translational study (Part B). If baseline blood sample shows presence of ctDNA and post-operative histopathology confirms high-risk stage II or stage III colorectal cancer, and patient is willing to take part in the ctDNA guided adjuvant	**Clarification that only those patients whose baseline blood test shows evidence of ctDNA will be included in**

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	management, then a further eligibility assessment will be undertaken specifically for Part C as per below criteria. Note for those patients who were not enrolled into the TRACC study before surgery and wish to specifically take part in Part C of the study, can take part in the study as long as they meet all eligibility criteria for part C of the study.	chemotherapy management, then a further eligibility assessment will be undertaken specifically for Part C as per below criteria.	**part C of the study and that they will be enrolled through Part B of the study**
20	9. Patients who were not recruited pre-surgery for the main study can take part as long as they fulfil all eligibility criteria for part C of the study	Patients must have detectable levels of ctDNA (i.e., ctDNA positive) in blood samples at baseline; this is collected pre-operatively if being treated with surgery alone, or before chemoradiotherapy in patients with locally advanced rectal cancer being treated with neoadjuvant chemoradiotherapy before surgery	**Clarification that only those patients whose baseline blood test shows evidence of ctDNA will be included in part C of the study and that they will be enrolled through Part B of the study**
26-28		Figures updated	
49	NGS based panels including Roche Avenio, Guardant Health panels are currently used as research and clinical tools in patients with metastatic disease. The amount of tumour derived cell-free DNA (ctDNA) in smaller, early to mid-stage tumors can be very low and is usually at or below 0.1%	NGS based panels including Roche Avenio, Guardant Health panels are currently used as research and clinical tools in patients with metastatic disease. The amount of tumour derived cell-free DNA (ctDNA) in smaller, early to mid-stage tumors can be very low and is usually at or below 0.1% VAF (variant allele frequency). With technological	**Explanation that we will be using a custom-made gene panel along with commercially available panels where necessary.**

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	VAF (variant allele frequency). The study will use Roche Avenio panel for detection of ctDNA in patients following surgery. The panel is highly specific with a variant -level specificity of 99.99% for the expanded panel, with each sample having a variant level specificity greater than 99.4%. Even with as low as 5ng of cfDNA the sensitivity was with expanded Kit was 93% sensitivity at a VAF of 0.5% with 50 million reads, and for early stage tumours, with lowest level of residual disease, with the higher reads, more sequencing allowed for higher sensitivity — with 200 million reads (3-4 samples per NextSeq High Output run) and 50ng input, the Targeted Kit showed >99% sensitivity at a VAF of 0.05% and the Expanded Kit gives near 90% sensitivity at a VAF of 0.05% (https://sequencing.roche.com/content/dam/rochesequence/worldwide/resources/SEQ100108_AVENIO%20ctDNA_Performance_White_Paper.pdf).	advances, NGS panels have a high degree of sensitivity and specificity. We will use a customised Royal Marsden GI panel with up to 20 genes for sequencing the plasma along with commercially available gene panels where necessary.	
52	Two different technological assays including ddPCR (digital droplet PCR) and Roche Avenio panel are being used to analyse samples, allowing for technical validation of ctDNA results in a	Two different technological asssays including ddPCR (digital droplet PCR) and NGS panels including Roche Avenio panel are being used to analyse samples, allowing for technical validation of ctDNA	**Clarification that NGS based gene panels including Roche Avenio panel will be used**

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	systematic and centralised manner. The use of these highly sensitive assays allows for detection of even small amounts of DNA from early stage tumours, which is at or below 0.1% variant allele frequency (VAF). With these technologies variant allele frequency of 0.05% to >1% can be assessed. Furthermore, the sensitivity of the Roche Avenio panel is 90% at 0.05% VAF, with a specificity of 99.9%.	results in a systematic and centralised manner.	
53	**For Part C (patients in the ctDNA guided adjuvant chemotherapy arm):**	**For Part C (patients in the ctDNA guided adjuvant chemotherapy group):**	**Correction of typo**
54	**For patients in the ctDNA guided adjuvant chemotherapy arm (Part C):**	**For patients in the ctDNA guided adjuvant chemotherapy group (Part C):**	**Correction of typo**
56		Participating centres may choose to enroll patients into Part B of the study alone or to Part B followed by Part C of the study.	**Clarification that participating centres can choose to take part B of study alone; participation in Part C of the study is optional.**
56	Part C of the study will include high risk stage II and stage III CRC patients who have had resection of their tumour and are due to receive adjuvant chemotherapy. Part C of the study will be a prospective, multi-centre study where patients will be randomised in a 1:1	Part C of the study will be a prospective, multi-centre study and will include high risk stage II and stage III CRC patients who have had resection of their tumour and are due to receive adjuvant chemotherapy. Patients who have ctDNA detected in their pre-op sample will be included and will be randomised in a 1:1	**Clarification that only those patients whose baseline blood test shows evidence of ctDNA will be included in**

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	manner to receive standard of care arm where patients are offered adjuvant chemotherapy according to national guidelines, or to the experimental arm, in which patients are treated based on ctDNA results.	manner to receive standard of care arm where patients are offered adjuvant chemotherapy according to national guidelines, or to the experimental arm, in which patients are treated based on ctDNA results.	**part C of the study.**
56	Following informed consent patients will have ctDNA samples collected during week 4-8 post-operatively and randomisation will then be performed.	Following informed consent patients will have ctDNA samples collected during week 4-8 post-operatively and screening completed following which randomisation will be performed.	**Clarification that patients are randomised after screening.**
57	Results of ctDNA analysis will be made available by week 8-10 following surgery in patients assigned to the ctDNA-guided adjuvant chemotherapy arm (Arm B) as follows: *Post-op ctDNA positive patients* Patients receive adjuvant chemotherapy if they have ctDNA detected and deemed positive for detection.	Results of post-operative ctDNA analysis will be made available by week 8-10 following surgery in patients assigned to the ctDNA- guided adjuvant chemotherapy arm (Arm B) as follows: *Post-op ctDNA positive patients* Patients receive standard of care adjuvant chemotherapy if they have ctDNA detected and deemed positive for detection.	**Clarification of post-operative sample and nature of chemotherapy in ctDNA positive patients**
57	Real time analysis of ctdNA with a two-week turn-around time will be performed at following time points: Post-operative ctDNA at month 0 (week 4-8 after surgery, before randomisation) Post-operative ctDNA at month 3	Real time analysis of ctdNA with a two-week turn-around time will further performed at month 3 following completion of adjuvant chemotherapy	**Clarification**

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58	For patients at The Royal Marsden Hospital who co-enrol into the TRIGGER trial [a multi-centre, prospective, translational study sponsored by the Royal Marsden NHS Foundation Trust exploring the magnetic resonance tumour regression grade (mrTRG) as a novel biomarker to stratify management of good and poor responders to chemo-radiotherapy] data generated from the molecular sub-classification of FFPE tumour tissue from pre-CRT biopsies and post-CRT resection specimens, described above, will be securely shared with the TRACC research team. In addition, translational data relating to ctDNA and re-sequencing results from FFPE tumour tissue, acquired in the TRACC trial for co-enrolled patients will be securely shared with the TRIGGER research team.	For patients who co-enrol into the TRIGGER trial [a multi-centre, prospective, translational study sponsored by the Royal Marsden NHS Foundation Trust exploring the magnetic resonance tumour regression grade (mrTRG) as a novel biomarker to stratify management of good and poor responders to chemo-radiotherapy] data generated from the molecular sub-classification of FFPE tumour tissue from pre-CRT biopsies and post-CRT resection specimens, described above, will be securely shared with the TRACC research team. In addition, clinical and translational data relating to ctDNA and re-sequencing results from FFPE tumour tissue, acquired in the TRACC trial for co-enrolled patients will be securely shared with the TRIGGER research team.	**Clarification that for patients co-enrolled within the TRACC and TRIGGER study both clinical and translational data will be shared.**
59	Part C of the study will include the ctDNA guided adjuvant chemotherapy group, a total of 1621 subjects (810 patients in each arm) would need to be randomized and 499 events required.	Part C of the study will include the ctDNA guided adjuvant chemotherapy group, a total of 1621 subjects (810 patients in each arm) would need to be randomized and 530 events required.	**Clarification of number of events needed**
62		4.Patients must have detectable levels of ctDNA (i.e., ctDNA positive) in blood samples at baseline; this is collected pre-operatively if being treated with surgery alone, or before	**Addition of a furtehr inclusion criteria defining that only those patients who**

