

IntAct: Intraoperative Fluorescence Angiography to Preve**nt A**nastomotic Leak in Re**ct**al Cancer Surgery

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3 TRIAL SUMMARY

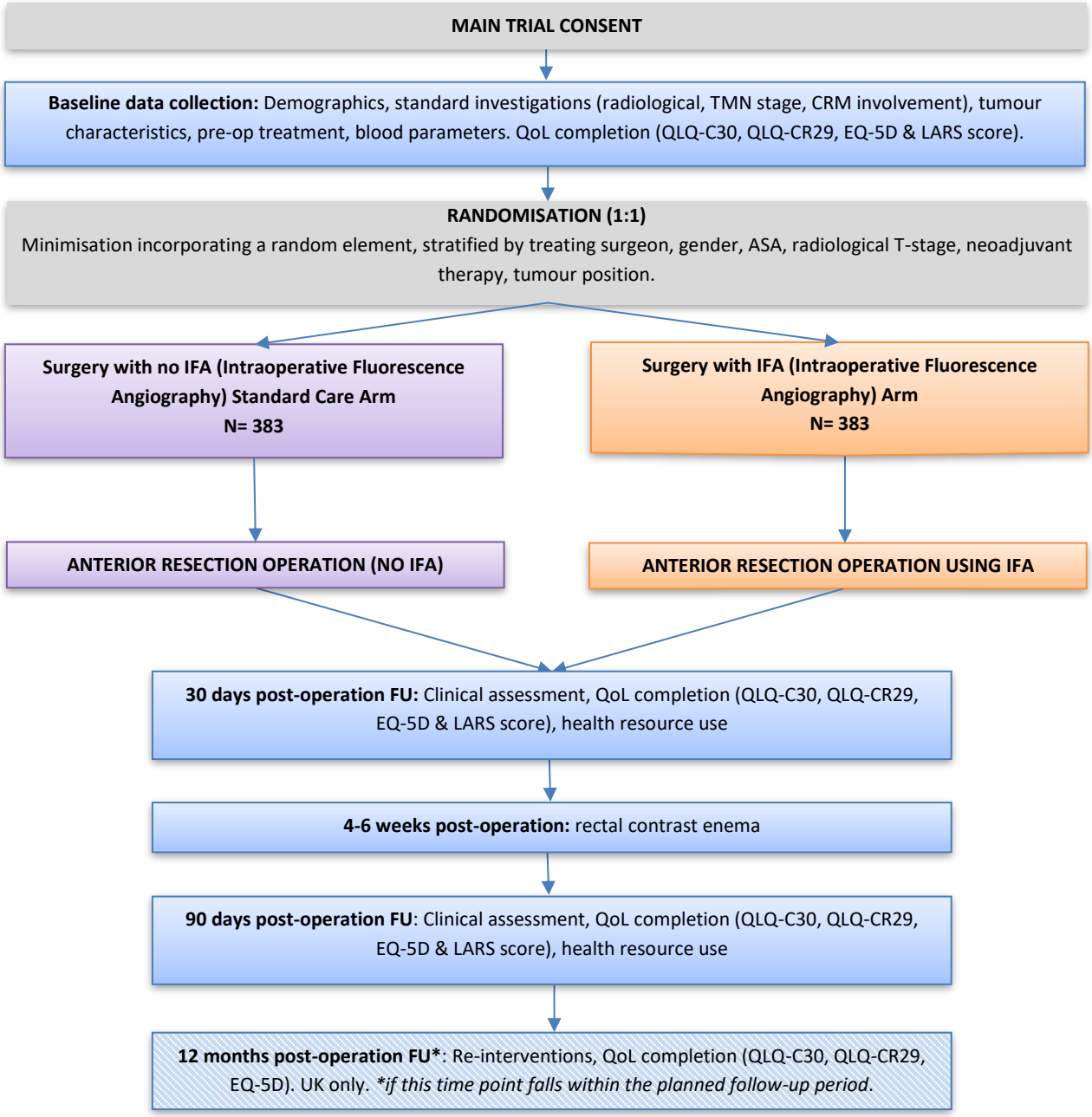
Trial Title	**I**ntraoperative Fluorescence Angiography to Preve**nt** **A**nastomotic Leak in Re**ct**al Cancer Surgery
Trial Acronym	IntAct
Trial Background	Colorectal cancer is the third most common cancer in the UK, with 30% of cases involving the rectum. Surgery can offer a cure, but comes at a cost in terms of morbidity and mortality. The most feared complication of rectal cancer surgery is anastomotic leak, which is reported in 10% to 15% of patients. Approximately 30,000 – 40,000 colorectal anastomoses are constructed each year in the NHS, most usually for colorectal cancer. Anastomotic leak is therefore a significant healthcare burden. It has a negative impact on patient recovery and consumes NHS resources for remedial interventions. Patients who survive anastomotic leak suffer long-term consequences with reduced quality of life, high rates of wound complications and permanent stoma, and increased risk of cancer recurrence. 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. Recently, intraoperative fluorescence angiography (IFA) has been introduced to evaluate anastomotic blood supply, with promising early results
Trial Design	A prospective, unblinded, parallel group, non-CTIMP, multicentre, European, randomized controlled trial comparing surgery with IFA against standard care (surgery with no IFA) to determine the effect on anastomotic leak in patients undergoing elective anterior resection for rectal cancer. A sub-study will explore the mechanisms of anastomotic leak.
Trial Aim	The aim of this research is to investigate the efficacy and mechanism of a new technology, intra-operative fluorescence angiography (IFA), in reducing anastomotic leak rate following elective rectal cancer surgery. The comparator will be standard white light endoscopic surgery, using either a laparoscopic or robotic technique.
Trial Endpoints	**Primary endpoint:** • Clinical anastomotic leak rate within 90 days post-operation **Secondary endpoints:** • Anastomotic leak rate within 90 days post-operation • Changes in planned anastomosis, i) including the decision to undertake a permanent stoma rather than an anastomosis, ii) the site of proximal bowel used for anastomosis, iii) the site of rectal remnant used for anastomosis and iv) the decision to undertake a diverting stoma. • Rate of stoma (temporary or permanent) • Operative and post-operative complications (Clavien-Dindo for complication-level classification and Comprehensive Complication Indicator for patient-level classification) within 90 days of operation • Length of post-operative hospital stay

	• Low Anterior Resection Syndrome (LARS) score at 30 days and at 90 days post-operation – participants without defunctioning ileostomy • Rate of re-interventions within 90 days and within 12 months[1] • Quality of life (QLQ-C30, QLQ-CR29, EQ-5D) at 30 days, 90 days and 12 months[1] post-operation • Health resource utilisation at 30 days, 90 days and 12 months[1] post-operation. • Death within 90 days of operation • Rectal microbiome profile (mechanistic sub-study)
Trial Population:	766 participants, aged ⩾ 18 years with a diagnosis of rectal cancer, suitable for curative resection by high or low anterior resection (laparoscopic or robotic) with anastomosis.
Randomisation:	Participants will be randomised on a 1:1 basis to receive either surgery with or without IFA. Randomisation will be performed by the Clinical Trials Research Unit (CTRU), Leeds.
Trial Intervention:	**Surgery with no IFA:** the anterior resection (high or low) will be performed according to the surgeon's usual technique, using either a laparoscopic or robotic approach, with white light assessment of bowel perfusion. The specifics of each operation will be at the discretion of the operating surgeon. **Surgery with IFA:** the anterior resection (high or low) will be performed according to the surgeon's usual technique, using either a laparoscopic or robotic approach. ICG will be administered intravenously at two points during the operation for perfusion assessment using near-infrared laparoscopy. The specifics of each operation, including the decision to make a change to the planned anastomosis following IFA assessment, will be at the discretion of the operating surgeon.
Duration:	All participants will be followed up to 90 days post-operation
Evaluation of outcome measures	Participants will be assessed at 30 days and 90 days post-operation, with a rectal contrast enema taking place between 4-6 weeks post-operation. Quality of Life (QoL) and participant reported outcomes (assessed using the EQ-5D-5L, EORTC-QLQC30, EORTC-QLQCR29 and Low Anterior Resection Score (LARS) questionnaires) and resource use will be measured at 30 days and 90 days post operation. Complications will be documented during trial treatment and follow-up.

[1] for UK participants only for whom the 12 months post-operation time point falls before the end of the planned follow-up period i.e. 90 days following the last participant's operation.

4 TRIAL SCHEMA

Setting: 25 centres throughout Europe. **Population:** Patients with a diagnosis of rectal cancer suitable for elective laparoscopic or robotic anterior resection with anastomosis**. Inclusion criteria:** Adult patients aged 18 years or older, diagnosis of rectal cancer (defined as a lower margin ≤15cm from the anal verge on endoscopic or radiological examination), suitable for curative high or low anterior resection, suitable for elective laparoscopic or robotic anterior resection with anastomosis, ASA ≤3. **Exclusion criteria**: Patients not undergoing colo-rectal/anal anastomosis, patients undergoing synchronous colonic resections, locally advanced rectal cancer requiring extended or multi-visceral excision, coexistent colorectal pathology, recurrent rectal cancer, previous pelvic radiotherapy. Hepatic dysfunction, renal dysfunction, known allergy to ICG, iodine, or iodine dyes, immunocompromised patients, Pregnancy.



5 BACKGROUND

Colorectal cancer is the third most common cancer in the UK, with 30% of cases involving the rectum. Surgery can offer a cure, but comes at a cost in terms of morbidity and mortality. The most feared complication of rectal cancer surgery is anastomotic leak, which is reported in 10% to 15% of patients. Approximately 30,000 – 40,000 colorectal anastomoses are constructed each year in the NHS, most usually for colorectal cancer. Anastomotic leak is therefore a significant healthcare burden. It has a negative impact on patient recovery and consumes NHS resources for remedial interventions. It increases morbidity from ~20% to ~60%, mortality from <5% to ~20%, and extends in-patient stay from an average of 7 to 19 days [1, 2]. The mean cost of care for patients undergoing uncomplicated colorectal surgery is ~£17,000, increasing to ~£45,000 in those who suffer complications [3]. Patients who survive anastomotic leak suffer long-term consequences with reduced quality of life, high rates of wound complications and permanent stoma, and increased risk of cancer recurrence [4].

