

Next Generation intraoperative Lymph node staging for Stratified colon cancer surgery (GLiSten): a multicentre, multinational feasibility study of fluorescence in predicting lymph node-positive disease

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***National Institute for
Health Research***

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Abstract

Next Generation intraoperative Lymph node staging for Stratified colon cancer surgery (GLiSten): a multicentre, multinational feasibility study of fluorescence in predicting lymph node-positive disease

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Background: 5-aminolevulinic acid (5-ALA) is used for fluorescence diagnosis (FD) in neurological, gynaecological and urological malignancies. The Medical Research Council/Efficacy and Mechanism Evaluation (EME) programme/National Institute for Health Research's Next Generation intraoperative Lymph node staging for Stratified colon cancer surgery (GLiSten) study investigated its use to predict lymph node (LN)-positive disease in colon cancer as an aid to stratified surgery.

Objectives: The primary objective was to optimise the dose of oral 5-ALA for intraoperative FD of metastatic LNs in colon cancer. Secondary objectives included standardisation of pre-operative computerised tomography (CT) LN reporting, intraoperative fluorescence detection, surgical resection with D3 lymphadenectomy and histopathological examination of resected specimens.

Design: This was a feasibility study to determine optimal strategies for 5-ALA positive LN detection. Patients with locally advanced disease identified using the Fluoropyrimidine, Oxaliplatin and Targeted-Receptor pre-Operative Therapy for patients with high-risk, operable colon cancer (FOXROT) criteria were recruited from two sites between October 2013 and June 2015. Cohort 1 received 20 mg/kg and cohort 2 received 30 mg/kg of oral 5-ALA, 1–6 hours preoperatively. Laparoscopic assessment of fluorescence was performed using the Storz D-Light system (KARL STORZ GmbH & Co. KG; Tuttlingen, Germany), with marking of fluorescent LNs, followed by oncological resection. The specimen was subjected to histological analysis with step sectioning of marked fluorescent LNs. Progression to an evaluation phase using the optimal dosing schedule was dependent on positively identifying at least 2 out of 10 patients with metastatic LN disease in either cohort.

Results: A total of 44 patients were recruited with a male to female ratio of 26 : 18 and a mean age of 71 years (range 52–88 years). Cohort 1 consisted of 18 patients, of whom six had fluorescent primary cancers and three of these had fluorescent LNs. One out of 10 patients with metastatic LN disease had a fluorescent involved LN. Cohort 2 consisted of 26 patients, of whom eight had fluorescent primary cancers and four of these had fluorescent LNs. None of the fluorescent LNs contained disease in this cohort. No serious adverse events (SAEs) occurred but two mild, self-limiting, photosensitivity reactions were observed in cohort 2. The sensitivity and specificity for 5-ALA detection of LN-positive disease were: cohort 1 11.1%, 75%; and cohort 2 0%, 75%.

Limitations: This was a feasibility study exploring the use of 5-ALA for LN disease in a select cohort of patients with advanced colorectal cancer. The study population was small and generalisation to other cancers is not possible. The study was limited by the ability to determine LN-positive patients on the basis of pre-operative CT staging, which is often inaccurate, resulting in our cohorts containing several patients without LN disease.

Conclusions: 5-ALA fluorescent diagnosis has poor sensitivity for discriminating LN-positive colon cancer. Its use as an aid to stratified colon cancer surgery is not supported. No SAEs were observed, suggesting that photosensitisers may be useful for intraoperative FD.

Future work: 5-ALA has poor sensitivity for detecting LN metastases and cannot be recommended for intraoperative staging. Other, more sensitive fluorescent probes are required if this strategy is to be used.

Study registration: Current Controlled Trials ISRCTN79949827 and EudraCT number 2012–002623–15.

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List of abbreviations

5-ALA	5-aminolevulinic acid	HDU	high-dependency unit
ASA	American Society of Anaesthesiologists	HEX	hexaminolaevulinate
CI	confidence interval	ICU	intensive care unit
CRF	case report form	IMP	Investigational Medicinal Product
CT	computerised tomography	LFT	liver function test
DMEC	Data Monitoring and Ethics Committee	LN	lymph node
EME	Efficacy and Mechanism Evaluation	PPI	patient and public involvement
EMVI	extramural vascular invasion	PpIX	protoporphyrin IX
FD	fluorescence diagnosis	SAE	serious adverse event
FOxTROT	Fluoropyrimidine, Oxaliplatin and Targeted-Receptor pre-Operative Therapy for patients with high-risk, operable colon cancer	SLN	sentinel lymph node
GLiSten	Next Generation intraoperative Lymph node staging for Stratified colon cancer surgery	TMG	Trial Management Group
		TSC	Trial Steering Committee

Plain English summary

Bowel cancer spreads along channels, called lymphatics, with cells becoming trapped in special glands, called lymph nodes (LNs). In surgery for bowel cancer it is necessary to remove the whole tumour with the lymphatics and LNs. To guide the extent of surgery, it is important to know whether or not spread to the LNs has happened. 5-aminolevulinic acid (5-ALA) is a drug that can make cancers fluoresce (glow). It has been used to guide surgery in other cancers, particularly brain cancer, but has not been used in bowel cancer. It is hoped that giving 5-ALA to patients with bowel cancer will make the cancers and cancer-containing LNs glow when viewed by a special blue-light camera and, thus, act as a guide to surgery.

Patients felt to have a high chance of cancer-containing LNs on computerised tomography were chosen. 5-ALA was given before surgery and cancer fluorescence was tested using a special blue-light keyhole camera. The presence of cancer within fluorescent LNs was confirmed by microscope testing.

Group 1 ($n = 18$ patients) received 20 mg/kg of 5-ALA. Six patients had fluorescent cancers and three patients had fluorescent LNs; only one patient had fluorescent LNs containing cancer. Group 2 ($n = 26$ patients) received 30 mg/kg. Eight patients had fluorescent cancers and four patients had fluorescent LNs; but none of the fluorescent LNs contained cancer. There were no major 5-ALA side effects.

It can be concluded that 5-ALA is safe, but does not allow cancer-containing LNs to be detected with enough accuracy to be useful in guiding bowel cancer surgery.

Scientific summary

Background

Colorectal cancer is the fourth most common cancer in the UK and represents a substantial burden on health-care resources. The current standard for colon cancer surgery is resection of the primary cancer along with the draining lymphatic field. The technique of complete mesocolic resection with extended lymphadenectomy reportedly has decreased rates of local recurrence and improved 5-year disease-free survival. Standard surgery would generally involve a 'D2 lymphadenectomy' whereby the second tier of draining lymph nodes (LNs) are removed but the central high ligation required for 'D3 lymphadenectomy' is not routinely practised. Emerging evidence suggests that survival outcomes following colon cancer surgery can be improved with D3 lymphadenectomy and by respecting oncological planes of resection (complete mesocolic excision). It has also been suggested that the standard of segmental colectomy performed in the UK is of variable quality and that improvement in technique may improve outcomes with a survival advantage of up to 27% in patients with LN involvement. Assuming this is correct, then a change in surgical technique to complete mesocolic resection and extended D3 lymphadenectomy might improve the prognosis of patients with colon cancer.

However, such a uniform radical approach fails to take into account the biological variation of colon cancer or the fitness of the patient. Only $\approx 25\%$ of cancers have metastatic disease to the LNs, meaning that D3 lymphadenectomy is overtreatment in the majority of colon cancer patients. There is the added concern that the majority of colorectal cancer patients are elderly with multiple comorbidities and a universal policy of radical resection will lead to unnecessary morbidity with an increased rate of post-operative complications. Another factor that needs to be taken into account is the changing pattern of disease presentation with the implementation of screening programmes. The introduction of a National Bowel Cancer Screening Programme in the NHS has seen a shift in incidence of early cancers from 10.1% prior to screening to 45.3% following implementation. As the incidence of LN metastases in Dukes' A cancer is $< 10\%$, a policy of D3 lymphadenectomy for all patients cannot be justified and is unlikely to produce any survival benefit.

Therefore, a potential strategy to improve patient survival outcomes in colon cancer would be a more selective approach whereby patients with LN involvement are offered D3 lymphadenectomy, while those without nodal involvement undergo a more limited lymphadenectomy.

The difficulty in implementing a selective strategy lies in accurately defining LN status prior to surgical resection. Unfortunately, there is no reliable method for determining LN status either pre- or intraoperatively. A novel approach to LN staging is therefore required so that the radicality of resection can be tailored to individual patient's needs.

A potential solution to intraoperative LN staging involves drugs used in photodynamic diagnosis. 5-aminolevulinic acid (5-ALA) is a pro-drug, taken up into the mitochondria of cells, where it serves as a precursor of protoporphyrin IX (PpIX). PpIX is a fluorescent molecule which, when exposed to blue-violet light of excitation wavelength 405 nm, emits a characteristic red fluorescence at a wavelength of 630–700 nm.

The 5-ALA is preferentially taken up by cancer cells and metabolised to PpIX. The tendency for cancer cells to accumulate PpIX is enhanced by exogenous administration of 5-ALA. When administered in high doses and irradiated with blue-violet light, 5-ALA is cytotoxic to cancer cells and has a photodynamic therapeutic effect. In lower doses, the emitted fluorescence can be used for photodynamic diagnosis.

There is a substantial body of work to support the use of 5-ALA and its derivatives in the fluorescence detection of solid cancers. In humans, 5-ALA fluorescence has been extensively used as a diagnostic aid in transitional cell carcinoma of the bladder, in neurosurgery to guide malignant glioma resection, and in gynaecological malignancies. There have been studies that suggest 5-ALA can distinguish involved from uninvolved LNs and, therefore, could be used in fluorescence-assisted surgery to guide the surgeon as to the level of lymphadenectomy required for oncological clearance.

The GLiSten study was a feasibility study to optimise 5-ALA intraoperative fluorescence diagnosis (FD) in LN-positive colon cancer, as a guide to surgical radicality.

Objectives

The primary objective was to optimise the dose of oral 5-ALA administration for intraoperative FD of metastatic LNs in colon cancer. Secondary objectives included standardisation of pre-operative computerised tomography LN reporting, intraoperative fluorescence detection, surgical resection with D3 lymphadenectomy and histopathological examination of resected specimens.

Methods

The GLiSten study was conducted under local ethical committee and Medicines and Healthcare Products Regulatory Agency approval between October 2013 and June 2015 at two sites: St James's University Hospital, Leeds, and The Mater Misericordiae University Hospital, Dublin, Ireland. The study was designed incorporating an initial developmental phase to optimise the use and dosing schedule of 5-ALA for intraoperative LN staging in colon cancer, followed by an evaluation phase in which patients with colon cancer would be recruited to determine sensitivity, specificity and diagnostic accuracy of 5-ALA intraoperative LN staging compared with in-depth histopathology. The study population consisted of adult patients undergoing elective surgery for colonic adenocarcinoma.

In the developmental phase, two cohorts of 10 patients with positive LNs, as verified on post-operative histology, were treated with different doses of 5-ALA in order to determine the optimal dose. Recruitment to the study was enriched to contain patients with LN disease using the Fluoropyrimidine, Oxaliplatin and Targeted-Receptor pre-Operative Therapy for patients with high-risk, operable colon cancer (FOxTROT) radiology criteria for locally advanced disease. As the presence of metastatic disease within the LNs can be verified only on post-operative histology, it was anticipated that > 10 patients would have to be recruited per cohort to identify 10 with positive LNs.

A total of 20 mg/kg was identified as the most commonly used dose in the literature and, therefore, the first cohort of patients was administered 20 mg/kg of oral 5-ALA prior to surgery. The dose administered to the second cohort of patients was modified to 10 mg/kg or 30 mg/kg according to the sensitivity observed in cohort 1. In this way, after the recruitment of 20 patients with LN-positive disease, the optimal dose of administration would be identified. To progress to the next part of the study, 5-ALA FD would need to detect positive nodes as compared with histology in at least 2 out of 10 patients in the same cohort.

If 5-ALA detected positive LNs in at least 2 out of 10 patients within the same cohort, a final cohort of 10 patients would receive the preferred dose of 5-ALA to optimise the technique, with flexibility to include further patients to confirm the validity of the technique before proceeding to the evaluation phase. The ability of 5-ALA FD to reliably detect LNs with metastatic disease as judged by histopathological evaluation would have been assessed by requiring that, to progress to the evaluation phase, the upper bound of the 99% (Clopper-Pearson) confidence interval (CI) of the sensitivity (in the patients with positive nodes recruited in this stage of the trial) was at least the target value of 80%. This analysis would be versus

histopathology but on a per-patient basis, considering whether or not a patient had at least one positive LN identified by 5-ALA. A 99% CI would have been used to reflect the additional uncertainty in a relatively low number of patients. However, to allow for the optimisation of the other variables (e.g. fluorescence detection system), this analysis would not have included patients treated with this dose before it was identified as the optimal dose.

During the process of identifying the optimal dose of 5-ALA, work was also carried out to standardise the pre-operative radiological assessment, the technique of laparoscopic D3 lymphadenectomy and the pathological LN mapping.

The study aimed to combine existing techniques in sentinel LN mapping using colorimetric dyes to provide an overall lymphatic map with the tumour-specific properties of 5-ALA FD. To our knowledge, this approach had never before been tried. This combination was particularly attractive in the context of laparoscopic surgery with both agents visible with the Storz D-Light Laparoscopic System (KARL STORZ GmbH & Co. KG; Tuttlingen, Germany), which combines white-light (colorimetric dyes) and blue-light (5-ALA) modes together with enhanced stereoscopic magnification. We focused on cancers of the right and sigmoid colon and performed segmental colectomy with D3 lymphadenectomy, if feasible and appropriate. Laparoscopic assessment of the cancer and draining lymphatic field was performed using the Storz D-light system. Any fluorescent LNs were marked with surgical clips to guide histopathological assessment and their site was documented. Resection specimens were scrutinised using routine and enhanced histopathological methods to determine the sensitivity, specificity and positive and negative predictive values for 5-ALA FD compared with histological analysis.

Results

A total of 44 patients were recruited to the trial in the developmental phase; 18 patients to cohort 1 and 26 patients in cohort 2. There were 26 male and 18 female patients recruited, with a mean age of 71 years (range 52–88 years). The mean body mass index was 27.3 kg/m² (range 19.1–37.8 kg/m²) with a median American Society of Anaesthesiologists grade of 2. The ratio of right-sided to left-sided cancers was 30 : 14. There were six conversions (14.3%) among the 42 patients who underwent surgical resection. The majority of patients had pT3, pN0/1 disease; three patients had metastatic disease involving either the liver or lungs, one of whom was in cohort 1 and two of whom were in cohort 2. There were no significant differences in the baseline characteristics between the two cohorts.

Three patients did not have blue-light laparoscopy as two were cancelled on the day of their operation and the Storz D-light equipment was not available for another day. One patient had unresectable disease and, therefore, no pathological assessment could be made. Therefore, out of the 44 patients recruited to the trial, 41 underwent blue-light laparoscopy and 40 patients had histopathological assessment performed on their specimens. Out of the 41 patients who underwent blue-light laparoscopy, 14 had fluorescent primary tumours and 7 out of these 14 patients also had fluorescent LNs. None of the patients had fluorescent LNs without having fluorescence of the primary tumour.

In cohort 1, 17 patients had blue-light laparoscopy. Fluorescence was observed in six primary cancers with three patients having fluorescent LNs. Only one fluorescent LN contained metastatic disease; therefore, out of the 10 patients with node-positive disease, only one patient had metastatic disease found in a fluorescent LN on standard histopathology.

Given the low sensitivity of 5-ALA for positive LN detection in cohort 1, the dose of 5-ALA was increased to 30 mg/kg for cohort 2 in line with the study protocol.

In cohort 2, 23 patients had blue-light laparoscopy. Fluorescence was observed in eight primary cancers with four patients having fluorescent LNs. However, none of the fluorescent LNs contained metastatic disease on standard histopathology; therefore, none of the nine patients with node-positive disease in cohort 2 had any detectable metastatic disease within fluorescent LNs.

There were no drug-related serious adverse events (SAEs) in this study. Two patients, both male in cohort 2 and admitted to a high-dependency unit (HDU) postoperatively, had a mild self-limiting photosensitivity reaction affecting the head and neck region. In both cases, this reaction was noted within the first 48 hours following surgery and lasted < 5 days. It is proposed that the brighter ambient environment in the HDU was the likely contributing factor. Four patients also had a transiently raised alkaline phosphatase in the post-operative period.

Conclusions

This was the first human clinical trial of a photosensitiser used for intraoperative fluorescence LN staging in colon cancer. The study found that 5-ALA has a poor sensitivity for detecting LN metastases and, therefore, cannot be recommended for intraoperative staging. However, the technique is safe with no SAEs related to 5-ALA. More sensitive fluorescent probes for colonic cancer are required if this strategy is to be perused.

Trial registration

This trial is registered as ISRCTN79949827 and EudraCT number 2012–002623–15.

Funding

This project was funded by the Efficacy and Mechanism Evaluation programme, a Medical Research Council and National Institute for Health Research partnership.

Chapter 1 Background

Colorectal cancer

Colorectal cancer is the fourth most common cancer in the UK with an incidence of > 41,581 new cases per annum and almost two-thirds occurring in the colon;^{1,2} as such it represents a substantial burden on health-care resources. In comparison with rectal cancer, which has seen improvement in survival over the past decade, the survival from colon cancer has remained largely unchanged with 5-year overall survival of 58.7%. In terms of segmental distribution, the right colon is the most common site, harbouring ≈25% of colorectal cancers, followed by the sigmoid colon with ≈20%.¹

Curative resection involves segmental colectomy to resect the primary cancer and the draining lymphatic field. It has been shown that the standard of segmental colectomy as performed in the UK is of variable quality and that improvement in technique may help to improve outcomes with a survival advantage of up to 27% in patients with lymph node (LN) involvement.² It has also been suggested that there is a difference in the oncological quality of the resected specimen between surgeons who routinely perform complete mesocolic resection and those who do not.^{3,4} If this is correct, then standardisation of surgical technique to complete mesocolic resection with D3 lymphadenectomy could make a substantial contribution to improving the prognosis of patients with colon cancer.⁵⁻⁸

The corollary is that only ≈25% of patients have LN involvement, meaning that for the other 75% of patients D3 lymphadenopathy potentially represents overtreatment and exposes patients to unnecessary additional morbidity. Ideally, one would want to tailor the radicality of surgery to the disease biology, such that patients with LN disease had the option of radical lymphadenectomy to maximally eradicate cancer spread, while patients without LN disease could have limited resection of their cancer with similar oncological outcomes to radical surgery. Presently, however, it is not possible to determine pre- or intraoperatively which patients have LN disease and, therefore, as a default all patients undergo varying degrees of lymphadenectomy regardless of the stage of their disease.

There is an unmet clinical need to develop strategies that accurately predict LN disease prior to or at surgery and so allow colorectal cancer surgery to be tailored to the oncological needs of the patient.

Strategies to improve colon cancer surgery

Segmental colectomy is the current standard of surgery for colon cancer based on resection of the primary cancer along with the draining lymphatic field. In this way, the primary cancer is removed along with any regional LN metastases so as to minimise the chance of local tumour recurrence. Emerging evidence suggests that survival outcomes following colon cancer surgery can be improved by increasing the radicality of lymphadenectomy and respecting oncological planes of resection.^{6,9} The technique of complete mesocolic resection with extended lymphadenectomy, as described by Hohenberger *et al.*,⁵ has reported local recurrence rates of 3.6% and 5-year disease-free survival of 89.1%, which has been attributed to removal of more tissue and LNs in the correct surgical planes. These figures compare favourably to accepted local recurrence rates of 8–10% reported elsewhere.¹⁰ Further, West *et al.*² showed a survival advantage from mesocolic plane surgery of 15% across all disease stages and up to 27% in patients with LN involvement. In the series reported by West *et al.*,² only 32% of the specimens appeared optimal in terms of pathological grade of mesocolic completeness, suggesting substantial room for improvement in surgical technique.⁸

Currently, the standard surgery for colon cancer in the UK differs from that described by Hohenberger⁵ in the extent of the lymphadenectomy performed. Standard surgery would generally involve a 'D2 lymphadenectomy', whereby the second tier of draining LNs are removed but the central high ligation required for 'D3 lymphadenectomy' is not routinely practised. Attempts to reproduce Hohenberger's⁵ results have been encouraging with a retrospective study from Copenhagen, Denmark, reporting that complete mesocolic excision was a significant independent factor for improved disease-free survival using multivariate Cox regression.⁶ This study compared 4-year disease-free survival between complete mesocolic excision and standard resection in patients with stage I–III disease and showed survival rates of 85.8% versus 75.9%, respectively. Recently, two systematic reviews have examined the evidence supporting complete mesocolic excision.^{3,9} They agree that radical resection removes more tissue and produces better-quality specimens; however, they stress that the available literature has fundamental limitations that make it difficult to recommend widespread implementation of the technique outside of expert centres.

However, this 'one-size-fits-all' approach fails to take into account the biological variation of colon cancer or the fitness and expectations of the patient. Only 25% of cancers have metastatic disease to the LNs, suggesting that D3 lymphadenectomy is overtreatment in the majority. There is the added concern that the majority of colorectal cancer patients are elderly with significant comorbidity and that a universal policy of radical resection will lead to unnecessary morbidity. Concerns regarding an increased complication rate owing to the technical demands of complete mesocolic resection have been raised,¹¹ although there is evidence in the literature to the contrary.^{4,9,12} Other series, such as that from Hillerød Hospital, Denmark, have reported improved oncological resection without any increase in morbidity.⁷ Another factor that needs to be taken into account when determining future surgical strategy is the changing pattern of disease presentation with the introduction of screening programmes. In the UK, the introduction of the National Bowel Cancer Screening Programme has seen a shift in incidence of early cancers (Dukes' stage A) from 10.1% prior to screening to 45.3% following implementation.¹³ As the incidence of LN metastases in Dukes' A cancer is < 10%, a policy of D3 lymphadenectomy for all cannot be justified and it is unlikely to produce any survival benefit. Therefore, it seems sensible to adopt a more selective approach whereby patients with LN involvement are offered D3 lymphadenectomy, while those without nodal involvement undergo a more conventional D2 lymphadenectomy. Currently, there is no reliable method for determining LN status either pre- or intraoperatively. The decision to carry out D2 or D3 lymphadenectomy therefore remains at the discretion of the operating surgeon. It is only with an accurate and objective system for LN staging that the level of resection can be tailored to individual patients. The proposed research will evaluate the merits of 5-aminolevulinic acid (5-ALA) fluorescence for this purpose.

Preoperative lymph node staging

The difficulty in implementing a selective strategy for surgical resection is in accurately defining pre-operative LN status. No clear radiological definition of a malignant LN is agreed. A common definition is any node > 1 cm or a cluster of three or more nodes < 1 cm. Some studies have used a cut-off size of 1.5 cm or have used contrast enhancement to distinguish positive nodes.¹⁴ The presence of micrometastases within normal sized LNs and benign enlargement of nodes due to inflammation are known to contribute to inaccuracies of size-based criteria. In a prospective audit of 84 patients with colon cancer undergoing pre-operative multidetector computerised tomography (CT) scanning, the accuracy, sensitivity and specificity for detection of LN disease was 58% [95% confidence interval (CI) 48% to 68%], 64% (95% CI 48% to 77%) and 53% (95% CI 39% to 67%), respectively, with poor interobserver agreement for node status.¹⁵ Further, when nodes were assessed according to tumour node metastasis classification by separating into N0, N1 and N2 disease, the accuracy, sensitivity and specificity fell to 50% for all values. A meta-analysis of 19 studies has reported an overall sensitivity and specificity for CT-detected malignant LNs to be 70% (95% CI 59% to 80%) and 78% (95% CI 66% to 86%), respectively, with a diagnostic odds ratio of 8.1 (95% CI 4.7 to 14.1).¹⁴ Attempts to improve LN staging by combining functional imaging, such as positron emission tomography, with CT or using functional diffusion weighted magnetic resonance imaging, have shown some promise but are not universally available and, therefore, as yet have limited application.