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		chemoradiotherapy in patients with locally advanced rectal cancer being treated with neoadjuvant chemoradiotherapy before surgery	**are ctDNA positive pre-operatively are eligible.**
67	Around 50mL of blood will be collected in cell free DNA blood collection tubes™ (Streck) or equivalent and processed in accordance with the ctDNA standard operating procedures (SOP) as outlined in the TRACC study laboratory manual.	Around 50mL of blood will be collected in cell free DNA blood collection tubes™ (Streck) or equivalent and processed in accordance with the ctDNA standard operating procedures (SOP) or equivalent SOPs for blood collection as outlined in the TRACC study laboratory manual.	**Clarification that Streck and similar tubes will be utilised for analysis of ctDNA and other relevant biomarkers.**
67-68	In patients who consent, of the 50mL of blood collected, 20mL (or 4 teaspoons) will be tested for plasma and serum protein biomarkers in centres where resources are available for centrifuging, processing and storing of blood. Known plasma and serum biomarkers including tumour markers such as CEA and CA19-9, and new exploratory protein biomarkers will be studied. Blood for protein biomarkers will be collected at month 0, month 3, month 6, end of year 1 and end of year 2 time-points and at the time of relapse.		**Deletion of collection of 20mL of blood for immunoassays.**
72		Figure updated	**Clarification that patients undergoing chemoradiotherapy will have bloods taken at end of**

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			chemoradiotherapy and before surgery.
77		For our a-priori hypotheses (section 4 and as below), we will include the following study population. **Hypothesis 1:** In patients with stage II and III CRC, detection of mutations in ctDNA in plasma can predict relapse. **Hypothesis 2**: We hypothesise that ctDNA directed adjuvant chemotherapy administration will enable biomarker driven selection of patients who would benefit from adjuvant chemotherapy and thereby reduce proportion of patients receiving adjuvant chemotherapy without compromising disease free survival	**Clarification of population for analysis**
77	All of the endpoints will be analysed in all of the patients based on disease stage	Endpoints will be analysed in as outlined below.	**Clarification of analysis of end points**
79	**Primary Endpoint for ctDNA guided adjuvant chemotherapy arm of the study (Part C)** Difference in 3 year disease-free survival from time of surgery to progression, between standard of care arm and ctDNA guided adjuvant chemotherapy arm **Secondary Endpoints for ctDNA guided adjuvant chemotherapy arm of the study (Part C)**	**Primary Endpoint for Part C of the study** Difference in 3 year disease-free survival from time of surgery to progression, between standard of care arm and ctDNA guided adjuvant chemotherapy arm **Secondary Endpoints for Part C of the study**	**Clarification of end-points for part C patients**

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80	**For ctDNA guided adjuvant chemotherapy Study (Part C)** Based on a standard 3-year DFS of 75% to demonstrate non-inferiority in survival with 80% power at α = 0.1 with non-inferiority hazards ratio of 1.25, a sample size of 810 subjects in each arm is estimated for a total number of 499 events. A total of 1621 subjects (810 patients in each arm) would need to be randomised, with 499 events required based on the following assumptions 5 year accrual	**For ctDNA guided adjuvant chemotherapy Study (Part C)** Based on a standard 3- year DFS of 75% to demonstrate non-inferiority in survival with 80% power at α = 0.1 with non-inferiority hazards ratio of 1.25, a sample size of 810 subjects in each arm is estimated for a total number of 530 events. A total of 1621 subjects (810 patients in each arm) would need to be randomised, with 499 events required based on the following assumptions 4 year accrual	**Updated statistics as we now wish to recruit our study population in 4 years.**
91	The study sponsor is the Royal Marsden NHS Foundation Trust, Downs Road, Sutton, Surrey. SM2 5PT, United Kingdom. There is support from the National Institute of Health Research for this study. Roche Hoffmann is funding the Avenio sequencing kits for ctDNA analysis and providing sequencing support. Roche are also analysing immunoassays in plasma and serum.	The study sponsor is the Royal Marsden NHS Foundation Trust, Downs Road, Sutton, Surrey. SM2 5PT, United Kingdom. There is support from the National Institute of Health Research and the Royal Marsden Cancer Charity for this study.	**Clarification of sponsors of the study; Roche are no longer funding sequencing kits, Royal Marsden Cancer Charity is providing funding instead.**
99		Addition of AJCC Colon and Rectal Cancer Staging 8th Edition	**To keep up with the updated version of AJCC staging of CRC**

Protocol changes from V8.0 to V9.0

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Through out	V8.0 dated 17.09.2019	V9.0 dated 01.09.2020	Version change
2 & 5-6		Addition of Dr Susana Slater Dr Charlotte Fribbens and Dr. Andrew Feber	Study team update
9	Part C (For ctDNA guided interventional group of the study)	Part C (randomized ctDNA guided versus standard of care adjuvant chemotherapy study	Clarification of Part C description
9	• To demonstrate de-escalation strategy of ctDNA guided adjuvant chemotherapy is non-inferior to standard of care treatment as measured by 3 year disease free survival in patients with high risk stage II or stage III colorectal cancer in patients with no evidence of minimal residual disease (ctDNA negative)	To demonstrate de-escalation strategy of ctDNA guided adjuvant chemotherapy is non- inferior to standard of care treatment as measured by 3 year disease free survival in patients with high risk stage II or stage III colorectal cancer with no evidence of minimal residual disease (ctDNA negative) .	Repetition deleted
10	Part C only (ctDNA guided interventional group of the study)	Part C only	Clarification
11	Detectable ctDNA is defined as the presence of at least one tumour-derived mutation above the limit of detection (LOD) threshold for that particular mutation assay in a duplicate investigation.	Detectable ctDNA is defined as the presence of at least one tumour-derived mutation above the limit of detection (LOD) threshold for that particular mutation assay.	Bu using NGS sequencing of plasma as the primary means of ctDNA detection over ddPCR, we do not need to perform duplicate investigation and hence has been deleted from the primary end-point

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13	• Proportion of patients in the ctDNA guided arm not receiving standard of care chemotherapy (i.e., proportion of patients who are ctDNA negative and therefore have de-escalation of adjuvant chemotherapy	• Proportion of patients in the ctDNA guided arm who are ctDNA negative and therefore have de-escalation of adjuvant chemotherapy	End-point description simplified
14	Only patients who were enrolled within Part B of the study and have had a baseline blood test taken before surgery or before chemo-radiotherapy will be eligible to be screened for Part C of the study. Only those patients whose baseline blood test shows presence of ctDNA will be allowed to continue within the Part C of the study.	Patients do not have to be enrolled in Part B in order to enroll in Part C of the study. Patients will be eligible to enroll regardless of whether their baseline ctDNA is positive or not.	Removed need to be enrolled in Part B in order to participate in Part C. Removed need for baseline positive ctDNA result to be eligible for Part C
14	Once informed consent is obtained in the oncology clinic from patients, prior to randomization.	Once informed consent is obtained in the oncology clinic from patients, either face to face or over the telephone or video consultations, prior to randomization.	Allowing for inclusion of virtual consent
Through out	Arm A	Standard of Care Arm	Clarification of terminology
Through out	Arm B	ctDNA guided arm	Clarification of terminology
14		**Standard of Care Arm** Patients will have blood collected post-operatively for ctDNA analysis at 8 ± 2 weeks after surgery. They will be offered standard of care capecitabine based adjuvant chemotherapy as per national guidelines (single agent capecitabine for 6 months or doublet CAPOX for 3 months).	