5.1 Anastomotic Leak

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 16% of patients who suffer an anastomotic leak die within 30 days of their operation, with the remainder suffering protracted hospital stays and significant long-term morbidity [5]. 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 10cm of the anal verge are up to 6.5-times more likely to leak than at more proximal sites [6-8]

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 [9]. 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 [10]. 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 [11].

In rectal cancer surgery, the current rate of AL is variously reported between 8% and 25% [12]. 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 [13]. 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 10.7% (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.) [14]. 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[15].

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 [16, 17]. Unfortunately, assessment of tissue perfusion is difficult at operation as demonstrated by the surgeon's inability to predict AL: in a study by Karliczek *et al*., surgeons were only able to correctly predict AL in 11% of cases, with low sensitivity (41%) and specificity (59%) [18].

5.2 Assessment of anastomotic integrity

The current standard for intraoperative testing of rectal anastomotic integrity involves "air-leak" testing and assessment of completeness of anastomotic "doughnuts". Air-leak testing is easy and cheap and has been shown to more than halve the radiological AL rate [18]. 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 [19]. 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 [20].

5.3 Emerging Technologies

5.3.1 Fluorescence angiography

Fluorescence angiography has been used to evaluate blood flow and tissue perfusion in many areas of medicine, including general surgery [21]. Recently, intraoperative fluorescence

angiography (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 1 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 1: 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* analysed 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.

5.4 Gut microbiome as an infective cause for AL

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 [12]. 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 *Enterococcus* and *Eschierichia/Shigella* species respectively [26]. Importantly, this difference was only apparent in anastomotic tissue and not luminal faecal samples (Figure 2).

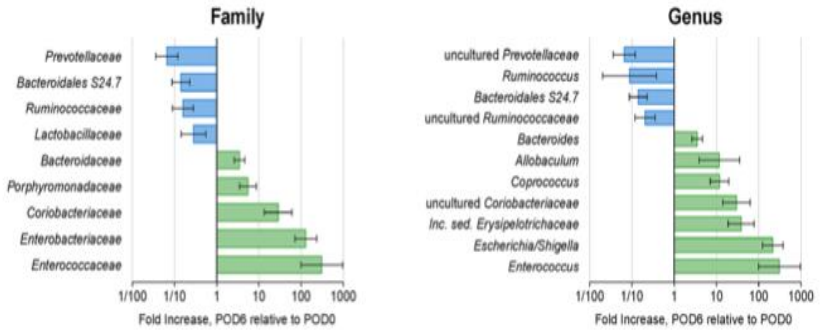


Figure 2: comparative analysis of bacterial abundance in anastomotic tissue between postop day-0 and day-6, showing marked elevation in Enterococcus and Escherichia/Shigella species [26].

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].

5.5 RATIONALE FOR INTACT

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 [6, 8] whilst those below 5cm from the anal verge have a 6.5-fold risk of AL [7]. 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.

IFA has become available in the NHS and has the potential to significantly reduce the rate of AL. It is a safe technology that involves intraoperative injection of a fluorescent molecule, Indocynanine Green, which can be visualized with a near-infrared laparoscope. It allows real-time, intraoperative assessment of tissue perfusion, which is the single most important factor in determining anastomotic healing. Initial results of IFA in colorectal surgery have been extremely promising. If the efficacy of IFA can be confirmed, the implications for safer surgery and cost reduction to the NHS will be considerable. The outputs from this research will enable us to better understand the evaluation of bowel perfusion and its role in determining AL. In turn, this may help to define optimal strategies to prevent its occurrence. In addition, we will gain valuable information from an important sub-study, exploring the contribution of the rectal microbiome to AL.

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 [1]. 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 [1, 2]. In patients undergoing cancer surgery, anastomotic leak has an adverse effect on local recurrence and cancer survival [4]. 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 [3]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.

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, intra-operative fluorescence angiography (IFA) 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 a sub-study evaluating the role of the rectal microbiome in AL, adds an exciting dimension that will further inform our understanding of the mechanisms underlying AL.

6 AIMS AND OBJECTIVES

6.1 AIM

The aim of this research is to investigate the efficacy and mechanism of a new technology, intra-operative fluorescence angiography (IFA), in reducing anastomotic leak rate following elective rectal cancer surgery. The comparator will be standard white light endoscopic surgery, using either a laparoscopic or robotic technique. A mechanistic sub-study will explore the rectal microbiome and association with anastomotic leak.

6.2 OBJECTIVES

Main trial objectives:

To assess whether surgery with IFA, compared to standard care:

1. Reduces anastomotic leak rate following rectal cancer surgery
2. Alters intra-operative decision making with regards to anastomosis construction.
3. Results in reduction in stoma rate (temporary or permanent)
4. Improves patients' quality of life
5. Results in cost savings for the NHS.

Sub-study objectives:

1. To assess changes in the rectal microbiome as a result of surgery and its association with anastomotic leak.

6.3 Outcomes

Primary outcome:

Clinical anastomotic leak rate within 90-days post-operation.

Clinical anastomotic leak is defined, as per the International Study Group definition[11], as a confirmed 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. In particular, an abscess or fluid collection in close proximity to the anastomosis will be deemed as an anastomotic leak. This equates to Grades B & C in the International Study Group definition of anastomotic leaks (see appendix 1 for a description of each grade).

Secondary outcomes:

- Anastomotic leak rate within 90 days post-operation.
  Anastomotic leak is defined, as per the International Study Group definition[11], as a confirmed 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. In particular, an abscess or fluid collection in close proximity to the anastomosis will be deemed as an anastomotic leak. This includes Grades A, B & C in the International Study Group definition of anastomotic leaks (see appendix 1 for a description of each grade).
- Changes in planned anastomosis, including i) the decision to undertake a permanent stoma rather than an anastomosis, ii) the site of proximal bowel used for anastomosis, iii) the site of rectal remnant used for anastomosis, and iv) the decision to undertake a diverting stoma.
- Rate of stoma (temporary or permanent)
- Operative and post-operative complications (Clavien-Dindo for complication-level classification and Comprehensive Complication Indicator for patient-level classification) within 90 days of operation
- Length of post-operative hospital stay
- Low Anterior Resection Syndrome (LARS) score at 30 days and at 90 days post-operation – participants without defunctioning ileostomy
- Rate of re-interventions within 90 days and within 12 months[2]
- Quality of life (QLQ-C30, QLQ-CR29, EQ-5D) at 30 days, 90 days and 12 months[2] post-operation
- Health resource utilisation assessed at 30 days 90 days, and 12 months[2] post-operation.
- Death within 90 days of operation
- Rectal microbiome profile (mechanistic sub-study)

7 DESIGN

7.1 Main Trial

A prospective, unblinded, parallel group, non-CTIMP, multicentre, European, randomised controlled trial comparing surgery with IFA against standard care (surgery with no IFA) to determine the effect on anastomotic leak 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.

880 participants will be randomised prior to surgery, on a 1:1 basis, to either surgery with IFA or surgery without IFA using minimisation (incorporating a random element). The follow-up period ends 90 days after the last participant's operation. The trial will not be blinded to participants, medical staff, or clinical trial staff.

[2] for UK participants only for whom the 12 months post-operation time point falls before the end of the planned follow-up period i.e. 90 days following the last participant's operation.

7.2 Rectal microbiome sub-study (UK participants only)

Recruitment to microbiome sub-study is now complete.

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 anastomotic leak, and ii) changes in collagenase activity in response to surgery and the relationship to anastomotic leak. The potential causal relationship between anastomotic leak and the microbiome and collagenase activity will be assessed via exploratory analyses of association, exploring how the estimated odds of anastomotic leak change with respect to microbiome characteristics and collagenase activity, adjusting for potential confounding factors.

The rectal microbiome sub-study will be performed in UK patients recruited into the trial up until January 2023, when the sample collection for this sub-study finished. Participants entered into the microbiome sub-study underwent faecal sampling (from the rectal mucosa and rectal lumen at baseline, operation, and day 3-5 postoperative, using rectal swabs (as detailed in Table 1).

Target	Sample	Sampling method
Luminal bacteria	Faeces in the lumen of the rectum	Rectal swab
Mucosal bacteria	Faeces at the rectal mucosal	Rectal swab

Table 1: Microbiome sub-study sample description.

Samples were transported to Leeds for processing. These samples will be used for analysis of bacterial16S rRNA and assays of collagenase activity.

Samples will be spun down and the pellet will undergo DNA extraction using our optimized protocol. The DNA yield will be measured by nanodrop. DNA from the V4 region of the 16S rRNA gene will be amplified according to the Earth Microbiome Project protocol and NGS will be performed. Quantitative PCR to specific species of interest will be performed.

Previous work has investigated the role of Enterococcus and Pseudomonas in anastomotic leakage and identified potential causal mechanisms in the development of anastomotic leak associated with their collagenase activity. Other species have not undergone such formal assessment for their role in anastomotic leakage. We will therefore screen faeces, collected by rectal swabs, for the presence of collagenase producing bacteria. Isolates with high collagenase activity will be speciated and have their collagenase activity quantified. These results will be analysed in relation to clinical outcomes i.e. anastomotic leakage.

Bioinformatics: Reads will be stripped of adaptor sequences using cutadapt. Quality plots will be examined and a decision made to truncate low quality bases using the DADA2 package within QIIME2. Reads will be filtered, de-noised, merged as pairs and representative sequences will be chosen using the DADA2 package within QIIME2.

Shannon index alpha diversity will be calculated and significance will be assessed by the Kruskal-Wallis test in QIIME2. Bray-Curtis beta diversity will be performed and plotted as

principal co-ordinate analysis (PCA) plots in QIIME2. The significance of differences in beta diversity between groups will be assessed by PERMANOVA analysis performed using the Adonis package within QIIME2 with 9999 iterations. Taxa which differ significantly according to metadata groups will be identified using MaAsLin2 (Multivariate Association with Linear Models).