The Japanese Society for Cancer of the Colon and Rectum has circumvented this dilemma to some extent by recommending surgical resection based on primary tumour T stage, with D3 lymphadenectomy recommended for T3/4 disease.¹⁶ A similar strategy was adopted in the National Cancer Research Institute the Fluoropyrimidine, Oxaliplatin and Targeted-Receptor pre-Operative Therapy for patients with high-risk, operable colon cancer (FOxTROT) study, with T3/4 cancers randomised to either surgery or pre-operative chemotherapy with or without antiepidermal growth factor receptor monoclonal antibody therapy.¹⁷ Although still not perfect, CT imaging is more accurate in determining T stage than it is for nodal status and within the context of FOxTROT has been shown to accurately select patients for pre-operative chemotherapy.¹⁸ The accuracy, sensitivity and specificity of CT scans in selecting patients with 'bad' T3/4-stage cancers was reported to be 74% (95% CI 64% to 82%), 78% (95% CI 65% to 87%) and 67% (95% CI 49% to 81%), respectively.¹⁵ Although this, to some extent, gets around the problem of inaccurate pre-operative staging of local disease, there is no absolute correlation between 'bad' T stage and LN status; approximately 50% of T4 cancers will not have metastatic disease to the LNs and in 'good' T-stage patients, approximately 30% will have node-positive disease.¹⁵

Strategies for intraoperative lymph node staging

Sentinel lymph node mapping

The ideal solution to the problem of accurate pre-operative LN staging is to develop a reliable means of intraoperative staging, such as sentinel lymph node (SLN) biopsy in breast cancer surgery. This would enable a real-time assessment of LN status. The concept of intraoperative LN staging is not new but has received much interest with the introduction of SLN mapping techniques. The SLN was defined by Morton *et al.*¹⁹ as the first regional LN encountered by metastasising tumour cells in a study using SLN mapping in melanoma. It was proposed that if the SLN was free of tumour, then it could be assumed that the remaining LNs would also be tumour free and, hence, unnecessarily extensive resections could be avoided in patients with early-stage disease. Early studies of SLN mapping were performed by injecting a dye, such as isosulphan blue or patent blue-V into the primary lesion,^{19,20} which was taken up into the lymphatics so that the LNs could be visualised at operation. Subsequently, lymphoscintigraphy was added to blue dye, whereby a locally injected radionuclide was detected intraoperatively using a gamma probe, enabling higher detection rates.^{21,22} This concept has proven validity in some cancers, most notably breast cancer, for which the absence of an involved sentinel node spares the patient more radical axillary clearance and associated morbidity. The Association of Breast Surgery Guidelines²³ now recommend SLN biopsy for the majority of patients with early invasive breast cancer. SLN mapping was first reported in colorectal cancer in 1999 with blue dye injected around the tumour prior to resection and histological examination of the LNs for cytokeratin expression. The results from early series were not encouraging with SLN detection rates of 58–98%, sensitivity rates of 40–100% and false-negative rates between 0% and 60%.²⁴ Further studies failed to improve a great deal on these results with large variations in the reported detection rates, sensitivity and false-negative rates, which have been variously attributed to heterogeneity in detection techniques, definition of the SLN, time interval between injection and SLN detection, histopathological techniques, and patient characteristics, including tumour stage and body mass index.²⁵ A recent meta-analysis showed a SLN identification rate of 92% with a pooled sensitivity rate for detecting LN metastases of 69.6% (range 33.3–100%) and a false-negative rate of 30.4%.²⁶

Importantly, concerns have been expressed regarding the high false-negative rates and whether metastases from colon cancer follow an orderly spread through tiers of LNs, or rather metastasise as skip lesions. The variability in anatomical site of the first metastatic LN was highlighted by Tan *et al.*,²⁷ who found that in 48% of cases the first metastatic LN was not adjacent to the tumour or was 5 cm beyond the longitudinal tumour margin in 18% of cases. Further, Park *et al.*²⁸ reported that in 6% of caecal cancers LN metastases occurred along the right branch of the middle colic artery, which might not be included in a standard D2 segmental resection.

Currently, therefore, SLN mapping is not in routine use in colon cancer, although it may have a role in focused histological ultra-staging, whereby positive nodes are subjected to detailed step sectioning in order to increase the detection of micrometastatic disease.²⁹ The main disadvantage of SLN mapping techniques is that, although they identify the tumour-draining lymphatic basin, they do not discriminate LNs with or without metastatic disease. Therefore, SLN cannot be used for guiding the extent of surgical resection in colon cancer.

Photodynamic diagnosis with 5-aminolevulinic acid

A potential solution to intraoperative LN staging involves drugs used in photodynamic diagnosis and therapy. Probably the most studied drug in this respect is 5-ALA, which is a pro-drug, taken up into the mitochondria of cells, where it serves as a precursor of protoporphyrin IX (PpIX), which, in turn, is the direct precursor of haem.³⁰ PpIX is a fluorescent molecule which, when exposed to blue-violet light of excitation wavelength 405 nm, emits a characteristic red fluorescence at a wavelength of 630–700 nm.

The 5-ALA is preferentially taken up by cancer cells, which exhibit altered levels of transporter molecules and catalytic enzymes, and metabolised to PpIX. The tendency for cancer cells to accumulate PpIX is enhanced by exogenous administration of 5-ALA. When administered in high doses and irradiated with blue-violet light, 5-ALA is cytotoxic to cancer cells (photodynamic effect), while in lower doses blue-violet light causes the cancers to emit a pink-red fluorescence (photodiagnosis effect).

The reasons for the selective uptake of 5-ALA in tumour cells have not been fully elucidated. In normal cells, 5-ALA synthesis is regulated by a feedback control system that is driven by high intracellular concentrations of free haem. This system is overridden when exogenous 5-ALA is administered. An accumulation of PpIX in a tumour relative to normal cells has been attributed to alterations in the activity of the rate-limiting enzymes porphobilinogen deaminase and ferrochelatase.³⁰ The former is increased and the latter decreased in tumour cells. Alterations in enzymatic activity may also be due to reduced availability of intracellular Fe²⁺ as a result of rapid cell division. In addition, several biochemical and structural changes in tumour cells have been linked to their avidity for PpIX, including reduced pH, up-regulation of low-density lipoprotein receptors and stromal abnormalities including large interstitial space, leaky vasculature and large amounts of newly synthesised collagens and lipids.³¹

Following systemic administration, 5-ALA is metabolised in the liver and excreted in the bile and urine.³⁰ Much of the 5-ALA is used by the liver to synthesise PpIX and the remaining 5-ALA circulates to other body sites where all other cell types (except those without mitochondria) convert it to PpIX. Intracellular PpIX returns to normal levels up to 48 hours post administration.

A substantial body of work has accumulated on the use of 5-ALA and its derivatives in the fluorescence detection of solid cancers. In humans, 5-ALA fluorescence has been extensively used as a diagnostic aid in transitional cell carcinoma of the bladder,^{32–35} in neurosurgery to guide malignant glioma resection,^{36–38} and in gynaecology to detect endometriosis,^{39,40} peritoneal metastases from ovarian cancer⁴¹ and cervical squamous intraepithelial lesions.⁴² In urology, several studies have shown the superior detection of transitional cell carcinoma and carcinoma in situ of the bladder using photodynamic diagnosis compared with white-light cystoscopy.³² Both 5-ALA and its hexyl-derivative, hexaminolaevulinate [(HEX) Hexvix®, GE Healthcare], have been evaluated in bladder cancer. Burger *et al.*³³ reported reduced residual tumour and increased recurrence-free survival in patients undergoing photodynamic diagnosis with 5-ALA or HEX than white light alone. Residual tumour was present in 33% of patients with white-light cystoscopy, 15% with 5-ALA and 9% with HEX. The 3-year recurrence-free survival was 67% with white light, 80% with 5-ALA and 82% with HEX and differences between 5-ALA and HEX were non-significant. Similar promising results were reported by Daniltchenko *et al.*³⁴ in 115 patients with superficial bladder cancer, but a larger randomised study of 300 patients failed to demonstrate a difference in tumour recurrence.³⁵ A 2013 systematic review³² showed strong evidence that using 5-ALA or HEX improves tumour detection rates and reduces the residual tumour rate. It also found that, although the majority of the randomised controlled studies demonstrated a reduced local recurrence rate, not all studies confirmed this finding and, therefore, the evidence for recurrence is less convincing.

In a study examining the clinical pharmacokinetics of 5-ALA in normal healthy volunteers and patients at high risk of recurrent bladder cancer, the peak plasma concentration of 5-ALA following oral administration was achieved at 0.83 ± 0.20 hours. Plasma 5-ALA concentrations declined with a terminal half-life of approximately 45 minutes.⁴³

In neurosurgery, a randomised controlled Phase III multicentre trial³⁶ was performed to investigate the safety and efficacy of fluorescence-guided resection in malignant gliomas. The study involved oral administration of 5-ALA (20 mg/kg) 3 hours (range 2–4 hours) before induction of anaesthesia. A modified neurosurgical microscope was used that allowed switching between conventional white xenon illumination and blue–violet excitation light. Follow-up (median 35.4 months) was available for 139 fluorescence-guided resections and 131 resections carried out with conventional white-light microsurgery. It was shown that 5-ALA fluorescence-guided surgery improved rates of complete resections³⁶ and progression-free survival.³⁷ It has also been demonstrated in a recent study that there was no difference in the number of adverse events between patients given 5-ALA and the controls.³⁷ However, a Cochrane review³⁸ of image-guided surgery for brain tumours found that there was low-quality evidence that 5-ALA-guided surgery improves complete tumour resection and the effect on survival is unclear.

In colorectal cancer and its metastases, PpIX has been identified as the predominant endogenous fluorophore and even in the absence of exogenous 5-ALA, it can distinguish involved from uninvolved LNs with a sensitivity and specificity of 62% and 78%, respectively.⁴⁴ A total of 33 patients were included in this study, which did not examine intraoperative fluorescence, and the primary tumours and LNs were examined *ex vivo*. In a study using a murine model of colon cancer, 5-ALA-induced PpIX fluorescence successfully detected LN metastases, whereas benign LNs did not exhibit any apparent fluorescence.⁴⁵

In a pilot study involving a mixed group of 18 patients with inoperable adenocarcinomas or adenomas of the oesophagus, duodenum and colon, patients were administered 30–60 mg/kg of 5-ALA orally.⁴⁶ In colorectal cancer patients, flexible sigmoidoscopy was performed with serial biopsies. Peak fluorescence in the large bowel tumours was achieved within 6 hours, with good selectivity between tumour and normal mucosa at a ratio of 5 : 1. Similarly effective results were seen in oesophageal and duodenal lesions, but with a lower dose of 30 mg/kg of 5-ALA. In a more recent pilot study, 5-ALA was administered orally at a dose of 20 mg/kg around 4 hours prior to patients undergoing endoscopic resection of early gastric or colorectal tumours.⁴⁷ Fluorescence was observed in 6 out of 10 gastric lesions and in one out of the three colorectal lesions. The study noted that fluorescence was observed only in areas of malignant tissue. In this series, four of the patients developed derangement of their liver function tests (LFTs), all of which spontaneously normalised.

A 2013 study examined the ability of 5-ALA to detect LN metastases in freshly excised LNs after *en bloc* resection of colorectal tumours.⁴⁸ The fluorescent images were analysed using a spectral unmixing method to specifically detect PpIX fluorescence. The accuracy, sensitivity and specificity of this method to detect LN metastases were 87.4%, 88.3% and 92.0%, respectively.

An important issue with the clinical use of any photosensitising agent is the potential for side effects. This problem appears to be confined to systemic administration when high doses are used for photodynamic therapy as opposed to lower doses used in fluorescence diagnosis (FD). When used systemically, it is important to establish a safe dose of photosensitiser according to the interval drug to light time to avoid photosensitivity side effects. Normal precautions include keeping the patient out of direct sunlight for 24–48 hours following drug administration. Occasionally reported side effects following systemic administration include nausea, vomiting, tachycardia, hypotension, photosensitivity for up to 48 hours, and elevated liver enzymes.³⁰ No safety concerns or serious adverse reactions have been highlighted in the literature following the use of 5-ALA in humans.

Proposed strategy for 5-aminolevulinic acid intraoperative lymph node detection

We aimed to combine existing techniques in SLN mapping using colorimetric dyes to provide an overall lymphatic map with the tumour-specific properties of 5-ALA FD. To our knowledge, this approach had never before been tried. This combination was particularly attractive in the context of laparoscopic surgery with both agents visible with the Storz D-Light Laparoscopic System (KARL STORZ GmbH & Co. KG; Tuttlingen, Germany), which combines white-light (colorimetric dyes) and blue-light (5-ALA) modes together with enhanced stereoscopic magnification. We focused on cancers of the right and sigmoid colon and performed segmental colectomy with D3 lymphadenectomy when appropriate. Resection specimens were scrutinised using routine and enhanced histopathological methods to determine the sensitivity, specificity and positive and negative predictive values for 5-ALA FD as compared with histological analysis.

We chose to use 5-ALA systemically via oral administration as ranges for the optimal dose and time of pre-operative administration in humans have been previously determined. The supplier of 5-ALA for oral administration was Photonamic GmbH & Co. KG, Wedel, Germany.

Chapter 2 Trial objectives

Purpose

The purpose of this study was to evaluate and optimise the use of 5-ALA for intraoperative FD of metastatic LNs in colon cancer.

Primary objective

To optimise the dose of oral 5-ALA administration for intraoperative FD of metastatic LNs in colon cancer.

Secondary objectives

To establish a reliable and repeatable methodology for FD of LN metastasis by standardisation of:

- pre-operative CT LN reporting
- intraoperative fluorescence detection system
- surgical technique for laparoscopic segmental colonic resection with D3 lymphadenectomy
- histopathological examination of resected specimens.

Chapter 3 Methods

Trial design

The GLiSten study was a feasibility study to assess the practicality of using fluorescence-assisted surgery and the ability of 5-ALA to accurately detect involved LNs intraoperatively. The study was designed in two parts:

1. a preliminary developmental phase
2. a larger evaluation phase.

The sites participating in the developmental phase were St James's University Hospital, Leeds, UK, and The Mater Misericordiae University Hospital, Dublin, Ireland.

Developmental phase

This would involve a small number of centres to optimise the use and dosage of 5-ALA for intraoperative LN staging in colon cancer and to determine if 5-ALA provided an accurate means for detecting LN metastasis. Two cohorts of 10 patients with positive LNs, as verified on post-operative histology, were treated with different doses of 5-ALA to determine the optimum dose. The end point for this phase was the ability of 5-ALA to detect positive nodes in at least 2 out of the 10 patients with involved LNs in a single cohort. This corresponded to the upper boundary of the 99% exact CI of the sensitivity being at least 60%, that is, a good indication of activity given that the technical protocols and techniques may not have been fully optimised. A 99% CI was chosen to reflect the additional uncertainty in 10 patients. In patients without obvious LN disease at surgery, fluorescence of the primary cancer was used as a positive control for the efficacy of 5-ALA FD in colon cancer. If the initial preliminary phase achieved this end point then the trial would proceed to the evaluation phase.

The most common dose of 5-ALA in the literature is 20 mg/kg and this was chosen as the starting dose given to patients within cohort 1. If 5-ALA produced good fluorescence at this dose, the second cohort was to be treated with a lower dose of 10 mg/kg to reduce the potential of 5-ALA-related side effects. If there was inadequate fluorescence in the first cohort then the dose of 5-ALA was to be increased to 30 mg/kg. The dosing schedule is illustrated below (*Figure 1*).

By assessing the first 20 patients with positive LNs with this regimen it was hoped that the dose of 5-ALA would be optimised.

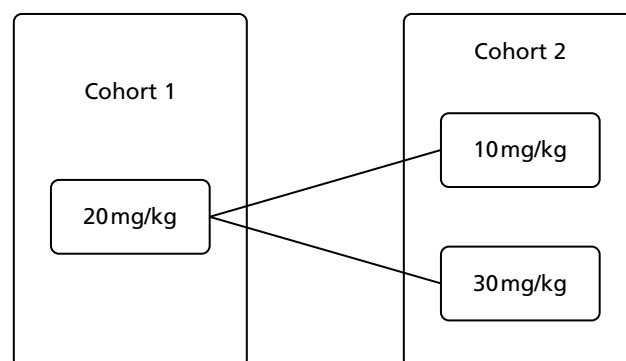


FIGURE 1 The GLiSten study dosing schedule.

For each cohort, particular attention was given to detecting and recording:

- any side effects related to 5-ALA
- intraoperative LN fluorescence
- histopathological confirmation of LN status.

If 5-ALA had detected positive LNs in at least 2 out of 10 patients in either cohort then the optimal dose of 5-ALA would have been identified. At least a further 10 patients with confirmed LN involvement would then be recruited and treated with this optimal dose, with flexibility to include further patients to confirm the validity of the technique before proceeding to the evaluation phase.

Evaluation phase

In this phase, several centres would recruit patients with colon cancer to determine sensitivity, specificity and diagnostic accuracy of 5-ALA intraoperative LN staging compared with in-depth histopathology, creating a much larger cohort.

Participant recruitment

Any adult patient undergoing elective surgery for right or sigmoid colon cancer amenable for laparoscopic resection, including those with metastatic disease, was eligible for the trial. The trial attempted to enrich the study population to contain patients with LN disease using the FOxTROT radiology criteria for locally advanced disease to obtain as much information as possible on 5-ALA for LN metastases. This included patients with radiological stage more than or equal to radiological stage T3 disease with either three or more visible LNs, at least one node of ≥ 10 mm in size or one irregularly enhancing node. Because of the inaccuracy of CT staging, > 10 patients were recruited to each cohort to achieve the required 10 with positive LN disease (*Figure 2*).

Participant eligibility

The predefined inclusion and exclusion criteria for the trial are described below.

Inclusion criteria

- Able to give informed consent and willing to follow trial protocol.
- Aged > 18 years.
- Patients with cancers of the right and sigmoid colon amenable to laparoscopic resection incorporating D3 lymphadenectomy, as agreed by multidisciplinary team discussion following histopathological confirmation of cancer diagnosis and radiological staging.
- Patients with distant metastatic disease will be eligible, provided laparoscopic resection of the cancer is part of routine clinical care.
- Fit for standard laparoscopic resection.
- American Society of Anaesthesiologists (ASA) classification of ≤ 3 .
- Normal hepatic and renal function on most recent blood tests (to be within 30 days prior to surgery).
For the purposes of the trial, normal hepatic and renal function were defined as:
 - total bilirubin within normal institutional limits
 - aspartate transaminase/alanine transaminase concentrations of $< 2.5 \times$ institutional upper limit of normal
 - glomerular filtration rate of ≥ 60 ml/minute/1.73m² or creatinine concentration within 10% of upper value for normal institutional limits.

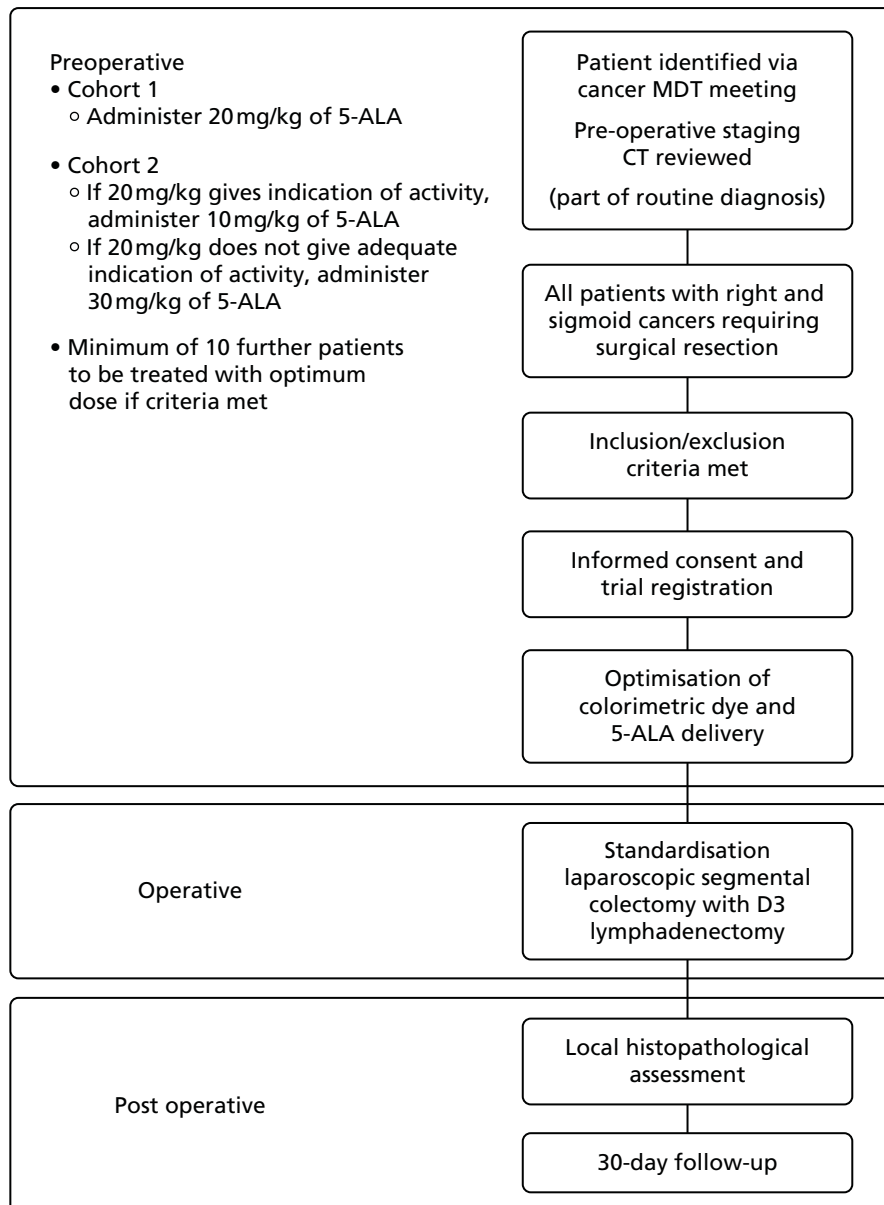


FIGURE 2 Trial flow diagram. MDT, multidisciplinary team.

Exclusion criteria

- Patients with cancers of the transverse and left colon, owing to difficulty in defining D3 lymphadenectomy in these anatomical locations.
- Past history of hypersensitivity reactions to 5-ALA or colorimetric dye.
- Acute or chronic porphyria or a family history of the same.
- Patients with synchronous colonic or rectal cancer (but patients with synchronous benign polyps are eligible).
- Patients with coexistent inflammatory bowel disease, such as Crohn's disease, ulcerative colitis or active diverticulitis, which may influence the lymphatic uptake of 5-ALA.
- Pregnant (positive pregnancy test) or breastfeeding.
- Received an Investigational Medicinal Product (IMP) at any dose within 28 days before registration.
- Poorly controlled or serious medical or psychiatric illness that, in the investigator's opinion, was likely to interfere with participation and/or compliance in this clinical trial.

Potential risks

Participants for this study were selected on the basis that they had a diagnosis of colon cancer that required surgical resection and were suitable for segmental colectomy with D3 lymphadenectomy. There were the normal risks of surgical complications associated with general anaesthesia and surgical resection. There was a small risk of photosensitivity reactions related to 5-ALA administration. This included transient derangement of LFTs (expected to return to baseline at 24–72 hours post administration) and skin hypersensitivity reactions owing to exposure to ultraviolet light. Other mild and transient side effects associated with 5-ALA administration in the literature include nausea, vomiting, tachycardia and hypotension. When 5-ALA is administered in conjunction with surgical resection of malignant brain tumours, a potential side effect is brain oedema and, although this was felt to be an unlikely occurrence in our trial patients, this information was included in the trial patient information leaflet.

In response to these potential adverse effects, all patients were kept out of direct sunlight for at least the first 48 hours following surgery and monitored for changes in vital signs and liver function (which is part of the standard post-operative care). During the operation, patient's eyes and skin were protected from the operating lights using standard methods such as sterile drapes and tape to keep their eyes closed.

Concurrent clinical trials

Patients were screened for participation in other clinical trials. Providing there was no conflict, then patients could be included in both GLiSten and other trials. Notable exceptions were patients recruited to FOxTROT in which pre-operative chemotherapy is given. This can downstage the disease and, therefore, may have reduced the probability of detecting LN metastases.

Duration of participant participation

Patients were followed up for the first 30 post-operative days and this completed their participation in the trial. After this point, patients continued with standard follow-up for their colon cancer as per their institution's normal clinical practice.

Withdrawals or removal of patient criteria

The right of the patient to refuse consent without giving reason was respected and all patients were free to withdraw from the study at any time without giving reasons and without prejudicing any further treatment.

Intervention details

Pre-operative care

All participants underwent a pre-operative staging CT of the thorax, abdomen and pelvis as standard practice within 8 weeks of surgery. All other pre-operative assessment was as per routine cancer practice including colorimetric tattooing of the tumour at colonoscopy. Oral 5-ALA was prepared as described in *Appendix 3* and administered to the patient 1–6 hours prior to surgery.

