1. Project title: IntAct: Intraoperative Fluorescence Angiography to Prevent Anastomotic Leak
EME Project No. 14/150/62

2. Background:

2.1. Existing research

The majority of patients suffering colorectal disease will require surgery to resect the diseased bowel and anastomosis to restore gastrointestinal continuity. If the anastomosis fails to heal an anastomotic leak (AL) occurs, leading to sepsis and possible multi-organ failure. Around 20% of patients who suffer an anastomotic leak do not survive, with the remainder suffering protracted hospital stays and significant long-term morbidity(1). Anastomotic leak is a devastating complication of any gastrointestinal surgery, but is particularly problematic in colorectal surgery. Within colorectal surgery, AL is highest following rectal resection and increases as the anastomotic site approaches the anal canal; rectal anastomoses within 5cm of the anal verge are up to 6.5-times more likely to leak than at more proximal sites (2,3,4).

Despite advances in surgery, with the introduction of stapling technology and laparoscopic/robotic techniques, there has been little progress in reducing the rate of AL and associated morbidity. A recent systematic review and meta-analysis of 98 prospective studies on rectal surgery, found no difference in leak rate between those studies published after 2003 compared to earlier investigations(5). The overall incidence of colorectal anastomotic leak varies widely, ranging from 1% to 24%, partly due to inconsistent definition and reporting. In a systematic review of 49 gastrointestinal studies, Bruce *et al* found 29 different definitions of anastomotic leak(6). To address this inconsistency, the International Study Group of Rectal Cancer has published a universal definition and grading system for AL, defining AL as a defect of the intestinal wall at the anastomotic site leading to a communication between the intra- and extraluminal compartments(7).

In rectal cancer surgery, the current rate of AL is variously reported between 8% and 20%(8). The most recent data, which takes into account new technologies, comes from the COLOR II and MRC/EME ROLARR studies. COLOR II was a large European randomised controlled comparison of laparoscopic versus open surgery for rectal cancer(9). In the 1044 patients randomised, there was no significant difference in AL between laparoscopic and open groups. AL rate varied depending on the height of the anastomosis, being 11%, 15%, and 11% for anastomoses in the upper, middle, or lower rectum respectively. The ROLARR trial has recently reported the results of a randomised comparison of robotic versus laparoscopic surgery in 471 rectal cancers. There was no difference between the two arms, with an overall AL rate of 10.2% (unpublished results).

Several risk factors have been implicated in AL and include technical aspects of anastomosis construction (poor blood supply, inadequate tissue approximation, tension on the anastomosis, distal obstruction etc.), and patient risk factors associated with poor tissue healing (malnutrition, cancer diagnosis, renal failure, immunosuppression etc.)(10). In a recent systematic review and meta-analysis, including 23 studies and 110,272 patients, the independent risk factors for AL following colorectal resection where low rectal anastomosis (OR 3.26, 95%CI 2.31, 4.62), male gender (OR 1.48, 95%CI 1.37, 1.60), and preoperative radiotherapy (OR 1.65, 95%CI 1.06, 2.51). ASA grade was also significant for AL on meta-analysis (OR 1.71, 95%CI 1.09, 2.67), but the grade of evidence was deemed to be very weak(11).

Of all the factors that contribute to AL, probably the most crucial, and the one that the surgeon has some influence over, is the blood supply to the anastomosis. Ensuring that both ends of the bowel to be anastomosed are adequately perfused is essential for healing(12,13). Unfortunately, assessment of tissue perfusion is difficult, if not impossible, at operation. Indeed, the surgeon's ability to predict AL is poor. In a study by Karliczek *et al,* surgeons were asked to predict the chance of AL following colorectal anastomosis(14). AL was correctly predicted in only 11% of cases, with low sensitivity (41%) and specificity (59%).

The current standard for intraoperative testing of rectal anastomotic integrity involves "air-leak" testing and completeness of anastomotic "doughnuts". Air-leak testing is easy and cheap and has been shown to more than halve the radiological AL rate(15). In some centres, this is combined with intraoperative endoscopic assessment of the anastomosis. Li *et al* showed that the routine use of intraoperative endoscopy reduced AL, as compared to selective use in cases where there was uncertainty about anastomotic integrity, but this failed to reach significance due to small patient numbers(16). Alternative strategies include intraoperative assessment of anastomotic tissue oxygenation. Using white light spectroscopy, Karliczek *et al* demonstrated that a reduction in bowel oxygen tension immediately after resection was predictive for AL, although the level of oxygen tension that led to irreversible necrosis was not defined(17).

There has been increasing interest in the use of functional imaging in the assessment of solid cancers and prediction of response to neoadjuvant therapies. Using perfusion CT scan in patients with rectal cancer, initial studies have shown that cancer blood flow, volume, and permeability-surface area product are significantly higher than normal rectal wall, and that cancer blood flow and volume significantly decrease following chemoradiotherapy(18,19) - (Figure 1).

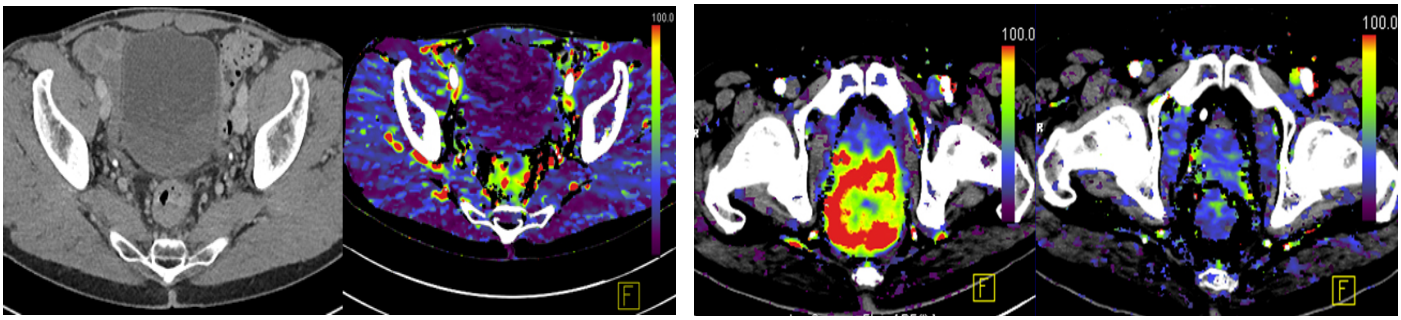


Figure 1: Left- perfusion CT scan showing: left -rectal tumour; right - functional map of blood flow; Right- perfusion CT pre and post CRT demonstrating a decrease in rectal cancer blood flow post chemoradiation. (Courtesy V Goh)

In addition, high baseline values of blood flow and volume are predictive of a good cancer response to neoadjuvant therapy. This difference is attributed to neoangiogenesis, with increased arterio-venous shunting, and better oxygenated cancers responding more favorably to radiotherapy. Assessment of normal large bowel perfusion is feasible, with rectal perfusion shown to be lower than at other sites(20). The ability of perfusion CT to quantify changes in rectal perfusion following radiotherapy makes it an attractive preoperative imaging tool for predicting anastomotic leak. Its use in combination with CT angiography, to determine anatomical variations in rectal blood supply, may provide valuable information for predicting risk of anastomotic leak.

Fluorescence angiography has been used to evaluate blood flow and tissue perfusion in many areas of medicine, including general surgery(21). Recently, IFA has been introduced to evaluate anastomotic blood supply, with promising early results. The technique involves intravenous administration of Indocyanine Green (ICG), which rapidly binds to plasma proteins and stays in the intravascular compartment. When irradiated with near-infrared light through an operating laparoscope, ICG fluorescence can be visualized on a standard visual display unit providing an image of tissue perfusion. Figure 2 illustrates the use of ICG-NIR angiography in selecting well-perfused bowel for anastomotic construction, and clearly demonstrates the possible advantage over white light assessment.

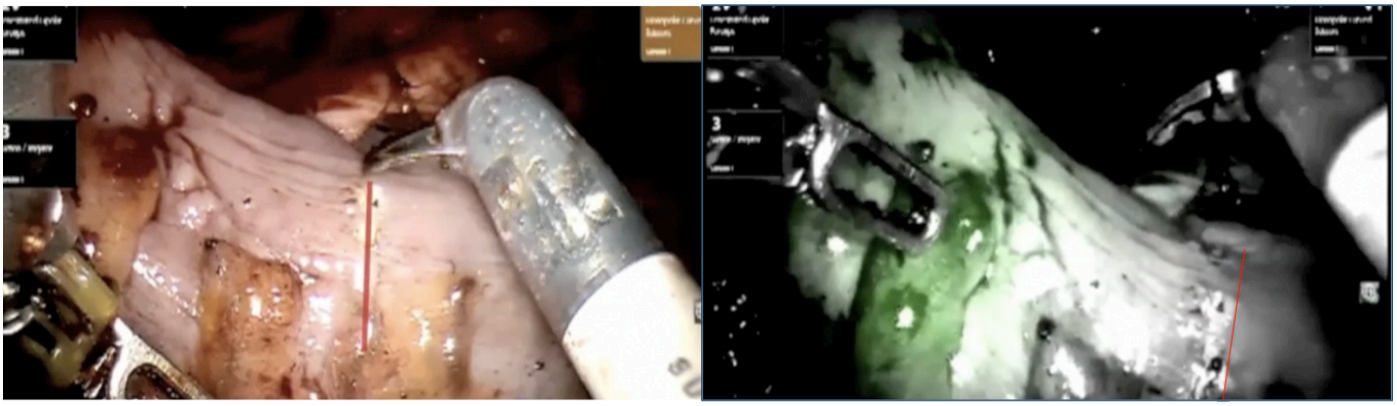


Figure 2: Left: the colon transection point (red line) appears well-perfused under white light laparoscopy. Right: poor bowel perfusion at the transection point under ICG-NIR laparoscopy (lack of green fluorescence), resulting in an ischaemic anastomosis.