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14, 15, 16	Results of ctDNA analysis will be made available within a 2 week turn- around period by week 6-10 following surgery in patients assigned to the ctDNA- guided adjuvant chemotherapy arm (Arm B) as follows:. <u>Post-op ctDNA positive patients</u> Patients receive standard of care adjuvant chemotherapy <u>Post-op ctDNA negative patients</u> If ctDNA is negative post-operatively, chemotherapy is de-escalated as follows: -if a doublet regimen was assigned to the patient before randomisation, patient receives either single agent chemotherapy or no chemotherapy as per clinician's decision prior to randomisation. -if single agent chemotherapy has been assigned, then patient receives no chemotherapy. A further real time analysis of ctdNA with a two–week turn-around time of ctDNA results will be performed at following time points: • Post-operative ctDNA at month 3 <u>Post-op ctDNA negative patients who become positive during follow-up</u>	ctDNA guided adjuvant chemotherapy Arm In patients assigned to the ctDNA- guided adjuvant chemotherapy arm, results of ctDNA analysis will be made available within a 2 week turn- around period by week 10± 1 week following surgery (Experimental arm) to allow for commencing adjuvant chemotherapy within 12 weeks after surgery. Based on the results, patients in this arm will be treated as follows: <u>Post-op ctDNA positive patients (ctDNA detected)</u> Patients receive standard of care adjuvant chemotherapy <u>Post-op ctDNA negative patients (ctDNA not detected)</u> If ctDNA is negative post-operatively (month 0), chemotherapy is de-escalated as follows: -if a doublet regimen (CAPOX) was assigned to the patient before randomisation, patient receives single agent chemotherapy (Capecitabine) -if single agent chemotherapy (capecitabine) has been assigned, then patient receives no chemotherapy. In patients in the ctDNA guided arm who are ctDNA negative at month 0), a further real time analysis of ctDNA with a two–week turn-	Study description has been expanded to make the description very clear on advice from participating centres.

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	In those patients who are ctDNA negative during month 0 but become positive at month 3, will undergo radiological imaging to assess for disease relapse (CT or MRI). If no evidence of macroscopic disease is noted, in this group of patients, systemic chemotherapy will be introduced with single agent capecitabine, or chemotherapy escalated to doublet regimen with CAPOX if they had capecitabine during the adjuvant setting. If there is evidence of macroscopic disease by radiological assessment, chemotherapy as per clinician's choice will be administered.	around time of ctDNA results will be performed at month 3. Based on the results, patients will be managed as follows: <u>Post-op ctDNA (month 0) negative patients who become positive during follow-up at month 3</u> In those patients who are ctDNA negative during month 0 (post-op) but become positive at month 3, will undergo radiological imaging to assess for disease relapse (CT or MRI). If no evidence of macroscopic disease is noted, in this group of patients, systemic chemotherapy will be introduced, or escalated to doublet regimen with CAPOX. If there is evidence of macroscopic disease by radiological assessment, chemotherapy as per clinician's choice will be administered. <u>Post-op ctDNA negative patients who remain negative at month 3 during follow-up</u> Those patients who are ctDNA negative at month 0 and continue to remain negative at month 3 will have clinical follow-up only and no further adjuvant chemotherapy will be administered. They will be followed up at months 6, 9, 12, 18, 24, 30, 36, 48 and 60 as per protocol schedule.	

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17	It is anticipated that the overall study population will be around 1800 (800 more than initially planned) and will be recruited over 5 years.	It is anticipated that the overall study population will be around 2600 (1600 more than initially planned) and will be recruited over 5 years.	Due to COVID-19 we have had to pause Part C whilst recruitment to Part B has continued. We will therefore need to recruit additional patients into Part C
17	For part C of the study, patients will be initially registered as part of the translational study (Part B). If baseline blood sample shows presence of ctDNA and post-operative histopathology confirms high-risk stage II or stage III colorectal cancer, and patient is willing to take part in the ctDNA guided adjuvant chemotherapy management, then a further eligibility assessment will be undertaken specifically for Part C as per below criteria.	For part C of the study, patients may be initially registered as part of the translational study (Part B), although this is not mandatory. A further eligibility assessment will be undertaken specifically for Part C as per below criteria	Clarification regarding eligibility criteria. Removed need for positive ctDNA baseline result in order to be eligible for Part C
19	For patients in the ctDNA guided interventional arm of the study only	For patients in Part C of the study only	Clarification of terminology
19	Subjects with histologically proven high risk stage II or stage III colon or rectal cancer treated with curative intent with surgery alone (any T, N1 or N2) with no evidence of metastatic disease.	Subjects with histologically proven high risk stage II or stage III colon or rectal cancer treated with curative intent with surgery alone (any T, N1 or N2) with no evidence of metastatic disease. High risk stage II is defined as having one or more of the following: T4	Clarification of high risk stage II definition

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		disease, tumour obstruction and/or perforation of the primary tumour during the pre-operative period, inadequate nodal harvest as indicated by <12 nodes examined, poorly differentiated grade on histology, perineural invasion, peritoneal involvement or extramural venous/lymphatic invasion. Subjects must be due to receive adjuvant chemotherapy following surgery.	
19	Subjects with histologically proven stage III rectal cancer are eligible, including patients with neoadjuvant chemoradiotherapy (any T, N1 or N2, M0) with no evidence of metastatic disease. Subjects must be due to receive adjuvant chemotherapy following surgery.	Subjects with histologically proven locally advanced rectal cancer treated with neoadjuvant chemoradiotherapy (any T, N1 or N2, M0) with no evidence of metastatic disease are eligible. Subjects must be due to receive adjuvant chemotherapy following surgery.	Clarification of the neoadjuvant chemotherapy group of patients
19	Fully surgically resected tumour with clear resection margins (i.e., >1 mm)	Fully surgically resected tumour (R0) with clear resection margins (i.e., >1 mm)	Clarification to include patients with R0 status
19	Patients must have detectable levels of ctDNA (i.e., ctDNA positive) in blood samples at baseline; this is collected pre-operatively if being treated		Removed requirement for ctDNA positive baseline result to be eligible for

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	with surgery alone, or before chemoradiotherapy in patients with locally advanced rectal cancer being treated with neoadjuvant chemoradiotherapy before surgery		participation in Part C
20	Adequate renal function as calculated by Cockcroft and Gault equation (GFR ≥ 30ml/min if FOLFOX chemotherapy chosen and GFR ≥ 50ml/min if single agent capecitabine or CAPOX chosen)	Adequate renal function as calculated by Cockcroft and Gault equation (GFR ≥ 50ml/min if single agent capecitabine or CAPOX being administered)	Clarification of inclusion criteria
20	Patients should be assessed by Oncology team for suitability and assessment for adjuvant chemotherapy, be able to have post-operative ctDNA sample collected and be randomised by week 4-8 after surgery	Patients should be assessed by Oncology team for suitability and assessment for adjuvant chemotherapy, be able to have post-operative ctDNA sample collected and be randomised by week 4-8 (±2) weeks after surgery and commenced adjuvant chemotherapy within 12 weeks after surgery	Protocol amended to allow for a window for randomisation. The goal to start adjuvant chemotherapy within 12 weeks after surgery remains unchanged
20	History of concurrent and previous malignancy within the last 3 years, with the exception of non-melanomatous skin cancer and carcinoma in situ	History of concurrent and previous malignancy within the last 5 years, with the exception of non-melanomatous skin cancer and carcinoma in situ	Eligibility amended
20	Hypersensitivity or contraindication to the drug(s) associated with the planned choice of systemic chemotherapy (CAPOX, FOLFOX or single agent 5FU	Hypersensitivity or contraindication to the drug(s) associated with the planned choice of systemic chemotherapy (CAPOX or	5FU based regimens deleted in

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	or capecitabine) as stated in the SPC for each of the drugs	capecitabine) as stated in the SmPC for each of the drugs	
20		Subjects due to receive 5-flurouracil (5FU) based adjuvant chemotherapy (either single agent 5FU or in combination with oxaliplatin) will not be eligible for Part C of the study, these patients will continue to be followed in the observational Part B of the study	Additional exclusion criteria added
21	Surveillance CT chest/ abdomen/ pelvis performed as part of routine surveillance post-operatively, at end of year 1, 2 and 3. Further imaging will be done if clinically indicated or ctDNA status or CEA show rise.	Surveillance CT chest/ abdomen/ pelvis performed as part of routine surveillance post-operatively, at end of year 1, 2 and 3. Further imaging will be done if clinically indicated or ctDNA status (in ctDNA negative patients of ctDNA guided arm at month 3) or CEA show rise.	Clarification of CT scan
22-26		Figures updated	
28-29		Table of abbreviations updated	
30-32		Table of contents updated	
46		Guardant Health (Redwood City, CA) has developed and validated its tumour-naive CLIA-certified LUNAR1 assay (recently commercialized under the name Guardant Reveal), an NGS-based assay for the qualitative detection of residual disease in individuals with CRC undergoing curative intent treatment. The assay evaluates the presence of ctDNA based on multiple	Background updated with new assay