Perioperative care

Participants were initially assessed with blue-light laparoscopy to detect any fluorescence within the tumour and draining lymphatic field within the mesentery. Fluorescent LNs were marked with Ligaclips [Ethicon Endo-Surgery (Europe) GmbH; Norderstedt, Germany] to facilitate subsequent pathological identification. Patients then underwent a segmental colectomy with D3 lymphadenectomy, if appropriate. For cancers of the right colon, segmental colectomy with D3 lymphadenectomy aimed to include central dissection at the origin of the ileocolic, right colic (when present) and middle colic vessels, with high vascular ligation and division. For cancers of the sigmoid colon, segmental colectomy with D3 lymphadenectomy included high ligation and division of the inferior mesenteric vessels proximal to the origin of the left colic vessels. Specimen extraction, anastomosis and formation of stoma were left to the discretion of the operating surgeon.

The sites of any fluorescent nodes were marked at operation with small surgical clips to facilitate the pathologists' assessment. Resection specimens were subjected to in-depth histopathology, as per the Royal College of Pathologists guidance⁴⁹ as well as additional assessment of fluorescent LNs in the form of step sectioning. This included:

- specimen photography and assessment of the completeness of mesocolic resection of the fresh and the fixed specimen
- distance of tumour and bowel wall to vascular ligation and length of vessels
- area of lymphadenectomy
- level of lymphadenectomy based on vascular anatomy
- histological analysis and mapping of all LNs.

Post-operative care

Participants received standard post-operative care with additional monitoring for side effects to 5-ALA, including measurement of liver and kidney function tests. Patients were reviewed at 30 days postoperatively, as per standard clinical practice. This marked the end of their participation within the trial but they continued to receive appropriate follow-up care as per guidelines.⁵⁰

5-aminolevulinic acid

5-aminolevulinic acid in the form of its hydrochloride, 5-ALA HCl, has been used in humans for FD and photodynamic therapy in a variety of cancers. It preferentially accumulates in cancer cells where it is metabolised to PpIX, a component of the haem pathway and an endogenous fluorophore.²²

5-aminolevulinic acid is safe for human administration and it is used topically in dermatological malignancies, intravesically to detect bladder cancers and orally in premalignant oesophageal lesions and malignant gliomas.²³ It has also been administered intradermally, intraperitoneally and intravenously in experimental studies without adverse effects. 5-ALA is available in various forms depending on the intended mode of application. 5-ALA is cleared by the liver and kidneys with a short half-life and return to baseline levels by 24 hours. Much smaller doses are required for FD – typically 20 mg/kg.

Investigational medicinal product supply, labelling, handling and investigational medicinal product preparation

5-aminolevulinic acid was supplied by photonamic GmbH & Co. KG with a trial-specific label in line with Directive 2001/20/EC and the Medicines for Human Use (Clinical Trials) Regulations 2004 (amended 2006). 5-ALA was prepared and handled in line with manufacturers' recommendations.

5-aminolevulinic acid administration

5-aminolevulinic acid was supplied in a sterile powder format within individual vials. Each vial contained 1.5 g of 5-ALA and was reconstituted in the vial by adding 50 ml of tap water using a needle and syringe, giving a concentration of 30 mg/ml. This was done immediately prior to oral administration.

The patient's weight was used to calculate the amount of 5-ALA to be given, which was checked and verified by the hospital pharmacy department prior to them dispensing the drug the day prior to surgery. On the morning of surgery, the drug was reconstituted as above by the clinical research fellow and witnessed by nursing staff who countersigned the prescription. The correct volume of the reconstituted 5-ALA was calculated and then given to the patient.

Concomitant medications

The only absolute contraindications to the use of 5-ALA are:

- a previous history of hypersensitivity to photosensitising drugs
- a previous history of acute or chronic porphyria
- hepatic or renal dysfunction
- pregnancy.

5-aminolevulinic acid should be used with caution:

- with other medications known to have a photosensitising effect, for example tetracyclines, sulphonamides, quinolones. Patients should not be exposed to any photosensitising agents up to 2 weeks after administration of 5 ALA
- with other medications associated with acute porphyria, for example diclofenac, barbiturates, carbamazepine, phenytoin
- with other medication associated with hepatic or renal dysfunction, for example non-steroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, loop diuretics, phenytoin.

Patient and public involvement

The study protocol was designed with patient and public input provided by the Leeds National Institute for Health Research Healthcare Technology Co-operative in Colorectal Therapies. The ability to identify metastatic LNs and, thus, tailor the radicality of surgery had been identified as an important unmet clinical need in Healthcare Technology Co-operative patient and public workshops. Specific input into the study protocol, in particular focusing on patient safety related to the interventional drug, was provided by Jean Gallagher, Richard Boards, David Wilkinson and Gillian Ivey. All four provided valuable feedback on the patient information leaflets and the plain English summary for the study. Jean Gallagher and David Wilkinson were also integral members of the Trial Management Group (TMG) with access to all data on trial progress. The TMG found the involvement of both Jean Gallagher and David Wilkinson particularly beneficial in commenting on patient recruitment issues and in providing solutions to help recruitment. Gillian Ivey and Richard Boards were major contributing members to the Trial Steering Committee (TSC) providing valuable insights during the temporary halt to the trial.

The decision to conclude the study after the developmental phase, in light of inadequate sensitivity and specificity of 5-ALA for LN detection meaning the end point was not achieved, was endorsed by the trial patient and public involvement (PPI) at the final TMG meeting.

Chapter 4 Data analysis

Primary objective

The primary objective for this study was to optimise the dose of oral 5-ALA administration for intraoperative FD of metastatic LNs in colon cancer.

The presence of any fluorescent LNs was documented at the time of surgery and marked with surgical clips to aid pathological identification. The pathological outcome (positive or negative for metastatic disease) for all LNs was documented. Patients were considered as node positive if metastatic disease was detected in at least one LN on histopathological examination. Similarly, if no metastatic disease was found in any of the LNs on standard histopathological examination, then the patient was considered node negative.

Assessment of diagnostic accuracy was performed on a patient level rather than node level for disease and fluorescence LN status. The sensitivity and specificity of 5-ALA at different doses within the two cohorts was calculated based on whether or not 5-ALA fluorescence detected the pathological outcome of a LN containing metastatic disease. The diagnostic test result was categorised as positive ('fluorescent LNs') if at least one of the positive nodes fluoresced.

The ability of 5-ALA to detect positive nodes in at least 2 out of 10 patients with involved LNs in either cohort was required to progress to the evaluation phase. A 99% CI was used to reflect the additional uncertainty in a relatively small number of patients.

Secondary objectives

Pre-operative staging was compared with the histopathological findings of the resected specimen to determine the sensitivity and specificity of pre-operative CT reporting. Histopathological analysis of the extent of lymphadenectomy, the plane and completeness of mesocolic resection and the resection margin clearance were used to demonstrate the oncological quality of the surgical specimen.

Any potential side effects secondary to 5-ALA were recorded together with conversion rates and any intraoperative or post-operative complications up to 30 days post surgery. Conversion refers to the changeover from the intended laparoscopic surgery to an open laparotomy, which can occur for a variety of reasons.

Chapter 5 Results

Trial recruitment

A total of 44 patients were recruited to the trial with no participants withdrawing during the study period. A total of 37 patients were recruited at St James's University Hospital, Leeds, UK, and seven were recruited from The Mater Misericordiae University Hospital, Dublin, Ireland. Cohort 1 patients were recruited between October 2013 and October 2014 and received an oral dose of 20 mg/kg of 5-ALA. Eighteen patients were required to achieve the target of 10 patients with metastatic LN disease as detected on standard histopathology. Cohort 2 patients were recruited between October 2014 and June 2015 and received an oral dose of 30 mg/kg of 5-ALA, in line with the dose escalation defined in the trial protocol. Twenty-six patients were treated within cohort 2 and nine of these patients had node-positive disease on standard histopathology.

Of the 44 trial patients, four did not undergo blue-light laparoscopy with subsequent histopathological examination. Two patients (one in each cohort) received their dose of 5-ALA and the operation was then cancelled. Another patient received the trial drug but did not have blue-light laparoscopy, as one of the components of the Storz D-light system had not returned from the sterilisation facility. The fourth patient received the trial drug and underwent blue-light laparoscopy, which demonstrated fluorescence of the tumour but no LN fluorescence. However, the tumour was determined to be unresectable and no histopathological examination of the tumour was possible. Therefore, 41 patients underwent blue-light laparoscopy, but 40 patients in the trial (17 in cohort 1 and 23 in cohort 2) underwent an operation with the aid of fluorescence detection and histopathological examination of their specimen was performed.

Patient demographics

Twenty-six male and 18 female patients participated in the trial (*Table 1*), with a mean age of 71 years (range 52–88 years). The mean body mass index was 27.3 kg/m² (range 19.1–37.8 kg/m²), with a median ASA grade of 2. The ratio of right-sided to left-sided cancers was 30 : 14. There were six (14.3%) conversions among the 42 patients who underwent surgery. The majority of patients had pT3, pN0/1 disease. Three patients had metastatic disease involving either the liver or lungs: one in cohort 1 and two in cohort 2. There were no significant differences in the baseline characteristics between the two cohorts.

TABLE 1 Patient demographics

Demographics	Cohort 1 (n = 18)	Cohort 2 (n = 26)
Sex, male : female	10 : 8	16 : 10
Age (years), mean (range)	69.3 (52–85)	71.8 (53–88)
BMI (kg/m ²), mean (range)	26.1 (21–35)	28 (19–38)
ASA grade		
I	9	5
II	8	13
III	1	8
Tumour site, right : left colon	12 : 6	18 : 8
Conversion, yes	4	2
5-ALA-related complication, yes	0	8

BMI, body mass index.

Primary objective: diagnostic accuracy of 5-aminolevulinic acid

Of the 41 patients who underwent blue-light laparoscopy, 14 had fluorescent primary tumours (34.1%) and seven of these 14 patients also had fluorescent LNs (*Table 2*). None of the patients had fluorescent LNs without having fluorescence of the primary tumour in either cohort.

Cohort 1

In the first cohort, 18 patients were treated with 20 mg/kg of 5-ALA but only 17 were operated on with blue-light laparoscopy, as one patient's operation was cancelled. Six out of the 17 patients had a fluorescent primary tumour (35.3%) and three of these patients had fluorescent LNs (see *Table 2*).

One out of the three patients with fluorescent LNs had confirmed metastatic nodal disease within the fluorescent node on standard histopathology. Therefore, there was only one confirmed case of a fluorescent positive node out of 10 patients with positive LN disease. The success detection criteria (the ability of 5-ALA to detect positive LNs in at least 2 out of 10 node-positive patients) for cohort 1 was therefore not met.

In cohort 1, 5-ALA has a sensitivity of 11.1% and specificity of 75% for determining the pathological outcome of LN assessment using fluorescence (*Table 3*).

Two other patients in cohort 1 had fluorescent nodes; however, none of these fluorescent nodes contain any metastatic disease on standard histopathological examination. One patient had no detectable metastatic disease in any of their LNs including the fluorescent node on standard histopathological examination. Therefore, this patient is counted within the fluorescent node-negative group. The third patient had metastatic disease present in 6 out of 23 nodes found within the pathological specimen; however, no metastatic disease was found within the fluorescent node on standard histopathological examination. This patient could occupy two groups in *Table 3*: the fluorescent node-negative group or the non-fluorescent node-positive group. As we are primarily interested in the outcome of the fluorescent nodes, this patient was counted in the fluorescent node-negative group as they are considered to be false positive for the purposes of statistical analysis. However, this does mean that the total number of node-positive patients in *Table 3* will appear to be nine instead of 10.

In summary, in cohort 1, 18 patients were treated with a dose of 20 mg/kg of 5-ALA and only one-third of patients had a fluorescent primary tumour. A total of 10 out of the 18 patients were found to be node positive. Only 1 out of these 10 patients had correctly identified node-positive disease via fluorescence, that is, a positive diagnosis. Therefore, cohort 1 did not meet the predefined criteria for acceptable 5-ALA sensitivity (2 out of 10) and the dosage was increased to 30 mg/kg, as per the protocol.

TABLE 2 Fluorescence results

Observed fluorescence	Cohort 1 (n = 17)	Cohort 2 (n = 24)	Total
Patients with fluorescent primary tumour	6	8	14
Patients with ≥ 1 fluorescent LN	3	4	7

TABLE 3 Comparison of fluorescence and pathology results in cohort 1

Cohort 1	Node positive on histology	Node negative on histology	Total
Patients with fluorescent LNs	1	2	3
Patients with non-fluorescent LNs	8	6	14
Total	9	8	17

Cohort 2

Twenty-six patients were recruited to cohort 2 and nine of these patients had confirmed nodal disease on standard histopathology. Only 24 patients underwent blue-light laparoscopy because one patient had their operation cancelled and the equipment was not available on the day of surgery for another. A third patient was found to have unresectable disease but blue-light laparoscopy was performed. The tumour exhibited fluorescence, however none of the LNs fluoresced in this patient; therefore, only 23 patients underwent blue-light laparoscopy and had histopathological examination of the tumour performed.

Eight out of the 24 patients who underwent blue-light laparoscopy had a primary fluorescent tumour (33.3%) and four of these cases also had fluorescent LNs. None of the patients with fluorescent LNs was found to contain metastatic disease on standard histopathological examination (*Table 4*).

In cohort 2, 5-ALA has a sensitivity of 0% and specificity of 75% for determining the pathological outcome of LN assessment using fluorescence (see *Table 4*).

Out of the four patients in cohort 2 with fluorescent LNs, two had no evidence of metastatic disease in any of the LNs within the specimens and are, therefore, counted in the fluorescent node-negative group. The other two patients had non-fluorescent nodes containing metastatic disease but the fluorescent nodes were benign on standard histopathological examination; therefore, similarly to the patient in cohort 1, they have been counted in the fluorescent node-negative group instead of the non-fluorescent node-positive group. This means that the total number of patients with node-positive disease in cohort 2 will appear to be seven instead of nine, according to *Table 4*.

In summary, in cohort 2, 26 patients were treated with a dose of 30 mg/kg of 5-ALA. Nine out of the 26 patients were found to be node positive. None of the nine patients had correctly identified node-positive disease via fluorescence, that is, a positive diagnosis. Recruitment to the trial ceased prior to reaching the target of 10 patients with node-positive disease in cohort 2; however, it is clear that the predefined criteria of at least two correct diagnoses in the 10 node-positive patients could not be reached as there had not been one correct diagnosis in nine node-positive patients.

Additional histopathological studies of all fluorescent benign LNs in both cohorts are continuing. Further staining of the LNs has not shown any evidence of metastatic disease but the results of immunohistochemistry are still awaited.

Summary

In conclusion, 7 out of 19 patients with node-positive disease exhibited LN fluorescence (36.8%). Neither dose of 5-ALA had adequate sensitivity to meet the defined end point and allow progression into the developmental phase of the trial.

TABLE 4 Comparison of fluorescence and pathology results in cohort 2

Cohort 2	Node positive on histology	Node negative on histology	Total
Patients with fluorescent LNs	0	4	4
Patients with non-fluorescent LNs	7	12	19
Total	7	16	23

Secondary objectives: pre-operative staging

Although 42 patients had surgery performed, one patient had unresectable disease and, therefore, comparison between pre-operative CT imaging and pathology staging is only possible in 41 patients, 19 of whom had nodal metastases.

In 23 out of the 41 patients, pre-operative staging of LN status correctly detected non-involved (N0) and involved (N1/2) nodes compared with histopathological findings, an accuracy of 56.1%. In 11 cases, the LN status was over staged and understaged in seven cases (*Table 5*).

The sensitivity and specificity for CT detection of LN stage in this study were 65% and 47.6%, respectively. This compares to reported sensitivity and specificity of 64–70% and 53–78% in the literature.^{14,15}

Pre-operative CT imaging successfully detected the presence of extramural vascular invasion (EMVI) in 14 out of 41 cases, showing an accuracy of EMVI detection across the two sites of 65.9% (*Table 6*). The sensitivity and specificity for CT detection of EMVI were 60.9% and 72.2%, respectively.

Pre-operative CT accurately predicted whether or not the tumour had invaded beyond the muscularis propria in 33 out of 41 cases (*Table 7*). It is important to remember that for the purposes of this study, patients were selected on the basis of having suspected locally advanced disease on CT imaging.

TABLE 5 Pre-operative CT prediction of LN stage compared with pathology

	LN stage on pathology	
	N1/2	N0
LN stage on CT		
N1/2	13	11
N0	7	10

TABLE 6 Pre-operative CT prediction of EMVI compared with pathology

	EMVI on pathology	
	Positive	Negative
EMVI on CT		
Present	14	5
Absent	9	13

TABLE 7 Pre-operative CT prediction of T stage compared with pathology

Pre-operative CT T stage	T stage on pathology					Total
	T0/1	T2	T3	T4		
T2	1	0	0	1		2
T3	0	6	16	9		31
T4a	0	1	2	4		7
T4b	0	0	0	1		1
Total	1	7	18	15		41

Secondary objectives: standardisation of surgical technique and histopathological examination

Quality of pathology specimens

The oncological quality of the resected specimen was judged on the plane of resection, which was reported in 40 pathology specimens. A total of 26 out of the 40 specimens were resected in the mesocolic plane (65%). There was a higher proportion of mesocolic resections in cohort 2 than cohort 1: 78.2% versus 47.1%, respectively (*Table 8*). These figures should be interpreted in light of the enriched cohort of locally advanced colon cancers. Although the 65% for complete mesocolic excision is lower than reported elsewhere in the literature, the advanced nature of the cancers in this study will have directly impacted on the ability to perform complete mesocolic plane surgery.

Resection margins

A total of 10 cases have been reported as having positive microscopic resection margins (R1), resulting in a positive resection margin rate of 23.8%, which is below the rate deemed to be excessive in the protocol (> 30%). Although this is higher than the R1 resection rate normally reported in colon cancer surgery, it probably reflects the enrichment of the cohorts with advanced stage disease and that some pathologists classify T4 disease with serosal involvement as a positive resection margin and would not be a cause for concern. There were no R2 resections in either cohort.

Complications

The overall anastomotic leak rate was 4.5%, which is below the rate deemed to be excessive in the trial protocol, but in keeping with the literature for this type of surgery. In the first cohort, four laparoscopic cases were converted to open laparotomies, resulting in a conversion rate of 22%, which is below the rate deemed to be excessive in the protocol (> 50%). There were two conversions in the second cohort giving a conversion rate of 8.3%. Although a conversion rate of 22% in cohort 1 would ordinarily be viewed as high, this cohort was enriched with advanced cancers and within this context a 22% conversion rate is probably acceptable.

There have been three intraoperative complications: one visceral perforation and two cases of intraoperative haemorrhage. In the first case, there was a perforation to the greater curve of the stomach following introduction of the Veress needle – this was repaired laparoscopically and the patient made an uneventful recovery. In the first case of intraoperative haemorrhage, there was bleeding from the inferior vena cava, which was clipped laparoscopically and no conversion was necessary; the patient made an uneventful recovery. In the second case of intraoperative haemorrhage, blood was seen in the abdominal cavity during closure of the laparoscopic port sites. The surgeon converted to an open procedure and found that the source of bleeding was from a peripancreatic vessel. This was oversewn and haemostasis achieved. The patient was admitted to the high-dependency unit (HDU) postoperatively for observation and made a good recovery. All three of these intraoperative complications are recognised within the type of surgery performed.

TABLE 8 Comparison of resection specimens between cohorts

Resection plane	Cohort 1 (n = 17)	Cohort 2 (n = 24)
Mesocolic	8	18
Intramesocolic	8	5
Muscularis propria	1	0
Missing data	0	1

Two other patients were managed for post-operative ileus in addition to a patient who had to be readmitted, all of whom had CT scans during the initial few days of their post-operative course. One of these patients was treated for a chest infection with intravenous antibiotics. The other patient with a post-operative ileus underwent CT and the scan did not show any pathology. However, the patient became seriously ill with septic shock in the intensive care unit (ICU). A decision was made to undertake exploratory laparotomy, despite the normal CT scan, and the patient was found to have a sigmoid volvulus causing ischaemic perforation of the bowel. A completion colectomy with end ileostomy was performed. The patient subsequently had a prolonged stay in the ICU, during which he sustained a left internal jugular vein thrombosis secondary to central line placement requiring treatment with low-molecular-weight heparin.

Thirty-day mortality

Unfortunately, one patient died within the first 30 days of surgery. This patient had significant comorbidities and was identified as a high-risk surgical candidate on pre-operative cardiopulmonary exercise testing. The risks were discussed with the patient and their family and the patient was keen to proceed with surgery, including participation within the GLiSten study. The patient developed a lower respiratory tract infection in the early post-operative period but responded well to conservative management. Unfortunately, the patient was found to be acutely peritonitic > 10 days post surgery and findings at relaparotomy were consistent with an anastomotic leak. The anastomosis was resected and a defunctioning stoma was created. Subsequently, the patient suffered a non-ST elevation myocardial infarction and went on to develop multiorgan failure, to which he succumbed. This case was reviewed by the Data Monitoring and Ethics Committee (DMEC) and TSC and 5-ALA was not felt to have been implicated in the patient's development of complications and demise.

Readmissions

Three patients had to be readmitted to hospital shortly following their discharge from the surgical ward. The first patient developed a lower respiratory tract infection that required treatment with intravenous antibiotics. This is a well-recognised post-operative complication and was not thought to be related to 5-ALA. The patient responded well to antibiotic treatment. The second patient requiring readmission presented with abdominal pain and acute kidney injury. The patient had urea and electrolyte levels checked for the first 5 post-operative days and, apart from a slight increase in creatinine on day 4 which had normalised by day 5, no other abnormalities were detected. The kidney injury was believed to be secondary to concomitant medications, which can cause renal impairment, and not as a consequence of receiving 5-ALA. The patient's renal function recovered with intravenous fluids and no other intervention was required. The third patient was managed for a post-operative ileus and a CT scan showed a small amount of intraperitoneal gas, with no demonstrable intra-abdominal collection. It is unclear whether or not this patient had a small anastomotic leak, but the patient was managed conservatively with oral antibiotics for 7 days and was readmitted to hospital for 2 days following their initial discharge.

Drug safety

There have been no drug-related serious adverse events (SAEs) but we did see some minor 5-ALA side effects in eight patients. There were two patients, both male in cohort 2, who developed mild self-limiting photosensitivity reactions that affected the head and neck region. Both of these patients were admitted to the HDU postoperatively for observation and it must be noted that HDU has a much brighter ambient environment than the normal surgical ward. In both cases, the reaction was noted within the first 48 hours following surgery and had resolved by the time of discharge (around post-operative day 5). It is proposed that the brighter ambient environment in the HDU is likely to be a contributing factor. No intervention was required in either patient other than a topical emollient cream for associated skin dryness.

Four patients were noted to have elevated LFTs in the immediate post-operative period. No intervention was required, apart from monitoring, and the LFTs normalised within 3 days post surgery. Two patients had a slight transient deterioration in their renal function but it is not clear whether this was related to the surgery or the 5-ALA. Either way, renal function returned to normal in both patients and without any intervention.

Chapter 6 Discussion

Recruitment to the trial was of a longer duration than originally anticipated. This was mainly due to a temporary halt to the trial from December 2013 to February 2014 as there had been concerns regarding the efficacy of the IMP. Initially, there was good fluorescence seen of the primary tumour but then in consecutive cases no fluorescence was apparent. This prompted a temporary suspension of the trial while the IMP and laparoscopic camera equipment were investigated. No fault was found with either product and the lack of fluorescence was felt to be due to tumour biology. There were no subsequent concerns during the remainder of the trial.

A 2014 study attempted to evaluate the use of 5-ALA FD of peritoneal metastases in patients with colon cancer.⁵¹ A total of 12 patients were selected on the basis of suspected peritoneal disease on CT scans and, in eight patients (66.7%), fluorescence of peritoneal metastases was shown. Unfortunately, this study did not comment on fluorescence of the primary tumour, LNs or any other metastatic deposits that would allow comparison with our study findings. In another recent study, in 2015, the ability of 5-ALA to assist with endoscopic resection of early-stage gastric and colorectal tumours was evaluated.⁴⁷ Only three patients in the study had colorectal lesions, of which one showed fluorescence (33.3%). This proportion fits with the pattern seen in this study, accepting that the numbers studied are very small. There are no other studies evaluating the use of 5-ALA FD for patients with colonic cancer for comparison. However, as discussed previously, several other surgical specialties utilise 5-ALA fluorescence for the detection of solid tumours. A systematic review of the use of 5-ALA in non-muscle-invasive bladder cancer surgery found that the sensitivity of tumour detection ranged from 76% to 97% with a specificity of 56%³² and with good evidence that FD reduced the rate of residual disease.

A Cochrane review³⁸ of image-guided surgery for brain tumours found that the evidence for the use of 5-ALA fluorescence-assisted surgery is possibly not as strong as expected. However, there was a significant improvement in complete resection of the tumour when using 5-ALA fluorescence compared with standard resection: 65% and 36%, respectively.