Proof of concept for IFA has been established, but the evidence is limited to a few case series and one multi-centre, non-randomized clinical study. Ris *et al* reported satisfactory assessment of bowel perfusion using IFA in 29/30 patients undergoing colorectal resection, with avoidance of stomas in 3 (10%) patients, and no anastomotic leaks(22). Kudszuz *et al* used IFA to study 402 patients undergoing colorectal cancer surgery and compared outcomes to a matched historical cohort(23). Twenty-two revisions were necessary; 7 (3.5%) in the intervention and 15 (7.5%) in the control group. Jafari *et al* analyzed 16 patients who underwent robotic low anterior resection using IFA in comparison to 24 patients without IFA(24). IFA resulted in 3 anastomotic revisions due to poor blood supply. The leak rate in the IFA group was 6% as

compared to 18% in the control group. The single multicentre study (PILLAR II: Perfusion Assessment in Laparoscopic Left Anterior Resection) recruited 147 patients from 12 centres across the USA(25). In 11 patients (8%), the anastomosis was revised. In the 139 patients available for analysis, 2 (1.4%) anastomotic leaks were observed. This represents an 8–9 fold reduction in the documented leak rate of 12% following anterior resection.

Although good surgical technique and optimal blood supply are paramount to anastomotic healing, there are some anastomoses that leak despite apparent perfect construction. It seems that other, as yet unexplained, factors might be involved the pathogenesis of AL. A recent concept that is attracting attention, and for which there is a growing body of evidence, is the role of intestinal microbiota and an infective aetiology to AL(8). Using a rat model of AL, Shogan *et al* have shown that anastomotic injury results in a change in anastomotic tissue-associated microbiota with a notable 500-fold and 200-fold increase in the relative abundance of *Enterococcus* and *Eschierichia/Shigella* species respectively(26). Importantly, this difference was only apparent in anastomotic tissue and not luminal faecal samples (Figure 3).

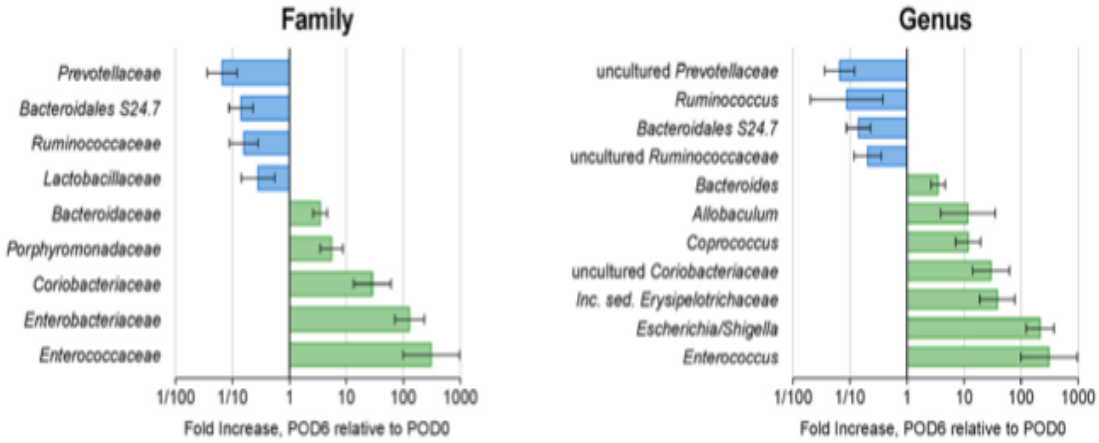


Figure 3: comparative analysis of bacterial abundance in anastomotic tissue between postop day-0 and day-6, showing marked elevation in Enterococcus and Escherichia/Shigella species(26).

AL was associated with increased bacterial virulence-associated pathways, including production of matrix degrading enzymes and cytotoxic necrotizing factors. Work by the same group, again in a rat model, has shown that *Enterococcus faecalis* contributes to AL by upregulation of collagenase activity and activation of tissue matrix metalloproteinase-9 (MMP-9), and that AL was prevented by administration of antibiotic enema or MMP-9 inhibitor(27). Furthermore, in a small cohort of 11 patients undergoing colonic surgery, *E. faecalis* and other bacteria with collagen degrading and MMP-9 activating ability could be isolated from anastomotic sites and were unaffected by the use of standard intravenous prophylactic antibiotics.

Another interesting observation, with relevance to rectal cancer surgery, is the change in composition and virulence of the rectal flora following radiotherapy(28). The adverse influence of radiotherapy on AL is usually attributed to tissue inflammation and microvascular injury, but it is possible that radiotherapy-induced changes in the rectal flora result in a pro-AL microenvironment. This is supported by the work of Olivas *et al* who showed in a model of low anterior resection that preoperative radiation and intestinal inoculation of *Pseudomonas aeruginosa* (a collagenase producing bacterium) resulted in high rates of AL, whereas radiation alone or *P. aeruginosa* alone did not cause leaks(29). Additional support for a causative role of the rectal microbiome in AL comes from studies documenting a beneficial role for intestinal decontamination in combination with oral antibiotics prior to surgery(30,31).

In summary, there has been no advance in eliminating the most feared complication of gastrointestinal surgery – anastomotic leak – in the past 50 years. This proposal evaluates a new technology that, for the first time, allows surgeons to easily assess intraoperative tissue perfusion and minimize one of the biggest risk factors for AL. The incorporation of two sub-studies evaluating rectal blood supply and perfusion in patients with and without neoadjuvant chemo/radiotherapy, and the role of the rectal microbiome in AL, adds exciting dimensions that will further inform our understanding of the mechanisms underlying AL.

2.2. Risks and benefits

2.2.i) Surgery: patients with a diagnosis of primary rectal cancer suitable for potentially curative surgery with restoration of gastrointestinal continuity will undergo laparoscopic or robotic anterior resection with or without preoperative chemo/radiotherapy. The surgery will not differ from normal practice and will not carry any additional risk. There will be the usual benefits from participation a in clinical trial, namely close

monitoring throughout the patient journey and for 90-days postoperative, and the satisfaction of contributing to a large UK and European study to improve outcomes for future rectal cancer sufferers.

2.2.ii) Intraoperative Fluorescence Angiography (IFA): patients will be randomised to receive IFA or standard white light laparoscopic assessment to evaluate bowel perfusion prior to anastomosis. Those patients randomised to IFA will require intravenous bolus injections of Indocyanine Green (ICG), administered in 3 (possibly 4) doses. ICG has an excellent safety profile, having been used in clinical practice for over 50-years. It has a short biphasic half-life and is excreted unmetabolised in the bile; following iv administration it is rapidly protein bound with an initial elimination half-life of 3-4 minutes, followed by a second, dose-dependent phase with a half-life of 60-80 minutes. Known adverse effects from ICG administration include rare anaphylactoid and anaphylactic reactions (<1/10,000) and urticarial skin reactions (<1/10,000). Allergic reactions appear to be more common in people with allergy to iodides and chronic renal failure. Medicinal products that interfere with liver metabolism may alter the clearance of ICG, and it should be used with caution in patients taking anticonvulsants, bisulphite-containing medications, methadone, nitrofurantoin etc. The total safe daily dose of ICG in adults is 5mg/kg body weight; in the current study 3 (or possibly 4) doses of ICG will be administered at 0.3 mg/kg, giving a maximal total daily dose of 1.2mg/kg – well below the 5mg/kg safety limit. The risk of allergy to ICG (<1/10,000) for patients in the study has to be weighed against the possible risk reduction from anastomotic leak (estimated 12% to 6%) and its consequences, including reduced risk of serious morbidity and even death. Advice from our patient representatives suggests that this is a risk that most participants would view as acceptable.

2.2.iii) Contrast enema examination: all patients will undergo rectal contrast enema examination at 4-6 weeks post-surgery, unless they have previously been diagnosed with a clinical AL or they are otherwise unable to undergo the investigation. This is standard of care for patients with a temporary defunctioning stoma and prior to considering reversal of stoma. In the 30-40% of patients without a defunctioning stoma, the contrast enema to determine radiological leak (secondary outcome measure) will be an additional investigation. It will incur an extra hospital visit and an examination that takes around 30mins. There is a low radiation dose of 5mSv, which equates to 2.1 years natural background radiation (2.4mSv/year).