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		analytic features, including detection of tumour-derived mutations and methylation signals and returns a result of “ctDNA detected” or “ctDNA not detected”. The integrated assay accurately reports genomic alterations down to allele frequencies of 0.01%, and effectively bioinformatically filters out biological noise sources such as mutations caused by clonal haematopoiesis. The addition of methylation analysis improved sensitivity by 36% over genomic assessment alone. In a study of 64 patients with colorectal cancer undergoing curative-intent treatment, the LUNAR1 assay showed 100% positive predictive value (PPV) for a ctDNA detected result to predict recurrence, 56% sensitivity to detect participants who will ultimately recur using a single four week post-treatment “landmark” timepoint, and >90% sensitivity for recurrence using serial ctDNA assessment in the surveillance setting detection in colorectal cancer (CRC) using a plasma-only integrated genomic and epigenomic circulating tumor DNA (ctDNA) assay [62].	
46-47		Reference 62 added – other refs amended for order	References updated
51	For Part C (Patients in ctDNA guided adjuvant chemotherapy study)	For Part C (Randomised ctDNA guided adjuvant chemotherapy versus SoC study)	Clarification of Part C

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51	For patients in the ctDNA guided adjuvant chemotherapy group (Part C)	For patients in the randomised ctDNA guided adjuvant chemotherapy versus standard of care adjuvant chemotherapy study (Part C)	Clarification of Part C
51	Assess proportion of patients who are ctDNA negative on post-operative ctDNA and not receiving adjuvant chemotherapy in interventional arm compared to standard arm	Assess proportion of patients who are ctDNA negative on post-operative ctDNA and receiving de-escalated adjuvant chemotherapy in interventional arm compared to standard arm	Description of secondary endpoint simplified
53		With rapid improvements in technology, we will adopt a tumour agnostic approach and test plasma directly for ctDNA detection using next generation sequening targeted gene panels. We will also aim to compare this with tumour informed approaches including whole exome and whole genome sequencing approached on tumour informing variants in plasma ctDNA. Other biomarker approaches which will enhance sensitivity such as methylation assays will also be used. A subset of samples from part B will be sent to Guardant Heath for testing with the LUNAR1 assay.	Change to assay method of ctDNA detection described
53	Patients who have ctDNA detected in their baseline sample will be included and will be randomised in a 1:1 manner to standard of care arm where patients are offered adjuvant chemotherapy according to national guidelines, or to the ctDNA guided arm, in which	Patients will be randomised in a 1:1 manner to standard of care arm where patients are offered adjuvant chemotherapy according to national guidelines, or to the ctDNA guided arm, in which patients are treated based on Month 0 ctDNA results.	Removed therequiremen t of ctDNA positive baseline result to be eligible to participate in Part C

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	patients are treated based on ctDNA results.		
54		Informed consent will be sought either face to face or over the telephone or video consultations.	Virtual consent process added to study procedures
54		Given recent improvements in technology and logistical challenges in coordinating tumor-informed ctDNA analyses across multiple centers, the Guardant Health LUNAR1 ctDNA assay (UKCA marked) will be utilized for Part C of the study. Whole blood samples from the following participants will be collected in four (4) 10mL Streck tubes in blood collection kits provided by Guardant Health and shipped to Guardant Health for processing and real-time return of ctDNA test results:	Added details of assay and blood tubes
54	Baseline samples from high-risk stage II/III patients recruited to TRACC Part B and who consent to TRACC Part C		Removed the need for real time assessment of baseline samples
54		Post-operative samples collected from patients recruited to Part C who are randomized to the standard of care arm will be banked for future analysis by the Guardant Health LUNAR1 assay. TRACC Part C participants recruited on prior protocol versions pre-dating implementation of the LUNAR1 assay will have additional plasma sample banked for retrospective analysis by the Guardant Health LUNAR1 assay	Added details of storage of blood from SoC arm for future assay assessment

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		(derivatives from at least 20mL whole blood preferred where possible).	
55	*Post-op ctDNA negative patients* If ctDNA is negative post-operatively, patients are de-escalated as follows: -if a doublet regimen was assigned to the patient before randomisation, patient receives either single agent chemotherapy or no chemotherapy as per clinician's discretion decided previously. -if single agent chemotherapy has been assigned, then patient receives no chemotherapy. Patients in both arms will have ctDNA levels measured during adjuvant chemotherapy at following time points. All patients are followed up every 3 months during first year, 6 months during 2nd and 3rd year and annually during years 4 and 5. Patients in both arms will have ctDNA levels collected during adjuvant chemotherapy at all the above time points or when disease recurrence is suspected clinically and confirmed by radiology. Real time analysis of ctdNA with a two-week turn-around time will further performed at month 3 following completion of adjuvant chemotherapyThe ctDNA results at month 0 will guide	*Post-op ctDNA negative patients* If ctDNA is negative post-operatively, patients are de-escalated as follows (outlined in Figs 5a and 5b): -if a doublet regimen (CAPOX) was assigned to the patient before randomisation, patient receives single agent chemotherapy (capecitabine) -if single agent chemotherapy (capecitabine) has been assigned, then patient receives no chemotherapy. Patients in both the SoC and ctDNA guided arms will have ctDNA levels collected during adjuvant chemotherapy at following time points: every 3 months during first year, 6 months during 2nd and 3rd year and annually during years 4 and 5. Patients in both arms will have ctDNA levels collected when disease recurrence is suspected clinically and/or confirmed by radiology. Only in patients in the ctDNA guided arm of the study whose month 0 results were negative (ctDNA negative), ctDNA at month 3 will tested in real-time and results made available, to guide decisions as follows: *Post-op ctDNA (month 0) negative patients who*	Chemotherapy details changed to capecitabine based regimens and study procedure in ctDNA negative patients explained.

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	decision of de-escalation of adjuvant chemotherapy in the ctDNA guided arm. Results of ctDNA at month 3 will guide decisions regarding escalation or starting systemic chemotherapy for microscopic disease in those patients who were initially negative but become positive at month 3 as follows. *Post-op ctDNA negative patients who become positive during follow-up* In those patients who are ctDNA negative during month 0 but become positive at month 3, will undergo radiological imaging to assess for disease relapse. If no evidence of macroscopic disease is noted, in this group of patients, systemic chemotherapy will be introduced with single agent capecitabine, or chemotherapy escalated to doublet regimen with CAPOX if they had capecitabine during the adjuvant setting. If there is evidence of macroscopic disease by radiological assessment, chemotherapy as per clinician's choice will be administered.	*become ctDNA positive at month 3 during follow-up* Those patients who are ctDNA negative at month 0 but become positive at month 3 will undergo radiological imaging to assess for disease relapse. If no evidence of macroscopic disease is noted, systemic chemotherapy will be escalated to doublet regimen with CAPOX. If there is evidence of macroscopic disease by radiological assessment, further management will be as per clinician's discretion within the advanced disease protocol as per local guidelines. *Post-op ctDNA (month 0) negative patients who remain ctDNA negative at month 3 during follow-up* Those patients who are ctDNA negative at month 0 and continue to remain negative at month 3 will have clinical follow-up only and no further adjuvant chemotherapy will be administered. They will be followed up at months 6, 9, 12, 18, 24, 30, 36, 48 and 60 as per protocol schedule.	
55-56	For patients who co-enrol into the TRIGGER trial [a multi-centre, prospective, translational study sponsored by the Royal Marsden NHS Foundation Trust exploring the magnetic resonance tumour regression grade (mrTRG) as a novel	For all TRACC patients who co-enrol into the TRIGGER trial [a multi-centre, prospective, translational study sponsored by the Royal Marsden NHS Foundation Trust exploring the magnetic resonance tumour regression grade	Clarification of sharing of clinical and bio-specimen data between TRACC and TRIGGER teams for co-