5-aminolevulinic acid appears to be more efficient in detecting urological and neurological malignancies than colorectal tumours, and other factors for the decreased sensitivity seen in our study must be considered. Timing and dose of 5-ALA administration were similar to other published studies with similar patient characteristics. Other possibilities include autofluorescence of the surrounding tissue rendering PpIX fluorescence of the tumour imperceptible and natural variability of fluorescence intensity depending on tumour biology. Another possibility is photobleaching of the tumour by white-light laparoscopy at the beginning of the procedure, diminishing the effect of fluorescence over time. Neither of these explanations is thought to have materially contributed to the low fluorescence rates (primary cancer and LNs) observed in our study.

In our series of 41 patients, 19 had nodal disease and, of these, seven exhibited intraoperative fluorescence of the LNs (36.8%). 5-ALA fluorescence studies in other malignancies have not focused on detection of LN metastases and, therefore, the only data for comparison are within animal studies of colorectal cancer.

In a small study using a murine model of colorectal cancer,⁴⁵ 10 mice were administered intraperitoneal 5-ALA. Of these, three had fluorescent mesocolic LNs present and all fluorescent LNs had metastatic disease present on histopathological examination. Unfortunately, the study did not perform histopathological examination of all LNs; therefore, sensitivity and specificity are not known. There has been a small study recently examining 5-ALA fluorescence and its ability to detect LN metastases in freshly excised colonic tumour resections.⁴⁸ Fourteen patients, the majority of whom had stage III disease ($n = 12$), were included in the study and nine had node-positive disease. The study used a spectral unmixing method to remove the possibility of masking true PpIX fluorescence from collagen autofluorescence. The sensitivity and specificity

for detecting LN metastatic disease in this study were 88.3% and 92%, respectively, which are much higher than our findings. The study also states that there were no fluorescent benign LNs; however, it must be stressed that fluorescence was tested *ex vivo* and further details of LN fluorescence, including any non-fluorescent LNs containing metastatic disease, were not provided.

Our results show that 5-ALA is not sensitive enough to detect LN metastasis and, therefore, cannot be recommended for use in intraoperative staging of colonic cancer. This is disappointing, but valuable knowledge has been gained because there is no other study that has used 5-ALA for intraoperative fluorescent diagnosis of colonic LN metastases.

Sensitivity and specificity of CT imaging to detect LN metastases preoperatively in the literature are 64–70% and 53–78%, respectively, bearing in mind that different studies use slightly different definitions of malignant LNs. In our study, CT imaging had a sensitivity of 68.4% and a specificity of 47.6%, which is comparable to published work in the literature^{14,15} (see *Table 5*). 5-ALA FD has not demonstrated an improved ability to detect LN metastases compared with pre-operative imaging; therefore, other strategies for improving the staging of LN involvement in colorectal cancer need to be investigated.

Patients with suspected locally advanced disease on CT imaging were selected in an attempt to enrich the study with patients who had node-positive disease. Thirty-three patients (80.5%) in the study were confirmed to have locally advanced disease on histopathological examination. As the literature suggests that around 75% of patients with 'bad' T-stage disease would have positive LNs, we would have expected to find approximately 30 patients with node-positive disease across the two cohorts. Instead, the total number of patients with LN disease was 19 (46.3%). None of our patients received pre-operative chemotherapy and so this lower rate of node-positive disease probably reflects inaccurate patient selection based on CT imaging.

Chapter 7 Conclusions

This is the first human clinical trial of a photosensitiser used for intraoperative fluorescence LN staging in colon cancer. There have been no safety concerns identified using 5-ALA or with regard to surgical procedure. All SAEs were recognised post-operative complications and not related to 5-ALA.

A distinct difference has been observed in the fluorescence of the primary colon cancers, with around 30% of cancers exhibiting fluorescence and 70% showing no fluorescence. This might indicate an underlying difference in the uptake and metabolism of 5-ALA, which could possibly be of prognostic benefit. Further work is planned to investigate the difference in fluorescence at the molecular level and to extend follow-up to include cancer recurrence and overall survival, which will be funded separately to the GLiSten study.

Another area of interest is the non-specific LN fluorescence (false-positive cases) seen in some patients. It is possible that this could be related to the host inflammatory/stromal response. Again, this will be investigated further.

5-aminolevulinic acid has been shown to have poor sensitivity for detecting LN metastases and cannot be recommended for intraoperative staging on the basis of this trial. However, the study design was acceptable to patients and it has demonstrated that fluorescence-assisted surgery using the Storz D-light system is feasible, which is important for the design of future studies using alternative fluorescent probes. Although our findings do not support the use of 5-ALA for intraoperative detection of malignant LNs, useful experience in fluorescence-assisted surgery has been gained and interesting areas for further research have been identified. Stratified surgery for colorectal cancer remains a 'holy grail' and will become increasingly important as the population ages and elderly and frail patients become less able to tolerate radical surgery. Ultimately, more sensitive and specific fluorescent probes are required if we are going to succeed in the challenge of providing personalised surgery for patients with colon cancer.

Recommendations for future research

An interesting finding of this trial is that only one-third of the primary tumours fluoresced and we feel that this warrants further investigation, as the existing literature would lead us to believe there should have been a higher rate of fluorescence. As discussed previously, the fluorescence from 5-ALA is due to its metabolite, PpIX. The most important element to ascertain is whether or not the 5-ALA and its metabolite were present within the tumour. We plan to perform an extraction procedure on each tumour specimen to determine if PpIX is present to see if this explains the low rate of fluorescence.

Another possibility is that the natural variability in tumour biology has had some effect on the fluorescence seen in the primary tumours. Other factors that could have had an impact are the effect of the inflammatory response incited by the tumour and potential underlying differences in cell metabolism. Further research investigating whether or not differences in tumour cell density, T-cell tumour infiltration and metabolic pathways could explain the low levels of fluorescence seen in the primary tumours is being conducted. This includes histological assessment of cellular and stromal components of the cancers with specific reference to immune profile and deoxyribonucleic acid gene array profiling.

The GLiSten study is the first human clinical trial using 5-ALA in laparoscopic colorectal cancer resection, and the majority of the work that has informed the existing literature on this area is based on laboratory animal work. It is difficult to know how representative the animal models were that demonstrated these significant fluorescence results. There is a need to develop better small-animal models for pre-clinical testing of molecular probes to facilitate translation to first-in-man translation.

The need to improve LN staging in colorectal cancer still exists and the use of fluorescence to achieve this aim is still a viable option. However, identification of better fluorescent probes for intraoperative visualisation of primary cancers and LN disease is required.

Nanomedicine is an exciting expanding area of research and there is particular interest in using nanoparticles in the imaging and treatment of tumours. Specific targeting of tumour tissue by nanoparticles is possible by combining them with tumour-specific cell surface receptor antibodies. Work on using antibodies to target fluorescent nanoparticles to create novel probes against colorectal cancer cell lines is currently ongoing at the University of Leeds (further information can be obtained from the corresponding author).

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Contributions of authors

Miss Helen Andrew contributed by fulfilling duties of the clinical research fellow responsible for the trial, active participation in data collection analysis and interpretation of the data as well as contributing to the writing of the report with final approval of the manuscript.

Miss Gemma Gossedge contributed by fulfilling duties of the clinical research fellow responsible for the trial as well as final approval of the manuscript.

Mrs Julie Croft provided support throughout the running of the trial as well as final approval of the manuscript.

Mr Neil Corrigan contributed to the conception and design of the trial, provided support throughout the running of the trial and assisted with analysis and interpretation of the data as well as final approval of the manuscript.

Professor Julia M Brown contributed to the conception and design of the trial as well as having final approval of the manuscript.

Dr Nicholas West contributed to the study by active participation in data collection, pathological assessment of the patient specimens as well as having final approval of the manuscript.

Professor Philip Quirke contributed to the conception and design of the trial and pathological assessment of the patient specimens as well as having final approval of the manuscript.

Dr Damien Tolan contributed to the trial by assessing all pre-operative CT imaging and providing radiological support.

Professor Ronan Cahill contributed to the conception and design of the trial, along with active participation of patient recruitment, execution of the study as well as having final approval of the manuscript.

Professor David G Jayne contributed to the conception and design of the trial, along with active participation of patient recruitment, execution of the study, analysis and interpretation of the data as well as contributing to the writing of the report with final approval of the manuscript.

Trial personnel

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Dr John Hartley: Senior Lecturer in Surgery and member of the DMEC.

Mrs Christina Wong: Consultant Pharmacist and member of the DMEC.

Publications

Gossedge G, Lowe C, West N, Tolan D, Brown J, Quirke P, *et al.* Next generation intraoperative lymph node staging for stratified colon cancer surgery (GLiSten). Belgrade: special issue – Abstracts of the ESCP (European Society of Coloproctology) 8th Scientific and Annual Meeting; 25–27 September 2013. *Colorectal Dis* 2013;**15**(Suppl. 3):1–129.

Data sharing statement

Patient information was prospectively collected and recorded on specifically designed case report forms (CRFs). The data from the CRFs were transferred onto a secure database managed through the Clinical Trials Research Unit at the University of Leeds. The paper CRFs are stored securely at St James's University Hospital. All trial documentation and data will be securely archived in accordance with the 1998 Data Protection Act and the principals of Good Clinical Practice and will be kept for a minimum of 15 years from the end of the trial as per the Sponsor's policy. Clinical trial summary results have been posted in the EudraCT. All available anonymised data can be obtained by contacting the corresponding author.

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Appendix 1 Literature review search strategies

Included in this appendix are the full electronic search strategies used to review the existing literature. All literature searches were conducted through the Ovid MEDLINE database and were last reviewed in November 2015.

Research question: has the National Bowel Cancer Screening Programme caused a downstaging effect in colorectal cancer?

Search string number	Search string (including any limits)	Total number of results found
1	national adj1 bowel adj1 cancer adj1 screening adj1 program\$	53
2	downstag\$	1997
3	1 and 2	1

Research question: does complete mesocolic excision/resection affect patient outcomes in colorectal cancer surgery?

Search string number	Search string (including any limits)	Total number of results found
1	exp Colorectal Neoplasms	163,580
2	colo\$	770,305
3	rect\$	168,199
4	2 or 3	889,325
5	cancer	1,114,158
6	malig\$	424,628
7	adeno\$	596,493
8	carcin\$	795,956
9	4 and 5	164,695
10	4 and 6	38,496
11	4 and 7	68,630
12	4 and 8	89,968
13	1 or 9 or 10 or 11 or 12	269,904
14	complete adj1 mesocolic adj1 excision	77
15	complete adj1 mesocolic adj1 resection	5
16	14 or 15	78
17	exp Recurrence or exp neoplasm Recurrence, Local	248,743
18	recurrence	361,374
19	local adj1 recurrence	102,965
20	17 or 18 or 19	361,374
21	exp disease-free survival	51,202
22	disease adj1 free adj1 survival	65,748
23	21 or 22	65,748
24	20 or 23	404,424
25	13 and 16 and 24	26
26	limit 25 to English language	22

Research question: does an extended D3 lymphadenectomy improve outcomes in patients with colorectal cancer?

Search string number	Search string (including any limits)	Total number of results found
1	exp Colorectal Neoplasms	163,580
2	colo\$	770,305
3	rect\$	168,199
4	2 or 3	889,325
5	cancer	1,114,158
6	malig\$	424,628
7	adeno\$	596,493
8	carcin\$	795,956
9	4 and 5	164,695
10	4 and 6	38,496
11	4 and 7	68,630
12	4 and 8	89,968
13	1 or 9 or 10 or 11 or 12	269,904
14	exp Recurrence or exp Neoplasm Recurrence, Local	248,743
15	recurrence	361,374
16	local adj1 recurrence	102,965
17	14 or 15 or 16	361,374
18	exp disease-free survival	51,202
19	disease adj1 free adj1 survival	65,748
20	exp Lymph Node Excision	38,894
21	lymphadenectomy	12,229
22	D3 adj3 lymphadenectomy	95
23	20 or 21 or 22	43,257
24	18 or 19	65,748
23	17 or 24	404,424
26	13 and 23 and 25	831
27	limit 26 to English language	629
28	13 and 22 and 25	22

Research question: does complete mesocolic excision/resection increase the surgical complication rate?

Search string number	Search string (including any limits)	Total number of results found
1	exp Colorectal Neoplasms	163,580
2	colo\$	770,305
3	rect\$	168,199
4	2 or 3	889,325
5	cancer	1,114,158
6	malig\$	424,628
7	adeno\$	596,493
8	carcin\$	795,956
9	4 and 5	164,695
10	4 and 6	38,496
11	4 and 7	68,630
12	4 and 8	89,968
13	1 or 9 or 10 or 11 or 12	269,904
14	complete adj1 mesocolic adj1 excision	77
15	complete adj1 mesocolic adj1 resection	5
16	14 or 15	78
17	exp Intraoperative Complications or exp Postoperative Complications	478,358
18	complica\$	1,161,022
19	intraoper\$ adj3 complica\$	34,562
20	postoper\$ adj3 complica\$	334,633
21	17 or 18 or 19 or 20	1,288,161
22	13 and 16 and 21	27

Research question: does extended D3 lymphadenectomy increase the surgical complication rate?

Search string number	Search string (including any limits)	Total number of results found
1	exp Colorectal Neoplasms	163,580
2	colo\$	770,305
3	rect\$	168,199
4	2 or 3	889,325
5	cancer	1,114,158
6	malign\$	424,628
7	adeno\$	596,493
8	carcin\$	795,956
9	4 and 5	164,695
10	4 and 6	38,496
11	4 and 7	68,630
12	4 and 8	89,968
13	1 or 9 or 10 or 11 or 12	269,904
14	exp Intraoperative Complications or exp Postoperative Complications	478,358
15	complica\$	1,161,022
16	intraoper\$ adj3 complica\$	34,562
17	postoper\$ adj3 complica\$	334,633
18	14 or 15 or 16 or 17	1,288,161
19	exp Lymph Node Excision	38,894
20	lymphadenectomy	12,229
21	D3 adj3 lymphadenectomy	95
22	19 or 20 or 21	43,257
23	13 and 18 and 22	674
24	limit 23 to English language	491

Research question: how accurate is pre-operative staging in detecting LN metastases in colorectal cancer?

Search string number	Search string (including any limits)	Total number of results found
1	exp Colorectal Neoplasms	163,580
2	colo\$	770,305
3	rect\$	168,199
4	2 or 3	889,325
5	cancer	1,114,158
6	malig\$	424,628
7	adeno\$	596,493
8	carcin\$	795,956
9	4 and 5	164,695
10	4 and 6	38,496
11	4 and 7	68,630
12	4 and 8	89,968
13	1 or 9 or 10 or 11 or 12	269,904
14	lymph node metas\$	32,188
15	nod\$ meta\$	40,469
16	exp neoplasm metastasis or exp lymphatic metastasis	169,914
17	lymp\$ met\$	77,565
18	nod\$ adj3 met\$	52,525
19	lym adj3 nod\$ adj3 met\$	43,104
20	lym\$ adj3 met\$	101,830
21	14 or 15 or 16 or 17 or 18 or 19 or 20	200,829
22	exp Neoplasm Staging	136,750
23	stag\$	849,852
24	preopera\$ adj3 stag\$	4254
23	22 or 23 or 24	849,852
26	13 and 21 and 25	8091
27	accuracy	227,249
28	accur\$	474,923
29	detection\$	611,530
30	detec\$	1,653,696
31	27 or 28 or 29 or 30	2,021,880
32	26 and 31	2811
33	limit 32 to English language	2335

Research question: is CT accurate in detecting LN metastases in colorectal cancer?

Search string number	Search string (including any limits)	Total number of results found
1	exp Colorectal Neoplasms	163,580
2	colo\$	770,305
3	rect\$	168,199
4	2 or 3	889,325
5	cancer	1,114,158
6	malig\$	424,628
7	adeno\$	596,493
8	carcin\$	795,956
9	4 and 5	164,695
10	4 and 6	38,496
11	4 and 7	68,630
12	4 and 8	89,968
13	1 or 9 or 10 or 11 or 12	269,904
14	lymph node metas\$	32,188
15	nod\$ meta\$	40,469
16	exp neoplasm metastasis or exp lymphatic metastasis	169,914
17	lymp\$ met\$	77,565
18	nod\$ adj3 met\$	52,525
19	lym adj3 nod\$ adj3 met\$	43,104
20	lym\$ adj3 met\$	101,830
21	14 or 15 or 16 or 17 or 18 or 19 or 20	200,829
22	accuracy	227,249
23	accur\$	474,923
24	detection\$	611,530
23	detec\$	1,653,696
26	22 or 23 or 24 or 25	2,021,880
27	exp Tomography, X-Ray Computed	334,528
28	CT adj3 imag\$	30,260
29	CT adj3 stag\$	2322
30	27 or 28 or 29	341,239
31	13 and 21 and 26 and 30	503
32	limit 31 to English language	399

Research question: can LN metastases be detected intraoperatively in colorectal cancer?

Search string number	Search string (including any limits)	Total number of results found
1	lymph node metas\$	32,188
2	nod\$ meta\$	40,469
3	exp neoplasm metastasis or exp lymphatic metastasis	169,914
4	lymp\$ met\$	77,565
5	nod\$ adj3 met\$	52,525
6	lym adj3 nod\$ adj3 met\$	43,104
7	lym\$ adj3 met\$	101,830
8	1 or 2 or 3 or 4 or 5 or 6 or 7	200,829
9	exp Colorectal Neoplasms	163,580
10	colo\$	770,305
11	rect\$	168,199
12	10 or 11	889,325
13	cancer	1,114,158
14	malig\$	424,628
15	adeno\$	596,493
16	carcin\$	795,956
17	12 and 13	164,695
18	12 and 14	38,496
19	12 and 15	68,630
20	12 and 16	89,968
21	9 or 17 or 18 or 19 or 20	269,904
22	intraoperative staging	119
23	intraop\$ adj3 stag\$	411
24	intraop\$	130,252
25	stag\$	849,852
26	detec\$	1,653,696
27	24 and 25	9958
28	24 and 26	11,875
29	intraop\$ adj3 detec\$	1471
30	intraoperative detec\$	419
31	22 or 23 or 27 or 28 or 29 or 30	20,186
32	8 and 21 and 31	431
33	limit 32 to English language	342

Research question: how accurate is SLN mapping in detecting LN metastases in colorectal cancer?

Search string number	Search string (including any limits)	Total number of results found
1	exp Colorectal Neoplasms	163,580
2	colo\$	770,305
3	rect\$	168,199
4	2 or 3	889,325
5	cancer	1,114,158
6	malig\$	424,628
7	adeno\$	596,493
8	carcin\$	795,956
9	4 and 5	164,695
10	4 and 6	38,496
11	4 and 7	68,630
12	4 and 8	89,968
13	1 or 9 or 10 or 11 or 12	269,904
14	exp Lymph Nodes or exp Sentinel Lymph Node Biopsy or exp Melanoma or exp Lymphatic Metastasis	211,417
15	sentinel lymph node	10,299
16	sentinel adj1 lymph	10,584
17	sentinel adj1 lymph adj1 node	10,300
18	sentinel adj3 mapping	1155
19	14 or 15 or 16 or 17 or 18	211,876
20	13 and 19	14,765
21	accuracy	227,249
22	accur\$	474,923
23	detection\$	611,530
24	detec\$	1,653,696
25	detection	610,414
26	accuracy\$	227,321
27	21 or 22 or 23 or 24 or 25 or 26	2,021,880
28	20 and 27	3724
29	limit 28 to English language	3152
30	exp Neoplasm Metastasis	169,914
31	exp Lymphatic Metastasis	75,941
32	lymphatic met\$	76,590
33	lymp\$ met\$	77,565
34	lym\$ adj3 met\$	101,830
35	lymph node	123,910
36	lymph node metas\$	32,188
37	lym\$ adj3 nod\$ adj3 met\$	43,104
38	30 or 31 or 32 or 33 or 34 or 35 or 36 or 37	260,653
39	28 and 38	2960
40	limit 39 to English language	2441

Research question: can photodynamic diagnosis be used to detect LN metastases intraoperatively in colorectal cancer?

Search string number	Search string (including any limits)	Total number of results found
1	lymph node metas\$	32,188
2	nod\$ meta\$	40,469
3	exp neoplasm metastasis or exp lymphatic metastasis	169,914
4	lymp\$ met\$	77,565
5	nod\$ adj3 met\$	52,525
6	lym adj3 nod\$ adj3 met\$	43,104
7	lym\$ adj3 met\$	101,830
8	1 or 2 or 3 or 4 or 5 or 6 or 7	200,829
9	exp Colorectal Neoplasms	163,580
10	colo\$	770,305
11	rect\$	168,199
12	10 or 11	889,325
13	cancer	1,114,158
14	malig\$	424,628
15	adeno\$	596,493
16	carcin\$	795,956
17	12 and 13	164,695
18	12 and 14	38,496
19	12 and 15	68,630
20	12 and 16	89,968
21	9 or 17 or 18 or 19 or 20	269,904
22	intraoperative staging	119
23	intraop\$ adj3 stag\$	411
24	intraop\$	130,252
25	stag\$	849,852
26	detec\$	1,653,696
27	24 and 25	9958
28	24 and 26	11875
29	intraop\$ adj3 detec\$	1471
30	intraoperative detec\$	419
31	22 or 23 or 27 or 28 or 29 or 30	20,186
32	8 and 21 and 31	431
33	photodynamic diagnosis	294
34	photody\$ adj3 diagno\$	395
35	exp Photosensitizing Agents	26,476
36	33 or 34 or 35	26,595
37	32 and 36	4

Research question: use of 5-ALA in photodynamic diagnosis of malignancy

Search string number	Search string (including any limits)	Total number of results found
1	exp Aminolevulinic acid	4496
2	aminolevulinic adj1 acid	6780
3	photodynamic diagnosis	294
4	photodyna\$ adj3 diagnos\$	395
5	1 or 2	6780
6	3 or 4	395
7	5 and 6	199
8	exp Neoplasms	2,805,544
9	7 and 8	179
10	limit 9 to English language	141

Research question: can 5-ALA detect LN metastases in colorectal cancer?

Search string number	Search string (including any limits)	Total number of results found
1	lymph node metas\$	32,188
2	nod\$ meta\$	40,469
3	exp neoplasm metastasis or exp lymphatic metastasis	169,914
4	lymp\$ met\$	77,565
5	nod\$ adj3 met\$	52,525
6	lym adj3 nod\$ adj3 met\$	43,104
7	lym\$ adj3 met\$	101,830
8	1 or 2 or 3 or 4 or 5 or 6 or 7	200,829
9	exp Colorectal Neoplasms	163,580
10	colo\$	770,305
11	rect\$	168,199
12	10 or 11	889,325
13	cancer	1,114,158
14	malig\$	424,628
15	adeno\$	596,493
16	carcin\$	795,956
17	12 and 13	164,695
18	12 and 14	38,496
19	12 and 15	68,630
20	12 and 16	89,968
21	9 or 17 or 18 or 19 or 20	269,904
22	intraoperative staging	119

Research question: can 5-ALA detect LN metastases in colorectal cancer?		
Search string number	Search string (including any limits)	Total number of results found
23	intraop\$ adj3 stag\$	411
24	intraop\$	130,252
25	stag\$	849,852
26	detec\$	1,653,696
27	24 and 25	9958
28	24 and 26	11,875
29	intraop\$ adj3 detec\$	1471
30	intraoperative detec\$	419
31	22 or 23 or 27 or 28 or 29 or 30	20,186
32	8 and 21 and 31	431
33	photodynamic diagnosis	294
34	photody\$ adj3 diagno\$	395
35	exp Photosensitizing Agents	26,476
36	33 or 34 or 35	26,595
37	32 and 36	4
38	exp Aminolevulinic acid	4496
39	aminolevulinic acid	6780
40	nod\$ adj3 met\$ adj1 aminolevulinic adj1 acid	0
41	38 or 39 or 40	6780
42	32 and 41	3
37	37 and 41	3

Appendix 2 Trial case report forms



FORM 01
Page 1 of 2

Participant Demographics

Participant Initials		Date of Birth	Day	Month	Year	Participant ID	Centre No	Trial No
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To be completed before registration and consent

Participant Measurements

Height . m

Weight . kg

BMI .