2.2.iv) CT angiography (CTA) and CT perfusion (CTp) sub-study: the CTA and CTp sub-studies will be performed in 75 UK patients randomized to the IFA group. This involves an additional CT scan performed after randomization and before surgery, and taking around 30 minutes. There is an inherent risk from intravenous contrast agent administration and additional radiation exposure of up to a 30mSv maximum. To put this in context, the 30mSv dose for the additional CT scan is negligible compared to the 54,000 mSv received by patients undergoing radiotherapy. This risk will be explained to the 75 patients involved in the sub-study and set against the benefits derived from better understanding the role of vascular anatomy and tissue perfusion in anastomotic leak.

2.2.v) Rectal microbiome sub-study: the rectal microbiome sub-study will be performed in 200 UK patients and requires the collection of rectal mucosal samples at 3 time points (pre-op, intra-op and 5 days post op). This will use a dedicated device, the Oricol™ system (Origin Sciences, Cambridge, UK), and a rectal swab. Sample collection with the Oricol™ system involves the transanal insertion of a proctoscope through which a balloon-sampling device is inserted and inflated to make contact with the rectal mucosal. The sample obtained is representative of the rectal mucosal microbiome and will be used for 16S rRNA bacterial analysis. A rectal swab will also be obtained for functional assays. There is a theoretical risk that inflation of the Oricol™ balloon could disrupt a low rectal anastomosis, but this is highly unlikely given that the balloon is inserted under vision, is highly compliant, and uses low pressures.

2.3. Rationale for current study:

Despite advances in surgery, there has been no progress in reducing the rate of anastomotic leak over the past 50-years. AL rates are particularly high following rectal cancer surgery, with the rate increasing as the level of the anastomosis approaches the anal verge; anastomoses below 10cm from the anal verge have a 5.4-fold increased risk of AL(2,4) whilst those below 5cm from the anal verge have a 6.5-fold risk of AL(3). The reason for this is usually attributed to poor blood supply to the rectal stump and the frequent use of preoperative radiotherapy in low rectal cancers. With the introduction of new intraoperative imaging technology to assess tissue perfusion (ICG-NIR laparoscopy), and new radiological methods to assess rectal perfusion, there is a golden opportunity to improve the way that anastomoses are constructed and reduce AL. Recent evidence that the rectal microbiome is implicated in AL demands that this important concept is also explored.

The proposed study is timely given the findings of a multicentre, non-randomised US clinical trial, which has established proof-of-concept for IFA, with a reduction in expected AL rate of 12% to an observed rate of 1.4%. If this 8-9 fold reduction can be replicated in a rigorous clinical evaluation, the impact for patient care and health resource utilisation will be considerable. It will be a major advance in colorectal surgery, facilitating safe anastomosis with reduced rates of stoma formation. It will eliminate a major source of risk for patients, improve quality of life, and produce immediate cost-savings for the NHS.

Although this research proposal focuses on anastomotic leak following rectal cancer surgery it has much wider implications. The findings will be readily transferable to any surgery involving an anastomosis, including other common colorectal diseases (inflammatory bowel disease, diverticular disease, ischaemic bowel etc.), and gastrointestinal diseases. It is estimated that around 30,000 – 40,000 colorectal anastomoses are constructed each year in the NHS. Assuming an overall 5% leak rate, this equates to around 1,500 – 2,000 anastomotic leaks per annum; an incidence supported by a recent national Dutch registry(32). Anastomotic leak increases the morbidity of elective colorectal surgery from ~20% to ~60%, and the mortality from ~5% to ~20%. It necessitates an average intensive care stay of 16 days and prolongs hospital stay by 7 to 19 days(33,34). In patients undergoing cancer surgery, anastomotic leak has an adverse effect on local recurrence and cancer survival(35). For those who survive an anastomotic leak, the consequences are long-term, with impact on quality of life and a high permanent stoma rate. The average additional cost of an anastomotic leak is estimated to be £28,000(36) or around £50M per annum to the NHS. It is apparent, therefore, that any intervention that reduces anastomotic leak will have a considerable impact on patient recovery, long-term morbidity and quality of life, and cancer survival, whilst producing immediate cost-savings for the NHS.

3. Research objectives:

The objective is to investigate the efficacy and mechanism of a new technology, intraoperative fluorescence angiography (IFA), in reducing AL rate following elective rectal cancer surgery. Rectal cancer surgery has been chosen as it has the highest rate of AL. The comparator will be standard white light laparoscopic or robotic rectal cancer surgery. It is hypothesized that surgery with IFA will result in a reduction in AL rate following rectal cancer surgery from 12.0% to 6.0%. To test this hypothesis, 880 patients with rectal cancer will be recruited from 13 UK and 12 European centres over a period of 36-months, allowing for a dropout rate of 10%.

The research questions that the main study address are:
i) does surgery with IFA reduce clinical AL rate following rectal cancer surgery?
ii) does surgery with IFA alter intraoperative decision-making with regard to anastomosis construction?
iii) does surgery with IFA result in a reduction in stoma rate (temporary and permanent stoma)?
iv) does surgery with IFA improve patients' quality of life through reduction in anastomotic leak?
v) does surgery with IFA result in cost-savings for the NHS through reduction in anastomotic leak?

Two sub-studies will explore the mechanisms of anastomotic leak:
i) evaluation of rectal vascular anatomy and perfusion using CT angiography and CT perfusion imaging. This will allow variations in IFA to be interpreted in light of individual patient anatomy and physiology. Quantitative data will be obtained on the effect of radiotherapy on rectal perfusion with implications for AL.
ii) changes in the rectal microbiome as a result of surgery and its association with anastomotic leak.

4. Research design:

4.1. *Surgery with IFA v's Surgery without IFA:* A prospective, unblinded, parallel group, multicentre, European, randomized controlled trial comparing surgery with IFA against standard care (surgery with no IFA) to determine the effect on AL in patients undergoing elective anterior resection for rectal cancer. Surgery can be performed using either a laparoscopic or robotic technique, depending on surgeon's preference - the technique has no bearing on the outcome measures.

Patients will be identified from colorectal outpatient clinics, endoscopy units, and colorectal cancer multidisciplinary team meetings and approached from inclusion in the study. Patients will be given a Patient Information Sheet and allowed to consider the study prior to giving consent. Patients will undergo standard surgical and oncological work-up, including visualization of the colorectum by colonoscopy or CT colonogram, staging CT and MRI scans, and assessment of fitness for surgery. Patients will be discussed in colorectal cancer multidisciplinary team meetings to determine optimal management based on institutional protocols, which might include chemo/radiotherapy. The type of chemo/radiotherapy will be included as a stratification factor for randomization to prevent treatment bias between interventions and will be recorded on CRFs along with the interval between completion of chemo/radiotherapy and surgery.

Consenting patients will be randomized prior to surgery, on a 1:1 basis, to either surgery with IFA or surgery without IFA using minimisation (incorporating a random element). Stratification factors will include: gender, ASA grade, T-stage, type and timing of neoadjuvant chemo/radiotherapy (long course chemoradiotherapy or short course radiotherapy with or without delay), high or low anterior resection, and surgeon.

All patients will undergo anterior resection in accordance with the surgeon's preferred technique, using either a laparoscopic or robotic approach. Those patients allocated to surgery with IFA will undergo ICG-PINPOINT (laparoscopic) or ICG-FIREFLY (robotic) assessment of bowel perfusion to aid anastomosis construction. Those patients allocated to surgery without IFA will undergo routine white light laparoscopic assessment of bowel perfusion.

Postoperative care will be according to usual institutional practice. Clinical AL (primary end-point) will be defined as a defect of the intestinal wall at the anastomotic site (including suture and staple lines of neorectal reservoirs) leading to a communication between the intra- and extraluminal compartments that has an impact on patient management. This definition aligns to the International Study Group definition of AL, including Grade B AL (AL requiring active therapeutic intervention but manageable without laparotomy), and Grade C (anastomotic leak requiring re-laparotomy)(7). It excludes Grade A AL (AL resulting in no change in patients' management), which would encompass radiological AL (secondary end-point).

Patients will be followed up at 4-weeks and 90-days postoperative. A contrast enema examination will be performed between 4-6 weeks to detect radiological AL. Radiological AL will be defined as AL resulting in no change in patients' management, in line with the International Study Group recommendations. Patients will exit the study following 90-day review.

4.2. CTA and CTp sub-study; 75 patients from UK centres will be entered into the CTA/CTp mechanistic sub-study. Patients randomized to the surgery with IFA will undergo additional CTA and CTp scans following randomization and prior to surgery.

4.3. Rectal microbiome sub-study; 200 patients from UK centres will undergo rectal mucosal sampling, using the Oricol™ device and rectal swabs, at 3 time points: baseline, operation, and day-5 postoperative. Samples will be preserved in buffer solution and transported to Leeds for 16S rRNA sequencing and assay of bacterial collagenase activity.