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	biomarker to stratify management of good and poor responders to chemo-radiotherapy] data generated from the molecular sub-classification of FFPE tumour tissue from pre-CRT biopsies and post-CRT resection specimens, described above, will be securely shared with the TRACC research team. In addition, clinical and translational data relating to ctDNA and re-sequencing results from FFPE tumour tissue, acquired in the TRACC trial for co-enrolled patients will be securely shared with the TRIGGER research team. This is to avoid duplication of work by research teams from the same sponsor and to optimise resource utilisation. Patients entering Part C of the study will not be eligible for the TRIGGER study.	(mrTRG) as a novel biomarker to stratify management of good and poor responders to chemo-radiotherapy] data generated from the molecular sub-classification of FFPE tumour tissue from pre-CRT biopsies and post-CRT resection specimens, described above, the TRIGGER team will securely shared with the TRACC research team. The clinical and translational data relating to ctDNA and any re-sequencing results from FFPE tumour tissue for the co-enrolled patients will be securely shared between the TRACC-TRIGGER research teams. For the co-enrolled patients randomised to the interventional arm of TRIGGER, the follow-up will be done as part of the TRIGGER trial and all clinical and translational bio-specimen data will be shared with the TRACC team. The data from this group of patients may be analysed as separate cohort and details specified in a statistical analysis plan. This is to avoid duplication of work by research teams from the same sponsor and to optimise resource utilisation.	enrolled patients
56	Part C of the study will include the ctDNA guided adjuvant chemotherapy group, a total of 1621 subjects (810 patients in each arm) would need to be randomized and 530 events required.	Part C of the study will include the ctDNA guided versus SoC adjuvant chemotherapy group, a total of 1621 subjects (810 patients in each arm) would need to be randomized and 530 events required.	Due to COVID-19 we have had to pause Part C whilst recruitment to Part B has continued. We will therefore

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	It is anticipated that the overall study population (Part B) will be around 1800 (800 more than initially planned) and will be recruited over 5 years. Once confirmation that we have recruited 1621 patients for part C of the study and that the main study has 500 stage II (low risk and high risk CRC not in part C) and 500 stage III (not in parts C), we will halt recruitment to that stage. We plan to over-recruit to account for drop-outs.	It is anticipated that the overall study population (Part B+C) will be around 2600 (1600 more than initially planned) and will be recruited over 5 years. There will be a proportion of patients who begin the study within Part B and proceed to Part C following surgery. Once it is confirmed that we have recruited 1621 patients for part C of the study and that the Part B study has 500 stage II and 500 stage III, we will halt recruitment to that stage. We plan to over-recruit to account for drop-outs.	need to recruit additional patients into Part B
57	9. ESTIMATED STUDY DURATION The study is planned to continue until a total of 2,000 patients have been enrolled. This is anticipated to take up to 7 years in total from start of study recruitment in 2016.	9. ESTIMATED STUDY DURATION The study is planned to continue until a total of 2,600 patients have been enrolled. This is anticipated to take up to 7 years in total from start of study recruitment in 2016.	Due to COVID-19 we had to pause Part C whilst recruitment to Part B has continued. We will therefore need to recruit additional patients into Part C
58	12.1 Additional eligibility criteria for rectal cancer patients following completion of pre-operative radiotherapy or chemo-radiotherapy Inclusion Criteria: • All patients proceeding to surgery Exclusion Criteria: • Patients scheduled to have further pre-operative treatment with chemotherapy	12.1 Additional eligibility criteria for rectal cancer patients following completion of pre-operative radiotherapy or chemo-radiotherapy Inclusion Criteria: • All patients proceeding to surgery Exclusion Criteria: • Patients scheduled to have further pre-operative treatment with chemotherapy	Additional exclusion criteria added

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	• Patients that are no longer proceeding with surgery i.e. those in whom surgery is considered too high risk	• Patients that are no longer proceeding with surgery i.e. those in whom surgery is considered too high risk • Patients that are no longer proceeding with surgery as they are proceeding with a deferral of surgery approach	
59	12.3 Eligibility criteria for ctDNA guided adjuvant chemotherapy study (Part C) only Inclusion Criteria: 1. Subject ≥ 18 years of age 2. Subjects with histologically proven high risk stage II or stage III colon or rectal cancer treated with curative intent with surgery alone (any T, N1 or N2) with no evidence of metastatic disease. Subjects with histologically proven stage III rectal cancer are eligible, including patients treated with neoadjuvant chemoradiotherapy (any T, N1 or N2, M0) with no evidence of metastatic disease.	12.3 Eligibility criteria Part C only Inclusion Criteria: 1. Subject ≥ 18 years of age 2. Subjects with histologically proven high risk stage II or stage III colon or rectal cancer treated with curative intent with surgery alone (any T, N1 or N2) with no evidence of metastatic disease. High risk stage II is defined as having one or more of the following: T4 disease, obstruction and/or perforation of the primary tumour during the pre-operative period, inadequate nodal harvest as indicated by <12 nodes examined, poorly differentiated grade on histology, perineural invasion, peritoneal involvement or extramural venous/lymphatic invasion. Subjects must be due to receive	Clarification of inclusion criteria

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		adjuvant chemotherapy after surgery or Subjects with histologically proven locally advanced stage III rectal cancer treated with neoadjuvant chemoradiotherapy (any T, N1 or N2, M0) with no evidence of metastatic disease are eligible. Subjects must be due to receive adjuvant chemotherapy after surgery 3. Fully surgically resected tumour with clear resection margins (i.e., >1 mm). 4. Adequate organ function - Absolute neutrophil function ≥1.0 x 109/ L - Platelet Count ≥ 75 x 109 / L - Haemoglobin ≥80g/L (blood transfusion before randomisation is allowed) - Adequate renal function (GFR ≥ 50ml/min if single agent capecitabine or CAPOX being administered) as calculated by Cockcroft and Gault equation	
	3. Fully surgically resected tumour with clear resection margins (i.e., >1 mm). 4. Patients must have detectable levels of ctDNA (i.e., ctDNA positive) in blood samples at baseline; this is collected pre-operatively if being treated with surgery alone, or before chemoradiotherapy in patients with locally advanced rectal cancer being treated with neoadjuvant chemoradiotherapy before surgery 5. Adequate organ function - Absolute neutrophil function ≥1.0 x 109/ L		

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	- Platelet Count ≥ 75 x 109 / L - Haemoglobin ≥80g/L (blood transfusion before randomisation is allowed) - Adequate renal function (GFR ≥ 30ml/min if FOLFOX chemotherapy chosen and GFR ≥ 50ml/min if single agent capecitabine or CAPOX chosen) as calculated by Cockcroft and Gault equation - Aspartate aminotransferase/ Alanine aminotransferase levels ≤ 2.5 upper limit of normal 6. Absence of major post-operative complications or other clinical conditions that, in the opinion of the investigator, would contraindicate adjuvant chemotherapy 7. Patients should be assessed by Oncology team for suitability and assessment for adjuvant chemotherapy, be able to have post-operative ctDNA sample collected and be randomised by week 8 after surgery.	- Aspartate aminotransferase/ Alanine aminotransferase levels ≤ 2.5 upper limit of normal 5. Absence of major post-operative complications or other clinical conditions that, in the opinion of the investigator, would contraindicate adjuvant chemotherapy 6. Patients should be assessed by Oncology team for suitability and assessment for adjuvant chemotherapy, be able to have post-operative ctDNA sample collected and be randomised by week 8 ± 2 weeks after surgery. 7. ECOG performance status 0- 2 8. Able to give informed consent	