Taxa will be assigned to the representative sequences by the QIIME2 feature classifier, using the BLAST+ algorithm, aligning sequences against the SILVA version 132 99% similarity database. Taxa which differ significantly between groups will be identified using LEfSe (Linear discriminant analysis Effect Size). Bioinformatic analysis will be updated to reflect ongoing QIIME2 developments. Analyses will be undertaken comparing microbiome populations in patients with and without leaks at baseline, operation, and 3-5 days postoperative to identify differences in microbial populations.

All samples collected for the microbiome sub-study will be destroyed at the end of the trial in accordance with the Human Tissue Authority's Code of Practice.

8 ELIGIBILITY

8.1 RESEARCH SITE ELIGIBILITY

The trial will open in at least 25 research sites throughout Europe. Each site must fulfil a set of pre-specified criteria and complete a registration form which verifies that the research site is willing and able to comply with the trial requirements. This will be signed by the proposed local Principal Investigator (PI) on behalf of all staff who will be affiliated with the trial. Research sites will be required to obtain local management approval, return all required essential documentation to CTRU and undertake a site initiation with the CTRU prior to the start of recruitment into the trial.

Participation of research sites will be dependent upon the following criteria:

1. Site must be able to perform robotic assisted or laparoscopic rectal cancer surgery with intra-operative fluorescence angiography using a near-infrared laparoscope (e.g. the PINPOINT™ (laparoscopic) or FIREFLY™ (robotic) systems etc.). Laparoscopic rectal cancer resection may be via the "top down" or "bottom-up" (taTME) approach.
2. Site must have experience in intra-operative fluorescence angiography (IFA)
3. Predicted capability to recruit a minimum of 12 patients per year into the IntAct trial.

8.2 SURGEON ELIGIBILITY

Prior to randomising participants, all participating surgeons must have performed a minimum of 3 relevant operations (high anterior resection or low anterior resection) using IFA. Surgeon experience level – number of relevant standard laparoscopic procedures performed, number of relevant robotic-assisted laparoscopic procedures performed, both with and without the use of IFA - at the point of entry into the trial will be recorded, in addition to ongoing collection of

surgeon experience throughout the trial (including relevant experience gained outside of the trial).

8.3 PATIENT ELIGIBILITY

Eligibility waivers to inclusion or exclusion criteria are not permitted.

8.3.1 Inclusion criteria

1. Aged ≥ 18 years.
2. Able to provide written informed consent.
3. Diagnosis of rectal cancer (defined as a lower margin ≤15cm from the anal verge as assessed by endoscopic or radiological assessment).
4. Suitable for curative resection by high or low anterior resection.
5. Suitable for elective laparoscopic[3] or robotic surgery.
6. ASA ≤3
7. Able and willing to comply with the terms of the protocol including QoL questionnaires.

8.3.2 Exclusion criteria

1. Patients not undergoing colo-rectal/anal anastomosis e.g. abdominoperineal excision of rectum (APER), Hartmann's procedure.
2. Patients undergoing synchronous colonic resections.
3. Locally advanced rectal cancer requiring extended or multi-visceral excision.
4. Recurrent rectal cancer
5. Coexistent colorectal pathology e.g. synchronous cancers, inflammatory bowel disease.
6. Previous pelvic radiotherapy for pathology unrelated to diagnosis with rectal cancer e.g. treatment for prostate cancer
7. Hepatic dysfunction, defined as bilirubin outside of institutional limits and/or ALT/AST >2.5 x institutional upper limit of normal.
8. Renal dysfunction defined as eGFR <40mL/min/1.73m^2 (or a serum creatinine value[4] >10% of upper value for normal institutional limits if eGFR is not performed locally)
9. Known allergy to ICG, iodine, iodine dyes, or taking drugs known to interact with ICG e.g. anticonvulsants, bisulphite containing drugs, methadone, nitrofuratoin.
10. Pregnant or likely to become pregnant within 3 months of surgery[5]
11. Immunocompromised patients e.g. taking steroids or receiving immunotherapy

[3] Laparoscopic surgery includes either the "top down" or bottom-up" (TaTME) approach.

[4] eGFR is the preferred method of renal function assessment, however if eGFR calculation is not performed locally, the serum creatinine measure can be used to assess renal function.

[5] It is the local surgeon's responsibility to ensure this is assessed in women of child-bearing potential according to local standard of care

8.3.3 Neo-adjuvant therapy

It is anticipated that many patients will require neo-adjuvant therapy (chemo/radiotherapy) prior to surgery. Patients undergoing neo-adjuvant therapy should be assessed for eligibility and consented following completion of neo-adjuvant therapy.

8.3.4 Concurrent clinical trials

A clinical trial is considered concurrent if the trial procedures occur, or are likely to occur, within 28 days prior to the IntAct operation, during the IntAct operation, or during the 90-day post operation follow-up period of IntAct.

Participation in concurrent clinical trials will be considered on a trial-by-trial basis. However, please note that participants will not be eligible for entry into concurrent clinical trials of surgical technique, which includes any other trial that would preclude delivery of either of the IntAct interventions as defined in section (see section 10.3). Note that IntAct allows the specifics of the operation to be at the discretion of the operating surgeon. Therefore, if a concurrent trial's protocol includes stipulations about the specifics of the operation that would require the operating surgeon to deviate from their usual practice, then a participant from that trial would not be eligible for IntAct.

In all cases where enrolment to a concurrent clinical trial is under consideration, please contact the Clinical Trials Research Unit (CTRU, University of Leeds) in the first instance to discuss participant co-enrolment further.

9 RECRUITMENT PROCESS

9.1 RECRUITMENT SETTING

Participants will be recruited from approximately 25 centres in Europe. A total of 766 participants (383 in each arm) will be recruited into the trial.

The rectal microbiome sub-study (see section 7.2) will involve UK participants recruited into the trial. Recruitment to the microbiome sub-study is now complete, no further participants will be entered into the sub-study and related microbiome samples no longer need to be taken from trial participants.

9.2 ELIGIBILITY SCREENING

Participating research sites will be required to complete a log of all patients screened for eligibility who are not randomised either because they are ineligible or because they decline participation. Anonymised information will be collected including:

- Age

- Gender
- Ethnicity
- ASA grade
- Tumour site (above peritoneal reflection, at peritoneal reflection, below peritoneal reflection)
- Radiological T-stage
- Neo-adjuvant therapy (none, short course with no delay; short course with delay; long course)
- Date screened
- Reason not eligible for trial participation, or
- Eligible but declined and reason for this, or
- Other reason for non-randomisation

This information will be requested from research sites on a regular basis (at least 3 monthly) by the CTRU.

9.3 INFORMED CONSENT

Patients with primary rectal cancer will be identified from outpatient clinics, endoscopy lists, and multidisciplinary colorectal cancer meetings. Patients will undergo standard preoperative work-up which may include 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 as per standard practice. 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. Where neo-adjuvant therapy is required, patients should be assessed for eligibility and consented for the trial upon completion of neo-adjuvant therapy.

Suitability for inclusion into the trial will be assessed according to the eligibility criteria and patients will be provided with verbal and written details. A verbal explanation of the trial along with the approved PIS/ICF will be provided by a suitably qualified member of the healthcare team for the patient to consider. The PIS will provide detailed information about the rationale, design and personal implications of the trial.

Following information provision, patients must be given the opportunity to discuss the trial with their family and healthcare professionals before they are asked whether they would be willing to take part in the trial. Patients will be given as much time as possible to consider their participation in the trial; ideally they will be allowed 24 hours as a minimum. The right of the patient to refuse consent without giving reasons will be respected.

Assenting patients will then be formally assessed for eligibility and invited to provide informed, written consent for their participation in the trial, including explicit consent for the transfer of a copy of their signed consent form to the CTRU.

Informed consent may only be obtained by the PI or an appropriate, delegated, healthcare professional. The healthcare professional must have knowledge of the trial interventions and have received training in the principles of GCP and the Declaration of Helsinki 1996. He/she must be fully trained in the trial according to the ethically approved protocol and be authorised and approved by the PI to take informed consent as documented in the trial APL. The PI retains overall responsibility for the informed consent of participants at their research site.

The patient consent form with all original signatures must be retained in the ISF. A copy of the signed consent form must be given to the participant, and a record of the consent process, detailing the date of consent and witnesses, must also be kept in the participant's medical notes (this may include a copy of the consent form as per local practice). A copy of the signed consent form must also be transferred to the CTRU.

Participants will remain free to withdraw from the trial at any time by revoking consent without giving reasons and without prejudicing any further treatment.

9.3.1 Timing of consent

Written informed consent should be obtained as close to randomisation as possible and must be no more than 28 days before randomisation. Where neo-adjuvant therapy is required, patients should be assessed for eligibility and consented for the trial upon completion of any neo-adjuvant therapy.

9.3.2 Loss of capacity following informed consent

Loss of mental capacity of a participant after giving informed consent for the trial is expected to be a rare occurrence. Should this eventuality occur, this should reported to CTRU via a withdrawal form with no further trial procedures or data collection occurring from this point. Any data collected up to the point of withdrawal will be kept on record and used in the trial analysis.

9.4 RANDOMISATION

Informed written consent for entry into the trial must be obtained prior to randomisation.

9.4.1 Timing of randomisation

Randomisation should take place as soon as possible after consent is obtained and after participants have completed their baseline participant-completed questionnaire (see section 13). Baseline participant-completed questionnaires must be collected immediately prior to

randomisation to avoid bias in questionnaires occurring due to patient knowledge of randomisation allocation. Randomisation should take place as close to the planned date of surgery as possible and must be no more than 28 days prior to the planned surgery date.

