

FLARE STUDY PROTOCOL

FLuorescent Angiography in REduction of Anastomotic Leak in Rectal Cancer Surgery: A Prospective Randomized Controlled Trial

Basavatarakam Indo-American Cancer Hospital and Research Institute

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Abbreviations

- AL: Anastomotic leakage
- ASA: American Society of Anesthesiologists
- CT: Computed tomography
- ICG: Indocyanine green
- ICG-FA: Indocyanine green fluorescence angiography
- mITT: Modified intention-to-treat
- NIR: Near-infrared
- RCT: Randomized controlled trial

Background and Rationale

Anastomotic leakage remains one of the most serious complications following rectal cancer surgery. Despite advances in minimally invasive techniques and perioperative care, the reported incidence of anastomotic leakage after rectal resection ranges from approximately 8% to 15%. Anastomotic leakage increases postoperative morbidity, mortality, reoperation rates, and length of hospital stay and may adversely affect long-term oncologic outcomes.

Adequate perfusion of the bowel ends is a critical determinant of anastomotic healing. Traditionally, surgeons assess bowel perfusion using subjective criteria such as bowel color, pulsation of mesenteric vessels, and bleeding from the bowel edge. However, these visual assessments may fail to detect subtle or segmental ischemia.

Indocyanine green fluorescence angiography (ICG-FA) enables real-time visualization of tissue perfusion using near-infrared imaging following intravenous administration of indocyanine green. The technique allows the operating surgeon to identify poorly perfused bowel and, when necessary, revise the planned transection line before construction of the anastomosis.

Recent randomized trials have produced important but not fully consistent results. The Japanese Essential phase 3 randomized clinical trial in minimally invasive rectal cancer surgery reported a significant reduction in all-grade anastomotic leakage with ICG fluorescence imaging compared with no ICG assessment. The reported leak rates were 7.6% in the ICG group and 11.8% in the non-ICG group, with a relative risk of 0.645 (95% confidence interval, 0.422-0.987). The rate of grade B plus C leakage and the reoperation rate were also lower in the ICG group [1].

The FLAG trial, a single-center Russian study, also reported that ICG fluorescence angiography was associated with a reduction in anastomotic leakage following low anterior resection [2].

The European IntAct randomized controlled trial evaluated ICG fluorescence angiography in rectal cancer surgery. It did not demonstrate a statistically significant reduction in the primary endpoint of clinical anastomotic leakage (grade B or C) within 90 days, although a signal toward benefit was observed. Clinical leaks occurred in 10% of participants in the ICG group and 15% in the standard-care group [3].

Similarly, the PILLAR III trial did not demonstrate a significant reduction in anastomotic leakage [4].

The Dutch AVOID multicenter phase 3 trial evaluated ICG near-infrared fluorescence bowel perfusion assessment across minimally invasive colorectal surgery. It did not demonstrate a significant reduction in the overall 90-day clinically relevant anastomotic leakage rate across all colorectal resections. However, subgroup findings suggested potential benefit in left-sided and rectosigmoid resections, and no adverse events related to ICG use were observed [5].

Given these mixed findings, the role of ICG-FA in minimally invasive rectal cancer surgery remains clinically relevant and incompletely resolved. Furthermore, randomized evidence from Indian surgical populations is limited. The FLARE study is designed to evaluate whether intraoperative ICG-FA can reduce anastomotic leakage in Indian patients undergoing minimally invasive rectal cancer surgery.

Study Objectives

Primary Objective

To determine whether intraoperative ICG-FA reduces the incidence of overall anastomotic leakage, including grade A, B, and C leakage, within 30 days after minimally invasive rectal cancer surgery.

Secondary Endpoints

1. Incidence of clinically significant anastomotic leakage, defined as grade B plus grade C leakage, within 30 days.
2. Thirty-day postoperative morbidity, graded according to the Clavien-Dindo classification.
3. Thirty-day postoperative mortality.
4. Rate of intraoperative modification of the proximal bowel transection line after ICG-FA assessment.
5. Rate of intraoperative modification of the distal rectal stump transection line after ICG-FA assessment.
6. Time to fluorescence appearance after ICG administration, measured in seconds.
7. Operative time, measured in minutes from skin incision to skin closure.
8. Reoperation rate within 30 days, defined as the proportion of participants requiring an unplanned surgical reoperation for a postoperative complication; planned procedures, including elective stoma closure, will be excluded.
9. Length of postoperative hospital stay, measured in days from surgery to discharge.