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	8. ECOG performance status 0- 2 9. Able to give informed consent 10. Patients who were not recruited pre-surgery for the main study can take part as long as they fulfil all eligibility criteria for part C of the study.		
60	Exclusion Criteria: 1. History of concurrent and previous malignancy within the last 3 years, with the exception of non-melanomatous skin cancer and carcinoma in situ 2. Any major post-operative complications or other clinical conditions that in the opinion of the investigator would contra-indicate adjuvant chemotherapy 3. Any subject not due to receive adjuvant chemotherapy will not be eligible for Part C of the study 4. Hypersensitivity or contraindication to the drug(s) associated with the planned choice of systemic chemotherapy	Exclusion Criteria: 1. History of concurrent and previous malignancy within the last 5 years, with the exception of non-melanomatous skin cancer and carcinoma in situ 2. Any major post-operative complications or other clinical conditions that in the opinion of the investigator would contra-indicate adjuvant chemotherapy 3. Any subject not due to receive adjuvant chemotherapy will not be eligible for Part C of the study 4. Hypersensitivity or contraindication to the drug(s) associated with the planned choice of systemic chemotherapy	Additional exclusion criteria added

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	(CAPOX, FOLFOX or single agent 5-FU or capecitabine) as stated in the SPC for each of the drugs	(CAPOX or single agent capecitabine) as stated in the SmPC for each of the drugs 5. Subjects due to receive 5-Flurouracil (5-FU) based adjuvant chemotherapy (either single agent 5-FU or in combination with oxaliplatin) will not be eligible for Part C of the study	
61	a qualified doctor who is authorised to make decisions regarding adjuvant chemotherapy can obtain consent. Patients will have the opportunity to discuss the study in detail prior to giving consent. Patients may consent on the same working day as receipt of the PIS provided they are willing and able to decide that they wish to participate. The patients will not be pressurised into making a decision and will be informed that their care will not be compromised if they choose not to participate. Patients will also be asked for permission to inform their GP if they consent to participate in the study. Additionally, patients will be asked to consent for their research tissue and blood samples to be stored and used in future research projects.	a qualified doctor who is authorised to make decisions regarding adjuvant chemotherapy can obtain consent. Patients will have the opportunity to discuss the study in detail prior to giving consent. Patients may consent to Part B on the same working day as receipt of the PIS provided they are willing and able to decide that they wish to participate. For Part C, patients will need at least 24 hours to read through the Part C specific patient information sheet and consider the study before signing an informed consent form. Informed consent can be obtained for Part B and C either during a face to face consultation or over the telephone or video consultations. Patients will not be pressurised into making a	Clarification of consenting procedures

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		decision and will be informed that their care will not be compromised if they choose not to participate. Patients will also be asked for permission to inform their GP if they consent to participate in the study. Additionally, patients will be asked to consent for their research tissue and blood samples to be stored and used in future research projects.	
62	15.1 Study Treatments (only applicable for patients in Part C of the study) All chemotherapeutic agents used for patients recruited in Part C of the study are standard of care treatments administered as per local policy. Patients in the experimental arm are de-escalated if ctDNA is negative on the post-operative blood sample. In those patients in whom ctDNA is negative in the post-operative sample, but become positive during surveillance, chemotherapy is started or escalated. *Single agent capecitabine regimen* Capecitabine: 2000mg/m2 per day in two divided doses, administered orally from days 1-14 of a 3 week cycle Total number of cycles will be as follows: Total of 6 months of treatment (8 cycles) in standard of care arm	15.1 Study Treatments (only applicable for patients in Part C of the study) All chemotherapeutic agents used for patients recruited in Part C of the study are standard of care treatments administered as per local policy. Patients in the ctDNA guided arm are de-escalated if ctDNA is negative on the post-operative blood sample. In those patients in whom ctDNA is negative in the post-operative sample, but become positive during surveillance, chemotherapy is started or escalated. *Single agent capecitabine regimen* Capecitabine: 2000mg/m2 per day in two divided doses, administered orally from days 1-14 of a 3 week cycle Total number of cycles will be as follows: Total of 6 months of treatment (8 cycles) in standard of care arm	Clarification of treatment cycles for patients receiving neoadjuvant chemotherapy

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	Total of 3 months of treatment (4 cycles) in experimental arm	Total of 3 months of treatment (4 cycles) in ctDNA guided arm In patients with locally advanced rectal cancer who have undergone neoadjuvant chemoradiotherapy with 2 cycles of concomitant capecitabine (6 weeks) will have the following number of cycles in the adjuvant period: Total of 18 weeks of treatment (6 cycles) in standard of care arm Total of 12 weeks of treatment (4 cycles) in ctDNA guided arm in case of de-escalation from CAPOX to capecitabine.	
	Capecitabine and Oxaliplatin doublet regimen A total of 4 cycles will be administered at following doses Oxalipatin: 130mg/m2 administered intravenously every 3 weeks of a 3 weekly cycles Capecitabine: 1700mg/m2 per day in two divided doses, administered orally from days 1-14 of a 3 weekly cycle Total number of cycles will be as follows: Total of 3 months of treatment (4 cycles) in	*Capecitabine and Oxaliplatin doublet regimen* A total of 4 cycles will be administered at following doses Oxalipatin: 130mg/m2 administered intravenously every 3 weeks of a 3 weekly cycles Capecitabine: 1700mg/m2 per day in two divided doses, administered orally from days 1-14 of a 3 weekly cycle Total number of cycles will be as follows: Total of 3 months of treatment (4 cycles) in standard of care and ctDNA	5FU based regimens deleted in

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	standard of care and experimental arms *5-Flurouracil and Oxaliplatin doublet regimen* A total of 12 cycles will be administered at following doses as per standard Oxaliplatin: 85mg/m2 administered intravenously every two weeks of a 2-weekly cycle 5-Fluro-uracil (5-FU): a bolus of 400mg/m2 administered on day 1 of chemotherapy and 5-FU at dose of 2400mg/m2 administered over two days (1200mg/m2 per day) of a 2-weekly cycle Total number of cycles will be as follows: Total of 6 months of treatment (12 cycles) in standard of care arm Total of 3 months of treatment (6 cycles) in standard of care arm	guided arms for those patients who need this at month 3	
62/63	15.1.1 Chemotherapy Drugs used in the trial for patients in Part C The following chemotherapy drugs are used in the clinical trial: Capecitabine, Oxaliplatin and 5-flurouracil. All chemotherapy agents must be sourced from local hospital stock and prepared as per local practice. All	15.1.1 Chemotherapy Drugs used in the trial for patients in Part C The following chemotherapy drugs are used in the clinical trial: Capecitabine with or without Oxaliplatin. All chemotherapy agents must be sourced from local hospital stock and prepared as per local practice. All	5FU based regimens deleted

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	chemotherapy agents used must be stored according to Summary of Product Characteristics and labelled as per local practice. *Drug Accountability* As per risk adapted approach, sites may use standard documents as drug accountability e.g. in-house preparation worksheets, orders from external partners. This should include batch numbers and expiries.	chemotherapy agents used must be stored according to Summary of Product Characteristics and labelled as per local practice. Drug Accountability As per risk adapted approach, sites may use standard documents as drug accountability e.g. in-house preparation worksheets, orders from external partners. This should include batch numbers and expiries.	
63/64	15.1.2 Dose Modifications for Toxicity for patients in Part C Expected toxicities as detailed in SmPC will form the reference safety information (RSI) for this study. The links to SmPC for individual drugs are as follows: Capecitabine: https://www.medicines.org.uk/emc/product/1319/smpc Oxaliplatin: https://www.medicines.org.uk/emc/product/6088/smpc 5-flurouracil: https://www.medicines.org.uk/emc/product/3791/smpc There will be an annual update of the SmPCs which will be submitted to the regulatory authorities. For toxicities or combinations of toxicities not specifically covered in detail in the SPC (see protocol appendix), doses of chemotherapy can be reduced at the discretion	15.1.2 Dose Modifications for Toxicity for patients in Part C Expected toxicities as detailed in SmPC will form the reference safety information (RSI) for this study. The links to SmPC for individual drugs are as follows: Capecitabine: https://www.medicines.org.uk/emc/product/1319/smpc Oxaliplatin: https://www.medicines.org.uk/emc/product/6088/smpc There will be an annual update of the SmPCs which will be submitted to the regulatory authorities. For toxicities or combinations of toxicities not specifically covered in detail in the SmPC (see protocol appendix), doses of chemotherapy can be reduced at the discretion	5FU based regimens deleted