9.4.2 Randomisation process

Following confirmation of written informed consent and eligibility, the participant-completed questionnaires should (wherever possible) be completed prior to randomisation, however where this is not possible, these must be completed prior to the participant being made aware of their randomised treatment. Participants will be randomised into the trial by an authorised member of staff at the research site. Randomisation will be performed centrally using the CTRU 24 hour randomisation service, either via the telephone or the CTRU website. Authorisation codes and PINs, provided by the CTRU, will be required to access the 24-hour randomisation telephone service, whilst authorised personnel will be able to use their email address and PIN to access the web based randomisation service.

Please complete the Randomisation Form prior to accessing the 24-hour registration/randomisation service. The following information will be required at randomisation:

- Participant details, including initials and date of birth
- Name and code of the research site
- Name of the person making the randomisation
- Name of the treating surgeon
- Confirmation of eligibility
- Confirmation of written informed consent
- Stratification factors (see section 9.4.3)
- Planned date of the operation

Once randomisation is complete, the randomisation service will allocate participants a unique 5 digit trial number and inform of the randomised treatment for that participant (standard care or IFA).

24-hr direct line for randomisation: 0113 343 2290

Web page for randomisation:
https://lictr.leeds.ac.uk/webrand/

9.4.3 Treatment allocation

Participants will be randomised on a 1:1 basis to receive either surgery with or without IFA and will be allocated a unique trial number. A computer-generated minimisation programme

that incorporates a random element will be used to ensure treatment groups are well-balanced for the following participant characteristics, details of which will be required for randomisation:

- Treating surgeon
- Participant gender (male or female)
- ASA grade (I, II, III)
- Radiological T-stage (T1, T2, T3, T4)
- Neo-adjuvant therapy (none, short course with no delay; short course with delay; long course)
- Tumour position (above peritoneal reflection; at peritoneal reflection; below peritoneal reflection)

10 INTERVENTION DETAILS

10.1 SCHEDULE OF CLINICAL ASSESSMENTS/DATA COLLECTION POINTS

The timing of clinical assessments and data collections points are summarised in Table 1. All participants will be followed up via clinic visits as per protocol until 90 days post-operation.

Table 1: Schedule of Events

	Events	Baseline/ Pre-op	Surgery	3-5 days post op	30 days post-op	4-6 weeks post op	90 days post-op	1 year post-op[6] (UK only)
Clinical Assessments/ investigations	Clinical examination	√			√		√	
	Pre-operative bloods	√						
	Operative details		√					
	Complications		√		√		√	
	Trial Consent	√						
	Rectal contrast enema scan					√		
Data collection time points	Eligibility CRF	√						
	Baseline CRF	√						
	Operative CRF		√					
	30 days post-operation f/up CRF				√			
	90 days post-operation f/up CRF						√	
	1 year post-operation f/up CRF							√[6]
Participant completed questionnaire	EQ-5D-5L	√			√		√	√[6]
	EORTC QLQ-C30 & QLQ-CR29	√			√		√	√[6]
	LARS	√			√		√	
	Resource Use (UK sites only)	√			√		√	

[6] Only required if this time point falls before the end of the planned follow-up period (i.e. 90 days following the last participant's operation).

10.2 PRE-OPERATION INVESTIGATIONS AND PREPARATION

Pre-operative investigations and preparation will be as per institutional protocol. Participants should undergo standard preoperative work-up which may include 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.

10.2.1 Pre-operative Bloods

FBC, LFTs & U&Es should be performed prior to treatment, details of which will be recorded on the trials CRFs.

10.3 INTERVENTION DETAILS

10.3.1 Surgery with no IFA (Standard care)

For participants randomised to the surgery with no IFA arm, the anterior resection (high or low) will be performed according to the surgeon's usual technique, using either a laparoscopic or robotic approach, with white light assessment of bowel perfusion. Laparoscopic technique might include either a "top down" or bottom-up" (TaTME) approach, dependent on surgeon's preference. High anterior resection is defined as resection and anastomosis above the peritoneal reflection. Low anterior resection is defined as resection and anastomosis below the peritoneal reflection. The specifics of each operation will be at the discretion of the operating surgeon. Colo-rectal/anal anastomosis will be performed according to surgeon's preference (hand-sewn, stapled, end-to-end, end-to-side, colo-pouch etc.)

The level of colonic transection, formation of colo-rectal/anal anastomosis, and defunctioning stoma will be performed according to normal practice.

10.3.2 Surgery with IFA

For participants randomised to the surgery with IFA arm, the anterior resection (high or low) will be performed according to the surgeon's usual technique, using either a laparoscopic or robotic approach. High anterior resection is defined as resection and anastomosis above the peritoneal reflection. Low anterior resection is defined as resection and anastomosis below the peritoneal reflection. The specifics of each operation, including the decision to make a change to the planned anastomosis following IFA assessment, will be at the discretion of the operating surgeon.

Two IFA assessments are required. The first will be prior to division of the bowel at the planned proximal transection point and the second will be an assessment of the constructed anastomosis. Additional assessments are allowed as per surgeon preference.

1. Proximal transection assessment

   The left colon and rectum will be mobilised and the rectum transected below the cancer. Then, dependent on surgeon preference, an intracorporeal *or* extracorporeal assessment technique can be used. The method used will be captured on the intraoperative CRF.

   First, the proximal colon will be assessed under white light and the point of planned transection marked. For extracorporeal methods, the white light (WL) assessment can be performed under direct vision without the use of the laparoscope if preferred. Additional aides to perfusion assessment, such as evaluation of the marginal artery supply, are allowed during WL assessment.

   A bolus of 0.1mg/kg of 5mg/ml ICG (reconstituted as per the manufacturer's instructions) will be administered intravenously via a peripherally sited cannula.

a) Intracorporeal; the colonic and rectal stump perfusion will be assessed using near-infrared laparoscopy (e.g. Novadaq PINPOINT - laparoscopic surgery; Firefly - robotic surgery etc.). The time to first visible fluorescence in the proximal colon and the rectal stump will be recorded (time recording should begin immediately after the full dose of ICG has been infused by rapid bolus push through a peripheral intravenous cannula). The maximum intensity of fluorescence in the proximal colon and rectal stump will be assessed subjectively as "clearly fluorescent", "borderline fluorescence", or "no fluorescence". Any change in the planned transection level or revision of the rectal stump as a result of IFA assessment will be recorded.

b) Extracorporeal; with the exteriorised bowel only IFA assessment of the proximal bowel is possible. The time to first visible fluorescence in the proximal colon will be recorded (time recording should begin immediately after the full dose of ICG has been infused by rapid bolus push through a peripheral intravenous cannula). The maximum intensity of fluorescence in the proximal colon will be assessed subjectively as "clearly fluorescent", "borderline fluorescence", or "no fluorescence". Any change in the planned transection level as a result of IFA assessment will be recorded.

If an intracorporeal method is used initially, and a surgeon wishes to perform an additional extracorporeal assessment, this is permissible but will be recorded separately on the CRF. A maximum of three IFA assessments can be performed.

2. Anastomosis assessment

   Colo-rectal/anal anastomosis will be performed according to surgeon's preference (hand-sewn, stapled, end-to-end, end-to-side, colo-pouch etc.), following which assessment of anastomotic perfusion will be undertaken with a further bolus of 0.1mg/kg of ICG administered via a peripheral cannula. The time to first visible fluorescence will be recorded along with any difference in fluorescence of the proximal colon and rectum (time recording should begin immediately after the full dose of ICG has been infused by rapid bolus push through a peripheral intravenous cannula). The intensity of fluorescence in the proximal colon and rectal stump will be subjectively recorded as "clear fluorescence", "borderline fluorescence", or "no fluorescence". 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.

A third dose of ICG will be permitted as preferred by the operating surgeon with the dose and timing recorded on the CRF.

10.4 POST-OPERATIVE CARE

Post-operative care will be as per institutional protocol and this can include enhanced recovery after surgery pathways.

10.4.1 Clinical Assessments

Participants will be reviewed in an outpatient clinic at:

- 30 days post-operation
- 90 days post-operation

Any further visits will be according to local standard clinical practice.

For patients still hospitalised at the 30 day time-point (due to complications, readmissions, or social factors preventing discharge etc.) assessment will take place in the hospital setting.

10.4.1.1 Definition of Clinical Anastomotic Leak

Clinical anastomotic leak is defined, as per the International Study Group definition [11], as a confirmed 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 equates to Grades B & C in the International Study Group definition of anastomotic leaks (see Appendix 1 for a description of each grade). In particular, an abscess or fluid collection in close proximity to the anastomosis will be deemed as an anastomotic leak.

10.4.1.2 Definition of Anastomotic Leak

Anastomotic leak is defined, as per the International Study Group definition[11], as a confirmed 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. This includes Grades A, B & C in the International Study Group definition of anastomotic leaks (see Appendix 1 for a description of each grade). In particular, an abscess or fluid collection in close proximity to the anastomosis will be deemed as an anastomotic leak.

10.4.2 Contrast Enema

If a rectal contrast study is not already routinely planned post-operation, participants should

undergo a rectal contrast study between 4-6 weeks post-operation to determine radiological evidence of anastomotic leak unless contraindicated or impractical due to comorbidity. A rectal contrast study does not need to be carried out at this time point if an anastomotic leak has already been confirmed e.g. radiologically or at re-laparotomy.

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.

10.5 WITHDRAWAL OF TREATMENT

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 participant themselves.

In the event that a participant withdraws prior to randomisation, no further data is required to be submitted. In the event that a participant withdraws after randomisation but prior to their operation, collection of follow-up data will still be required. For participants withdrawing from the trial after their operation, they will still attend follow-up visits unless unwilling to do so and safety data and follow-up data will continue to be collected.

If a participant explicitly states they do not wish to contribute further data to the trial or to complete any further participant questionnaires, the CTRU must be informed in writing.

The PI or delegate must make every effort to ensure that the specific wishes of any participant who wishes to withdraw consent for further involvement in the trial are defined and documented using the Withdrawal CRF in order that the correct processes are followed by the CTRU and research site following the withdrawal of consent.