A Go/NoGo time point is included at 24 months (12 months into the recruitment period following set-up; see Gantt Chart, Section 14), at which point 15 of the 25 centres should be open to recruitment and a minimum of 150 patients recruited to the study. Trial Steering Committee (TSC) meetings will be held approximately 6-monthly and Data Monitoring and Ethics Committee (DMEC) meeting annually during recruitment. The DMEC will consider data on patient safety, including AL rate, adverse events related to IFA (including ICG side-effects), CT angiography/CT perfusion (including contrast allergy), and Oricol™ rectal mucosal sampling.

An interim analysis with the potential for early stopping due to evidence of efficacy will be performed once primary endpoint data has been collected on 554 patients. The O'Brien-Flemming alpha spending function has been used to determine the test boundaries. Note that the costings for the trial are based on the full sample size of 880 patients, but if the interim analysis does yield significant evidence of efficacy then the trial will stop early and any surplus funds will be reimbursed. It is estimated that there is a 46% chance of stopping early if 12.0% and 6.0% are the true AL rates in non-IFA and IFA arms respectively.

5. Study population:

Inclusion criteria:

- Adults, 18 years or older.
- Able to provide written informed consent
- Diagnosis of rectal cancer (defined as a lower margin 15cm from the anal verge).
- Suitable for curative resection.
- Suitable for elective laparoscopic or robotic surgery.

Exclusion criteria:

- Patients not undergoing colo-rectal/anal anastomosis e.g. APER.
- Patients undergoing synchronous colonic resections.
- Locally advanced rectal cancer requiring extended or multi-visceral excision.
- Recurrent rectal cancer

- Coexistent colorectal pathology e.g. synchronous cancers, inflammatory bowel disease.
- Previous pelvic radiotherapy e.g. treatment for prostate cancer
- Hepatic dysfunction, defined as Model for End-Stage Liver Disease (MELD) Score >10(37).
- Renal dysfunction, defined as eGFR <40mmol/l
- Known allergy to ICG, iodine, iodine dyes, or drugs known to interact with ICG e.g. anticonvulsants, bisulphite containing drugs, methadone, nitrofuratoin.

Withdrawal criteria: Given the short follow-up period, we are not expecting many withdrawals. In line with usual clinical care, cessation or alteration of treatment at any time will be at the discretion of the attending clinician or the patient. Patients will be informed about their right to withdraw from the study and that this will not affect their subsequent care. In case of patient withdrawal, follow-up and safety data will continue to be collected unless the patient explicitly states they do not wish to contribute further data to the study. Any withdrawals will be reported to CTRU on a study specific withdrawal form to ensure that this is documented.

6. Planned interventions:
Patients with primary rectal cancer will be identified from outpatient clinics, endoscopy lists, and multidisciplinary colorectal cancer meetings and approached for participation. They will be given a Patient Information Sheet and Consent Form Document and allowed a minimum of 24 hours to consider participation before being asked to sign a the consent form.

Patients will undergo standard preoperative work-up, including colonic visualisation by either colonoscopy or CT colonogram, staging CT scan of the chest, abdomen and pelvis, MRI of the rectum, and assessment of fitness for surgery. Patients will be discussed in colorectal cancer multidisciplinary team meetings and optimal management determined based on institutional protocols. It is anticipated that ~50% of patients will require neoadjuvant chemo/radiotherapy, which will be dictated by local policy and might include long-course chemoradiotherapy or short-course radiotherapy with or without delay to surgery. Consent for participation in the trial will be taken prior to surgery, and after chemo/radiotherapy in those patients requiring adjuvant therapy.

6.1. Surgery.
6.1.i. Surgery with IFA: anterior resection will be performed according to the surgeon's usual technique, using either a laparoscopic or robotic approach. The left colon and rectum will be mobilised and the rectum transected below the cancer. In the IFA group, the proximal colon will be assessed under white light and the point of planned transection marked. 0.3mg/kg of 2.5mg/ml ICG will be administered intravenously and colonic and rectal stump perfusion assessed using near-infrared laparoscopy (PINPOINT - laparoscopic surgery; FIREFLY - robotic surgery). Any change in the planned transection level or revision of the rectal stump as a result of IFA assessment will be recorded. The mobilized bowel will be exteriorised and a second bolus of 0.3mg/kg ICG administered to confirm the optimal level for transection. When a colonic reservoir is constructed a further 0.3mg/kg ICG may be used. Colo-rectal/anal anastomosis will be performed according to surgeon's preference (hand-sewn, stapled, end-to-end, end-to-side etc.), following which endoluminal assessment of anastomotic perfusion will be undertaken with a final bolus of 0.3mg/kg of ICG. Any anastomotic revision will be recorded. Use of a defunctioning stoma will be at the discretion of the surgeon, with the reason for defunctioning and the relation to IFA assessment will be documented.

Quality assurance in the performance of IFA will include a standardized protocol and review of video evidence of the technique in a selected number of cases from each centre.

6.1.ii. Surgery without IFA: in the standard care group (no IFA) the operation will proceed in the usual manner using either a laparoscopic or robotic approach, with white light assessment of bowel perfusion. The level of colonic transection, formation of colo-rectal/anal anastomosis, and defunctioning stoma will be performed according to normal practice.

Postoperatively, patients will be cared for according to institutional protocol. This can include enhanced recovery after surgery pathways. Complications, including clinical AL, will be diagnosed and treated according to local practice. Patients will be discharged according to institutional policy.

Patients will be followed-up at 4-weeks and 90-days post-surgery.

6.2 Rectal contrast enema examination:
All patients will undergo a rectal contrast study between 4-6 weeks post-surgery to determine evidence of radiological leak, unless contraindicated or impractical due to comorbidity, in which case clinical evidence of anastomotic leak will be relied upon.

Single contrast enema examinations are a routine radiological technique. Anteroposterior and lateral control exposures will be acquired to assess the position of the anastomosis. A flexible catheter will be placed in the rectum below the level of the anastomosis. Iodinated contrast with a concentration of 125-200mg/ml will be instilled into the rectum under gravity and the anastomosis distended. A minimum of 2 images will be obtained in an anteroposterior and lateral orientation to prove the anastomosis is intact with additional oblique views as required. A final image will be obtained at the end of the examination when the rectum has been drained of contrast. For quality control, images from the first 5 contrast enemas from each institution will be subjected to central review.

6.3. CTA/ CTp sub-study:

75 UK patients from the IFA arm will be recruited into the CTA/CTp sub-study. A non-contrast abdomino-pelvic study (120kV, variable mA, 0.75/5mm slice thickness, from liver dome to pubic symphysis) will be performed for localization of bowel segments, CT acquisition planning and assessment of vascular calcification. CTp will be centred on the site of the distal rectal anastomosis. Following an iv injection of an anti-peristaltic (e.g. Buscopan, unless contraindicated) an iv bolus injection of iodinated contrast agent (50ml, >300mg/ml iodine concentration, 4ml/s iv bolus with saline chaser) will be administered. The dynamic contrast enhanced acquisition will be performed (100kV, 100-150mAs, 1.5s temporal resolution, 50s acquisition, 3-5mm slice thickness, >4cm z-coverage, 20mSv maximum dose proposed) to allow the changes in Hounsfield Unit attenuation over time to be plotted for the rectal wall and regional blood flow, blood volume and permeability surface area product to be derived by distributed parameter kinetic modelling for the region of interest using standard manufacturer software, dependent on CT scanner type. CTA will be performed following CTp (120kV, dose modulated mA, 0.75/5mm slice thickness, breathheld, bolus triggered, from liver dome to pubic symphysis matching the non-contrast acquisition) and reconstructed in the sagittal and coronal planes, with MIP and 3D volume rendering.

The CTA/CTp sub-study will benefit from the investigators' previous experience of running the multicenter NIHR funded PROSPeCT study of CTp in 445 patients with colorectal cancer. A team from KCL will be sent to participating sites with ≥64 MDCT capable of delivering CTp for study set up, standardization of protocols, quality control & training.

6.4. Rectal microbiome sub-study:

200 patients, recruited from UK sites, will undergo rectal mucosal sampling at baseline, operation, and day-5 postoperative, using the Oricol™ balloon system and rectal swabs. Baseline samples may be taken at any time preoperatively (clinic, hospital admission), but prior to the administration of mechanical bowel preparation or rectal enema for surgery. Samples will be preserved in a preservation solution and transported to Leeds for storage and processing. The Oricol™ system samples the rectal mucosa, which has been shown to have a different microbiota to the rectal lumen. These samples will be used for analysis of bacterial16S rRNA and rectal swabs for assays of collagenase activity. Oricol™ rectal sampling is a straightforward and quick procedure that will be familiar to all colorectal surgeons, who regularly undertake proctoscopy.