Co-morbidities

Please indicate comorbidities present by ticking yes or no for each:

	Yes	No
Cardiovascular	<input type="checkbox"/>	<input type="checkbox"/>
Peripheral vascular disease	<input type="checkbox"/>	<input type="checkbox"/>
Respiratory	<input type="checkbox"/>	<input type="checkbox"/>
Renal Failure	<input type="checkbox"/>	<input type="checkbox"/>
Liver Failure	<input type="checkbox"/>	<input type="checkbox"/>

Is the participant diabetic? Yes No

If yes, are they taking insulin? Yes No

Are they taking tablets? Yes No

Please list tablets

Completed by	<input type="text"/>	Date	Day	Month	Year	Form continues on next page ►►
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	Initials	

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FORM 01
Page 2 of 2

Participant Demographics

Participant Initials		Date of Birth	Day	Month	Year	Participant ID	Centre No	Trial No
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Medications

Please list the medications the participant is receiving: N/A – No other medications
(Excluding any diabetes medication noted on page 1)

Medication name	Dose	Units	Route	Frequency

Participant Assessment

Only participants with grade ≤3 are eligible for the trial

- ASA grade I 1 – A normal healthy patient
 II 2 – A patient with mild systemic disease
 III 3 – A patient with severe systemic disease
 4 – A patient with severe systemic disease that is a constant threat to life
 5 – A moribund patient who is not expected to survive without the operation
 6 – A declared brain-dead patient whose organs are being removed for donor purposes
<http://www.asahq.org/clinical/physicalstatus.htm> Retrieved 26/04/2010

Completed by Date

Day	Month	Year
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	Date	Initials	Date	Initials

Participant Initials		Date of Birth	Day	Month	Year	Participant ID	Centre No	Trial No
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To be completed prior to registration.
Participants must fulfil ALL eligibility criteria in order to be registered into the trial.

Inclusion Criteria

Please tick yes/no for all questions

If any shaded boxes are ticked, the participant is ineligible

	Yes	No						
1. Is the patient 18 years of age or over?	<input type="checkbox"/>	<input type="checkbox"/>						
2. Has the patient provided written informed consent?	<input type="checkbox"/>	<input type="checkbox"/>						
3. Is the patient willing to follow trial protocol?	<input type="checkbox"/>	<input type="checkbox"/>						
4. Does the patient have a histologically confirmed colonic carcinoma?	<input type="checkbox"/>	<input type="checkbox"/>						
Date of histology report	<table border="1"> <tr> <td>Day</td> <td>Month</td> <td>Year</td> </tr> <tr> <td></td> <td></td> <td></td> </tr> </table>		Day	Month	Year			
Day	Month	Year						
5. Does the patient have radiological evidence of colonic carcinoma?	<input type="checkbox"/>	<input type="checkbox"/>						
6. Does the patient have a right sided or sigmoid cancer?	<input type="checkbox"/>	<input type="checkbox"/>						
7. Is the patient's colon cancer suitable for resection by laparoscopic procedure?	<input type="checkbox"/>	<input type="checkbox"/>						
8. Is the patient fit for laparoscopic D3 resection?	<input type="checkbox"/>	<input type="checkbox"/>						
9. Has the patient management been agreed at MDT discussion? <i>(N.B. Distant metastatic disease should not preclude patients from the developmental phase of the trial provided laparoscopic resection is part of routine clinical care)</i>	<input type="checkbox"/>	<input type="checkbox"/>						
10. Does the patient have an ASA grade ≤3?*	<input type="checkbox"/>	<input type="checkbox"/>						
11. Does the patient have normal hepatic function?***	<input type="checkbox"/>	<input type="checkbox"/>						
12. Does the patient have normal renal function?***	<input type="checkbox"/>	<input type="checkbox"/>						

***ASA grade**

- 1 – A normal healthy patient
 - 2 – A patient with mild systemic disease
 - 3 – A patient with severe systemic disease
 - 4 – A patient with severe systemic disease that is a constant threat to life
 - 5 – A moribund patient who is not expected to survive without the operation
 - 6 – A declared brain-dead patient whose organs are being removed for donor purposes
- <http://www.asahq.org/clinical/physicalstatus.htm> Retrieved 26/04/2010

****Definition of normal hepatic and renal function**

- Total bilirubin within normal institutional limits
- AST/ALT <2.5 × institutional upper limit of normal
- GFR ≥60 mls/min/1.73 m² or creatinine within 10% of upper value for normal institutional limits. Any concerns should be raised with the SJUH Research Fellow.

Completed by	<input type="text"/>	Date	Day	Month	Year
Investigator signature	<input type="text"/>	Date	Day	Month	Year

Form continues on next page ►

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		Initials

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Participant Initials		Date of Birth	Day	Month	Year	Participant ID	Centre No	Trial No
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Exclusion Criteria

**Please tick yes/no for all questions
 If any shaded boxes are ticked, the participant is ineligible**

	Yes	No	
1. Does the patient have a PMH of a hypersensitivity reaction to ALA?	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
2. Does the patient have a PMH of a hypersensitivity reaction to colourimetric dye?	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
3. Does the patient have a PMH of acute or chronic or a family history of porphyria?	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
4. Does the patient have a carcinoma of the transverse colon? <i>(Distal to the proximal border of the falciform ligament to the initial angulation of the splenic flexure)</i>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
5. Does the patient have a carcinoma of the descending colon? <i>(From the initial angulation of the splenic flexure to the level of the left iliac crest)</i>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
6. Does the patient have a PMH of Crohn's disease?	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
7. Does the patient have a PMH of ulcerative colitis?	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
8. Does the patient have a PMH of any additional on-going colitis, e.g. ischaemic/active diverticulitis?	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
9. Does the patient have a PMH of synchronous colonic or rectal cancer (but not benign polyps)?	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
10. Is the patient pregnant?	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> N/A – The patient is male
11. Is the patient breastfeeding?	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> N/A – The patient is male
12. Has the patient received any investigational medicinal product at any dose within 28 days before registration?	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
13. Does the patient have any poorly controlled medical illness that, in the Investigator's opinion, is likely to interfere with participation and/or compliance in this clinical trial?	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
14. Does the patient have any poorly controlled psychiatric illness that, in the Investigator's opinion, is likely to interfere with participation and/or compliance in this clinical trial?	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
15. Is the patient involved in the FOxTROT trial?	<input checked="" type="checkbox"/>	<input type="checkbox"/>	

Completed by	<input type="text"/>	Date	Day	Month	Year
Investigator signature	<input type="text"/>	Date	Day	Month	Year

Last Page ■

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GLISTEN Version 2.0 03/10/2014

Participant Initials		Date of Birth	Day	Month	Year	Participant ID	Centre No	Trial No
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**To be completed following informed consent and assessment of eligibility.
 Complete Section A and B before telephoning the CTRU to register the participant.**

Section A – Caller and Participant Details

Caller name			
Name of treating surgeon			
Glisten centre number/ NIHR site code			
Centre name			
Participant initials	Please use the phonetic alphabet for participant initials		
Participant gender	<input type="checkbox"/> Male	<input type="checkbox"/> Female	
Participant date of birth	Day	Month	Year
NHS number			
<input type="checkbox"/> N/A for Irish hospitals			

A Alpha	H Hotel	O Oscar	V Victor
B Bravo	I India	P Papa	W Whiskey
C Charlie	J Juliet	Q Quebec	X X-ray
D Delta	K Kilo	R Romeo	Y Yankee
E Echo	L Lima	S Sierra	Z Zulu
F Foxtrot	M Mike	T Tango	
G Golf	N November	U Uniform	

Has the eligibility checklist (Form 02) been completed?	<input type="checkbox"/> Yes	<input type="checkbox"/> No	All answers must be YES to proceed with registration
Does the participant satisfy all the eligibility criteria?	<input type="checkbox"/> Yes	<input type="checkbox"/> No	
Has the participant provided written informed consent to enter the trial?	<input type="checkbox"/> Yes	<input type="checkbox"/> No	
Date of written informed consent	Day	Month	

Section B – Planned Operation

Planned operation	<input type="checkbox"/> Right hemicolectomy		
(Please tick one only)	<input type="checkbox"/> Extended right hemicolectomy		
	<input type="checkbox"/> Sigmoid colectomy		
	<input type="checkbox"/> High anterior resection		
	<input type="checkbox"/> Hartmann's procedure		
Planned operation date	Day	Month	Year

**Please ensure all details in Section A and B are completed.
 TO REGISTER THE PARTICIPANT, PLEASE CALL THE CTRU OFFICE-HOURS
 REGISTRATION SERVICE ON 0113 343 4930.
 (Monday–Friday 9 am–5 pm except public and university holidays)**

Section C – Registration Details

This information will be given at registration			
Participant ID	Centre No / Trial No		
Date of registration	Day	Month	Year

You will only be given the **5-digit trial number** at registration. This forms the second part of the participant ID number.

Completed by	Date	Day	Month	Year	Last Page
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Participant Initials		Date of Birth	Day	Month	Year	Participant ID	Centre No	Trial No
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To be completed prior to the planned operation

CT Scan Details

Was a CT chest abdomen and pelvis performed? Yes → Date of scan

Day	Month	Year

 No Is this within 8 weeks of the actual date of surgery? Yes No

Was a CT colonography performed? Yes → Date of scan

Day	Month	Year

 No Is this within 8 weeks of the actual date of surgery? Yes No

Which scan was used to locate nodes? CT CAP
 CT colon

Was a scan performed in portal venous phase at 65 seconds? Yes
 No

Was a reconstructed slice thickness 5 mm axial & 3 mm coronal planes for the abdomen performed? Yes
 No → Slice thickness

--

Tumour Details

Site of tumour (Tick one only)
 Caecum (Segment proximal to or involving ileocaecal valve)
 Ascending colon (Segment distal to ileocaecal valve and proximal to the initial angulation of the hepatic flexure)
 Hepatic flexure (Segment distal to the initial angulation of the hepatic flexure to the proximal border of the falciform ligament)
 Sigmoid colon (Segment distal to the level of the left iliac crest to 15 cm proximal to the anal verge)

Tumour morphology (Tick one only)
 Polypoidal
 Flat
 Semi annular
 Annular

Tumour length

--

 mm

Completed by

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 Date

Day	Month	Year

Form continues on next page ▶

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Day	Month	Year											
Day	Month	Year											

Participant Initials	Date of Birth	Day	Month	Year	Participant ID	Centre No	Trial No
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Tumour Details (Continued)

Radiological T stage
(Tick one only)

T2 or less (limited by muscularis propria)
 T3 not breaching serosa
 T4a penetration of serosa with extension into adjacent organs
 T4b penetration of serosa and peritoneal surface with perforation of bowel

Radiological N stage
(Tick one only)

Number of visible nodes

Number of malignant nodes

N0 – none
 N1 – 1-3 regional lymph nodes appear malignant
 N2 – 4 or more regional lymph nodes appear malignant

Size of largest malignant node mm

V stage
(Tick one only)

V1 – Vascular invasion present or probably present
 V0 – Vascular invasion probably absent or absent

M stage
(Tick one only)

M0 – no distant metastases
 M1a – distant metastases one organ
 M1b – peritoneal or distant metastases to more than one organ or distant nodes

Please specify other location(s)

Liver
 Lung
 Peritoneum

Right colon vascular anatomy assessment
(Tick as many as apply)

Ileocolic artery present
 Right colic artery present (arising directly from the SMA not ileocolic)
 Middle colic artery present
 Artery crosses anterior to SMV
 Artery crosses posterior to SMV

N/A – Not a right-sided cancer

Additional Pathology

Additional pathology present?

Yes → Please give details

No

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Lymph Node Assessment

Figure 1

For right-sided cancers

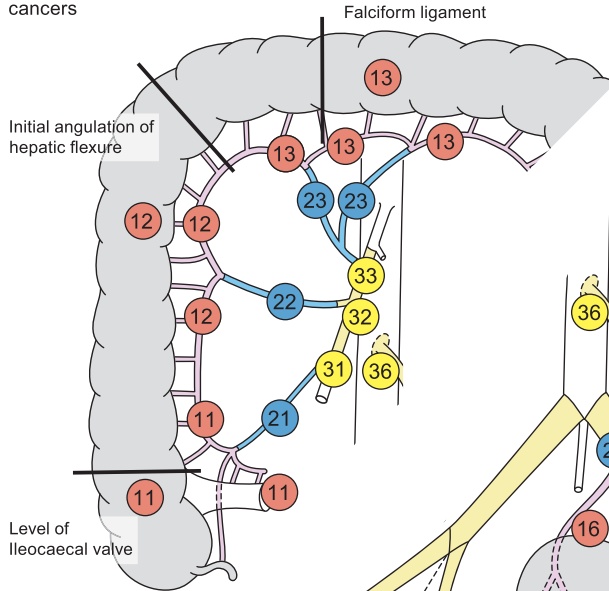
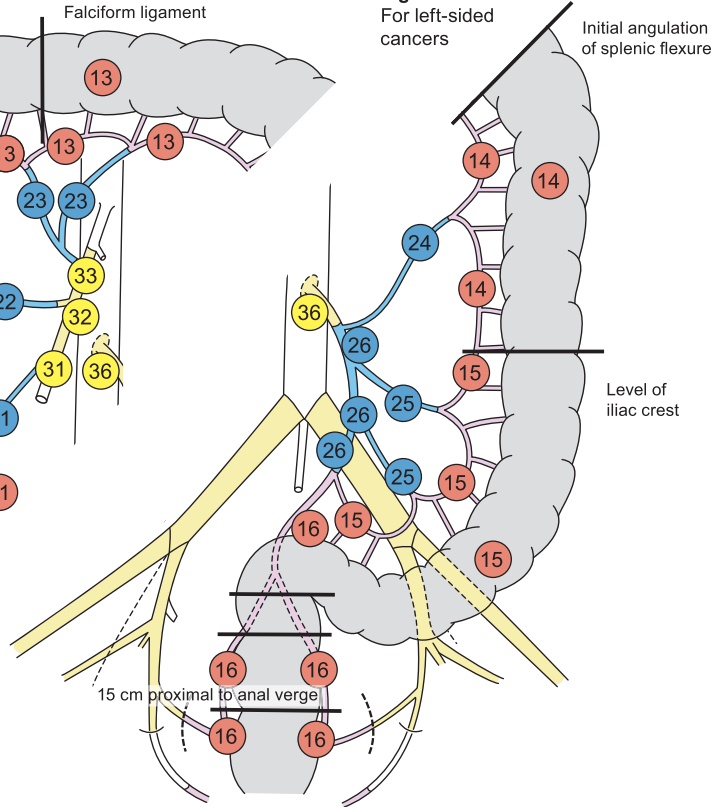


Figure 2

For left-sided cancers



Instructions

- Please mark nodes (by crossing the lymph node station) considered to be malignant on the specimen diagram (Figure 1 for right sided cancers and Figure 2 for left sided cancers) and note nodes considered to be malignant, plus give an estimation of their size on page 4
- Size of nodes to be recorded as the maximum short axis diameter on the table on page 4

Modified Japanese staging subgroups

Pericolic, D1 lymph nodes (red); Intermediate D2 lymph nodes (blue); Main, D3, lymph nodes (yellow).

Coding for lymph node stations

- In the superior and inferior mesenteric arterial system, the first figure of the code indicates the position of the lymph nodes, expressing the epicolic and paracolic (D1) nodes as 1Δ (marked in red on figure 1), the intermediate (D2) nodes as 2Δ (marked in blue on figure 1), the main (D3) nodes as 3Δ (marked in yellow on figure 1) and the para-aortic nodes as 4Δ (marked in white on figure 1).
- The second figure indicates the position of the lymph nodes along the main trunk artery; Δ1 is used for the nodes along the ileo-colic artery, Δ2 is used for nodes long the right colic artery, Δ3 for those along the middle colic artery, Δ4 for those along the left colic artery and Δ5 for the sigmoid artery and Δ6 for the superior rectal artery.
- The inferior mesenteric nodes are expressed as 36.

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Participant Initials		Date of Birth	Day	Month	Year	Participant ID	Centre No	Trial No
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Lymph Node Assessment (Continued)

Station number of malignant node – comment on malignant nodes only	Node (e.g. 1, 2, 3, 4)	Estimate size of each node: maximum short axis diameter (mm)
11		
12		
13		
14		
15		
16		
21		
22		
23		
24		
25		
26		
31		
32		
33		
36		

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To be completed prior to the planned operation

Colonoscopy Details

Has a colonoscopy been performed? Yes No

If yes, date of colonoscopy

Day	Month	Year

Please indicate the site of of tumour (*tick yes for one only*):

Right? Yes → Approximate site of tumour Caecum
(Segment proximal to or involving ileocaecal valve)
 No Ascending colon
(Segment distal to ileocaecal valve and proximal to the initial angulation of the hepatic flexure)
 Hepatic flexure
(Segment distal to the initial angulation of the hepatic flexure to the proximal border of the falciform ligament)

Sigmoid? Yes → Approximate site of tumour Sigmoid colon
(Segment distal to the level of the left iliac crest to 15 cm proximal to the anal verge)
 No

Recto-sigmoid? Yes → Distance from anal verge

	.	

 cm
 No

Is diverticular disease present? Yes No

Is colitis present? Yes No

Are benign polyps present? Yes No

Administration of Indian Ink

Was indian ink administered to the tumour? Yes No

Completed by

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 Date

Day	Month	Year

Last Page ■

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Participant Initials		Date of Birth	Day	Month	Year	Participant ID	Centre No	Trial No
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To be completed on the day of the participant's planned operation

5 ALA Details

Dose of 5 ALA
 10 mg/kg
 20 mg/kg
 30 mg/kg

Total dose of 5 ALA mg

Timing of 5 ALA prior to surgery hours (To nearest hour; round up or down from time 5 ALA taken to time of first incision)

Storz D-light Laparoscopic System

Settings of the Storz D-light laparoscopic system

Operation Details

Date of operation Day Month Year

Name of operating surgeon

Initial Laparoscopy Findings

Adhesions?
 Yes → Few Yes No
 No Single quadrant Yes No
 No Multi-quadrant Yes No

Locoregional tumour spread?
 Yes → Description
 No

Tumour perforation/ abscess?
 Yes → Description
 No

Other organ involvement?
 Yes → Description
 No

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Initial Laparoscopy Findings (Continued)

Tumour marked preoperatively (indian ink)? Yes No → Tumour visible intraoperatively? Yes No

Lymphatic anatomy visible from colorimetric dye tattoo? Yes No → Was tumour tattooed at colonoscopy? Yes No

Presence of fluorescence (5-ALA) of lymph nodes? Yes No → Assessment of intensity of fluorescence: 1 = barely visible 2 = easily visible 3 = intense fluorescence

Presence of fluorescence (5-ALA) of tumour? Yes No → Assessment of intensity of fluorescence: 1 = barely visible 2 = easily visible 3 = intense fluorescence

Presence of fluorescence (5-ALA) of parietal or visceral peritoneum? Yes No → Assessment of intensity of fluorescence: 1 = barely visible 2 = easily visible 3 = intense fluorescence

Presence of fluorescence on visible liver capsule? Yes No → Assessment of intensity of fluorescence: 1 = barely visible 2 = easily visible 3 = intense fluorescence

Any other findings of note? Yes No → Description

Actual Mode of Surgery

Please tick one:

Laparoscopic

Laparoscopic converted to open

Conversion to an open procedure will not affect the initial laparoscopic assessment and marking of fluorescent nodes; such patients will still be included in the trial.

Theatre Timings

Laparoscopic start time Hours Minutes

Laparoscopic finish time Hours Minutes

Total operative time hours minutes

Please use 24 hr clock

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Operation Performed

Right hemicolectomy Yes No

Extended right hemicolectomy Yes No

Sigmoid colectomy Yes No

High anterior resection Yes No

Hartmann's procedure Yes No

Other operation Yes → Description

No

Operative Details

Were fluorescent lymph nodes marked with ligaclips to facilitate subsequent pathological identification? Yes No
 (See diagrams on page 7 & 8 and table on page 9)

Was a D3 lymphadenectomy performed? Yes

No → Why not?

Cancers of the Right Colon

Complete this section for cancers of the right colon (caecum to the medial border of the falciform ligament) for participants undergoing a right hemicolectomy:

High, central ligation of ileocolic artery and vein? Yes No

High, central ligation of the right colic vessels, when present as separate branches? Yes No

If hepatic flexure cancers, were the middle colic vessels taken at their origin? Yes No N/A (No hepatic flexure cancers)

If caecal or ascending colon cancer, were the right branches of the middle colic vessels taken at their origin? Yes No N/A (Not caecal or ascending colon cancer)

Completed by

Date

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Operative Details (Continued)

Cancers of the Sigmoid Colon

Complete this section for cancers of the sigmoid colon (distal to the level of the left iliac crest to 15 cm proximal to the anal verge) for participants undergoing a Sigmoid colectomy/left sided resection:

High ligation and division of the inferior mesenteric artery proximal to the origin of the left colic vessels? Yes No

High ligation and division of the inferior mesenteric vein immediately below the inferior border of the pancreas? Yes No

Mobilisation of the splenic flexure? Yes No **→ Please specify (Tick one only)**
 Complete mobilisation
 Partial mobilisation
 No mobilisation

Rectal dissection? Yes No **→ Please specify (Tick one only)**
 Total mesorectal excision
 Partial mesorectal excision

Anastomosis? Yes No **→**
 Extracorporeal Yes No
 Intracorporeal Yes No
 Doughnuts intact Yes No
 Air-tight Yes No
 Hand-sewn Yes No
 Stapled Yes No

Further Details

For all participants:

Anastomotic complication? Yes No

End stoma? Yes No **→**
 Colostomy Yes No
 Ileostomy Yes No

Defunctioning stoma? Yes No **→**
 Colostomy Yes No
 Ileostomy Yes No

Estimated blood loss ml

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Operative Outcome

Unresectable? Yes
 No

Curative resection (R0)? Yes
 No

Palliative? Yes → Local disease remaining (R1 & R2) Yes No
 No Peritoneal disease Yes No
 Liver metastases Yes No

Latrogenic tumour perforation? Yes
 No

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Intraoperative Complications

Did any intraoperative complications occur? Yes No

If yes, please record details below, ticking yes or no for each complication:

Damage to organ/structure? Yes No

Bowel
 Bladder/ureter
 Major vessel
 Nerves

Faecal contamination? Yes No

Local
 Widespread

Haemorrhage? Yes No

Action taken?

Failure of surgical equipment? Yes No

Failure of laparoscopic equipment/hardware? Yes No

Give details

Cardiac event? Yes No

Action taken?

Respiratory event? Yes No

Action taken?

Surgical emphysema? Yes No

Action taken?

Other complication? Yes No

Please specify

Action taken?

Video Recording

Was a video made of the procedure that was suitable for use to produce guidance for evaluation phase? Yes No

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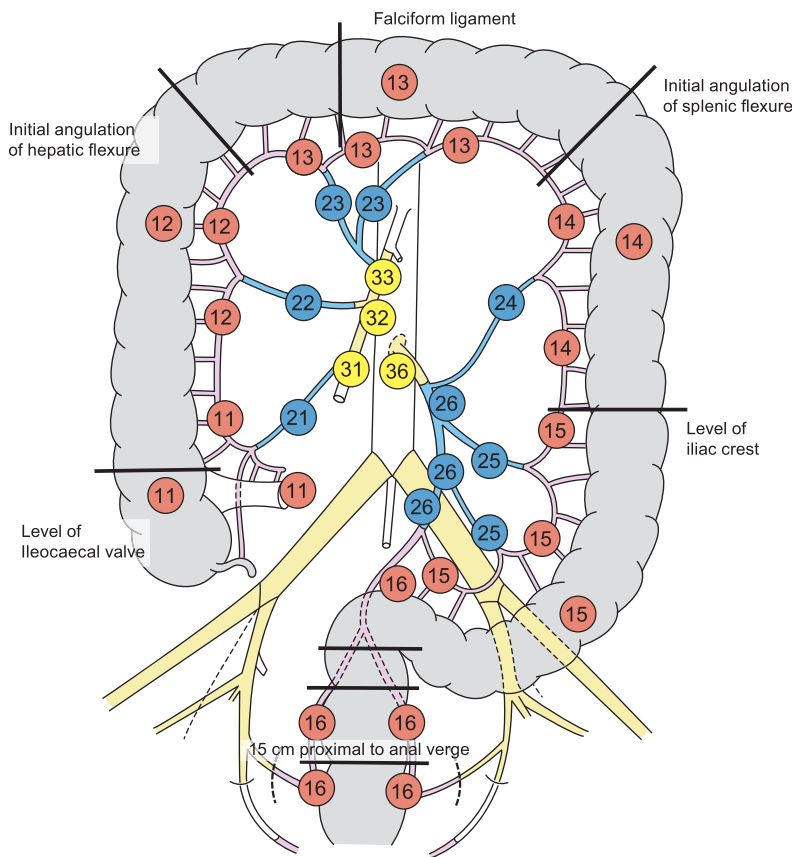
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Lymph Node Assessment

Figure 1



Instructions

- Please mark fluorescent nodes marked with a surgical clip intra-operatively with a cross

Modified Japanese staging subgroups

Pericolic, D1 lymph nodes (red); Intermediate D2 lymph nodes (blue); Main, D3, lymph nodes (yellow).