11 DATA COLLECTION

Participating research sites will be expected to maintain a file of essential trial documentation (ISF), which will be provided by the CTRU, and keep copies of all completed CRFs for the trial. The CRFs and participant-completed questionnaires will contain the participant's unique trial number, date of birth, and initials. Clinical data will be collected at baseline, operation, and at 30 days and 90 days post-operation; participant-completed data will be collected at baseline, and at 30 days and 90 days post-operation.

11.1 SUBMISSION OF TRIAL DATA

Participating research sites will record trial participant data on trial-specific paper CRFs and submit them to the CTRU. Missing and discrepant data will be flagged and additional data validations raised as appropriate from the CTRU data management team.

11.2 PRE-TREATMENT DATA COLLECTION

Participants must be screened, assessed for eligibility and have provided written informed consent before they can then be randomised (Section 9.4).

Data collected on the pre-treatment CRFs (Eligibility Checklist, Baseline and Randomisation Forms) will include (but will not be limited to):

- Personal details and demographics including height, weight, gender, and American Society of Anesthesiologists (ASA) grade (I, II, III),
- Clinical assessment: to include co-morbidities (e.g. diabetes, cardiorespiratory disease), clinical assessment of anal sphincter function (normal, sub-normal).
- Results of pre-treatment investigations, to include staging details of the rectal cancer (radiological TNM stage, CRM involvement), location in relation to peritoneal reflection (above, at, or below peritoneal reflection), blood parameters (haemoglobin, eGFR, albumin).
- Other information required to confirm eligibility

Following written informed consent and wherever possible prior to randomisation (where this is not possible this must be prior to the participant being made aware of their randomised treatment) participants will also be asked to complete the baseline participant-completed questionnaires:

- EQ-5D-5L
- EORTC-QLQC30
- EORTC-QLQCR29
- Low Anterior Resection Score (LARS)
- Health Resource Use

11.3 OPERATIVE DATA COLLECTION

An operative CRF will be completed which will collate data relating to the surgical operation including (but not limited to):

- Operating surgeon
- Performed operation: to include: i) robotic or laparoscopic anterior resection (laparoscopic to include TaTME), ii) conversion to open surgery, iii) laparoscopic or open anastomosis, iv) high or low anterior resection as defined by the relationship of the anastomosis to the peritoneal reflection

- Details of IFA (IFA group only): to include i) dose and time of ICG administered, ii) time to observed fluorescence in proximal colon and rectal remnant, and iii) visual assessment of fluorescent intensity of the proximal colon and rectal stump.
- Changes in planned anastomosis, including i) the decision to undertake a permanent stoma rather than an anastomosis, ii) the site of proximal bowel used for anastomosis, iii) the site of rectal remnant used for anastomosis, and iv) the decision to undertake a diverting stoma.
- Anastomosis details: to include construction of anastomosis (hand-sewn or stapled, single or double-stapled), configuration of anastomosis (end-to-end, side-to-end, colo-pouch)
- Level of anastomosis in centimetres above anal verge and in relation to the peritoneal reflection (above, below)
- Use of defunctioning stoma
- Any intra-operative complications[7]

11.4 FOLLOW-UP DATA COLLECTION

11.4.1 Data Collection for clinical assessments

At 30 days and 90 days post-operation, a clinical assessment must be carried out for all participants.

Data collected during follow up will include (but will not be limited to):

- Anastomotic leaks (as defined in section 10.4.1)
- Length of hospital stay
- Post-operation complications, and severity (see section 12.3.1 for classification)[2]
- Re-interventions, to include stoma reversal.

11.4.2 Longer term data collection (UK participants only)

Hospital re-admission details from medical notes will be collected for UK participants at 1 year post-operation, if this time point falls before the end of the planned follow-up period (i.e. 90 days following the last participant's operation). Participants do not need to be seen at this time point for trial purposes.

[7] Some complications will require expedited reporting to CTRU, please see Section 12 for more details

11.5 PARTICIPANT-COMPLETED QUESTIONNAIRES

11.5.1 Participants in the UK

Participant- completed questionnaires measuring quality of life (EQ-5D, EORTC QLQ-C30, EORTC QLQ-CR29 and LARS) and Health Resource Use will be completed in clinic at baseline and at 30 days[8] post-operation and posted to participants for completion at 90 days post-operation. There will be an additional quality of life questionnaire pack (EQ-5D, EORTC QLQ-C30, EORTC QLQ-CR29) posted to UK participants at 1 year post-operation if this time point falls before the end of the planned follow-up period (i.e. 90 days following the last participant's operation). See section 13 for more details.

11.5.2 International participants

Participant- completed questionnaires measuring quality of life (EQ-5D, EORTC QLQ-C30, EORTC QLQ-CR29 and LARS) will be completed in clinic at baseline and at 30 days[9] post-operation and at 90 days[4] post-operation (see section 13).

11.6 CENTRAL REVIEW

11.6.1 Operative videos

The IFA parts of the operation involving ICG/NIR fluorescence perfusion for participants in the IFA arm should be videoed. Randomly selected videos will be requested and reviewed by the central research team for quality assurance. Any discrepancies will be fed back to sites. All personal identifiable data should be removed from the video prior to sending. Videos should be labelled with the trial number, DOB and initials.

11.6.2 Contrast Enema Scan Central Review

Images from the first 5 contrast enemas from each institution will be subjected to central review. All personal identifiable data should be removed from the scan prior to sending. Scans should be labelled with the trial number, DOB and initials.

[8] Where this is not possible due to clinic visit time constraints, the participant may complete at home and return directly to CTRU using the provided stamped addressed envelope.

[9] Where this is not possible due to clinic visit time constraints, the participant may complete at home and return directly to site using the provided addressed envelope.

11.7 PREGNANCY

Any suspected or confirmed pregnancies between the date of randomisation to the date of surgery must be reported to the CTRU **within 7 days** of the research site becoming aware. All further protocolised treatment must be stopped immediately if a pregnancy occurs or is suspected during this time; it is the responsibility of the treating surgeon/radiologist to decide what course of action should be taken in relation to ensuring the participant's ongoing treatment outside of the trial protocol.

The CTRU will inform the Sponsor of all reported pregnancies.

11.8 DEATH

All deaths must be recorded on the Notification of Death CRF. Data collected will include (but will not be limited to):

- Date of death
- Cause of death

Deaths occurring in the trial population from randomisation to 90 days post operation must be reported on the Notification of Death CRF. A completed Notification of Death CRF must be faxed **within 7 days** of site becoming aware of the event. The original form must then be posted to the CTRU and a copy retained at the research site.

11.9 DEFINITION OF END OF TRIAL

The end of the trial is defined as the date of the last participant's last data item.

12 SAFETY REPORTING

For the purpose of this surgical trial, the safety reporting terms adverse events and serious adverse events have been translated into complications.

12.1 GENERAL DEFINITIONS

A **complication** is defined as an untoward medical event in a participant, which has a causal relationship to the trial. The trial includes the trial intervention as defined in section 10.3 and any further treatment related to the trial intervention (such as treatment of complications caused by the trial intervention and any trial-specific interventions e.g. the consent process and completion of questionnaires).

An untoward medical event can include:

- any unintentional, unfavourable clinical sign or symptom

- any new illness or disease or the deterioration of existing condition
- any clinically relevant deterioration in any clinical tests

A **serious complication (SC)** is defined as a complication which:

- results in death
- is life-threatening[10]
- requires in-patient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability or incapacity
- consists of a congenital anomaly or birth defect, or
- is otherwise considered medically significant by the investigator

An **Unexpected Serious Complication (USC)** is a **serious** complication which is **related** and **unexpected** and will require expedited reporting (see section 12.3.2) to enable reporting to the main Research Ethics Committee (REC) and Sponsor.

The Health Research Authority (HRA) defines the terms **related** and **unexpected** as:

- **Related**: that is, it resulted from administration of any research procedures. All complications by definition are related to the trial procedures. (Untoward medical events which are unrelated to the trial procedures are not being collected in this trial.)
- **Unexpected**: that is, the type of event that in the opinion of the investigator is not considered expected. Examples of expected complications are provided in section 12.2; note this is not an exhaustive list.

Medical and scientific judgement must be exercised in deciding whether an event is serious (see section 12.4 for Responsibilities). These characteristics/ consequences must be considered at the time of the event and do not refer to an event which hypothetically may have caused one of the above.

12.2 EXPECTED COMPLICATIONS

Operative

- Damage to organ/structure e.g.
  - Bowel
  - Bladder/ureter
  - Major vessel

[10] Life-threatening refers to an event in which the participant was at risk of death at the time of the event, NOT an event which hypothetically may have caused death had it been more severe.

  - Nerves
- Faecal contamination
- Haemorrhage
- Surgical emphysema
- Failure of surgical equipment laparoscopic equipment or robotic system including hardware/software malfunction

Post-operative Complications

- Gastrointestinal
  - Anastomotic leak
  - Gastrointestinal fistula
  - Gastrointestinal ischaemia/necrosis
  - Gastrointestinal obstruction
  - Gastrointestinal perforation
  - Gastrointestinal stricture/stenosis
  - Gastrointestinal ulceration
  - Protracted Ileus (>3 days)
- GI Infection
  - Intra-abdominal/pelvic abscess
  - Post-operative peritonitis
  - Pseudomembranous colitis
- Stoma
  - Stoma prolapse/retraction
  - Stoma dehiscence
  - Stoma necrosis
  - Overactive stoma (>1.5 L per 24 hours for >1 week)

- Renal / Urinary
  - Acute renal failure
  - Urinary retention
  - Urinary tract infection
- Vascular
  - Cerebrovascular accident/stroke
  - Distal limb ischaemia/compartment syndrome
  - Deep vein thrombosis (DVT)
- Wound
  - Wound infection
  - Wound dehiscence
  - Incisional hernia
- Miscellaneous
  - Back pain
  - Cholecystitis

- Delirium
- Haemorrhage
- Pancreatitis
- Pressure sore
- Subcutaneous emphysema

Cardiorespiratory Complications
(May be operative or post-operative)

- Respiratory, including
  - Respiratory failure
  - Aspiration
  - Pleural effusion
  - Pneumonia/chest infection
  - Pulmonary embolus
- Cardiac, including
  - Arrhythmia
  - Cardiac failure
  - Ischaemic heart disease/ myocardial infarction
- Cardio-respiratory arrest

Related to ICG administration

- Anaphylactoid reactions
  - Pruritis
  - Urticaria
  - Bronchospasm
  - Flush

- Anaphylaxis

12.3 REPORTING OF COMPLICATIONS

Information on all complications will be collected for this trial whether volunteered by the participant, discovered by investigator questioning or detected through physical examination or other investigation.