Oricol™ samples will be spun down and the pellet will undergo DNA extraction using the QIAamp DNA microbiome kit (Qiagen) as per our optimized protocol. The DNA yield will be measured by nanodrop. DNA from the 240bp V4 region of the 16S rRNA gene will be amplified by polymerase chain reaction (PCR) using Q5 Hot Start High-Fidelity DNA Polymerase (New England Biolab, Hitchin, UK). The products will undergo library preparation using the NEBNext ultra DNA library prep kit for Illlumina (Illumina, Fulbourn, Cambridge, UK) with in-house index primers and next generation sequencing using the Illumina Miseq platform.

Rectal swabs will be analysed to determine if specific bacterial species are associated with anastomotic leak. In addition, rectal colonization with, and the collagenase activity of, Enterococcus and pseudomonas species, will be determined via culture and an ELISA method. Enterococcus and pseudomonas have previously been causally implicated in the development of anastomotic leakage when these strains have a high collagenase activity. If the microbiome analysis implicates other bacterial species in anastomotic leakage, their collagenase activity will also be determined.

Bioinformatics: Data taken from the MiSeq will be used to produce consensus sequences using fastq-join (http://code.google.com/p/ea-utils/) and the QIIME software package. Operational taxonomic units (OTUs) will be picked using UCLUST following the default closed reference clustering algorithm and aligned to the Greengenes database using PyNAST. Taxonomy will be assigned using the ribosomal database project (RDP) classifier 2.2. Diversity of OTUs will be assessed using various species diversity indices and

principle component analysis. Analyses will be undertaken comparing microbiome populations in patients with and without leaks at base line, operation, and 5-days postoperative to identify differences in microbial populations.

6.5. Problems with compliance and likely loss to follow-up:
6.5.i. Surgery with IFA v's surgery without IFA: participation in the main study is expected to be high because there is little risk or additional inconvenience for patients and potential gains from reduced risk of AL far outweigh the remote risk of side effects from ICG administration.

Compliance with data collection, including 90-day follow-up data, is expected to be high, with the aim to replicate the almost 100% compliance with short-term data collection achieved in the ROLARR trial. A 10% loss to follow-up has been built into the sample size to allow for any unforeseen events.

6.5.ii. CTA/CTp sub-study: participation in the CTA/CTp study will be optional and may be lower due to the need for an additional CT scan, requiring an additional hospital visit. But as only 75 patients, out of a total of ~450 UK patients, are required it is not envisaged that recruitment will be a problem. It is possible that patients who initially agree to partake in the CTA/CTp study subsequently withdraw or for logistical reasons there is insufficient time to undertake the additional scans prior to surgery. Further patients will be approached to make up the 75 patient cohort.

6.5.iii. Rectal microbiome sub-study: As only 200 patients, out of a total of ~450 UK patients, are required for this sub-study it is not envisaged that recruitment will be a problem. Detailed Patient Information Sheets explaining the study and what it involves will be provided.

7. Proposed outcome measures:
Primary outcome:
- clinical AL rate up to 90-days post-surgery.

The clinical AL rate has been chosen as the primary outcome measure because it reflects the incidence of leaks that cause clinically important morbidity and mortality. This is not to dismiss radiological leaks, which can have an impact on long-term bowel function, and are included as a secondary outcome measure. Clinical AL is defined as a defect of the intestinal wall at the anastomotic site (including suture and staple lines of neorectal reservoirs) leading to a communication between the intra- and extraluminal compartments that has an impact on patient management – see Section 4.1.

Secondary outcomes:
- change in planned anastomosis
- rate of defunctioning stoma
- postoperative complications (Clavien-Dindo classification)(38)
- length of hospital stay
- radiological anastomotic leak rate
- Low Anterior Resection Syndrome (LARS) score – patients without defunctioning ileostomy(39)
- rate of re-interventions
- quality of life (QLQ-C30, QLQ-CR38, EQ-5D)(40,41)
- health resource utilisation
- death within 90 days of operation
- Vascular anatomy and rectal perfusion and response to radiotherapy (mechanistic sub-study)
- Changes in rectal microbiome and correlation to AL (mechanistic sub-study)

Health economics: In line with NICE guidance(42), the within trial cost effectiveness study will take the perspective of the health and social care sector. Analyses will report the differences in the cost of health and social care service utilization between groups and the incremental cost effectiveness ratios using (i) the same primary outcome as the trial; and (ii) quality adjusted life years derived from the EQ-5D-5L. Resource use will be collected through the CRF (investigations, drugs, referrals for other services) and participant completed forms at the 4-week and 90-day assessment. The latter will be adapted from those used for cost effectiveness analysis in previous colorectal surgical trials (e.g. ROLARR). Unit costs for resources will be obtained from national sources such as the PSSRU, the BNF and NHS Reference cost database. Where national unit costs are not available the finance departments of hospitals participating in the study will be asked to provide local cost data. The mean of these costs will be used as the unit cost estimate in the analysis. The non-parametric bootstrap method will be used to produce a within-trial probabilistic sensitivity analysis of the incremental cost effectiveness ratio. In addition to presenting the expected incremental cost

effectiveness ratio, we will present the scatterplot on the cost effectiveness plane, the 95% cost effectiveness ellipse and the cost effectiveness acceptability curve(43).

8. Assessment and follow up
Data collection will occur at 4 time-points: baseline, operation, and 4-weeks and 90-days follow-up.

8.1. Assessment of efficacy/effectiveness:
Assessment of primary outcome – clinical AL rate: evidence of clinical AL will be collected prospectively. The majority of clinical ALs occur within 5-7 days of surgery, and will be detected during the inpatient stay, and recorded on the 4-week CRF. A few clinical ALs will occur following discharge and necessitating readmission. These will be recorded on the 4-week or 90-day follow-up CRFs. Clinical AL will align to the International Study Group category grade B or C leak and might be diagnosed as the passage of faecal content through an abdominal drain, radiological evidence of AL (by CT scan or contrast enema), or findings at laparotomy/laparoscopy. Quality assurance in the diagnosis of clinical AL will involve review of evidence (CT scan report, operation note, or contrast enema report) on a selected number of cases from each centre. The efficacy of IFA to prevent clinical AL will be assessed by adjusted estimation of the odds ratio of clinical AL in the IFA arm vs the non-IFA arm via a multi-level logistic regression model (see section 10 for more detail).

Assessment of secondary outcomes:
i) change in the planned anastomosis and use and rationale for defunctioning stoma will be recorded on the operative CRF
ii) length of hospital stay will be recorded on the perioperative CRF, which will also capture inpatient postoperative complications, categorized by the Clavien-Dindo classification.
iii) radiological anastomotic leak will be detected by rectal contrast enema performed between 4–6 weeks post-surgery and captured on the 90-day follow-up CRF.
iv) Low Anterior resection Syndrome will be determined using the validated LARS score(39), a patient reported outcome questionnaire collected at 4-weeks and 90-days follow-up
v) Quality of Life will be assessed at 4-week and 90-day follow-up using the validated QLQ-C30, QLQ-CR38, and EQ-5D-5L questionnaires along with the out- patient Health Resource Utilisation
v) death will be recorded at 90-days follow-up.

Mechanistic sub-studies:
i) CTA/CTp sub-study: variations in vascular anatomy and rectal perfusion will be analysed from 75 patients in the early part of recruitment. Measures of rectal blood supply and perfusion will be used to assess the effects of pre-operative characteristics of perfusion on the IFA assessment. The effect of radiotherapy on perfusion will also be explored by a comparison between those who did and did not receive chemo/radiotherapy, adjusting for potential confounding factors.
ii) Rectal microbiome: the rectal microbiome will be determined on 200 UK patients. Two exploratory investigations will be performed: i) bacterial 16S rRNA analysis to determine how the microbiome changes in response to surgery (samples taken at baseline, operation, postoperative) and its relationship to radiological anastomotic leak, and ii) changes in *Enterococcus faecalis* collagenase activity in response to surgery and the relationship to anastomotic leak. The potential causal relationship between clinical anastomotic leak and the microbiome and collagenase activity will be assessed via exploratory analyses of association, exploring how the estimated odds of clinical anastomotic leak change with respect to microbiome characteristics and collagenase activity, adjusting for potential confounding factors.

8.2. Assessment of safety:
For the purposes of this surgical trial, adverse events will be classed as complications. A complication will be defined as an untoward medical event in a participant, which has a causal relationship to the study. Information on all complications will be collected throughout the trial whether volunteered by the participant, or discovered by the investigator. As per Health Resource Authority (HRA) guidance, a serious complication will be defined as an untoward occurrence that meets any of the following criteria: (a) results in death; (b) is life-threatening; (c) requires hospitalisation or prolongation of existing hospitalisation; (d) results in persistent or significant disability or incapacity; (e) consists of a congenital anomaly or birth defect; or (f) is otherwise considered medically significant by the investigator.

Complications will be collected on trial case report forms and graded using the Clavien-Dindo classification. Any complications meeting the definition of a serious complication will require expedited reporting to CTRU within 24 hours of a research site being aware of an event. Any serious complications that are reported to be 'unexpected' (termed unexpected serious complication) will be reviewed by the Chief Investigator

and reported to the Research Ethics Committee (REC) and Sponsor. The type and duration of any complication will be according to local institutional policy.