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	of the Investigator as per local practice. All dose modifications documented in the CRF including reasons. Dose modifications due to neurotoxicity should be documented separately. Crossover from capecitabine to infusional 5-FU and vice versa is allowed if clinically indicated. At all times all endeavours should be made to keep the total number of weeks of treatment as determined by randomisation. Please do not hesitate to contact your coordinating trial office for advice.	of the Investigator as per local practice. All dose modifications documented in the CRF including reasons. Dose modifications due to neurotoxicity should be documented separately. Dose banding of chemotherapy agents as per local practice based on National Guidelines are to be followed. Local guidelines for DPYD (dihydroprymidine dehydrogenase) testing and dose modifications should be followed. Crossover from capecitabine to infusional 5-FU is not allowed but if clinically indicated, please discuss with the Chief Investigator or Trial Physician. Use of alternative chemotherapeutic agent in case of toxicity (e.g., raltitrexed in case of cardic side effects with capecitabine) is allowed. Please do not hesitate to contact your coordinating trial office for advice.	
64	15.1.3 Neurosensory Toxicity (applicable for patients in Part C) Neurosensory toxicity due to oxaliplatin may require dose reduction and dose adjustments according to local protocol may be followed as long as the dose given is carefully annotated in the CRF. Wherever possible, oxaliplatin should be dose-reduced (as per Investigator discretion) rather than	15.1.3 Neurosensory Toxicity (applicable for patients in Part C) Neurosensory toxicity due to oxaliplatin may require dose reduction and dose adjustments according to local protocol may be followed as long as the dose given is carefully annotated in the CRF. Wherever possible, oxaliplatin should be dose-reduced (as per Investigator discretion) rather than	5FU based regimens deleted

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	discontinued and can be given over a longer period of time if it is the hyperacute neurotoxicity which is particularly a problem. In the situation where oxaliplatin is discontinued due to toxicity, adjuvant treatment can continue with 5-FU or capecitabine alone if deemed appropriate by the local Investigator. In this case, the dose per m^2 of the single agent 5-FU/capecitabine can be increased as per local practice at the discretion of the Investigator and documented in the CRF.	discontinued and can be given over a longer period of time if it is the hyperacute neurotoxicity which is particularly a problem. In the situation where oxaliplatin is discontinued due to toxicity, adjuvant treatment can continue with capecitabine alone if deemed appropriate by the local Investigator. In this case, the dose per m^2 of the single agent capecitabine can be increased as per local practice at the discretion of the Investigator and documented in the CRF.	
65	For part C of the study only, real time analysis of ctdNA with a twoweek turn-around time of ctDNA results will be performed at following time points: • Post-operative ctDNA at month 0 (week 4-8 following surgery, before randomisation) • Post-operative ctDNA at month 3	For part C of the study only, real time analysis of ctdNA with a two-week turn-around time of ctDNA results will be performed at following time points: • Post-operative ctDNA at month 0 (by 4-8 (± 2) weeks following surgery, before randomisation) • Post-operative ctDNA at month 3	Protocol amended to allow for a window for radnomisation. The goal to start adjuvant chemotherapy within 12 weeks after surgery remains unchanged
70	Table 1: Summary of Study assessments for Part A and Part B (Translational Study) Footnote C: 4-12 weeks post-surgery, (for patients with complete response to CRT who do not proceed to surgery, sample to be taken 4-12 weeks after previous sample or prior to starting adjuvant chemotherapy if this is sooner)	Table 1: Summary of Study assessments for Part A and Part B (Translational Study) Footnote C: 4-8 (± 2) weeks post-surgery, (for patients with complete response to CRT who do not proceed to surgery, sample to be taken 4-12 weeks after previous sample or prior to starting adjuvant chemotherapy if this is sooner)	Post-surgery sampling timepoint amended

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71	Table 2: Summary of Study assessments in ctDNA guided adjuvant chemotherapy (Part C) only Footnote C: 4-6 weeks post-surgery, (for patients with complete response to CRT who do not proceed to surgery, sample to be taken 4-6 weeks after previous sample or prior to starting adjuvant chemotherapy if this is sooner)	Table 2: Summary of Study assessments in ctDNA guided adjuvant chemotherapy (Part C) only Footnote C: 4-8 (± 2) weeks post-surgery, (for patients with complete response to CRT who do not proceed to surgery, sample to be taken 4-6 weeks after previous sample or prior to starting adjuvant chemotherapy if this is sooner)	Post-surgery sampling timepoint amended
83	18. REGULATORY OBLIGATIONS 18.0 Informed Consent Before a patient's participation in the clinical study, the principal investigator (or a medically qualified member of the study team named in the site signature log and authorised by the site principal investigator) is responsible for obtaining written informed consent from the patient after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific screening procedures or any investigational products are administered.	18. REGULATORY OBLIGATIONS 18.0 Informed Consent Prior to participation in the clinical study, the principal investigator (or a member of the study team named in the site signature log and authorised by the site principal investigator – for Part C this individual must be medically qualified) is responsible for obtaining written informed consent from the patient after adequate explanation of the aims, methods, anticipated benefits, and potential risks of the study. This must take place before protocol-specific screening procedures or treatment is administered. Where patients are being seen in person, face-to-face consent should proceed as usual. Due to the COVID-19 pandemic the patient pathway has changed and routine care now involves telephone or video consultations. For patients	Virtual consent process added to study procedures

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		with early colorectal cancers, remote consultations may take place prior to curative surgery, and/or prior to commencing adjuvant chemotherapy. Remote consent for parts B and C of the TRACC trial can be taken by telephone or video consultation. A member of the study team will contact the patient to discuss the trial. Should the patient wish to participate and agree to remote consent, this should be documented in their notes. The remote consent will follow a structured process, details of which can be found in the guidance note on Remote Consent. For Part B, informed consent can be obtained on the same day that the PIS is issued. 24 hours must be allowed for the patient to read the PIS/ICF prior to consenting for Part C of the study. The acquisition of informed consent should be documented in the patient's medical records, and the ICF should be signed and personally dated by the patient and by the authorised person who conducted the informed consent discussion. The original signed informed consent form should be retained in accordance with institutional policy and GCP, and a copy of the signed consent form should be provided to the patient or	
	acquisition of informed consent should be documented in the patient's medical records, and the informed consent form should be signed and personally dated by the patient and by the authorised person who conducted the informed consent discussion. The original signed informed consent form should be retained in accordance with institutional policy and GCP, and a copy of the signed consent form should be provided to the patient or		

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	legally acceptable representative. If a potential patient is illiterate or visually impaired and does not have a legally acceptable representative, the investigator must provide an impartial witness to read the informed consent form to the patient and must allow for questions. Thereafter, both the patient and the witness must sign the informed consent form to attest that informed consent was freely given and understood.	legally acceptable representative. If a patient is illiterate or visually impaired and does not have a legally acceptable representative, the investigator must provide an impartial witness to read the informed consent form to the patient and must allow for questions. Thereafter, both the patient and the witness must sign the informed consent form to attest that informed consent was freely given and understood. If a patient requires help with language, an interpreter can be present on the call for remote consent to translate. A witness must be present and this must be documented in the patient's notes.	
85	18.3 Patient Confidentiality The investigator must ensure that the patient's confidentiality is maintained in compliance with the UK Data Protection Act of 1998.	18.3 Patient Confidentiality The investigator must ensure that the patient's confidentiality is maintained in compliance with the UK Data Protection Act of2018.	Data Protection Act amended
86	19. SAFETY DATA COLLECTION, RECORDING AND REPORTING Adverse Events Reporting Data on neurotoxicity related to oxaliplatin treatments as per CTCAE v5.0 will be collected and reported in the CRFs. Only those toxicities listed in the SPC leading to dose reductions in chemotherapy need to be recorded in CRFs. All other toxicities listed in the SPC for	19. SAFETY DATA COLLECTION, RECORDING AND REPORTING Adverse Events Reporting Data on neurotoxicity related to oxaliplatin treatments as per CTCAE v5.0 will be collected and reported in the CRFs. Only those toxicities listed in the SmPC leading to dose reductions in chemotherapy need to be recorded in CRFs. All other toxicities listed in the SmPC	5FU based regimens deleted