Coding for lymph node stations

- In the superior and inferior mesenteric arterial system, the first figure of the code indicates the position of the lymph nodes, expressing the epicolic and paracolic (D1) nodes as 1Δ (marked in red on figure 1), the intermediate (D2) nodes as 2Δ (marked in blue on figure 1), the main (D3) nodes as 3Δ (marked in yellow on figure 1) and the para-aortic nodes as 4Δ (marked in white on figure 1).
- The second figure indicates the position of the lymph nodes along the main trunk artery; Δ1 is used for the nodes along the ileo-colic artery, Δ2 is used for nodes along the right colic artery, Δ3 for those along the middle colic artery, Δ4 for those along the left colic artery and Δ5 for the sigmoid artery and Δ6 for the superior rectal artery.
- The inferior mesenteric nodes are expressed as 36.

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Lymph Node Assessment (Continued)

Figure 2

Note the anatomy of the right colic artery – draw in

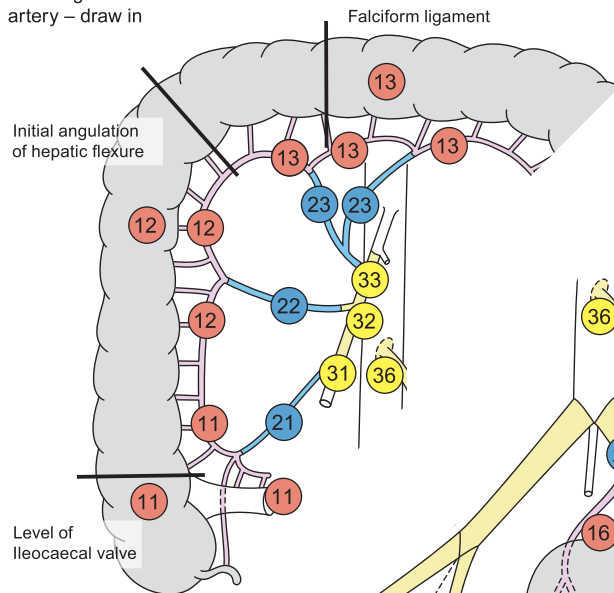
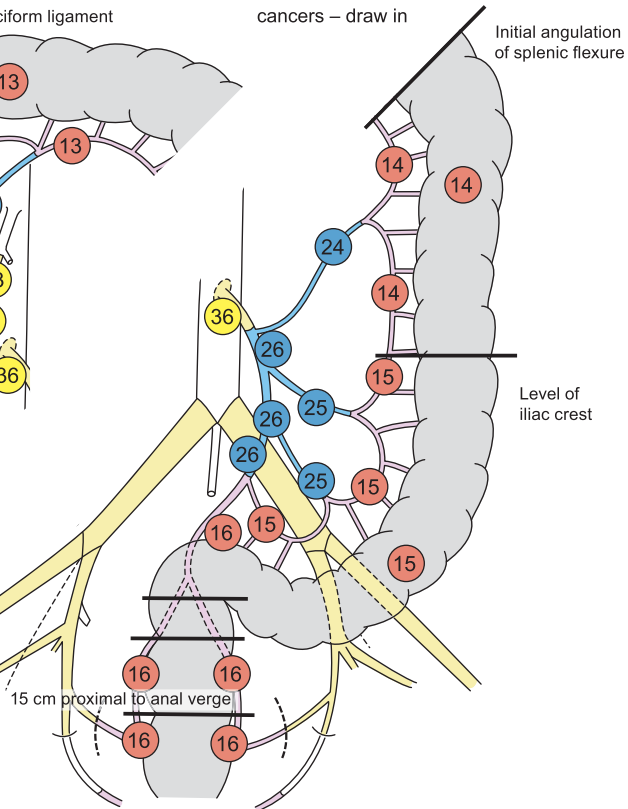


Figure 3

Note any anomalies for left sided cancers – draw in



Instructions

- Please mark nodes (by crossing the lymph node station) considered to be malignant on the specimen diagram (Figure 2 for right sided cancers and Figure 3 for left sided cancers) and note nodes considered to be malignant, plus give an estimation of their size on page 9
- Size of nodes to be recorded as the maximum short axis diameter on the table on page 9

Modified Japanese staging subgroups

Pericolic, D1 lymph nodes (red); Intermediate D2 lymph nodes (blue); Main, D3, lymph nodes (yellow).

Coding for lymph node stations

- In the superior and inferior mesenteric arterial system, the first figure of the code indicates the position of the lymph nodes, expressing the epicolic and paracolic (D1) nodes as 1Δ (marked in red on figure 1), the intermediate (D2) nodes as 2Δ (marked in blue on figure 1), the main (D3) nodes as 3Δ (marked in yellow on figure 1) and the para-aortic nodes as 4Δ (marked in white on figure 1).
- The second figure indicates the position of the lymph nodes along the main trunk artery; Δ1 is used for the nodes along the ileo-colic artery, Δ2 is used for nodes along the right colic artery, Δ3 for those along the middle colic artery, Δ4 for those along the left colic artery and Δ5 for the sigmoid artery and Δ6 for the superior rectal artery.
- The inferior mesenteric nodes are expressed as 36.

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Lymph Node Assessment (Continued)

N/A – no fluorescent nodes present

	Station number of fluorescent node – comment on fluorescent nodes only	Node (e.g. 1, 2, 3, 4)	Vessel	Estimate size of node: maximum short axis diameter (mm)	Comments	Colorimetric dye visible?	
						Yes	No
11			Ileocolic D1			<input type="checkbox"/>	<input type="checkbox"/>
12			Right colic D1			<input type="checkbox"/>	<input type="checkbox"/>
13			Middle colic D1			<input type="checkbox"/>	<input type="checkbox"/>
14			Left colic D1			<input type="checkbox"/>	<input type="checkbox"/>
15			Sigmoid branches D1			<input type="checkbox"/>	<input type="checkbox"/>
16			Superior rectal D1			<input type="checkbox"/>	<input type="checkbox"/>
21			Ileocolic D2			<input type="checkbox"/>	<input type="checkbox"/>
22			Right colic D2			<input type="checkbox"/>	<input type="checkbox"/>
23			Middle colic D2			<input type="checkbox"/>	<input type="checkbox"/>
24			Left colic D2			<input type="checkbox"/>	<input type="checkbox"/>
25			Sigmoid branches D2			<input type="checkbox"/>	<input type="checkbox"/>
26			IMA			<input type="checkbox"/>	<input type="checkbox"/>
31			Ileocolic D3			<input type="checkbox"/>	<input type="checkbox"/>
32			Right colic D3			<input type="checkbox"/>	<input type="checkbox"/>
33			Middle colic D3			<input type="checkbox"/>	<input type="checkbox"/>
36			Origin of IMA			<input type="checkbox"/>	<input type="checkbox"/>
TOTAL							
Any additional areas of fluorescence; size, approximate location and whether excised as separate specimen							

Estimated tumour location (Tick one only)

Caecum (Segment proximal to or involving ileocaecal valve)

Ascending colon (Segment distal to ileocaecal valve and proximal to the initial angulation of the hepatic flexure)

Hepatic flexure (Segment distal to the initial angulation of the hepatic flexure to the proximal border of the falciform ligament)

Sigmoid colon (Segment distal to the level of the left iliac crest to 15 cm proximal to the anal verge)

Completed by Date

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To be completed post the planned operation.
(Post-op care defined as until participant's discharge from hospital or until 10 days post-op if not discharged)

Liver Function Tests (LFTs)

	Day 1 post-op		Day 2 post-op		Day 3 post-op		Day 4 post-op <input type="checkbox"/> Tick if N/A		Day 5 post-op <input type="checkbox"/> Tick if N/A	
	Result	Abnormal?	Result	Abnormal?	Result	Abnormal?	Result	Abnormal?	Result	Abnormal?
		Yes No		Yes No		Yes No		Yes No		Yes No
Bilirubin (µmol/L)	<input type="text"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
ALT or AST (IU/L)	<input type="text"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
ALP (IU/L)	<input type="text"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Urea and Electrolytes (U&Es)

	Day 1 post-op		Day 2 post-op		Day 3 post-op		Day 4 post-op <input type="checkbox"/> Tick if N/A		Day 5 post-op <input type="checkbox"/> Tick if N/A	
	Result	Abnormal?	Result	Abnormal?	Result	Abnormal?	Result	Abnormal?	Result	Abnormal?
		Yes No		Yes No		Yes No		Yes No		Yes No
Urea (mmol/L)	<input type="text"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Creatinine (µmol/L)	<input type="text"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Was the participant kept in ward environment for 72 hrs? Yes No → Reason

Potential Side-effects

In the opinion of doctors involved in post-op care, has the participant experienced any of the following symptoms **due to 5 ALA administration**? Yes → Please tick yes or no for each complication below and give details where applicable No

Photosensitivity reactions	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	<input type="text"/>
Skin hypersensitivity reactions	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	<input type="text"/>
Nausea	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	<input type="text"/>
Vomiting	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	<input type="text"/>
Tachycardia	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	<input type="text"/>
Hypotension	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	<input type="text"/>
Other, please specify	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	<input type="text"/>

If any of these fulfil the seriousness criteria as listed in protocol section 14.1, please also complete **F10 SAE / F11 SUSAR** report as appropriate within 24 hours of becoming aware

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To be completed at the time of the pathological assessment post the planned operation.
N.B. specimens should be sent to the lab fresh (Acceptable to refrigerate for 24 hrs post surgery)

Assessment Details

Date of assessment	Day	Month	Year
Operation date	Day	Month	Year
Pathology reference	<input type="text"/>		
Name of pathologist completing work	<input type="text"/>		

Photography Details

Digital photographs taken of surface

Required:

Anterior Yes No

(Prior to inking of non-peritonealised surfaces – clips should be clearly visible)

Posterior Yes No

(Prior to inking of non-peritonealised surfaces – clips should be clearly visible)

Mesocolic defects Yes No N/A

Perforations Yes No N/A

The site of the tumour and high vascular tie should be clearly marked (e.g. with forceps) and the photograph should include a ruler/tape measure to enable sizing of the specimen. The whole specimen should be visible in the image and mesentery should be laid out flat (not folded or over stretched). The proximal and distal aspects can also be labelled if not obvious.

Photographs should be taken directly above the specimen to reduce distortion and while a white background is ideal, any other plain colour is acceptable. The photographs should not contain any direct identifiers (e.g. name or date of birth) but should be identifiable by trial number, histopathology number and patient initials.

Photography following formalin filtration for 48 hours but prior to Indian Ink application to all non-peritonealised surfaces

Digital photographs taken of surface

Required:

Anterior Yes No

(Prior to inking of non-peritonealised surfaces – clips should be clearly visible)

Posterior Yes No

(Prior to inking of non-peritonealised surfaces – clips should be clearly visible)

Mesocolic defects Yes No N/A

Perforations Yes No N/A

It should be remembered that the circumferential margin only applies to the surgically incised mesocolic planes (e.g. the retroperitoneal margin in right sided specimens and the upper mesorectal margin in left sided specimens) and not to the peritonealised surfaces.

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Photography Details (Continued)

Photography of the serial cross-sectional slices from the resection specimen

Specimen cross-sectional slices photographed? Yes No *It should be clear which are the most proximal and distal slices (e.g. by using labels)*
(Thinly (3–5 mm) sliced transversely from 2 cm below to 2 cm above the tumour)

Additional close ups of all tumour bearing slices Yes No

Plane of Dissection (See protocol Appendix 3)

Please indicate the plane of dissection Mesocolic Plane
(Tick one only) Intramesocolic Plane
 Muscularis propria Plane

Position of the Tumour

Site of tumour Caecum
(According to the pre-operative assessment, Tick one only) *(Segment proximal to or involving ileocaecal valve)*
 Ascending colon
(Segment distal to ileocaecal valve and proximal to the initial angulation of the hepatic flexure)
 Hepatic flexure
(Segment distal to the initial angulation of the hepatic flexure to the proximal border of the falciform ligament)
 Sigmoid colon
(Segment distal to the level of the left iliac crest to 15 cm proximal to the anal verge)

Maximum dimension of tumour	<input type="text"/>	mm
Distance of direct tumour spread beyond the muscularis propria*	<input type="text"/>	mm
Distance from the tumour to the nearest non-peritonealised margin*	<input type="text"/>	mm
Distance of the primary tumour to the closest high tie vessel	<input type="text"/>	mm
Distance from tumour to distal margin	<input type="text"/>	mm
Is there involvement of the distal margin? <i>(This should be defined as 0 mm)</i>	<input type="checkbox"/> Yes <input type="checkbox"/> No	
Distance from tumour to proximal margin	<input type="text"/>	mm
Is there involvement of the proximal margin? <i>(This should be defined as 0 mm)</i>	<input type="checkbox"/> Yes <input type="checkbox"/> No	

**These should initially be measured macroscopically and then confirmed histologically (please alter the histological measurement if necessary)*

Perforations

Is there evidence of perforation within tumour? Yes No

Is there evidence of bowel perforation away from tumour? Yes No

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Tumour Morphology

Is the tumour type adenocarcinoma? Yes
 No → Specify type

What is the differentiation? (By predominate type, tick one only)
 Poor
 Well / Moderate

Local Invasion

Please stage the maximum extent of tumour invasion:

Submucosa
 Inner circular muscle layer
 Outer longitudinal muscle layer
 Mesocolic fat
 Through peritoneum
 Adjacent organ(s) → Specify organ(s)

Is there peritoneal involvement? Yes No

Extramural Vascular Invasion

Is there extramural vascular invasion? Yes No

Metastatic Spread

Number of lymph nodes examined*

Number of positive lymph nodes*

**Excluding tumour deposits outside lymph nodes, irrespective of their size*

Is the apical node positive? Yes
 No

Are there tumour deposits outside lymph nodes? Yes → Number of deposits measuring 3 mm or more in maximum diameter
 No

Are there any deposits measuring less than 3 mm in diameter? Yes No

Likely level of lymphadenectomy based on vascular anatomy
 D1
 D2
 D3

*All of the local lymph nodes in the specimen should be identified, mapped, embedded and examined.
 The high tie (apical node) should be blocked separately to allow Dukes' staging to be conducted as should any lymph nodes marked (e.g. with a clip) by the surgeons to allow comparison with the intra-operative staging.
 Lymph nodes should be embedded in their entirety either by submitting whole, bisecting or serial slicing if large.*

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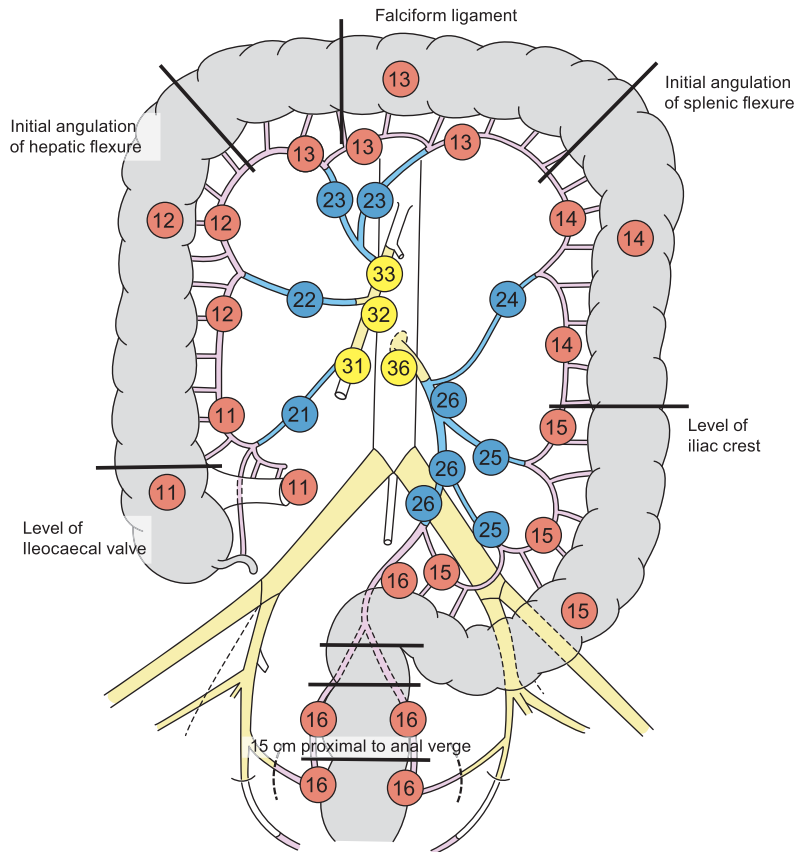
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Lymph Node Assessment

Figure 1



Instructions

The pathologist will mark on a specimen diagram and lymph node mapping table the number of nodes in each station and how many of these were fluorescent intra-operatively.

Key

Please mark on modified Japanese station sub-groupings diagram

If node is...		Mark as
Fluorescent &	Malignant	FM
	Benign	FB
Non-fluorescent &	Malignant	NFM
	Benign	NFB

Modified Japanese staging subgroups

Pericolic, D1 lymph nodes (red); Intermediate D2 lymph nodes (blue); Main, D3, lymph nodes (yellow).

Coding for lymph node stations

- In the superior and inferior mesenteric arterial system, the first figure of the code indicates the position of the lymph nodes, expressing the epicolic and paracolic (D1) nodes as 1Δ (marked in red on figure 1), the intermediate (D2) nodes as 2Δ (marked in blue on figure 1), the main (D3) nodes as 3Δ (marked in yellow on figure 1) and the para-aortic nodes as 4Δ (marked in white on figure 1).
- The second figure indicates the position of the lymph nodes along the main trunk artery; Δ1 is used for the nodes along the ileo-colic artery, Δ2 is used for nodes long the right colic artery, Δ3 for those along the middle colic artery, Δ4 for those along the left colic artery and Δ5 for the sigmoid artery and Δ6 for the superior rectal artery.
- The inferior mesenteric nodes are expressed as 36.

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Lymph Node Assessment (Continued)

No fluorescent nodes present

Station number	Node number	Notes (See guidance below)	Fluorescent?		Cassette	Involved?	
			Yes	No		Yes	No
			<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
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			<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>

Completion guidance

- Please list each individual node on a separate line.
- Please state if the node is an apical node (there may be more than one apical node if the tumour lies between two vascular ties)
- Describe the distance of the pericolic D1 nodes from the tumour centre (either within 5 cm, between 5 cm and 10 cm, or more than 10 cm)
- Add any other comments as necessary.

Please use another copy of this page if you need more space

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Metastatic Spread

Please indicate TNM (Version 5) staging:

pT	<input type="checkbox"/> 0	pN	<input type="checkbox"/> 0	pM	<input type="checkbox"/> 0	<input type="checkbox"/> R0
	<input type="checkbox"/> 1		<input type="checkbox"/> 1		<input type="checkbox"/> 1	<input type="checkbox"/> R1 (0 mm)
	<input type="checkbox"/> 2		<input type="checkbox"/> 2		<input type="checkbox"/> X	<input type="checkbox"/> R1 (<1 mm)
	<input type="checkbox"/> 3					<input type="checkbox"/> R2
	<input type="checkbox"/> 4					

(Peritoneal involvement (pT4) should be diagnosed if tumour cells penetrate the peritoneal surface)

Dukes' (Tick one only)

Dukes' A (Tumour growth limited to the bowel wall (pT1 or pT2) but no further, nodes -ve)

Dukes' B (Tumour growth beyond the bowel wall (pT3 or pT4), nodes -ve)

Dukes' C1 (Any pT stage, nodes +ve and apical node -ve)

Dukes' C2 (Any pT stage, apical node +ve)

Stage D (Presence of distant metastases)

Are there pathologically-proven metastases present?

Yes → Please specify type (Tick all that apply)

No

Liver

Lung

Peritoneal

Other, please specify:

Other Comments

Submitting for Central Review

	Yes	No
Photographs – intact fresh resection specimen?	<input type="checkbox"/>	<input type="checkbox"/>
Photographs of the whole formalin-fixed resection specimen?	<input type="checkbox"/>	<input type="checkbox"/>
Photographs of the serial cross-sectional slices from the resection specimen?	<input type="checkbox"/>	<input type="checkbox"/>
Specimen sketch detailing the estimated position of all lymph nodes (positive and negative) according to station number ?	<input type="checkbox"/>	<input type="checkbox"/>
Submitting the final histopathology report with full histopathological staging data for review?	<input type="checkbox"/>	<input type="checkbox"/>
Submitting all of the H&E stained glass slides (or copies) for central review?	<input type="checkbox"/>	<input type="checkbox"/>
Submitting the formalin-fixed paraffin-embedded tissue blocks of all of the lymph nodes (and the additional two blocks of tumour and one of normal mucosa if the participant has consented)?	<input type="checkbox"/>	<input type="checkbox"/>

Completed by Date

Day	Month	Year
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Last Page

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	Date	Initials	Date	Initials

Participant Initials		Date of Birth	Day	Month	Year	Participant ID	Centre No	Trial No
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To be completed 30 days post the planned operation

Assessment Details

What is the participant's status? Alive
 Dead → Date of death

Day	Month	Year

Was the 30 day assessment undertaken? Yes → Date of assessment

Day	Month	Year

 Location of assessment In clinic
 By telephone
 On ward
 No → Reason not undertaken

--	--	--

 Date last known to be alive

Day	Month	Year

 N/A – participant has died

Has the participant been discharged? Yes → Date fit for discharge

Day	Month	Year

 No
 Date of actual discharge

Day	Month	Year

 Reason for delay in discharge

--	--	--

 N/A – No delay

Length of postoperative hospital stay

--

 days

Has the participant had further surgery? Yes → Date of further surgery

Day	Month	Year

 No
 Was the surgery cancer-related? Yes No
 Was the surgery a consequence of the original surgical procedure? Yes No
 Description of further surgery

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Completed by

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 Date

Day	Month	Year

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	Date	Initials

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Participant Initials		Date of Birth	Day	Month	Year	Participant ID	Centre No	Trial No
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Postoperative (30-day) Complications

Operative Complications

Has the participant experienced any operative complications following surgery? Yes No **→ Please tick yes or no for each complication below and give details where applicable**

Altered bowel habit/diarrhoea Yes No **→ C. difficile related?** Yes No
 Action taken? Yes No **→ Description**

Anastomotic leak Yes No **→ Intervention required?** Yes No **→ Description**

Post-operative peritonitis Yes No **→ Intervention required?** Yes No **→ Description**

Cardiac event Yes No **→ Specify**

Specify	Action taken?	Description
<input type="checkbox"/> Arrhythmia	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="text"/>
<input type="checkbox"/> Cardiac failure	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="text"/>
<input type="checkbox"/> Myocardial infarction/ Ischaemic heart disease	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="text"/>
<input type="checkbox"/> Cardio-respiratory arrest	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="text"/>

Respiratory event Yes No **→ Specify**

Specify	Action taken?	Description
<input type="checkbox"/> Acute respiratory distress syndrome/ respiratory failure	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="text"/>
<input type="checkbox"/> Aspiration	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="text"/>
<input type="checkbox"/> Atelectasis	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="text"/>
<input type="checkbox"/> Pleural effusion	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="text"/>
<input type="checkbox"/> Bronchospasm	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="text"/>
<input type="checkbox"/> Pneumonia/ chest infection	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="text"/>
<input type="checkbox"/> Pulmonary embolus	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="text"/>

Cerebrovascular attack/stroke Yes No **→ Action taken?** Yes No **→ Description**

Completed by Date **Form continues on next page →**

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Participant Initials		Date of Birth	Day	Month	Year	Participant ID	Centre No	Trial No
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Postoperative (30-day) Complications (Continued)

Deep Vein Thrombosis	<input type="checkbox"/> Yes → Action taken?	<input type="checkbox"/> Yes → Description	
	<input type="checkbox"/> No	<input type="checkbox"/> No	
Gastrointestinal fistula	<input type="checkbox"/> Yes → Intervention required?	<input type="checkbox"/> Yes → Description	
	<input type="checkbox"/> No	<input type="checkbox"/> No	
Gastrointestinal ischaemia/necrosis	<input type="checkbox"/> Yes → Intervention required?	<input type="checkbox"/> Yes → Description	
	<input type="checkbox"/> No	<input type="checkbox"/> No	
Gastrointestinal obstruction	<input type="checkbox"/> Yes → Intervention required?	<input type="checkbox"/> Yes → Description	
	<input type="checkbox"/> No	<input type="checkbox"/> No	
Gastrointestinal perforation	<input type="checkbox"/> Yes → Intervention required?	<input type="checkbox"/> Yes → Description	
	<input type="checkbox"/> No	<input type="checkbox"/> No	
Gastrointestinal stricture/stenosis	<input type="checkbox"/> Yes → Intervention required?	<input type="checkbox"/> Yes → Description	
	<input type="checkbox"/> No	<input type="checkbox"/> No	
Gastrointestinal ulceration	<input type="checkbox"/> Yes → Intervention required?	<input type="checkbox"/> Yes → Description	
	<input type="checkbox"/> No	<input type="checkbox"/> No	
Haemorrhage	<input type="checkbox"/> Yes → Transfusion required?	<input type="checkbox"/> Yes → No. units transfused	<input type="text"/>
	<input type="checkbox"/> No	<input type="checkbox"/> No	
	Intervention required?	<input type="checkbox"/> Yes → Description	<input type="text"/>
	<input type="checkbox"/> No	<input type="checkbox"/> No	
Hernia	<input type="checkbox"/> Yes → Specify	Action taken?	Description
	<input type="checkbox"/> No	<input type="checkbox"/> Port	<input type="checkbox"/> Yes →
			<input type="checkbox"/> No
	<input type="checkbox"/> Wound	<input type="checkbox"/> Yes →	<input type="text"/>
		<input type="checkbox"/> No	
Intra-abdominal/pelvic abscess	<input type="checkbox"/> Yes → Intervention required?	<input type="checkbox"/> Yes → Description	
	<input type="checkbox"/> No	<input type="checkbox"/> No	
Lower limb ischaemia/compartment syndrome	<input type="checkbox"/> Yes → Intervention required?	<input type="checkbox"/> Yes → Description	
	<input type="checkbox"/> No	<input type="checkbox"/> No	
Protracted ileus	<input type="checkbox"/> Yes → Intervention required?	<input type="checkbox"/> Yes → Description	
	<input type="checkbox"/> No	<input type="checkbox"/> No	
Renal failure (acute)	<input type="checkbox"/> Yes → Intervention required?	<input type="checkbox"/> Yes → Description	
	<input type="checkbox"/> No	<input type="checkbox"/> No	
Stoma prolapse/necrosis	<input type="checkbox"/> Yes → Intervention required?	<input type="checkbox"/> Yes → Description	
	<input type="checkbox"/> No	<input type="checkbox"/> No	

Completed by	<input type="text"/>	Date	Day	Month	Year	Form continues on next page ►
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Postoperative (30-day) Complications (Continued)

Stoma – high output	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	
Urinary incontinence	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	
Urinary retention	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	
Urinary tract infection	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	
Wound infection	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	
Wound dehiscence	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	
Back pain	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	
Disseminated intra-vascular coagulation	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	
Necrotising fasciitis	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	
Pressure sore	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	
Metabolic acidosis	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	
Pseudomembranous colitis	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	
Scrotal swelling	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	
Sepsis	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	
Delirium	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	
Other, please specify	<input type="checkbox"/> Yes → Action taken? <input type="checkbox"/> No	<input type="checkbox"/> Yes → Description <input type="checkbox"/> No	

Completed by Date

Day	Month	Year
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Stoma

Does the participant currently have a stoma?