12.3.1 Classification of complications

All complications should be graded using the Clavien-Dindo Classification scale[32] where appropriate (see Appendix 2)

12.3.2 Serious Complication (SCs) and Unexpected Serious Complications (USCs) occurring within 30 days of the operation – Expedited reporting

All Serious Complications (SCs) and Unexpected Serious Complications (USCs) (see section 10.1) occurring within 30 days of the operation are subject to expedited reporting requirements and must therefore be notified to the CTRU **within 24 hours** of the clinical research staff becoming aware of the event. Notifications must be sent to CTRU by fax or email using the SC / USC CRF. Once all resulting queries have been resolved, the CTRU will request the original form is posted to the CTRU and a copy retained at site.

24 hr fax for reporting SC & USCs: 0113 343 6774 or INTACT@leeds.ac.uk

For each SC and USC, the following data will be collected:

- Start and end dates of event, if resolved
- Full details of complication in medical terms with a diagnosis (if possible)
- Action/intervention
- Outcome
- An identifiable and authorised reporting source (i.e. the signature of the investigator or other medic authorised by the investigator at the reporting research site)

Any follow-up information on SCs and USCs must be faxed or emailed to the CTRU as soon as it is available. Events will be followed up until resolution or a final outcome has been reached. All USCs will be reviewed by the Chief Investigator (CI) and will be subject to expedited reporting to the Sponsor and the REC by the CTRU on behalf of the CI in accordance with current HRA guidance, CTRU Standard Operating Procedures (SOPs), and Sponsor requirements.

SCs and USCs with an onset date greater than 30 days post-operation are not subject to expedited reporting, but must be reported with all other types of complication (i.e. non-serious expected and unexpected complications) via a post-operative complication form submitted with the Follow Up Assessment CRFs, as appropriate (see section 12.3.3).

12.3.3 All other complications – Non-expedited reporting

Information about the incidence and severity of all other complications (this includes all non-serious expected and unexpected complications) which occur from the date of initial treatment until 90 days post-operation will be collected for all participants on the treatment CRF or -on the Post-operation Follow Up Assessment CRFs, as appropriate. This also applies to any SCs or USCs with an onset date greater than 30 days post-surgery.

These events will **not** be subject to expedited reporting requirements.

12.3.4 Untoward medical events unrelated to the trial – Not reportable

It is anticipated that there will be minimal additional risks associated with the interventions in this trial. Participants treated may have co-morbidities and in recognition of this, untoward medical events will only be reported if they are classified as related to trial procedures (including the surgical intervention and related procedures or trial-specific procedures such as consent and questionnaire completion).

12.4 RESPONSIBILITIES FOR SAFETY REPORTING

Principal Investigator (PI) (i.e. lead trial clinician at each recruiting research site or appropriate clinical individual identified in the APL)

- Checking for complications during admission and follow-up, including judgment in assigning:
    - Causality, i.e. whether an untoward medical event is related (i.e. a complication which therefore needs to be reported) or unrelated (i.e. not a complication and therefore does not need to be reported)
    - Seriousness
    - Expectedness
- To ensure all SCs and USCs up to 30 days post-treatment are recorded and initially reported to the CTRU within 24 hours of the research site team becoming aware and to provide further follow-up information as soon as available.
- To report SCs and USCs to the CTRU in-line with the protocol.
- To report USCs to local committees in line with local arrangements.

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

- Assign relatedness and expected nature of reported complications/untoward medical events where it has not been possible to obtain local assessment.
- Undertake review of SCs and USCs (see section 12.3.2).

- In the event of disagreement between local assessment and the CI, local assessment may be upgraded or downgraded by the CI prior to reporting to the REC.

Clinical Trials Research Unit (CTRU)

- Expedited reporting of USCs occurring within 30 days post-operation to the REC and Sponsor within required timelines.
- Preparing annual safety reports to the REC and periodic safety reports to the Trial Steering Committee (TSC) and Data Monitoring & Ethics Committee (DMEC) as appropriate.
- Notifying Investigators of SCs and USCs which compromise participant safety.

Trial Steering Committee (TSC)

- Periodic review of safety data in accordance with the TSC Terms of Reference, and liaising with the DMEC regarding safety issues.

Data Monitoring & Ethics Committee (DMEC)

- In accordance with the DMEC Terms of Reference, periodic review of unblinded overall safety data to determine patterns and trends of events and to identify any safety issues which would not be apparent on an individual case basis.

12.5 ONWARD REPORTING

Safety issues will be reported to the REC as part of the annual progress report.

An annual summary of complications will be reported to the TSC and Sponsor.

Expedited reporting of events (as detailed in section 12.3.2) to the REC and Sponsor will be subject to current HRA guidance, CTRU SOPs and Sponsor requirements.

Non-UK sites will be responsible for onward reporting of safety events occurring at their own sites as per local requirements.

13 PARTICIPANT QUESTIONNAIRES

Participants will complete a number of health related quality of life questionnaires

- **EQ-5D-5L:** a validated questionnaire which provides a simple descriptive profile and a single index value for health status. [33]
- **EORTC QLQ-C30**: a validated questionnaire used to assess the quality of life of cancer patients [34]

- **EORTC QLQ-CR29:** a validated questionnaire specifically for patients with colorectal cancer[35]
- **Low Anterior Resection Syndrome (patients without defunctioning ileostomy):** The LARS score is a 5-item scoring system to evaluate bowel function in patients after anterior resection for rectal cancer. The score is available in a number of translations, including: English, German, Spanish, Swedish and Chinese, with some translations being validated. [36]
- **Health and social care resource use (UK participants only)**: is composed of questions related to contact with primary, community and social care services including medications, plus time off work.

All participants will complete the health related quality of life questionnaire packs at baseline and at 30 days and 90 days post-operation. All participants will be followed up as per protocol until 90 days post-operation. There will be an additional quality of life questionnaire pack (EQ-5D-5L, EORTC QLQ-C30, EORTC QLQ-CR29) posted to UK participants at 1 year post-operation, if this time point falls before the end of the planned follow-up period.

13.1 UK Participants

Questionnaire packs will be completed at clinic at baseline[11] and 30 days[12] post operation (wherever possible) and participants will be asked to seal the questionnaires in pre-supplied stamped addressed envelopes prior to being given to research staff. Research staff will then send the sealed envelopes to the CTRU for entry into the database.

Participant questionnaires at 90 days post-operation and at 1 year post-operation (if applicable) will be received by the participants via post (these will be posted directly from the CTRU) who complete them at home and return them to the CTRU using a pre-supplied stamped addressed envelope. A thank you letter will be sent to participants by CTRU upon receipt of a completed questionnaire. Should a completed questionnaire not be received at CTRU by the required timepoint, CTRU will send a reminder letter to the participant.

13.2 International Participants

Questionnaire packs will be completed at clinic at baseline[7] and at 30 days[13] and 90 days[9] post operation (wherever possible) and participants will be asked to seal the questionnaires

[11] Baseline questionnaires must be completed after consent and, wherever possible, prior to randomisation (where this is not possible, they must be completed prior to the participant being made aware of their randomised treatment).

[12] Where this is not possible due to clinic visit time constraints, the participant may complete at home and return directly to CTRU using the provided stamped addressed envelope.

[13] Where this is not possible due to clinic visit time constraints, the participant may complete at home and return directly to site using the provided addressed envelope.

in pre-supplied stamped addressed envelopes prior to being given to research staff. Research staff will then send the sealed envelopes to the CTRU for entry into the database.

14 ECONOMIC EVALUATION

In line with NICE guidance, the within trial cost effectiveness study for UK participants 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 30 day and 90-day post operation 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 trusts 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 [37].

A second cost effectiveness analysis will be undertaken at 12 months from the perspective of the health and social care sector. In addition to the patient completed resource use data (30 and 90 days) the analysis will use CRF data collected at 12 months (see 11.4.2). Incremental cost effectiveness ratios will use QALYs derived from the EQ-5D-5L. The analysis will replicate that of the within trial analysis reported above.

15 ENDPOINTS

15.1 PRIMARY ENDPOINT

The primary endpoint is clinical anastomotic leak rate within 90-days post-operation.

Clinical anastomotic leak is defined, as per the International Study Group definition[11], as a confirmed 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. In particular, an abscess in close proximity to an anastomosis will be deemed an anastomotic leak. This equates to Grades B & C in the International Study Group definition of anastomotic leaks (see appendix 1 for a description of each grade).

15.2 SECONDARY ENDPOINTS

Secondary end-points include:

- *Anastomotic leak rate within 90 days post-operation:*
  Anastomotic leak is defined, as per the International Study Group definition [11], as a confirmed 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. In particular, an abscess or fluid collection in close proximity to the anastomosis will be deemed as an anastomotic leak.This includes Grades A, B & C in the International Study Group definition of anastomotic leaks (see appendix 1 for a description of each grade).

- *Change in planned anastomosis:*
  Changes in planned anastomosis, including i) the decision to undertake a permanent stoma rather than an anastomosis, ii) the site of proximal bowel used for anastomosis, iii) the site of rectal remnant used for anastomosis, and iv) the decision to undertake a diverting stoma.