Study Design

The FLARE study is a prospective, randomized, open-label, parallel-group clinical trial comparing ICG fluorescence-guided perfusion assessment with conventional visual perfusion assessment during minimally invasive rectal cancer surgery.

Participants will be randomized in a 1:1 ratio into two groups:

- ICG-FA Group: Intraoperative bowel perfusion assessment using ICG-FA in addition to standard surgical assessment.
- Control Group: Conventional visual bowel perfusion assessment without ICG-FA.

The primary analysis will follow the modified intention-to-treat principle.

Study Setting

The study will be conducted at Basavatarakam Indo-American Cancer Hospital and Research Institute, Hyderabad, Telangana, India.

Study Population

Inclusion Criteria

1. Age 18 to 75 years, inclusive.
2. Histologically confirmed rectal adenocarcinoma, treated with or without neoadjuvant therapy.
3. Planned minimally invasive anterior rectal resection, either laparoscopic or robotic, with anastomosis 2-15 cm from the anal verge.
4. Planned colorectal or coloanal anastomosis.
5. Ability to provide written informed consent.

Exclusion Criteria

1. Emergency surgery or palliative procedure.

2. Known allergy to indocyanine green or iodine.
3. Pregnancy or lactation.
4. Severe renal or hepatic failure.
5. Any serious contraindication to minimally invasive surgery.
6. Planned open rectal surgery.
7. Intraoperative discovery of unresectable disease before randomization.
8. Inability to construct the planned colorectal or coloanal anastomosis before randomization.

Randomization and Allocation Concealment

Participants will be randomized in a 1:1 allocation ratio using a computer-generated randomization sequence.

Randomization will occur intraoperatively after rectal mobilization but before transection of the proximal bowel.

Allocation concealment will be maintained using sequentially numbered, sealed, opaque envelopes.

Participants in whom conversion from minimally invasive to open surgery occurs before randomization will not proceed to randomization.

Masking

This is an open-label surgical trial. Masking of the operating surgeon and operating room team is not feasible because they must know whether ICG-FA is being used during the procedure.

Outcome definitions, postoperative surveillance, and follow-up assessments will be standardized according to the study protocol.

Surgical Procedure

All participants will undergo minimally invasive anterior rectal resection according to standard oncologic principles, including total mesorectal excision where indicated.

Laparoscopic procedures will be performed using standard minimally invasive laparoscopic platforms. Robotic procedures will be performed using the da Vinci X Surgical System (Intuitive Surgical, Sunnyvale, CA, USA).

The following intraoperative decisions will remain at the discretion of the operating surgeon:

- Level of vascular ligation.
- Splenic flexure mobilization.
- Creation of a diverting stoma.
- Type and technique of anastomosis.

ICG-FA Group

In participants randomized to the ICG-FA group, a single ICG fluorescence angiographic assessment will be performed before construction of the anastomosis.

ICG Administration and Imaging Platform

ICG (Aurogreen, Aurolabs, Madurai, India) will be administered intravenously at a dose of 0.1 mg/kg body weight as a bolus, followed by a normal saline flush.

For laparoscopic procedures, near-infrared fluorescence imaging will be performed using a compatible fluorescence imaging system, including the Stryker 1688 or 1788 HD laparoscopic system or the Stryker SPY-PHI device, depending on availability.

For robotic procedures, surgery will be performed using the da Vinci X Surgical System, and ICG-based perfusion assessment will be performed using the integrated Firefly fluorescence imaging mode (Intuitive Surgical, Sunnyvale, CA, USA).

Perfusion Assessment

Perfusion will be assessed separately at both components of the planned anastomosis:

- Proximal large bowel end.
- Distal rectal stump.

The time from ICG administration to the first visible appearance of fluorescence will be recorded separately for the proximal large bowel end and distal rectal stump.

Perfusion will be assessed according to the appearance, distribution, and adequacy of fluorescence as judged intraoperatively by the operating surgeon.

If satisfactory perfusion is demonstrated at both bowel ends, the planned transection lines will be maintained.