Yes → Is it: Permanent } → Have they had a colostomy or ileostomy? Yes → Which? Colostomy
 No Temporary } No Ileostomy
 What type? Loop
 End

Complications Related to 5 ALA after Discharge from Hospital

Has the participant experienced any complications related to 5 ALA after discharge from hospital? Yes → Please tick yes or no for each complication below and give details where applicable
 No

Photosensitivity reactions Yes → Action taken? Yes → Description No No

Skin hypersensitivity reactions Yes → Action taken? Yes → Description No No

Nausea Yes → Action taken? Yes → Description No No

Vomiting Yes → Action taken? Yes → Description No No

Tachycardia Yes → Action taken? Yes → Description No No

Hypotension Yes → Action taken? Yes → Description No No

Other, please specify Yes → Action taken? Yes → Description No No

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The Leeds Teaching Hospitals

NHS Trust

John Goligher Colorectal Unit
 Research Office
 Ground Floor Lincoln Wing
 St James University Hospital
 Beckett Street
 Leeds
 LS9 7TF
 Tel: 0113 20 64672



5-ALA in Bowel Cancer Surgery

PARTICIPANT INFORMATION SHEET AND INFORMED CONSENT DOCUMENT

A large-print version of this sheet is available on request.

You have been invited to take part in a research study called “GLiSten”. Before you decide if you want to take part, we would like to explain why the research is being done, how we will use the information we have about you, and what the study will involve.

Please read this information carefully, and discuss it with others if you like. Ask us if anything is unclear, or if you would like more information.

Once you have read this information, the study team will talk to you about the study again and you can ask any questions you like.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
 - Part 2 gives you more detailed information about the conduct of the study.

Take time to decide whether or not you wish to take part.

Thank you for reading this information sheet.

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Part 1

What is the purpose of the study?

The purpose of this study is to find a new way of looking at bowel cancer to determine whether it has spread beyond the bowel. The most common place that bowel cancer spreads to is the small nodes next to the bowel. These are called “lymph nodes” and help to stop the spread of cancer. It is important to know if cancer has spread to lymph nodes as this can affect the extent of surgery you require, and whether you need further treatment, such as chemotherapy.

The study will use a substance called 5-ALA. This will be given as a liquid you will drink 4 to 6 hours prior to your operation. 5-ALA will detect the cancer along with any spread to lymph nodes that surround your bowel by causing them to glow red under blue light during your operation. This study aims to find the best dose of 5-ALA. The best dose will be the lowest dose that causes the cancer and any spread to lymph nodes to glow. This study will involve approximately 50 participants. Once the best dose is known, the results of this study will feed into a larger evaluation study involving approximately 300 participants.

Why have I been chosen?

You have been chosen for this study because you have a bowel cancer that can be removed by an operation. Your surgeon has suggested that your cancer is suitable for a “key-hole” or laparoscopic operation.

Do I have to take part?

No, your participation in GLiSten is voluntary and you may withdraw your consent to take part at any time, without giving us a reason.

If you decide to take part you will be given this document to keep. You will be asked to sign a consent form, but you are still free to withdraw at any time and without giving a reason. If you decide not to take part, your doctor will be happy to talk through how your cancer will be treated. Your treatment and care will not be affected in any way.

If I want to, will I definitely be able to take part?

Although your doctor thinks you might be suitable to take part, they still need to ask you some questions about your medical history and any medications you take to make sure you are suitable.

What is the standard treatment?

You have already had a colonoscopy to diagnose and mark your bowel cancer with a special dye. A CT scan has been performed to ensure the cancer can be removed with an operation.

If you haven't already had some blood tests, these will be required. However, these tests are not specific to the study and would need to be performed prior to this type of operation.

Standard treatment for your bowel cancer involves an operation to remove part of the bowel containing the cancer and the surrounding lymph nodes. This is to minimise the chance of the cancer coming back.

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This study will not change any further treatment you may require after recovering from the operation.

Your CT scan gives us an impression of whether cancer has spread to the lymph nodes but we only know for definite after the lymph nodes have been removed and have been examined by a pathologist.

What is being tested?

The substance being tested is called 5-aminolevulinic acid (5-ALA).

5-ALA will not be used to “treat” your cancer, but will be used during your operation to detect the cancer along with any spread to lymph nodes that surround your bowel.

The purpose of the study is to find the lowest dose of 5-ALA that causes the cancer and any spread to lymph nodes to glow. To do this different doses of 5-ALA will be given to different groups (or cohorts) of participants.

The first group of participants will be given a dose of 20mg/kg of 5-ALA. Depending on how well this detects the cancer and spread to the lymph nodes, the second group will be given either 30mg/kg or 10 mg/kg.

A final group of participants will be given the most successful dose to confirm how well it detects the cancer and spread to lymph nodes.

Although only the dose of 20mg/kg is licensed for use, doses of up to 50mg/kg have been used in clinical studies. If you have private medical insurance you should check whether this will be affected by taking an unlicensed dose.

Your doctor will be able to tell you what dose of 5-ALA you will be given before your operation.

What will happen to me if I take part?

If you choose to take part in the study the management of your bowel cancer will differ only slightly from the standard treatment in that you will take 5-ALA before your standard operation.

5-ALA will be given to you as a liquid to drink (about 100mls) approximately 4 to 6 hours before your operation. 5-ALA is naturally occurring in human cells. When this substance is given in higher doses it is preferentially taken up into cancer cells. The drink is clear and slightly yellowish in colour. It tastes slightly acidic, similar to lemon juice diluted in water.

During the operation we will shine blue light from the camera used in keyhole surgery, and any cancer cells in the bowel and in the lymph nodes will glow red when you have taken 5-ALA. This might help us to identify the cancer and any spread to the lymph nodes.

The parts of your bowel and surrounding lymph nodes that glow red will be marked with surgical clips and will be removed as part of your standard operation. This study does not involve removing additional tissue.

The tissue removed will be examined in detail, by a pathologist to confirm whether cancer cells are present. This is part of the standard procedure following bowel cancer surgery. However, as part of the study the pathologist will assess whether the areas with confirmed cancer cells glowed red during the operation. This will not affect the standard pathology process, or how the results from your removed tissue are interpreted. It will however, allow us

to see how accurately the substance (5-ALA) detects bowel cancer and its spread to lymph nodes. If 5-ALA detects bowel cancer and its spread to lymph nodes accurately it might be used in the future to decide how much tissue a surgeon needs to remove during an operation.

5-ALAHas been used extensively before in other cancers, such as bladder cancer, brain tumours, and ovarian cancer. It has only been used before on a very small scale in colorectal cancer. This is one of the reasons why this study is important, as we plan to test the substance in a large number of patients with colorectal cancer.

In order to see how effective this substance is at detecting bowel cancer we will perform a standard cancer operation with the aim of removing all the cancer, including any cancer that might have spread to lymph nodes. Participating in this study will not mean that extra tissue is removed.

In order to help the researchers obtain as much useful information as possible from the study, videos and photographs may be taken during your operation. These will not identify you by name, and will be anonymised.

How long does treatment go on?

Participants in GLiSten will undergo standard postoperative care, with monitoring for any unwanted effects to the 5-ALA. This will include daily blood tests following surgery, whilst you are in hospital. These blood tests are part of standard practice.

At the end of the study, 30 days after the operation, you will be reviewed in the outpatient clinic (as is standard practice). Your participation in the study will then end.

What are the unwanted effects of treatment?

5-ALA can cause photosensitivity (make your skin more sensitive to bright lights). This means you should stay away from bright lights for 24-48 hours after taking the substance. The standard ward environment after the operation will be satisfactory as long as you avoid bright sunlight. During your operation your eyes and skin will be protected from the operating lights.

Occasional unwanted effects include nausea, vomiting and fast heart rate for 48 hours after taking 5-ALA. You will be monitored for all of these effects. However, this happens as part of standard post-operative monitoring and to experience nausea after an operation is quite common. In addition, biochemical testing occasionally show raised levels of certain enzymes (chemicals) made by the liver. If this occurs, it is usually a mild change for the first 48 hours following surgery, with the enzymes returning to normal as the effect of the drug wears off. Blood tests will be performed on a daily basis when in hospital (as is standard care) to monitor for this. Very occasionally, when 5-ALA has been given prior to brain tumour surgery side effects have included excess accumulation of fluid within the brain. Whether this applies to bowel surgery is not known.

Studies that have used this substance in the past have not seen any greater frequency of these possible unwanted effects in patients who took the substance compared to those who did not.

Women of childbearing potential and men with partners of child bearing potential should use adequate contraception (hormonal or barrier method of birth control or abstinence) prior to study entry and for the duration of study participation. We will perform a pregnancy test on any woman of child bearing potential (any woman who has experienced menarche and who is

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not postmenopausal or permanently sterilized) and will need evidence of a negative result prior to entry into the study.

The following drugs should be avoided before participating in the study and for 30 days after your operation:

- Medicines known to have a photosensitising effect e.g. tetracyclines, sulphonamides, quinolones
- Medicines associated with acute porphyria e.g. diclofenac, barbiturates, carbamazepine, phenytoin
- Medicines associated with hepatic or renal dysfunction e.g. NSAIDs, ACE-inhibitors, loop diuretics, phenytoin

Your doctor will go through your medication prior to your entry into the study to ensure it does not include any of the above. If you have any concerns about any medication prescribed to you during your involvement in the study your doctor will be happy to discuss it with you.

How is my condition monitored?

During your stay in hospital you will be seen on a ward round on a daily basis to ensure you are recovering at a satisfactory pace after the operation. At this time all your routine observations (pulse, blood pressure and temperature) will be reviewed. Again, this is no different to standard post-operative care.

You will be seen in the outpatient clinic at roughly 30 days after the operation to again check on your recovery.

This study will not change any further treatment you may require after recovering from the operation. Neither will the study change any long-term follow up including regular checks in the outpatient clinic.

What are the possible disadvantages and risks of taking part?

The disadvantages and risks of taking part in GLiSten include the unwanted effects mentioned above. As stated, if these are experienced there are usually mild and do not require any intervention. The most frequent unwanted effects are

- nausea, vomiting, and fast heart rate (common after any operation),
- sensitivity to bright sun-light (specific to 5-ALA).

What are the possible benefits of taking part?

Participants will benefit from high quality keyhole surgery by experienced surgeons, with proven short-term benefits, including less post-operative discomfort, quicker recovery, improved cosmetic result and possible shorter hospital stay. Tissue removed at the time of the operation will be subject to in-depth analysis by experienced pathologists. You will also be monitored closely following your surgery.

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By participating in a clinical trial you will receive at least the best treatment currently available. However, there is no guarantee that you will benefit from taking 5-ALA before your operation. 5-ALA may or may not be effective in detecting the spread of cancer to lymph nodes. Whatever the outcome of this research, information from this study will benefit patients who develop bowel cancer in the future by allowing doctors to learn more about the disease.

The main beneficiaries, should 5-ALA prove to be effective in detecting lymph node spread, will be future generations of colon cancer sufferers. The ability to accurately determine lymph node spread may enable surgeons to vary surgery to suit each patient's needs; patients with no lymph node spread may benefit from less extensive surgery compared to patients with lymph node spread who need a more extensive operation to eradicate their cancer.

What if something goes wrong?

If you become unwell whilst taking part in the study you should contact your clinical care team as soon as possible for advice. Any serious unexpected unwanted effect you may have will be reported to the GLiSten research team immediately. A Steering Group will be set up which will closely monitor the study on an ongoing basis so that if there are any problems then they will be detected as soon as possible so that the study can be changed or stopped if necessary.

You will find detailed information in Part 2 about what procedures are available to you if you have a complaint about the way you have been dealt with during the study, or if you suffer harm as result of being in this study.

What happens when the research study stops?

Your involvement in the GLiSten study will stop 30 days following your operation. After this your follow up will be as standard treatment with outpatient appointments on a regular basis for up to 5 years following your operation.

An outpatient appointment usually includes a physical examination by your doctor and some blood tests. As part of the standard practice following bowel cancer treatment you will also undergo regular CT scans and a colonoscopy. These tests will not be part of this study.

Will my taking part be kept confidential?

If you decide to participate in GLiSten the information collected about you will be handled strictly in accordance with the consent that you have given and also the 1998 Data Protection Act. Please refer to Part 2 for further details.

Contact Details

If you have any further questions about your illness or clinical studies, please discuss them with your doctor.

You may also find it helpful to contact Macmillan Cancer Support, an independent cancer information charity (Tel: +44 (0)808 808 00 00; address: 89 Albert Embankment, London, SE1 7UQ; website www.macmillan.org.uk)

or

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CancerHelp, an information service about cancer and cancer care for people with cancer and their families by [Cancer Research UK](http://www.cancerhelp.org.uk) (Tel: +44 (0)20 7061 8355; website www.cancerhelp.org.uk).

If you would like further information about clinical research, the UK Clinical Research Collaboration (a partnership of organisations working together on clinical research in the UK) have published a booklet entitled 'Understanding Clinical Trials'. Contact UKCRC: Tel: +44 (0)207 670 5452; website www.ukcrc.org

Your contact telephone numbers:

.....
.....

This completes Part 1 of the Information Sheet. If the Information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What will happen if I don't want to carry on with the study?

If you withdraw consent from further study treatment, and/or follow-up, your data and samples will remain on file and will be included in the final study analysis.

If you leave the study and do not wish for any further information to be collected, you should inform your clinical care team of this in order that no further follow-up information is collected from your medical records.

Please note the GLiSten study team may be required to continue to collect some limited information about you in the case of any unwanted effects you may have as a result of taking part in the trial. This will only be collected if required by the regulatory authorities. In line with Good Clinical Practice guidelines, at the end of the study, your data will be securely archived for a minimum of 15 years. Arrangements for confidential destruction will then be made.

What will happen if a patient loses mental capacity during the study period?

There is no reason why taking part in this study should affect mental capacity and so this is expected to be an exceptionally rare occurrence. It could however happen to any patient whether or not they are a participant in this study, for example due to an entirely separate event (e.g. a head injury). If this did occur, your doctor would discuss any changes in your treatment with your family/ carer including whether you should be withdrawn from the study. In any event, the GLiSten study team would continue to collect safety and follow up data about you from your medical records via your clinical care team until the end of the study.

Who has organised, funded and reviewed the research and who will be supervising it?

The GLiSten study is being organised by St James's University Hospital, Leeds, UK in collaboration with the Clinical Trials Research Unit at the University of Leeds, UK.

The study is funded by the National Institute for Health Research (NIHR) Efficacy and Mechanism Evaluation (EME) Programme. The study was also reviewed by experts on behalf of the funder.

The study has been reviewed by a Research Ethics Committee, the Medicines and Healthcare products Regulatory Agency (MHRA), Irish Medicine Board (IMB) and the Research and Development Department at your hospital. A Data Monitoring & Ethics Committee and Steering Committee will monitor and supervise the study. These committees are independent of the researchers and funder.

What if there is a problem?

Complaints:

If you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal local complaints services are available to you.

Leeds:

Please contact the Patient Advice and Liaison Service (PALS) at Leeds Teaching Hospitals NHS Trust on (0113) 2066261 or (0113) 2067168 or email patient.relations@leedsth.nhs.uk Complaints will be dealt with via the National Health

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Service. These are unique to your local NHS trust and your doctor or nurse can give you their information,

Dublin:

Please contact the patient representatives at Beaumont Hospital on (01) 809 3234 or email patientrepresentative@beaumont.ie

Harm:

If you are harmed by taking part in this research project compensation arrangements are in place. If you have grounds for legal action you may have to pay your legal costs.

Any claims will be subject to UK law and must be brought in the UK.

If you have private medical insurance, you should tell your insurer that you are taking part in research. They will let you know if it affects your policy.

Will my taking part in this study be kept confidential?

If you decide to participate in GLiSten, the information collected about you will be handled in accordance with the consent that you have given and also the 1998 Data Protection Act.

- The information needed for study purposes will be recorded on paper forms and collected by or sent to (usually using standard post but in some cases by fax or email) the researcher at St James's University Hospital Leeds. Some data will also be sent to the researchers at Clinical Trials Research Unit (CTRU)
- You will be allocated a study number, which will be used along with your date of birth and initials to identify you on each paper form. Your full name will be included on your consent form and a copy of this will be collected by or sent to the researchers by fax, post or email.
- Every effort will be made to ensure that any further information about you that leaves the hospital will have your name and address removed so that you cannot be recognised from it; this information will usually be removed by a member of the study team at your hospital, but may also be removed by the researchers upon receipt.

Your data will be entered onto a secure database held at St James's University Hospital in accordance with the 1998 Data Protection Act.

Your healthcare records may be looked at by authorised individuals from the research team, the University of Leeds (the study Sponsor) or the regulatory authorities to check that the study is being carried out correctly.

The information collected about you may be shared with other research teams to answer new research questions in the future. Wherever possible, information will be anonymised (for example; your full name will not be disclosed)

Your name, date of birth, and NHS number and address/postcode will be submitted to standard NHS patient registries (e.g. Medical Research Information Service; Hospital Episodes Statistics etc) held at the NHS Information Centre for Health and Social Care, and cancer registry. This is so that information about your health status may be obtained by the researchers if necessary.

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- Your data may be passed to other organisations (possibly in other countries where the data protection standards and laws are different to the UK) to monitor the safety of the treatment(s) that you are receiving; this data will have your name removed.
- CT scans and pathology blocks will be sent for central review to ensure that results are consistent across hospitals. These will be sent via standard hospital processes (such as Royal Mail or courier). Wherever possible, this data will be anonymised and your name removed
- Tissue that is removed will be held in a Human Tissue Act compliant storage facility at the University of Leeds.

Involvement of the General Practitioner/Family Doctor (GP):

Your GP, and the other doctors involved in your healthcare, will be informed of your participation in this study.

Additional research

Bowel cancer research is very important. We do not know all of the important questions which need to be researched at the present time. Therefore, with your permission, the surplus specimens from your cancer operation that will be stored in the hospital pathology laboratory may be used in the future for cancer research.

Strict confidentiality will be maintained at all times and your name and individual details will not be stored with your tissue samples (i.e. they will be anonymised). However, a unique reference number will be allocated to the samples which may allow them to be linked back to data we have collected about your condition in future for research purposes; this will be in strict confidence and you would not be identified in any way.

The samples and information you give may be made available to researchers in the UK or overseas. They may work in universities, hospitals, or in private/commercial companies that do medical research. You will not receive any personal financial award for your gift.

Your donation will be used only for medical research and will not be provided for any other purpose. The people who will store your tissue may ask researchers for fees to cover some of the costs it incurs. This is known as 'cost recovery' as it is for reinvestment to ensure the highest standards of safety and professionalism and to enable further medical research. The samples you have gifted will never be sold for profit.

If you have questions or concerns about the donation of samples and information or the possible uses of them, please ask the person discussing donation with you and seeking consent.

If you do not want to your surplus tissue to be used in this way, you can still take part in the study

Will any genetic tests be done?

No.

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What will happen to the results of the research study?

When the study is complete the results will be published in a medical journal, but no individual participants will be identified. If you would like to obtain a copy of the published results, please ask your doctor.

The Leeds Teaching Hospitals

NHS Trust

John Goligher Colorectal Unit
 Research Office
 Ground Floor Lincoln Wing
 St James University Hospital
 Beckett Street
 Leeds
 LS9 7TF
 Tel: 0113 20 64672

Participant ID:	Initials:
Date of Birth:	NHS/Hospital Number:
EudraCT Number: 2012-002623-15	Principal Investigator:

GLiSten

5-ALA in Bowel Cancer Surgery

PARTICIPANT CONSENT FORM

Please initial each box

1.

I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions.

2.

I understand that my participation in this study is voluntary and that I am free to withdraw at any time without my medical care or legal rights being affected. I understand that even if I withdraw from the above study, the data and samples collected from me will be used in analysing the results of the study. In some cases further information about any unwanted effects of my treatment may need to be collected by the study team.

3.

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I understand that my healthcare records may be looked at by authorised individuals from the study team, regulatory bodies or Sponsor in order to check that the study is being carried out correctly.

4. I agree to allow any information or results arising from this study to be used for healthcare and/or further medical research upon the understanding that my identity will remain anonymous wherever possible.

5. I agree for my details (which will include my name, date of birth, NHS number and address) to be submitted to the e.g. Medical Research Information Service; Hospital Episodes Statistics via the NHS Information Centre for Health and Social Care, so that information about my health status may be obtained by St James's University Hospital Leeds if necessary.

6. I agree to a copy of this Consent Form being sent to St James's University Hospital.

7. I agree that my GP, or any other doctor treating me, will be notified of my participation in this study.

8. I agree to take part in the study.

The following points are OPTIONAL.

Even if you agree to take part in this study, you do not have to agree to this section:

I give permission for surplus samples from my cancer that have been stored in the hospital pathology laboratory to be retrieved and used in the future for bowel cancer research.

No

Yes

I understand that my tissue sample is a 'gift' that may be used in future research that receives ethical approval. I understand that my sample and data collected from it may be shared on a collaborative basis with researchers in the UK and potentially, centres abroad, including outside the European Economic Area.

No

Yes

Patient:

Signature.....

Name (block capitals).....

Date.....

Investigator:

I have explained the study to the above named patient and he/she has indicated his/her willingness to participate.

Signature.....

Name (block capitals).....

Date.....

(If used)Translator:

Signature.....

Name (block capitals).....

Date.....

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Appendix 3 5-aminolevulinic acid prescription and guidance for reconstitution



Clinical Trial Prescription

Patient Name			
Date of Birth		Consultant	
Hospital No		Ward	
Trial ID number	Assigned retrospectively	Cohort	
Allergies			

Original: retain in the pharmacy site file

Copy: return to ward with 5-ALA

5-aminolevulinic acid (5-ALA) powder for oral solution

Please also prescribe the dose on the patient's drug chart			
Date Requested	/ /	Date Required	/ /
Patient weight	(kg)	Time surgery scheduled for	:
Dose level required: (please circle)	10mg/kg	20mg/kg	30mg/kg
Dose	(mg)	Rounded to (if applicable)	(mg)
Prescribed by:		Date	
For Pharmacy Use Only			
Prescription Validated By		Date	
Quantity dispensed (number of vials)		Batch number	
Dispensed by (sign and print)		Date	
Checked by (sign and print)		Date	
GLiSten 5-ALA guidance leaflet issued (please tick): <input type="checkbox"/>			
Administration			
Please refer to the GLiSten Trial protocol before giving this medicine <i>I confirm I have read and understand the GLiSten 5-ALA information leaflet prior to administration:</i>			
Ward		Nurse Signature (1)	
Date of administration	/ /	Nurse Signature (2)	
Time of administration	:		
Give the calculated amount of 5-ALA in tap water at 30mg/ml between 4-6 hours before the scheduled beginning of anaesthesia			

Trial Sponsor: University of Leeds, Ref: GS11/9681
 EudraCT number: 2012-002623-15
 GLiSten Trial – oral 5-ALA Prescription v2 29.10.2013

CI: Prof D Jayne
 PI: Dr Gemma Gossedge



5-ALA in Bowel Cancer Surgery

GUIDANCE FOR 5-ALA RECONSTITUTION ON THE WARD

One vial contains 1.5 g 5-aminolevulinic acid hydrochloride (5-ALA HCl). The powder is a white to off white cake.