- *Rate of stoma (temporary or permanent)*

- *Operative and post-operative complications (Clavien-Dindo for complication-level classification and Comprehensive Complication Indicator for patient-level classification) within 90 days of operation*

- *Length of post-operative hospital stay*

- *Low Anterior Resection Syndrome (LARS) score at 30 days and at 90 days post-operation – patients without defunctioning ileostomy*

- *Rate of re-interventions within 90 days and within 12 months*[14]

- *Quality of life (QLQ-C30, QLQ-CR29, EQ-5D) at 30 days, 90 days and 12 months* [14] *post-operation*

- *Health resource utilisation assessed at 30 days, 90 days and 12 months*[14] *post-operation.*

- *Death within 90 days of operation*

- *Changes in rectal microbiome and correlation to AL (mechanistic sub-study)*

End-points relating to the economic evaluation can be found described in section 14

[14] for UK participants only for whom the 12 months post-operation time point falls before the end of the planned follow-up period i.e. 90 days following the last participant's operation.

16 STATISTICAL CONSIDERATIONS

16.1 SAMPLE SIZE

Anastomotic leak following rectal cancer resection is reported between 8% and 25%. Recent evidence, which takes into account innovation in laparoscopic technique and stapler technology, documents a leak rate between 10% and 15%[38, 39] The most relevant data is from the COLOR II study [13], which 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. We will assume an overall anastomotic leak rate of 12.0% as a value that most colorectal surgeons would accept. The only data on anastomotic leak rate using IFA is from the PILLAR II study [25], 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 clinical anastomotic leak rate from 12.0% to 6.0% at a two-sided 5% level of significance with 80% power allowing for a 10% drop-out rate. If an interim analysis, after primary endpoint data is available for 554 patients, shows significant evidence of efficacy with respect to the primary endpoint then the trial will stop.

16.2 SAMPLE SIZE (Post-interim analysis)

As a result of the sample size re-estimation (see section 17.2), the target sample size has been revised to 766 patients.

17 STATISTICAL ANALYSIS

Statistical analysis is the responsibility of the CTRU Statistician, not including the mechanistic sub-study (see section 7.2) and Economic Evaluation analysis (see section14). A full statistical analysis plan will be written before any analyses are undertaken and in accordance with CTRU standard operating procedures. Analysis and reporting will be in line with CONSORT.

The primary analysis will be conducted using the principles of intention-to-treat (ITT) meaning participants will be analysed in the group to which they were randomised irrespective of whether or not they receive their allocated intervention.

17.1 Final analyses

17.1.1 Primary endpoint analyses

The rate of clinical AL in each trial arm will be summarised by trial arm alongside measures of uncertainty. The primary analysis will compare leak 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 95% confidence interval and the p-value yielded by a Wald test of the treatment allocation regression coefficient.

17.1.2 Secondary endpoint analyses

Secondary endpoints with binary measures (change in planned anastomosis, stoma, complications, anastomotic leak, rate of re-intervention 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 quality of life 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 quality of life 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.

17.2 Interim analysis

A blinded review of data was originally planned to allow re-estimation of assumptions employed in the initial sample size calculation. This was to be conducted prior to unblinding for the formal interim analysis as recommended by Gould [40] and would have been conducted as per the methodology of Shih & Zhao [41]. However, in order to adequately monitor and comment on the safety profiles of the two treatment arms, the DMEC requested to review data in an unblinded manner and so a blinded review of the sample size parameters was no longer applicable. In place of the originally planned blinded sample size recalculation, appropriate summaries of key sample size parameters will be provided to informally assess the validity of the original sample size assumption. The sample size re-estimation aspect of the interim analysis remains non-binding as per the original design. This amendment was documented and justified fully in the statistical analysis plan prior to any analysis being undertaken.

The trial was designed to include an interim analysis that would be conducted on unblinded data for 554 participants with a p-value less than 0.0146 considered as sufficiently strong evidence of efficacy for early stopping (as per the O'Brien-Flemming alpha spending function). Following funder request and with approval from the DMEC, the formal interim analysis was conducted on unblinded data once primary endpoint data was available for 546 participants and the alpha-spending function was recalculated accordingly and documented in the statistical analysis plan.

The interim analysis will formally compare randomised groups with regard to the primary endpoint only. Secondary endpoints will be formally assessed at the final analysis only. Note that in the event of early stopping for efficacy, recruitment will cease and the final analysis (as

detailed in Section 17.1) will be performed upon completion of follow up on all patients recruited.

17.3 Interim reports

A DMEC will be set up to independently review data on safety and recruitment. Interim reports will be presented to the DMEC in strict confidence, in at least yearly intervals. This committee, in light of the interim data, and of any advice or evidence they wish to request, will advise the TSC if there is proof beyond reasonable doubt that one treatment is better or whether there are any safety concerns.

17.4 Exploratory analyses

Exploratory analyses, including cautious exploration of causal pathway analysis and analysis of the sub-study, will be fully detailed in a statistical analysis plan.

18 TRIAL MONITORING

Trial supervision will be established according to the principles of GCP and in-line with the NHS Research Governance Framework (RGF). This will include establishment of a core Project Team, Trial Management Group (TMG), an independent TSC and independent DMEC. A Trial Monitoring Plan will be developed based on the trial risk assessment; this may include site monitoring.

18.1 TRIAL STEERING COMMITTEE (TSC) & DATA MONITORING AND ETHICS COMMITTEE (DMEC)

An independent DMEC will be appointed to review the safety and ethics of the trial, alongside trial progress and the overall direction as overseen by the TSC. Detailed un-blinded reports will be prepared by the CTRU for the DMEC at approximately yearly intervals.

The DMEC will be provided with detailed un-blinded reports containing the following information:

- Rates of occurrence of unexpected serious complications (USCs; see section 12.1) by treatment group
- Time between randomisation and trial treatment by treatment group for each participating research site
- Rates of operative and post-operative complications by treatment group for each participating surgeon.

Trial progress will be closely monitored by the independent DMEC, who will report to the TSC, and the overall direction overseen by the TSC (ensuring regular reports to the NIHR Efficacy and Mechanism Evaluation (EME) Programme).

18.2 DATA MONITORING

Data will be monitored for quality and completeness by the CTRU. Missing data will be chased until they are received, until confirmed as not available, or until the trial is at analysis.

The CTRU or Sponsor will reserve the right to intermittently conduct source data verification (SDV) exercises on a sample of participants, which will be carried out by staff from the CTRU or Sponsor. SDV will involve direct access to participant medical notes at the participating research sites and the ongoing central collection of copies of consent forms and other relevant investigation reports.

A Trial Monitoring Plan will be developed.

18.3 CLINICAL GOVERNANCE ISSUES

To ensure responsibility and accountability for the overall quality of care received by participants during the trial period, clinical governance issues pertaining to all aspects of routine management will be brought to the attention of the TSC and, where applicable, to individual research sites.

19 QUALITY ASSURANCE, ETHICAL CONSIDERATIONS, AND CONFIDENTIALITY

19.1 QUALITY ASSURANCE

The trial will be conducted in accordance with the principles of GCP in clinical trials, the UK NHS Research Governance Framework (RGF) and through adherence to CTRU SOPs.

19.2 SERIOUS BREACHES

The CTRU and Sponsor have systems in place to ensure that serious breaches of GCP or the trial protocol are picked up and reported. Investigators are required to **immediately** notify the CTRU of a serious breach (as defined in the latest version of the HRA SOP) that they become aware of. A 'serious breach' is defined as a breach of the protocol or of the conditions or principles of GCP (or equivalent standards for conduct of non-CTIMPs) which is likely to affect to a significant degree-

a) the safety or physical or mental integrity of the trial subjects, or

b) the scientific value of the research

In the event of doubt or for further information, the Investigator should contact the Senior Trial Manager at the CTRU.

19.3 ETHICAL CONSIDERATIONS

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, amended at the 64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013. Informed written consent will be obtained from the participants prior to randomisation into the trial. The right of a patient to refuse participation without giving reasons must be respected. The participant must remain free to withdraw at any time from the trial without giving reasons and without prejudicing his/her further treatment.

19.3.1 Ethical approval within the UK

Ethical approval will be sought through the Health Research Authority (HRA). The trial will be submitted to and approved by a REC, the HRA and the appropriate Site Specific Assessor for each participating research site prior to entering participants into the trial. The CTRU will provide the REC with a copy of the final protocol, participant information sheets, consent forms and all other relevant trial documentation.

19.3.2 Ethical approval outside of the UK

For non-UK sites, 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. Non-UK sites will be provided with a copy of the protocol, translated patient documents (e.g. participant information sheet/consent form document, quality of life questionnaires etc.) and other relevant trial documentation. It will be the responsibility of the local site to ensure country-specific ethical approval (and any other local approvals required) is obtained as per local clinical trial legislation prior to opening to recruitment. Non-UK sites will be required to provide the CTRU with a copy of the local ethical approval document and complete a Regulatory Approval Confirmation document prior to participant recruitment.

20 CONFIDENTIALITY

All information collected during the course of the main trial will be kept strictly confidential. Information will be held securely on paper at the CTRU. In addition, the CTRU will hold electronic information on all trial participants. The CTRU will have access to the entire database for monitoring, co-ordinating, and analysis purposes.

The CTRU will comply with all aspects of the UK 1998 Data Protection Act. Operationally this will include:

- Explicit written consent from participants to record personal details including name, date of birth, NHS number (UK participants).

- Appropriate storage, restricted access and disposal arrangements for participants' personal and clinical details.
- Consent from participants for access to their medical records by responsible individuals from the research staff or from regulatory authorities, where it is relevant to trial participation.
- Consent from participants for the data collected for the trial to be used to evaluate safety and develop new research.
- Copies of participants consent forms, which will include participants names, will be collected when a participants is randomised into the trial by the CTRU. In addition participant name and address will be collected for questionnaire posting. All other data collection forms that are transferred to or from the CTRU will be coded with a unique participant trial number and will include two participant identifiers, usually the participant's initials and date of birth.
- Where central monitoring of source documents by CTRU (or copies of source documents) is required (such as scans or local blood results), the participant's name must be obliterated by site before sending.
- Where anonymisation of documentation is required, research sites are responsible for ensuring only the instructed identifiers are present before sending to CTRU.

If a participant withdraws consent from further trial treatment and/or further collection of data, their data will remain on file and will be included in the final trial analysis.