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	these drugs do not need to be reported or recorded. Serious Adverse Events Reporting The study will use chemotherapy drugs that are routinely used as standard of care, adverse events and serious adverse events will not need to be reported for any known adverse reaction to Oxaliplatin and/or Capecitabine/5FU as listed in the Summary of Product Characteristics (SPC) for each drug does not need to be reported.	for these drugs do not need to be reported or recorded. Serious Adverse Events Reporting The study will use chemotherapy drugs that are routinely used as standard of care, adverse events and serious adverse events will not need to be reported for any known adverse reaction to Oxaliplatin and/or Capecitabine as listed in the Summary of Product Characteristics (SmPC) for each drug does not need to be reported.	
87	Any toxicity not listed in the SPC or not covered by the above exemptions will need to be reported as a serious unexpected serious adverse reaction (SUSAR). <u>Suspected Unexpected Serious Adverse Reaction (SUSAR):</u> A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question as outlined in the SPC of the medication qualifies for a SUSAR and will need to be reported to the RMH Clinical Trial Units team.	~~Any toxicity not listed in the SPC or not covered by the above exemptions will be need to be reported as a serious unexpected serious adverse adverse reaction (SUSAR).~~ ~~Suspected Unexpected Serious Adverse Reaction (SUSAR): A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question as outlined in the SPC of the medication qualifies for a SUSAR and will need to be reported to the RMH Clinical Trial Units team.~~ If any unexpected serious adverse reactions deemed related to either of the	SUSAR reporting amended

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		chemotherapy agents used in the study are identified, they should be reported to the MHRA using the Yellow card system.	

Protocol changes from V9.0 to V10.0

Page No	Version 9.0	Version 10.0	Explanation
Page 71	Table 2: Summary of Study assessments for Part C (ctDNA guided adjuvant chemotherapy study)	Table 2: Summary of Study assessments for Part C (ctDNA guided adjuvant chemotherapy study)	Update to Table 2 schedules of events
Page 72		Scenarios	Inclusion of the 'Scenarios' box to offer further clarification over ctDNA blood sample timepoints

Protocol changes from V10.0 to V11.0

Substantial Amendment 7, Protocol V11 dated 01.02.2023

The following changes have been made to the study:

- Funding sources have been acknowledged.
- The study duration has been increased from 7 to 10 years.
- Patients with rectal cancer who have undergone neo-adjuvant radiotherapy followed by curative surgery can be enrolled in Part C of the study.
- Inclusion criteria 2 for locally advanced rectal cancer patients with neoadjuvant radiotherapy or chemoradiotherapy have been clarified.
- Inclusion criteria 6 for adjuvant chemotherapy regimen and duration have been clarified
- The post-op ctDNA sampling time has been changed from 4-8 (±2) to 4-8 (+2) weeks.
- The Post-ACT ctDNA sampling time has been changed to 3-5 weeks after the last chemotherapy tablet/dose.
- Specialist health care professional with the required qualifications of their Trust who is authorised to prescribe, consent and make decisions regarding adjuvant chemotherapy can now consent patients to the study and prescribe ACT
- All chemotherapeutic agents used for patients recruited in Part C of the study are standard of care treatments administered as per local hospital policy, including standard doses for the Trust. This includes dose reductions before and during treatment, for example, those used for patients with DPYD variants and toxicity.
- If re-escalation treatment is required, systemic chemotherapy will be escalated to doublet regimen with CAPOX for 3 months or capecitabine for 6 months in patients unsuitable to receive oxaliplatin.

Protocol changes from V11.0 to V12.0

Summary of proposed changes

TRACC changes (general):

- Trial Managers Hsiang-Chi Chen and Abiramy Neduncheliyan added to the team list. Annette Bryant removed.
- TRACC team statisticians Maria Aresu and Abena Glover added to the team list.
- Professor Thereasa Wiseman changed to Professor Susanne Cruikshank as Strategic Lead for Health Service Research.
- Applied Health Research Group team members added; Emma Hainsworth and Sarah Stapleton.
- Study period amended to *a minimum of* 5 years from surgery.
- Availability of FFPE tumour tissue can be from either biopsy or surgery.
- Patients should be contacted annually by telephone from relapse to confirm their clinical status until they have reached a minimum of 5 years from their surgery.
- Relapse should be by clinical or radiological confirmation.
- The wording and figures for the overall accrual target has been updated. It is anticipated that the overall study population will be at least 2700 (1700 more than initially planned). This is anticipated to take approximately 10 years in total from the start of study recruitment in 2016. Once confirmation that we have 1620 evaluable patients for Part C of the study and that Part B of the study has 500 evaluable stage II (low risk and high risk CRC) and 500 evaluable stage III patients, we will consider halting recruitment to that stage. We plan to over-recruit to account for drop-outs.
- Plasma samples from the ongoing TRACC study are currently being analysed as per protocol on behalf of the Royal Marsden Hospital. This has been changed to reflect samples processed outside the Centre for Molecular Research laboratory.
- Clarification given to study withdrawal procedures.
- Process evaluation and implementation protocol attached to the main TRACC protocol.
- Sensitivity and specificity have been defined for the population.
- Minor spelling and grammatical updates as well as minor updates made for the purposes of clarity.

TRACC Part B specific changes:

- Disease free survival (DFS) endpoint changed to recurrence free survival (RFS) for Part B analysis.
- Wording around Part B eligibility changed: ‘Patients with histology consistent with adenocarcinoma or those with high grade dysplasia whose imaging is strongly suggestive of colorectal carcinoma (CRC).’
- NB: Synchronous CRC primaries are allowed in Part A and B.

TRACC Part C specific changes:

- Wording of inclusion criteria updated to be clear that high risk features for stage II malignancies include *extramural* perineural/venous/lymphatic invasion.
- Wording added to first exclusion criteria: History of concurrent and previous malignancy within the last 5 years, *including those on anti-cancer therapy (e.g.,*

adjuvant endocrine therapy), except for curatively treated superficial malignancies, (e.g., non-melanomatous skin cancer and carcinoma in situ)

- Patients with synchronous CRC primary tumours added to exclusion criteria for Part C
- History of concurrent and previous malignancy within the last 5 years, including those on anti-cancer therapy (e.g., adjuvant endocrine therapy), except for curatively treated superficial malignancies (e.g., non-melanomatous skin cancer and carcinoma in situ
- Statistical considerations updated to outline that 1620 patients (810 in each arm) would need to be randomised with 499 events required based on the following assumptions:
    - 5 year accrual
    - 3 year minimum follow up on all
    - 5% 1 sided significance level
    - 80% power
    - 1, 2, 3, 4, 5, 6 year DFS estimated from SCOT study as 0.9, 0.8, 0.75, 0.725, 0.7, 0.68
    - Non inferiority margin = 1.25 (ruling out 69.8% 3 year DFS)
- The accrual target will be inflated by ~5% to account for drop-outs, therefore the overall total accrual target for Part C will be 1700 patients.
- The primary population for analysis will be the Intention to Treat (ITT) population, defined as eligible randomised patients. A sensitivity per protocol (PP) analysis will also be performed, defined as defined as all patients randomised to study treatments (standard of care or ctDNA guided adjuvant chemotherapy) who received the treatment assigned at randomisation without major protocol deviations. Patients with resected MMRd/MSI-H CRC who are eligible to enrol in the study will have treatment de-escalated according to a different schema; 3 months CAPOX de-escalated to no chemotherapy in patients randomised to ctDNA guided adjuvant chemotherapy in whom ctDNA is not detected post-operatively (at month 0). The primary endpoint for this exploratory cohort has been added. The randomisation procedure for these patients has been outlined.
- Clarity given around processing/analysis of blood samples in the SOC arm for Part C, 'Blood samples for ctDNA will be banked for future analysis, hence patients will not receive ctDNA results if randomized to this arm.'
- Health economic analysis pilot study on 40 patients who completed at least 6 months follow up details added.
- Details regarding the improved version of the Guardant Reveal assay have been added in line with GH recommendations.
- Schedule of events updated to outline documents required to be submitted for sponsor to confirm eligibility.