If fluorescence is absent, delayed, or demonstrates a clearly demarcated area considered to have inadequate perfusion at either bowel end, the affected segment will be reassessed to identify a better-perfused area.

The corresponding proximal and/or distal transection line may be revised, as clinically appropriate, to ensure that the anastomosis is constructed between adequately perfused bowel ends.

The following parameters will be recorded separately, where applicable:

- Time to first visible fluorescence at the proximal large bowel end.
- Time to first visible fluorescence at the distal rectal stump.
- Presence of delayed, absent, or visibly inadequate fluorescence.
- Modification of the planned proximal bowel transection line.
- Modification of the planned distal rectal stump transection line.
- Number of transection-line modifications.
- Additional length of bowel resected following ICG-FA assessment.

A single ICG administration and fluorescence assessment will be performed before construction of the anastomosis. No additional ICG fluorescence assessment is planned after completion of the anastomosis.

Control Group

In the control group, bowel perfusion will be assessed without ICG-FA.

Conventional intraoperative parameters may be used to assess perfusion, including:

- Bowel color.
- Active bleeding from the cut bowel edge.
- Mesenteric arterial pulsation.
- Overall tissue appearance.

The method and interpretation of conventional perfusion assessment will remain at the discretion of the operating surgeon.

For robotic procedures in the control group, surgery will be performed using the da Vinci X Surgical System; Firefly fluorescence perfusion assessment will not be used.

No ICG-FA will be performed in the control group.

Anastomotic Leak Grading

Anastomotic leakage will be classified according to clinical severity and the therapeutic intervention required.

Grade A: Radiological leakage without clinical symptoms and requiring no active therapeutic intervention.

Grade B: Symptomatic anastomotic leakage requiring non-operative treatment, such as antibiotics, percutaneous drainage, or transanal drainage, without reoperation.

Grade C: Symptomatic anastomotic leakage requiring surgical intervention or reoperation.

For the primary endpoint, overall anastomotic leakage will include grade A, B, and C leakage.

Clinically significant anastomotic leakage will include grade B and grade C leakage.

Diagnostic Protocol for Anastomotic Leakage

All participants will undergo standardized radiological assessment of the colorectal or coloanal anastomosis.

A contrast enema examination will be performed routinely on postoperative day 5 to assess anastomotic integrity and identify early or subclinical leakage.

A second contrast enema examination will be performed at approximately 4 weeks after surgery as part of scheduled postoperative follow-up to identify delayed or clinically occult leakage.

Radiological findings suggestive of anastomotic leakage may include:

- Extravasation of contrast from the anastomotic site.
- Perianastomotic collection.
- Sinus tract formation.

Computed tomography will be performed when clinically indicated. Indications may include:

- Fever.
- Tachycardia.
- Abdominal pain.
- Purulent or feculent drain output.
- Elevated inflammatory markers.
- Clinical deterioration.

Computed tomography may also be performed when appropriate contrast examination of the anastomosis is not feasible.

The diagnosis of anastomotic leakage will be based on radiological findings, clinical presentation, and the therapeutic intervention required. Anastomotic leaks will subsequently be graded according to the predefined classification system.

Follow-up

All participants will be followed for 30 days after surgery.

Outcome assessment will occur:

- During the index hospital admission.
- Following discharge, where applicable.
- At the 30-day postoperative outpatient follow-up visit.

The second contrast enema examination planned at approximately 4 weeks will form part of the 30-day postoperative follow-up assessment.

Sample Size Calculation

The sample size was calculated for a dichotomous primary endpoint comparing two independent groups.

Based on previous literature:

- Expected anastomotic leakage rate in the control group: 13%.
- Expected anastomotic leakage rate in the ICG-FA group: 6%.

- Expected absolute risk reduction: 7%.

Using a two-sided alpha of 0.05, statistical power of 80%, and an allocation ratio of 1:1, the required sample size is 274 participants per group, giving a total of 548 participants.

To account for approximately 10% attrition and prespecified exclusions from the analysis population, the target enrollment will be increased to approximately 305 participants per group, giving a total planned enrollment of 610 participants.

Analysis Population

Modified Intention-to-Treat Population

The primary analysis will follow the modified intention-to-treat principle.

The modified intention-to-treat population will include randomized participants who undergo surgery and in whom a colorectal or coloanal anastomosis is constructed.