20.1 ARCHIVING

20.2 Trial data and documents held by CTRU

At the end of the trial, all data held by the CTRU and all trial data will then be securely archived at the University of Leeds in line with the Sponsor's procedures for a minimum of 15 years.

20.3 Trial data and documents held by research sites

Research sites are responsible for archiving all trial data and documents (ISF and all essential documents therein, including CRFs) at the participating research site until authorisation is issued from the Sponsor for confidential destruction.

20.4 Participant medical records held by research sites

Research sites are responsible for archiving trial participant medical records in accordance with the site's policy and procedures for archiving medical records of patients who have participated in a clinical trial. However, participant medical records must be retained until authorisation is received from the Sponsor for confidential destruction of trial documentation.

21 STATEMENT OF INDEMNITY

The University of Leeds is able to provide insurance to cover for liabilities and prospective liabilities arising from negligent harm. Clinical negligence indemnification for UK sites will rest with the participating NHS Trust or Trusts under standard NHS arrangements. Clinical negligence indemnification for sites outside of the UK will rest with the institution of the participating site.

22 TRIAL ORGANISATIONAL STRUCTURE

Research sites will liaise with the CTRU for advice and support on trial set-up and operation, and submission of trial data. In turn, the CTRU will be responsible for data chasing.

22.1 RESPONSIBILITIES

The CI is responsible for the design, management and reporting of the trial.

The CTRU will have responsibility for overall conduct of the trial in accordance with the NHS RGF and CTRU SOPs.

The responsibility for ensuring clinical management of participants is conducted in accordance with the trial protocol ultimately remains with the PI at each research site.

22.2 OPERATIONAL STRUCTURE

Chief Investigator (CI): As defined by the NHS Research Governance Framework, the CI is responsible for the design, conduct, co-ordination and management of the trial.

Trial Sponsor- University of Leeds: The sponsor is responsible for trial initiation management and financing of the trial as defined by the Directive 2001/20/EC. The sponsor delegates some of these responsibilities to CTRU as detailed in the trial contract.

Clinical Trials Research Unit (CTRU): the CTRU at the University of Leeds will have responsibility for the conduct of the trial in accordance with the NHS Research Governance Framework (RGF) and CTRU SOPs. The CTRU will provide set-up and monitoring of trial conduct to CTRU SOPs including randomisation design and service, database development and provision, protocol development, CRF design, trial design, source data verification, ongoing management including training, monitoring reports and trial promotion, monitoring schedule and statistical analysis for the trial. In addition, the CTRU will support ethical approval submissions, any other site-specific approvals, and clinical set-up. The CTRU will be responsible for the overall day-to-day running of the trial including trial administration, database administrative functions, data management, safety reporting, and statistical analyses (not including the mechanistic sub-study and Economic Evaluation analysis). At the end of the trial, CTRU will be responsible for archiving all data and trial data held by the CTRU in line with the Sponsor's procedures for a minimum of 15 years.

Leeds Institute of Cancer and Pathology (LICAP, Leeds Institute of Biomedical and Clinical Sciences (LIBACS) and Leeds Institute of Medical Research (LIMR): Researchers from these institutes are responsible for the micro-biome sub-study as outlined in section 7.2.

Academic Unit of Health Economics (AUHE): The researchers at the AUHE are responsible for the Economic Evaluation as outlined in section 14. CTRU will input the data from the CRFs and participant completed forms.

22.3 OVERSIGHT/ TRIAL MONITORING GROUPS

Trial Management Group (TMG): the TMG, comprising the CI, CTRU team, other key external members of staff involved in the trial, and a patient representative will be assigned responsibility for the clinical set-up, on-going management, promotion of the trial, and for the interpretation of results. Specifically the TMG will be responsible for:

- Protocol completion
- CRF development
- Obtaining approval from the HRA, UK REC and supporting applications for Site Specific Assessments (SSAs)
- Completing cost estimates and project initiation
- Nominating members and facilitating the TSC and DMEC
- Reporting of complications
- Monitoring of screening, recruitment, treatment and follow-up procedures
- Auditing consent procedures, data collection, trial end-point validation and database development.

Trial Steering Committee (TSC): the TSC will provide overall supervision of the trial, in particular trial progress, adherence to protocol, participant safety and consideration of new information. It will include an Independent Chair, not less than two other independent members, and a consumer representative. The CI and other members of the TMG may attend the TSC meetings and present and report progress. The Committee will meet annually as a minimum.

Data Monitoring and Ethics Committee (DMEC): the DMEC will review the safety and ethics of the trial by reviewing interim data during recruitment and follow-up. The Committee will meet annually as a minimum.

22.4 FUNDING

This project is funded by the National Institute for Health Research. (NIHR) Efficacy and Mechanism Evaluation (EME) Programme (Grant Ref: 14/150/62)

23 PUBLICATION POLICY

The trial will be registered with an authorised registry, according to the International Committee of Medical Journal Editors (ICMJE) Guidelines, prior to the start of recruitment.

The success of the trial depends upon the collaboration of all participants. For this reason, credit for the main results will be given to all those who have collaborated in the trial, through authorship and contributorship. Authorship decisions will be guided by standard requirements for authorship relating to submission of manuscripts to medical journals. These state that authorship credit should be based only on the following conditions being met (http://www.icmje.org):

- Substantial contribution to conception and design, or acquisition of data, or analysis and interpretation of data
- Substantial contribution to drafting the article or revising it critically for important intellectual content
- Substantial contribution to final approval of the version to be published.

In light of this, the CI, other grant co-applicants, and relevant senior CTRU staff will be named as authors in any publication, subject to journal authorship restrictions. In addition, all collaborators will be listed as contributors for the main trial publication, giving details of roles in planning, conducting and reporting the trial. IntAct publications will be published on behalf of the IntAct Group. PIs from participating sites who recruit at least 10 participants into the IntAct trial will included as members of the IntAct Group.

To maintain the scientific integrity of the trial, data will not be released prior to the first publication of the analysis of the primary endpoint, either for trial publication or oral presentation purposes, without the permission of the TSC. In addition, individual collaborators must not publish data concerning their participants which is directly relevant to the questions posed in the trial until the first publication of the analysis of the primary endpoint.

On completion of the research project a draft final report will be submitted to the EME programme (trial funder) by the CTRU, within 14 days. This will be peer reviewed and then published on the EME website. The CTRU is obliged to provide NIHR/EME with advanced notice of any publication relating to the trial. Copies of any materials intended for publication will be provided to NIHR/EME at least 28 days prior to submission for publication.

24 ABBREVIATIONS USED

ACRONYM	DEFINITION
AL	Anastomotic leak
APL	Authorised Personnel Log
ASA	American Society of Anesthesiologists
BNF	British National Formulary
CI	Chief Investigator
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CT	Computerised Tomography
CTIMP	Clinical Trial of an Investigation Medicinal Product
CTRU	Clinical Trials Research Unit
DMEC	Data Monitoring and Ethics Committee
DNA	Deoxyribonucleic Acid
DOB	Date of Birth
DVT	Deep Vein Thrombosis
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme-linked Immunosorbent Assay
EME	Efficacy and Mechanism Evaluation Programme
EORTC	European Organisation for Research and Treatment of Cancer
FBC	Full Blood Count
GCP	Good Clinical Practice
GI	Gastrointestinal
HRA	Health Research Authority
ICF	Informed Consent Form
ICG	Indocyanine Green
ICMJE	International Committee of Medical Journal Editors (ICMJE)
IFA	Intraoperative Fluorescence Angiography
IMA	Inferior Mesenteric Artery
ISF	Investigator Site File
IV	Intravenous
LARS	Low Anterior Resection Syndrome

LFT	Liver Function Test
MIP	Maximum Intensity Projection
MRI	Magnetic Resonance Imaging
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NIHR	National Institute of Health Research
NIR	Near infra-red
OTA	Operational taxonomic units
PCR	Polymerase chain reaction
PI	Principal Investigator
PIS	Patient Information Sheet
PSSRU	Personal Social Services Research Unit
QIIME	Quantitative Insights Into Microbial Ecology
QLQ	Quality of Life Questionnaire
RDP	Ribosomal database project
REC	Research Ethics Committee
RGF	Research Governance Framework
SC	Serious Complication
SOP	Standard Operating Procedure
SSA	Site Specific Assessment
SSOP	Site Standard Operating Procedure
TMG	Trial Management Group
TSC	Trial Steering Committee
U&E	Urea & Electrolytes
USC	Unexpected Serious Complication

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APPENDIX 1: Definition & Grading of Anastomotic Leak[11]

Definition	Defect of the intestinal wall integrity at the colorectal or colo-anal anastomotic site (including suture and staple lines of neorectal reservoirs) leading to a communication between the intra- and extraluminal compartments. A pelvic abscess close to the anastomosis is also considered as anastomotic leakage.	
Grade	A	Anastomotic leakage requiring no active therapeutic intervention
	B	Anastomotic leakage requiring active therapeutic intervention but manageable without re-laparotomy
	C	Anastomotic leakage requiring re-laparotomy

APPENDIX 2: Clavien-Dindo Classification of Complications

Grade	Definition
Grade I	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions. Allowed therapeutic regimens are: drugs as antiemetics, antipyretics, analgetics, diuretics and electrolytes and physiotherapy. This grade also includes wound infections opened at the bedside.
Grade II	Requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusions and total parenteral nutrition are also included.
Grade III Grade IIIa Grade IIIb	Requiring surgical, endoscopic or radiological intervention Intervention not under general anesthesia Intervention under general anesthesia
Grade IV: Grade IVa Grade IVb	Life-threatening complication (including CNS complications)‡ requiring IC/ICU-management Single organ dysfunction (including dialysis) Multi organ dysfunction
Suffix “d”	If the patients suffers from a complication at the time of discharge, the suffix “d” (for ‘disability’) is added to the respective grade of complication. This label indicates the need for a follow-up to fully evaluate the complication.

‡ brain haemorrhage, ischemic stroke, subarachnoidal bleeding, but excluding transient ischemic attacks (TIA);IC: Intermediate care; ICU: Intensive care unit.