The Trial Management Group, the Data Monitoring and Ethics Committee and Trial Steering committee will monitor safety. A progress report containing safety issues will be sent annually to the REC.

9. Proposed sample size:
Anastomotic leak following rectal cancer resection is variously reported between 8% and 25%. Recent evidence, taking into account advances in laparoscopic technique and stapler technology, documents a leak rate between 10% and 15%(44,45). The most relevant data available is from the COLOR II study(9) and the MRC/EME ROLARR trial (unpublished data). The COLOR II study randomised 1103 patients from 30 European centres to either laparoscopic and open rectal cancer surgery. The overall anastomotic leak rate was 13% in the laparoscopic group, but varied with the height of anastomosis, being 11%, 15%, and 11% in the upper, middle, and lower rectum, respectively. In the ROLARR trial, which compared laparoscopic with robotic rectal resection in 471 patients, the overall anastomotic leak rate was 10.2%.

We will assume an overall anastomotic leak rate of 12.0%, which is mid-way between that reported in COLOR II and that observed in ROLARR, and is a realistic value that most colorectal surgeons would accept. The only data on anastomotic leak rate using IFA is from the PILLAR II study, which reported 1.4% in 139 patients undergoing anterior resection. A conservative estimate of sample size has been calculated at 880 patients to show a reduction in leak rate from 12.0% to 6.0% - a 50% relative reduction in leak rate is considered to be the minimum clinically important difference - at a 5% level of significance with 80% power, allowing for a 10% drop-out rate.

A planned interim analysis will be undertaken once primary endpoint data has been collected for 554 patients. The interim analysis will allow the possibility of early stopping due to evidence of efficacy with respect to the primary endpoint. The O'Brien-Flemming spending function - a well-established, conservative approach to alpha spending - has been used to determine the test boundaries whilst maintaining the overall type I error rate of the trial.

To recruit 880 patients, 25 centres will be involved – 13 from the UK and 12 from Europe. We are keen to build on the success of the MRC/EME ROLARR trial, and the productive collaborations established, so as to maximize the chances of successful recruitment. To do so, we plan to involve European ROLARR centres that demonstrated an ability to recruit large volumes of patients and comply with data follow-up. There are more centres in Europe than the UK with experience in IFA (PINPOINT/laparoscopic and FIREFLY/robotic), which will greatly help recruitment. We have identified 18 robotic centres (15 European, 3 UK) and 23 laparoscopic centres (5 European, 18 UK) interested in participating. 9 of the 18 robotic centres already have the FIREFLY technology, and 7 of the 23 laparoscopic centres already have the PINPOINT technology. From these 41 centres we will prioritize 25 with a demonstrable track record of high patient recruitment, commitment to collaborative research, and resources for data collection and follow-up, leaving 16 reserve centres in case of slower than expected recruitment. Each centre will be expected to recruit 12 patients per year for 3-years (total of 36 patients per centre) to achieve the recruitment target; this was achieved by high volume recruiting centres in ROLARR. By way of example, Leeds Teaching Hospital NHS Trust is a high volume UK centre for rectal cancer, serving a population of ~900,000 and undertaking ~120 rectal cancer resections per year. Recruitment of 1 in 10 of the annual rectal cancer population should be very achievable.

The MHRA have indicated that the use of ICG in this study does not constitute a CTIMP.

Commercial support has been obtained to supply PINPOINT laparoscopic systems (Elemental Healthcare/Novadaq Technologies Inc.) and FIREFLY robotic systems (Intuitive Surgical Inc.) to centres without the technology and at no additional cost for the duration of the study – see Letters of Support.

10. Statistical analysis:
Analysis and reporting will be in line with CONSORT. The primary analysis will compare clinical AL rates between the arms using multi-level logistic regression incorporating random effects with respect to surgeon and adjusting for the stratification factors. This approach will be used to test the two-sided hypothesis that the anastomotic leak rate is equal in both arms (i.e. an odds ratio of 1), considering the confidence interval and the p-value yielded by a Wald test of the treatment allocation regression coefficient. At the interim analysis, a p-value less than 0.0146 will be considered to be sufficiently strong evidence of efficacy for early stopping (as per the O'Brien-Flemming alpha spending function). At the final primary analysis, a p-value less than 0.0456, rather than 0.05, will be considered as "significant" in order to maintain the overall

type I error rate (as per O'Brien-Flemming).

Only the primary endpoint will be assessed at the interim analysis. Secondary endpoints will be assessed at the final analysis.

Secondary endpoints with binary measures (change in planned anastomosis, defunctioning stoma, complications, radiological anastomotic leak and death) will also be analysed using multi-level logistic regression adjusting for the stratification factors, incorporating random effects with respect to surgeon.

Secondary endpoints with continuous measures – length of stay, LARS score, other QoL scores – will be analysed using multi-level generalised linear models incorporating random effects with respect to surgeon and assuming Normal errors at the patient level. If the assumption of Normal errors is clearly violated by the observed response data, then transformations of the response variable as well as alternative distributional assumptions (e.g. Gamma) will be considered, and the choice of a transformation and/or an alternative distribution will be driven by comparative measures of model fit. Models for the LARS score and other QoL measures, which are measured at multiple time points, will also include an additional level to account for repeated measures - i.e. repeated measures (level 1) nested within patient (level 2) nested within surgeon (level 3) – so that longitudinal effects can be assessed.

11. Ethical arrangements:

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964, and subsequent amendments.

A Patient Information Sheet will contain detailed information about the rationale, design and personal implications (including any possible benefits and known risks). Patients will be allowed as much time as necessary to consider their participation. Informed written consent will be obtained from assenting patients prior to any trial specific investigation or randomisation into the study. The right of a patient to refuse participation without giving reasons will be respected. The patient remains free to withdraw from the study at any time without giving reasons and without prejudicing his/her further treatment.

The study will be submitted to and approved by a main REC and by the R&D department for each participating centre prior to entering patients into the study. Approval will also be sought for any substantial amendments required during the course of the study. A detailed trial protocol, patient information and consent forms will be developed in the pre-set-up period (<0 months) and will form the basis of the ethics application which will be completed by the Trial Manager for review and approval by the wider project team and Chief Investigator prior to submission. The trial manager will work closely with the PPI representatives in development of the patient information which will be submitted as part of the application for ethical approval.

12. Research Governance

The University of Leeds has agreed to take on sponsorship of this trial. The trial will be conducted in accordance with the principals of GCP in clinical trials and the NHS Research Governance Framework (RGF). The MHRA have indicated that this proposal is not a Clinical Trial of an Investigational Medicinal Product (IMP) as defined by the EU Directive 2001/20/EC therefore no submission to the MHRA is required. Ethical approval will be sought through the Health Research Authority (HRA). The trial will be submitted to and approved by a REC and the appropriate Site Specific Assessor for each participating centre prior to entering patients into the study. For non UK centers, it will be the contracted responsibility of the Principal Investigator at each site to ensure compliance to local standards of Clinical Governance and ethical approval.

Trial supervision will be established according to the principals of GCP and in-line with the NHS (RGF). This will include establishment of a core Project Team, Trial Management Group (TMG), an independent Trial Steering Committee (TSC) and an independent Data Monitoring and Ethics Committee (DMEC). The trial will be led by the Chief Investigator, Professor David Jayne. The day-to-day management, trial coordination, data management and statistical oversight will be provided by the project team at CTRU. The Clinical Research Fellow will manage the clinical set-up at sites. A Trial Management Group comprising the CI, Clinical Research Fellow, CTRU team, co-applicants and PPI representative (Mr Andrew North) will be established to implement the trial and manage the ongoing running of the trial including the clinical and practical aspects. The TMG will meet either face to face or via teleconference monthly during pre set-up, 3-6 monthly during set-up, recruitment and follow-up (or as required by trial progress) and will report on progress to the independent TSC (who will meet during set-up and then 6 monthly) and to the DMEC

(meeting during set-up and then annually during recruitment). All oversight groups will pull together expertise from the necessary organizations and disciplines, both clinical and methodological, to ensure the smooth running of the project and adherence to key milestones.

At the end of the study, all data held by CTRU and all study data will be securely archived in line with the Sponsor's procedures for a minimum of 10 years.

13. Project timetable and milestones:
The project milestones are illustrated on the Gantt chart (Section 14), and include:

Pre-set-up: <0 months: IRAS application and protocol and related document development, ethical approval, contractual requirements identification, formal confirmation of sponsorship. Identification of project team, TMG, TSC and DMEC members.

Set-up: 1 – 12 months: contracts finalized, oversight groups confirmed, design of CRFs, development and testing of trial database, establishment of the 24 randomisation system, centre approvals and initiations, development of trial specific guidance and work instructions, writing statistical analysis plan, first meeting of the TSC/DMEC. A launch meeting will be held for all collaborators during this period.