Participants will be excluded from the modified intention-to-treat analysis only if:

- Surgery is not performed.
- Unresectable disease precludes the planned resection.
- A colorectal or coloanal anastomosis is not created.

Statistical Analysis Plan

Categorical variables will be presented as numbers and percentages.

Continuous variables will be expressed as mean with standard deviation or median with interquartile range, depending on the distribution of the data.

Categorical outcomes, including the primary endpoint, will be compared between the ICG-FA group and control group using the chi-square test or Fisher's exact test, as appropriate.

Relative risks with 95% confidence intervals will be reported for binary outcomes.

Continuous variables will be compared using Student's t-test or the Mann-Whitney U test, depending on data distribution.

All statistical tests will be two-sided. A p value of less than 0.05 will be considered statistically significant.

Exploratory subgroup analyses may be performed to assess whether the effect of ICG-FA differs according to clinically relevant variables such as:

- Sex.
- Body mass index.
- Neoadjuvant therapy.
- Anastomosis height.
- Diverting stoma.
- Tumor height from the anal verge.

These subgroup analyses will be considered hypothesis-generating.

Statistical analyses will be conducted using R or SAS software.

Data Collection and Data Management

Data will be collected prospectively using standardized case record forms.

Data collection will include the following domains:

- Screening and eligibility.

- Baseline demographic and clinical risk factors.
- Intraoperative details.
- Postoperative outcomes.
- Imaging findings.
- Thirty-day follow-up outcomes.

The master data collection chart will include, where applicable:

- Screening ID.
- Study ID.
- Age.
- Sex.
- Diagnosis.
- Tumor distance from the anal verge.
- Neoadjuvant treatment.
- Body mass index.
- ASA physical status.
- Smoking status.
- Diabetes.
- Steroid use.
- Hemoglobin.
- Serum albumin.
- Tumor stage.
- Procedure type.
- Anastomosis height.
- Stapler details.
- Intraoperative leak-test result.
- Diverting stoma status.
- ICG dose.
- Time to first fluorescence at the proximal bowel end.
- Time to first fluorescence at the distal rectal stump.
- Perfusion assessment.
- Modification of the proximal transection line.
- Modification of the distal transection line.
- Additional bowel resection length.
- Operative time.
- Anastomotic leakage grade.
- Diagnostic method used to identify leakage.
- Intervention required for anastomotic leakage.
- Reoperation.
- Clavien-Dindo grade.
- Wound infection.
- Postoperative ileus.
- Readmission.
- Intensive care unit stay.
- Length of postoperative hospital stay.
- Thirty-day mortality.
- Follow-up imaging findings.

Data flow will proceed from screening, eligibility confirmation, baseline assessment, initiation of surgery and rectal mobilization, intraoperative randomization, study-group assessment, completion of surgery, postoperative surveillance, 30-day follow-up, imaging review, data lock, and final statistical analysis.

No names or directly identifying information of research participants will be included in documents uploaded to ClinicalTrials.gov.

Safety and Adverse Event Reporting

Adverse events and surgical complications will be recorded during the hospital stay and up to 30 days after surgery.

Serious adverse events, including death, reoperation, life-threatening events, prolonged hospitalization, or events requiring major medical or surgical intervention, will be reported to the Institutional Ethics Committee according to institutional requirements.

No separate Data Monitoring Committee has been constituted.

Study safety and data oversight will be performed by the Principal Investigator and study team, with reporting to the Institutional Ethics Committee as required.

Ethical Considerations

The study will be conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Ethical approval was obtained from the Institutional Ethics Committee on 08-December-2025, prior to initiation of the study.

Written informed consent will be obtained from all participants before enrollment.

Individual Participant Data Sharing Statement

Individual participant data will not be made publicly available because of concerns regarding participant privacy and confidentiality.

The study includes sensitive clinical, operative, imaging, and postoperative data from surgical participants.

De-identified aggregate study findings will be reported through scientific publications and trial-registry reporting as applicable.

Expected Significance

The FLARE study aims to clarify the role of ICG fluorescence angiography in reducing anastomotic leakage during minimally invasive rectal cancer surgery.

By evaluating fluorescence-guided bowel perfusion assessment in an Indian surgical population, the study may help address conflicting evidence from previous randomized trials and contribute additional evidence to guide intraoperative perfusion assessment and surgical practice.

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