Recruitment: 13 – 48 months: recruitment of 880 patients from 25 centres; 13 UK and 12 European. The recruitment phase will involve collection, monitoring and cleaning of data (including clinical, QoL and health economics data), report generation. A Go/NoGo time point has been set at month 25 to include the opening of at least 15 centres and recruitment of at least 150 patients. It is anticipated that the CTA/CTp substudy, involving 75 UK patients, will be completed within the first 18-months of recruitment (study month 30). Data will be analysed separately from the main study, allowing publication of the results by study month 38. It is anticipated that collection of samples for the rectal microbiome sub-study, involving 200 UK patients, should be completed within the first 24-months of recruitment (study month 36). Data will be analysed separately from the main study, allowing publication of the results by study month 45. The team recognizes that this would involve revealing information about the overall radiological AL rate in these 200 patients while the main trial is ongoing. However, we do not believe that this is cause for concern, since this interim data will not offer information about the primary endpoint (clinical AL) or allow inferences about how the rates compare between arms to be made with any certainty. It involves a small subpopulation only, and therefore we do not believe that it would influence the behavior of investigators in the main trial since it would not reveal critical information about the efficacy of IFA.

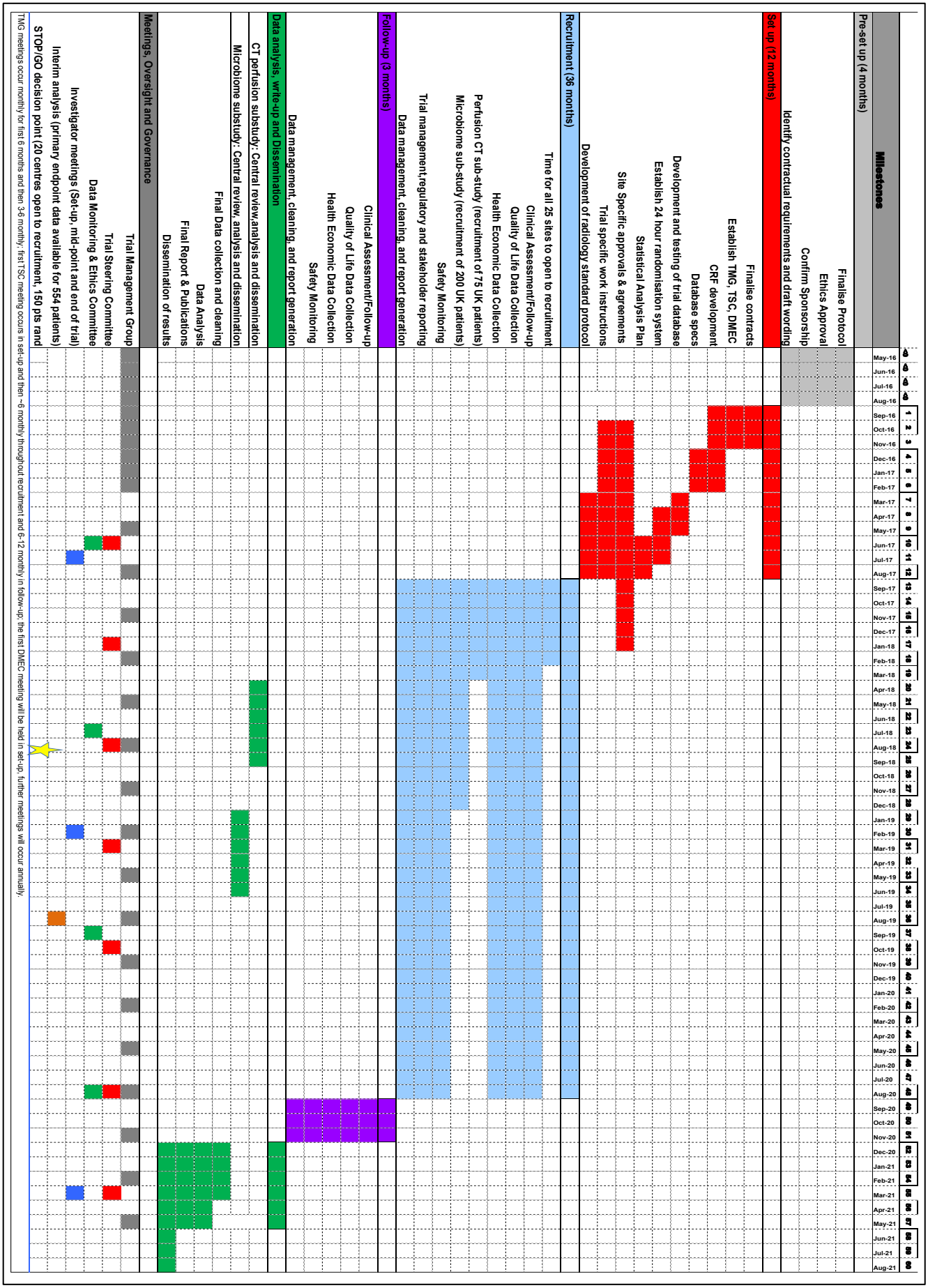
An interim analysis will be undertaken once complete primary endpoint data has been obtained for 554 patients.The interim analysis allows for the potential of early stopping due to efficacy. Therefore there is a possibility that, given significant evidence of efficacy at this interim point (which is expected to be around month 36 - month 24 of recruitment), the trial will finish recruiting and move into the follow-up period earlier than planned and the funder reimbursed accordingly.

Progress of the study will be kept under constant review by the TMG, TSC, and DMEC. TSC meetings will be 6-monthly and DMEC meetings 12-monthly throughout recruitment. A mid point investigator meeting is also planned during this stage of the trial.

49 – 51 months: completion of 90-day clinical follow-up, follow-up data collection, monitoring and cleaning report generation.

52– 57 months: data cleaning, data analysis, write-up, and dissemination. It is anticipated that dissemination will continue beyond the formal end of the grant.

14. Gantt chart:



15. Deliverability

Ensuring sample size is realistic and achievable: The research team believes the sample size is realistic and achievable based on experience from the ROLARR trial and that recruitment of only 36 patients over 3-years from high volume sites is very conservative.

Appropriate recruitment strategy: the project team, TMG and oversight committees will closely monitor recruitment. Screening logs will be completed on a 3-monthly basis to identify any potential barriers to recruitment.

Consideration of subject attrition: The sample size calculation allows for a rate of attrition of 10%. Our experience in ROLARR, which was conducted in a similar patient population and had excellent compliance, combined with the short follow-up period of 90-days, suggests that 10% is a prudent estimate. It is considered unlikely that the study will be left underpowered by a higher than anticipated rate of attrition.

Ensuring sufficient resource, expertise and facilities at each site: 41 potential sites have already been identified. Prior to initiating set-up at a site, a feasibility assessment will be undertaken to ensure sufficient resource, expertise and facilities. We will work closely with the Yorkshire and Humber CRN to resolve any issues experienced with UK sites, particularly with identification of local research support. We have successfully worked with a number of the UK and European sites in the NIHR EME ROLARR trial.

Potentially competing studies for recruitment: review of the NCRI Colorectal Cancer Clinical Studies Group site specific portfolio map identifies 9 studies in rectal cancer: three involve the use of neoadjuvant regimens, one investigates a preoperative physical fitness programme, one tests the use of drug treatments to minimize long-term consequences of radiotherapy, one is recruiting patients with locally advanced cancers for pelvic exenteration, and one is an observational study of rectal irrigation for anterior resection syndrome. Of the other two studies, both are pilot studies that have completed recruitment. This includes the only other study to investigate a surgical technique (TREC), which recruited patients with early stage rectal cancer. The research team is aware that a follow-on study from TREC, STAR-TREC, is due to commence setup in 2015/16. This will investigate the treatment of early rectal cancer using local or radical excision with or without radiotherapy. Thus, there are no current rectal cancer studies that would compete with IntAct for patients; any studies involving preoperative investigation or treatment would still be eligible.

16. Expertise:

D Jayne is Prof. of Surgery, Uni of Leeds, Consultant Surgeon at Leeds Teaching Hospitals NHS Trust (LTHT), and NIHR Research Professor. As CI he will be responsible for the conduct and deliver of the study. He brings expertise in laparoscopic and robotic surgery and experience as CI for MRC/EME ROLARR, HTA/NIHR FIAT, MRC/EME GLiSten, and HTA/NIHR SaFaRi. He is Clinical Director of the NIHR HTC in Colorectal Therapies, a national network to accelerate the translation of new technologies into clinical practice. He will supervise a 1.0FTE research fellow to facilitate coordination with Leeds CTRU and UK and European sites, help with troubleshooting, training events, recruitment, and trial promotion etc.

D Miskovic is Clin. Assoc. Prof, Uni. of Leeds, and Consultant Surgeon at LTHT. He brings expertise in laparoscopic surgery for cancer and a research interest in anastomotic leak. He will assist in coordination of the UK and European recruitment.

M Coleman is Assoc. Prof. at Plymouth Uni. Peninsula School of Medicine and Dentistry and Consultant Surgeon at Derriford Hospital. He brings expertise in laparoscopic surgery, including National Lead for LAPCO National Training Programme.

R Cahill is Prof. of Surgery, Uni. College Dublin and Consultant Surgeon at Mater Misericordiae Uni. Hospital. He brings expertise in laparoscopic surgery, including interests in NIR imaging technologies.

PR O'Connell is Prof. of Surgery, Uni. College Dublin and Consultant Surgeon at St. Vincent's University Hospital. He brings expertise in colorectal surgery and international leadership as Past President of the European Society of Coloproctology. Research interests include the role of the microbiome in GI disease.

D Tolan is Consultant GI Radiologist at LTHT. He brings expertise in gastrointestinal radiology with clinical trials radiology expertise as Co-I on MRC/EME GLiSten, HTA/NIHR FIAT, and MeTRIC.

V Goh is Prof. of Cancer Imaging at King's College London and Past President of the European Society of Oncologic Imaging. She brings expertise in preclinical and clinical functional & molecular imaging in anorectal cancer, including CI for HTA/NIHR PROSPeCT and Co-I on HTA/NIHR STREAMLINE,

EME/NIHR MALIBO, CRUK BACCHUS & PLATO. She will be responsible, along with D Tolan, for training, coordination, delivery, and quality assurance of the CT angiography/CT perfusion sub-study.

A North is a Chartered Accountant who has undergone rectal cancer surgery complicated by an anastomotic leak. He has contributed to the design and reviewing of this proposal and will be an integral member of the TMG. He will be support by the NIHR HTC PPI group and Bowel Cancer UK.

J Brown is Prof. of Clinical Trials Research and Director of the Leeds CTRU. She brings expertise in the design and delivery of surgical trials, including MRC/EME ROLARR, MRC/EME GLISTEN and HTA/NIHR SaFaRI. She will take overall responsibility for statistical and clinical trials input for the study. She will be assisted by *N. Corrigan*, Senior Medical Statistician at the Leeds CTRU, who specializes in the design and analysis of surgical trials and *J. Croft*, Senior Clinical Trials Manger, who will be responsible for day-to-day trial coordination. Both N. Corrigan and J. Croft have extensive experience in surgical trials research as Co-I's for MRC/EME ROLARR, MRC/EME GLiSten and HTA/NIHR SaFaRi.

*C Hulm*e is Prof. of Health Economics, Uni of Leeds, with expertise in economic evaluation, including lead health economist on HTA/NIHR FIAT and HTA/NIHR SaFaRi. She will be responsible for the conduct and deliver of the health economics evaluation.

P Quirke is Prof. of Histopathology, Uni. of Leeds and an NIHR Senior Investigator. He has extensive experience in pathological assessment and molecular biology within clinical trials as Co-I for ClasicC, Rolarr, Enrol, Foxtrot, CR07, Piccolo, Focus 3 and FOCUS 4 He brings the expertise, next generation sequencing capability, and laboratory facilities to undertake the laboratory investigation for the rectal microbiome sub-study. *A Kirby*, Clin. Assoc. Prof. in Microbiology, brings expertise in GI microbiology with responsibility for the rectal microbiome bacterial assays.

17. Service Users:

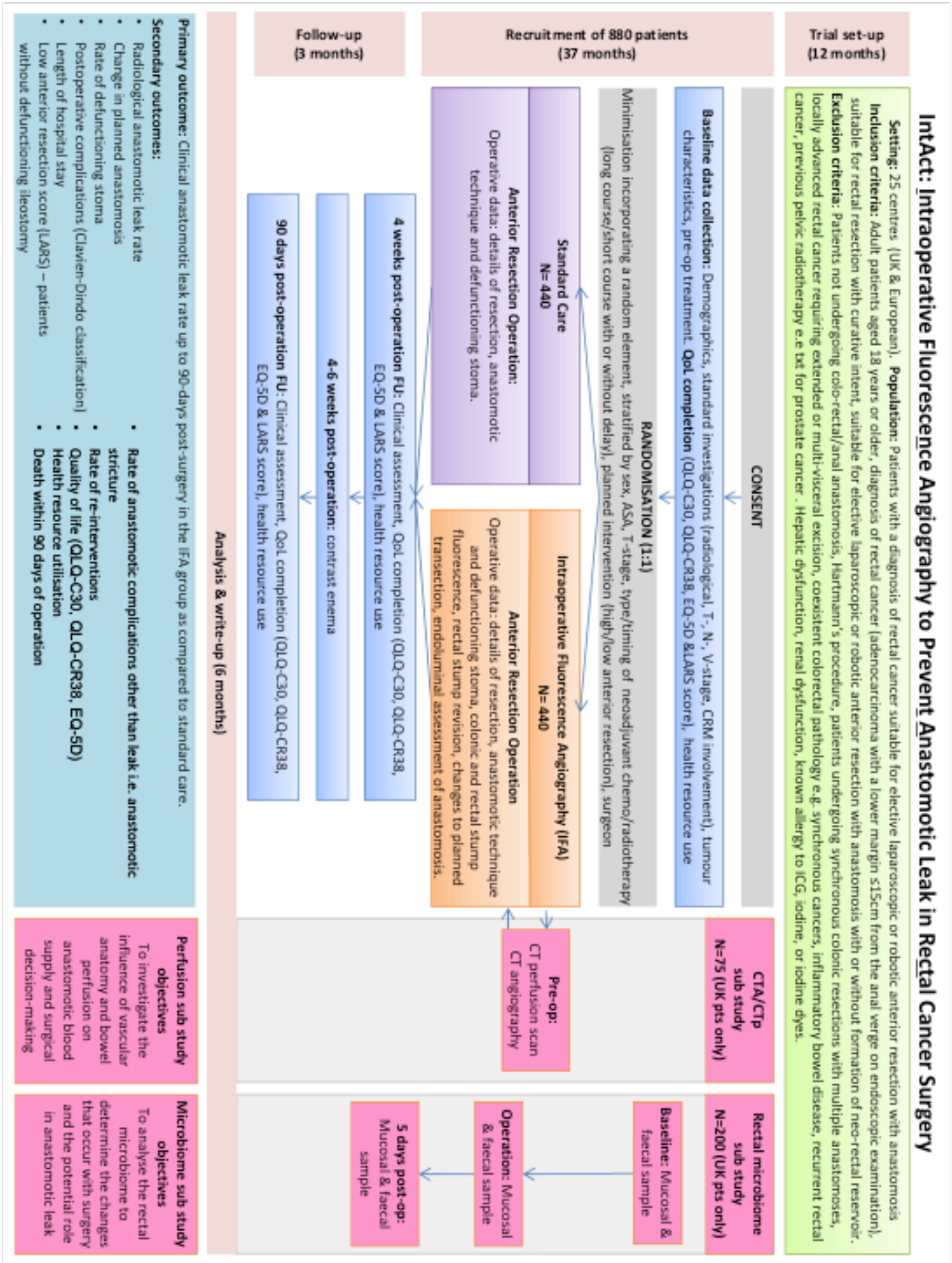
The research team has worked closely with the NIHR HTC in Colorectal Therapies in developing this proposal. The HTC is a national network of partners interested in colorectal disease, and includes patient and public representatives, clinicians, healthcare managers, and commercial partners. Several of the research team are members of the HTC. Involvement of the HTC PPI group, and other PPI groups, such as Leeds Cancer PPIR, has been instrumental in structuring the design of the study. The inclusion of two sub-studies has posed logistical dilemmas, involving additional investigations and hospital visits. The research team is particularly grateful for PPI input in making the study as acceptable as possible to patients. The research team has also worked closely with Bowel Cancer UK, the largest forum for patients and public affected by bowel cancer. The proposal has been reviewed by Bowel Cancer UK, who are fully supportive.

The research team has consulted with other service users, including surgeons, operating theatre personnel, hospital managers, and GPs. A warm response has been received from surgeons, who recognize the urgent need to address anastomotic leak, and see potential in the IFA technology. We have support from 41 surgeons who are interested in participating. Operating theatre personnel are also supportive. Those that have seen the technology in practice recognize the potential benefits for little additional burden on theatre time or resources. The business case for IFA is highly persuasive, given the resource implications and cost of an anastomotic leak, and hospital managers are interested in the potential cost-benefit of IFA. The views of these service users have been taken into account when designing the various components of the study.

We will continue to work with all service users throughout the study. Mr Andrew North, and other PPI representatives, will be actively involved as members of the TMG, TSC, and DMEC. We have good clinical representation with 5 surgeons, 2 radiologists, a pathologist, and a microbiologist as Co-I's and members of the TMG. We will seek to include operating personnel, nursing staff, and hospital managers on the various committees to ensure we gain a comprehensive perspective of service implications.

We will continue to work with Bowel Cancer UK, and other PPI groups, throughout the study to make the study as patient-centred as possible. Costings have been included for PPI and Bowel Cancer UK time in accordance with INVOLVE recommendations. Bowel Cancer UK, in particular, will be very useful in the dissemination of research outputs given their large membership and UK-wide reach. To ensure maximal breath of dissemination we will liaise with professional organisations (e.g. ACPGB&I), healthcare manager organisations, and national commissioning and advisory bodies (NHS England, NICE).

Appendix 1: Flow Chart



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