The solution should be administered **orally** 4-6hrs prior to surgery.

The reconstituted solution is a clear and colourless to slightly yellowish fluid.

Gliolan is for single use only and any content remaining after first use must be discarded.

Reconstituted solution The reconstituted solution is physically-chemically stable for 24 hours at 25°C.

To calculate the dose of 5-ALA

1. **Find which dose cohort the patient is in from the patient case notes**
2. **Weigh the patient – in kg**
3. **Calculate the total dose in mg**
 - Patients weight in kg X 20 (If in 20mg/kg cohort) = total dose in mg required
 - Patients weight in kg X 30 (If in 30mg/kg cohort) = total dose in mg required
 - Patients weight in kg X 10 (If in 10mg/kg cohort) = total dose in mg required
4. **Dissolve the contents of the vial in 50mls of sterile water**
 - 1ml of the reconstituted solution contains 30mg of 5-ALA HCL
5. **Divide the total dose required in mg by 30.**

Answer = volume of reconstituted solution needed to give total dose in mg

i.e. the volume to give to the patient (round up or down to nearest ml)

N.B. for some patients more than 1 vial may be required

Additional information on 5-ALA

Aim of the trial

The aim of the glisten trial is to see if 5 ALA can detect malignant spread within the lymph nodes surrounding the bowel during a patient's surgery. 5ALA is preferentially taken up into malignant cells. During the operation when blue light from the laparoscope is shone onto the bowel and surrounding tissue any malignant cells should glow red.

The purpose of this phase of the trial is to find the lowest dose of 5-ALA that causes the cancer and any spread to lymph nodes to glow. To do this different doses of 5-ALA will be given to different cohorts of participants.

If 5 ALA is able to detect malignant spread accurately it may be used in the future to guide how much tissue should be removed during bowel cancer operations.

Treatment of patients on the ward following administration of 5ALA

After administration of 5-ALA, exposure of eyes and skin to strong light sources (e.g. operating illumination, direct sunlight or brightly focused indoor light) should be avoided for 48 hours. Patients should be prevented from going outside in sunny weather conditions; the ward environment is suitable. During the operation patient's eyes and skin will be protected from the operating lights, using standard methods such as sterile drapes and tape to keep their eyes closed.

5-ALA Routine blood tests including Urea and Electrolytes and Liver Function Tests will be performed on a daily basis for 5 days post-operatively

Adverse Effects N.B. Likely to only occur within 48hrs of administration of the drug

- Nausea
- Vomiting
- Tachycardia
- Hypotension
- Transiently deranged LFT's
- Photosensitivity (see above)

Medication to avoid with 5-ALA

Medicines to avoid before participation on the study and for 30 days following administration of 5 ALA

- Medicines known to have a **photosensitising effect**
 - **tetracyclines** (e.g. doxycycline, minocycline and oxytetracycline)
 - **sulphonamides** (e.g. Antibiotics - sulfadiazine, trimethoprim, sulfasalazine, Antidiabetic drugs - glipizide, gliclazine, Thiazide diuretics - hydrochlorothiazide, indapamide, metolozone, Loop diuretics - frusemide, Carbonic anhydrase inhibitors - acetazolamide, COX2 inhibitors - celecoxib)
 - **quinolones** (e.g. ciprofloxacin, levofloxacin)
- Medicines associated with **acute porphyria**
 - **Diclofenac**

- **Barbiturates**
- **Carbamazepine**
- **Phenytoin**

- Medicines associated with **hepatic or renal dysfunction**
 - **NSAIDs**
 - **ACE-inhibitors**
 - **Loop diuretics**
 - **Phenytoin**

- Medicine containing **hypericin extracts** (e.g. St John's wort)

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Appendix 4 Summary of product characteristics

Information reproduced from European Medicines Agency: EMEA/H/C/000744-IAIN/0008/G – Gliolan: EPAR – Product Information March 2014. www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000744/human_med_000807.jsp&mid=WC0b01ac058001d124⁵² (accessed November 2015). © EMA [1995-2016].

ANNEX I

SUMMARY OF PRODUCT CHARACTERISTICS

1. NAME OF THE MEDICINAL PRODUCT

Gliolan 30 mg/ml powder for oral solution.

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

One vial contains 1.17 g of 5-aminolevulinic acid (5-ALA), corresponding to 1.5 g 5-aminolevulinic acid hydrochloride (5-ALA HCl).

One ml of reconstituted solution contains 23.4 mg of 5-ALA, corresponding to 30 mg 5-ALA HCl.

3. PHARMACEUTICAL FORM

Powder for oral solution.

The powder is a white to off-white cake.

4. CLINICAL PARTICULARS

4.1 Therapeutic indications

Gliolan is indicated in adult patients for visualisation of malignant tissue during surgery for malignant glioma (WHO grade III and IV).

4.2 Posology and method of administration

This medicinal product should only be used by experienced neurosurgeons conversant with surgery of malignant gliomas and in-depth knowledge of functional brain anatomy who have completed a training course in fluorescence-guided surgery.

Posology

The recommended dose is 20 mg 5-ALA HCl per kilogram body weight.

Patients with renal or hepatic impairment

No trials have been performed in patients with clinically relevant hepatic or renal impairment. Therefore, this medicinal product should be used with caution in such patients.

Elderly patients

There are no special instructions for use in elderly patients with regular organ function.

Paediatric population

The safety and efficacy of Gliolan in children and adolescents aged 0 to 18 years have not yet been established. No data are available.

Method of administration

The solution should be administered orally three hours (range 2-4 hours) before anaesthesia. Use of 5-ALA under conditions other than the ones used in the clinical trials entail an undetermined risk.

Precautions to be taken before handling or administering the medicinal product

For instructions on reconstitution of the medicinal product before administration, see section 6.6.

4.3 Contraindications

- Hypersensitivity to the active substance or porphyrins.
- Acute or chronic types of porphyria.
- Pregnancy (see sections 4.6 and 5.3).

4.4 Special warnings and precautions for use

5-ALA-induced fluorescence of brain tissue does not provide information about the tissue's underlying neurological function. Therefore, resection of fluorescing tissue should be weighed up carefully against the neurological function of fluorescing tissue.

Special care must be taken in patients with a tumour in the immediate vicinity of an important neurological function and pre-existing focal deficits (e.g. aphasia, vision disturbances and paresis) that do not improve on corticosteroid treatment. Fluorescence-guided resection in these patients has been found to impose a higher risk of critical neurological deficits. A safe distance to eloquent cortical areas and subcortical structures of at least 1 cm should be maintained independent of the degree of fluorescence.

In all patients with a tumour in the vicinity of an important neurological function, either pre- or intraoperative measures should be used to localise that function relative to the tumour in order to maintain safety distances.

After administration of this medicinal product, exposure of eyes and skin to strong light sources (e.g. operating illumination, direct sunlight or brightly focused indoor light) should be avoided for 24 hours.

Co-administration with other potentially phototoxic substances (e.g. tetracyclines, sulfonamides, fluoroquinolones, hypericin extracts) should be avoided (see also section 5.3).

Within 24 hours after administration, other potentially hepatotoxic medicinal products should be avoided.

In patients with pre-existing cardiovascular disease, this medicinal product should be used with caution since literature reports have shown decreased systolic and diastolic blood pressure, pulmonary artery systolic and diastolic pressure as well as pulmonary vascular resistance.

4.5 Interaction with other medicinal products and other forms of interaction

Patients should not be exposed to any photosensitizing agent up to 2 weeks after administration of Gliolan.

4.6 Fertility, pregnancy and lactation

Pregnancy

There are no or limited amount of data from the use of 5-ALA in pregnant women. Some limited animal studies suggest an embryotoxic activity of 5-ALA plus light exposure (see section 5.3). Therefore, Gliolan should not be used during pregnancy.

Breast-feeding

It is unknown whether 5-ALA or its metabolite protoporphyrin IX (PPIX) is excreted in human milk. The excretion of 5-ALA or PPIX in milk has not been studied in animals. Breast-feeding should be interrupted for 24 hours after treatment with this medicinal product.

Fertility

There are no data available regarding the influence of 5-ALA on fertility.

4.7 Effects on ability to drive and use machines

Not relevant, the treatment itself will have an influence on the ability to drive and use machines.

4.8 Undesirable effects

Summary of the safety profile

Adverse reactions observed after the use of this medicinal product for fluorescence-guided glioma resection are divided into the following two categories:

- immediate reactions occurring after oral administration of the medicinal product before anaesthesia (= active substance-specific side effects)
- combined effects of 5-ALA, anaesthesia, and tumour resection (= procedure-specific side effects).

Most serious side effects include anaemia, thrombocytopenia, leukocytosis, neurological disorders and thromboembolism. Further frequently observed side effects are vomiting, nausea and increase of blood bilirubin, alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase and blood amylase.

Tabulated summary of adverse reactions

Very common ($\geq 1/10$)

Common ($\geq 1/100$ to $< 1/10$)

Uncommon ($\geq 1/1,000$ to $< 1/100$)

Rare ($\geq 1/10,000$ to $< 1/1,000$)

Very rare ($< 1/10,000$)

Not known (cannot be estimated from the available data).

Within each frequency grouping, adverse reactions are presented in order of decreasing seriousness.

Substance-specific side effects:

Cardiac disorders	<u>Uncommon:</u> hypotension
Gastrointestinal disorders	<u>Uncommon:</u> nausea
Skin and subcutaneous tissue disorders	<u>Uncommon:</u> photosensitivity reaction, photodermatosis

Procedure-related side effects

The extent and frequency of procedure-related neurological side effects depends on the localisation of the brain tumour and the degree of resection of tumour tissue lying in eloquent brain areas (see section 4.4).

Blood and lymphatic system disorders	<u>Very common:</u> anaemia, thrombocytopenia, leukocytosis
Nervous system disorders	<u>Common:</u> neurological disorders (e.g. hemiparesis, aphasia, convulsions, hemianopsia) <u>Uncommon:</u> brain oedema <u>Very rare:</u> hypesthesia
Cardiac disorders	<u>Uncommon:</u> hypotension
Vascular disorders	<u>Common:</u> thromboembolism
Gastrointestinal disorders	<u>Common:</u> vomiting, nausea <u>Very rare:</u> diarrhoea

Hepatobiliary disorders	Very common: blood bilirubin increased, alanine aminotransferase increased, aspartate aminotransferase increased, gamma glutamyltransferase increased, blood amylase increased
-------------------------	---

Description of selected adverse reactions

In a single-arm trial including 21 healthy male volunteers, erythema of the skin could be provoked by direct exposure to UVA light up to 24 hours after oral application of 20 mg/kg body weight 5-ALA HCl. An adverse drug reaction of mild nausea was reported in 1 out of 21 volunteers.

In another single-centre trial, 21 patients with malignant glioma received 0.2, 2, or 20 mg/kg body weight 5-ALA HCl followed by fluorescence-guided tumour resection. The only adverse reaction reported in this trial was one case of mild sunburn occurring in a patient treated with the highest dose.

In a single-arm trial including 36 patients with malignant glioma, adverse drug reactions were reported in 4 patients (mild diarrhoea in one patient, moderate hypesthesia in another patient, moderate chills in another patient, and arterial hypotension 30 minutes after application of 5-ALA in another patient). All patients received the medicinal product in a dose of 20 mg/kg body weight and underwent fluorescence-guided resection. Follow-up time was 28 days.

In a comparative, unblinded phase III trial (MC-ALS.3/GLI), 201 patients with malignant gliomas received 5-ALA HCl in a dose of 20 mg/kg body weight and 176 of these patients underwent fluorescence-guided resection with subsequent radiotherapy. 173 patients received standard resection without administration of the medicinal product and subsequent radiotherapy. Follow-up time comprised at least 180 days after administration. At least possibly related adverse reactions were reported in 2/201 (1.0 %) patients: mild vomiting 48 hours after surgery, and mild photosensitivity 48 hours after trial surgery. Another patient accidentally received an overdose of the medicinal product (3000 mg instead of 1580 mg). Respiratory insufficiency, which was reported in this patient, was managed by adaptation of ventilation and resolved completely. A more pronounced transient increase of liver enzymes without clinical symptoms was observed in the 5-ALA-treated patients. Peak values occurred between 7 and 14 days after administration. Increased levels of amylase, total bilirubin, and leukocytes, but decreased levels of thrombocytes and erythrocytes were observed, however differences between treatment groups were not statistically significant.

Reporting of suspected adverse reactions

Reporting suspected adverse reactions after authorisation of the medicinal product is important. It allows continued monitoring of the benefit/risk balance of the medicinal product. Healthcare professionals are asked to report any suspected adverse reactions via [the national reporting system listed in Appendix V](#).

4.9 Overdose

Within a clinical trial, a 63-year old patient with known cardiovascular disease was accidentally given an overdose of 5-ALA HCl (3000 mg instead of 1580 mg). During surgery he developed respiratory insufficiency, which was managed by adaptation of ventilation. After surgery the patient also displayed facial erythema. It was stated that the patient had been exposed to more light than permitted for the trial. Respiratory insufficiency and erythema completely resolved.

In the event of overdose, supportive measures should be provided as necessary, including sufficient protection from strong light sources (e.g. direct sunlight).

5. PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

Pharmacotherapeutic group: Antineoplastic agents, sensitisers used in photodynamic therapy, ATC code: L01XD04

Mechanism of action

5-ALA is a natural biochemical precursor of heme that is metabolised in a series of enzymatic reactions to fluorescent porphyrins, particularly PPIX. 5-ALA synthesis is regulated by an intracellular pool of free heme via a negative feedback mechanism. Administration of excess exogenous 5-ALA avoids the negative feedback control, and accumulation of PPIX occurs in target tissue. In the presence of visible light, fluorescence of PPIX (photodynamic effect) in certain target tissues can be used for photodynamic diagnosis.

Pharmacodynamic effects

Systemic administration of 5-ALA results in an overload of the cellular porphyrin metabolism and accumulation of PPIX in various epithelia and cancer tissues. Malignant glioma tissue (WHO-grade III and IV, e.g. glioblastoma multiforme, gliosarcoma or anaplastic astrocytoma) has also been demonstrated to synthesise and accumulate porphyrins in response to 5-ALA administration. The concentration of PPIX is significantly lower in white matter than in cortex and tumour. Tissue surrounding the tumour and normal brain may also be affected. However, 5-ALA induced PPIX formation is significantly higher in malignant tissue than in normal brain.

In contrast, in low-grade tumours (WHO-grade I and II, e.g. medulloblastoma, oligodendroglioma) no fluorescence could be observed after application of the active substance. Brain metastases revealed inconsistent or no fluorescence.

The phenomenon of PPIX accumulation in WHO grade III and IV malignant gliomas may be explained by higher 5-ALA uptake into the tumour tissue or an altered pattern of expression or activity of enzymes (e.g. ferrochelatase) involved in haemoglobin biosynthesis in tumour cells. Explanations for higher 5-ALA uptake include a disrupted blood-brain barrier, increased neo-vascularisation, and the overexpression of membrane transporters in glioma tissue.

After excitation with blue light ($\lambda=400-410$ nm), PPIX is strongly fluorescent (peak at $\lambda=635$ nm) and can be visualised after appropriate modifications to a standard neurosurgical microscope.

Fluorescence emission can be classified as intense (solid) red fluorescence (corresponds to vital, solid tumour tissue) and vague pink fluorescence (corresponds to infiltrating tumour cells), whereas normal brain tissue lacking enhanced PPIX levels reflects the violet-blue light and appears blue.

Clinical efficacy and safety

In a phase I/II trial including 21 patients, a dose-efficacy relationship between the dose levels and the extent and quality of fluorescence in the tumour core was detected: higher doses of 5-ALA enhanced the fluorescence quality and the fluorescence extent of the tumour core compared to demarcation of the tumour core under standard white illumination in a monotone, non-falling fashion. The highest dose (20 mg/kg body weight) was determined to be the most efficient.

A positive predictive value of tissue fluorescence of 84.8 % (90 % CI: 70.7 %-93.8 %) was found. This value was defined as the percentage of patients with positive tumour cell identification in all biopsies taken from areas of weak and strong fluorescence. The positive predictive value of strong fluorescence was higher (100.0 %; 90 % CI: 91.1 %-100.0 %) than of weak fluorescence (83.3 %; 90 % CI: 68.1 %-93.2 %). Results were based on a phase II trial including 33 patients receiving 5-ALA HCl in a dose of 20 mg/kg body weight.

The resulting fluorescence was used as an intraoperative marker for malignant glioma tissue with the aim of improving the surgical resection of these tumours.

In a phase III trial with 349 patients with suspected malignant glioma amenable to complete resection of contrast-enhancing tumour were randomised to fluorescence-guided resection after administration of 20 mg/kg body weight 5-ALA HCl or conventional resection under white light. Contrast-enhancing tumour was resected in 64 % of patients in the experimental group compared to 38 % in the control-group ($p < 0.0001$).

At the visit six months after tumour resection, 20.5 % of 5-ALA-treated-patients and 11 % of patients who underwent standard surgery were alive at the six-month visit without progression. The difference was statistically significant using the chi-square test ($p = 0.015$).

No significant increase in overall survival has been observed in this trial; however, it was not powered to detect such a difference.

5.2 Pharmacokinetic properties

General characteristics

This medicinal product shows good solubility in aqueous solutions. After ingestion, 5-ALA itself is not fluorescent but is taken up by tumour tissue (see section 5.1) and is intracellularly metabolised to fluorescent porphyrins, predominantly PPIX.

Absorption

5-ALA as drinking solution is rapidly and completely absorbed and peak plasma levels of 5-ALA are reached 0.5–2 hours after oral administration of 20 mg/kg body weight. Plasma levels return to baseline values 24 hours after administration of an oral dose of 20 mg/kg body weight. The influence of food has not been investigated because this medicinal product is generally given on empty stomach prior to induction of anaesthesia.

Distribution and biotransformation

5-ALA is preferentially taken up by the liver, kidney, endothelials and skin as well as by malignant gliomas (WHO grade III and IV) and metabolised to fluorescent PPIX. Four hours after oral administration of 20 mg/kg body weight 5-ALA HCl, the maximum PPIX plasma level is reached. PPIX plasma levels rapidly decline during the subsequent 20 hours and are not detectable anymore 48 hours after administration. At the recommended oral dose of 20 mg/kg body weight, tumour to normal brain fluorescence ratios are usually high and offer lucid contrast for visual perception of tumour tissue under violet-blue light for at least 9 hours.

Besides tumour tissue, faint fluorescence of the choroid plexus was reported. 5-ALA is also taken up and metabolised to PPIX by other tissues, e.g. liver, kidneys or skin (see section 4.4). Plasma protein binding of 5-ALA is unknown.

Elimination

5-ALA is eliminated quickly with a terminal half-life of 1-3 hours. Approximately 30 % of an orally administered dose of 20 mg/kg body weight is excreted unchanged in urine within 12 hours.

Linearity/non-linearity

There is dose proportionality between AUC_{0-inf} of 5-ALA values and different oral doses of this medicinal product.

Renal or hepatic impairment

Pharmacokinetics of 5-ALA in patients with renal or liver impairment has not been investigated.

5.3 Preclinical safety data

Standard safety pharmacology experiments were performed under light protection in the mouse, rat and dog. 5-ALA administration does not influence the function of the gastrointestinal and central nervous system. A slight increase in saluresis cannot be excluded.

Single administration of high doses of 5-ALA to mice or rats leads to unspecific findings of intolerance without macroscopic abnormalities or signs of delayed toxicity. Repeat-dose toxicity studies performed in rats and dogs demonstrate dose-dependent adverse reactions affecting changes in bile duct histology (non-reversible within a 14 day recovery period), transient increase in transaminases, LDH, total bilirubin, total cholesterol, creatinine, urea and vomiting (only in dogs). Signs of systemic toxicity (cardiovascular and respiratory parameters) occurred at higher doses in the anaesthetised dog: at 45 mg/kg body weight intravenously a slight decrease in peripheral arterial blood pressure and systolic left ventricular pressure was recorded. Five minutes after administration, the baseline values had been reached again. The cardiovascular effects seen are considered to be related to the intravenous route of administration.

Phototoxicity observed after 5-ALA treatment *in vitro* and *in vivo* is obviously closely related to dose- and time-dependent induction of PPIX synthesis in the irradiated cells or tissues. Destruction of sebaceous cells, focal epidermal necrosis with a transient acute inflammation and diffuse reactive changes in the keratinocytes as well as transient secondary oedema and inflammation of dermis are observed. Light exposed skin recovered completely except for a persistent reduction in the number of hair follicles. Accordingly, general light protective measures of eyes and skin are recommended for at least 24 hours after administration of this medicinal product.

Although pivotal studies on the reproductive and developmental behaviour of 5-ALA have not been performed, it can be concluded that 5-ALA induced porphyrin synthesis may lead to embryotoxic activity in mouse, rat and chick embryos only under the condition of direct concomitant light exposure. This medicinal product should, therefore, not be administered to pregnant women. Excessive single dose treatment of rats with 5-ALA reversibly impaired male fertility for two weeks after dosing.

The majority of genotoxicity studies performed in the dark do not reveal a genotoxic potential of 5-ALA. The compound potentially induces photogenotoxicity after subsequent irradiation or light exposure which is obviously related to the induction of porphyrin synthesis. Long-term *in vivo* carcinogenicity studies have not been conducted. However, considering the therapeutic indication, a single oral treatment with 5-ALA might not be related to any serious potential carcinogenic risk.

6. PHARMACEUTICAL PARTICULARS

6.1 List of excipients

None.

6.2 Incompatibilities

In the absence of compatibility studies, this medicinal product must not be mixed with other medicinal products.

6.3 Shelf life

Unopened vial

3 years.

Reconstituted solution

The reconstituted solution is physically-chemically stable for 24 hours at 25°C.

6.4 Special precautions for storage

Keep the vial in the outer carton in order to protect from light.

For storage conditions after reconstitution of the medicinal product, see section 6.3.

6.5 Nature and contents of container

Colourless type II glass vial with butyl rubber stopper containing 1.5 g powder for reconstitution in 50 ml of drinking water.

Pack sizes: 1, 2 and 10 vials.

Not all pack sizes may be marketed.

6.6 Special precautions for disposal and other handling

The oral solution is prepared by dissolving the amount of powder of one vial in 50 ml of drinking water. The reconstituted solution is a clear and colourless to slightly yellowish fluid.

Gliolan is for single use only and any content remaining after first use must be discarded.

Any unused medicinal product or waste material should be disposed of in accordance with local requirements.

7. MARKETING AUTHORISATION HOLDER

medac

Gesellschaft für klinische

Spezialpräparate mbH

Theaterstr. 6

22880 Wedel

Germany

Tel. +49 4103 8006 0

Fax: +49 4103 8006 100

8. MARKETING AUTHORISATION NUMBER(S)

EU/1/07/413/001-003

9. DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

Date of first authorisation: 07 September 2007

Date of latest renewal: 12 September 2012

10. DATE OF REVISION OF THE TEXT

Detailed information on this medicinal product is available on the website of the European Medicines Agency <http://www.ema.europa.eu>.

ANNEX II

- A. MANUFACTURER RESPONSIBLE FOR BATCH RELEASE**
- B. CONDITIONS OR RESTRICTIONS REGARDING SUPPLY AND USE**
- C. OTHER CONDITIONS AND REQUIREMENTS OF THE MARKETING AUTHORISATION**
- D. CONDITIONS OR RESTRICTIONS WITH REGARD TO THE SAFE AND EFFECTIVE USE OF THE MEDICINAL PRODUCT**

A. MANUFACTURER RESPONSIBLE FOR BATCH RELEASE

Name and address of the manufacturer responsible for batch release

medac
Gesellschaft für klinische
Spezialpräparate mbH
Theaterstr. 6
22880 Wedel
Germany

B. CONDITIONS OR RESTRICTIONS REGARDING SUPPLY AND USE

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

C. OTHER CONDITIONS AND REQUIREMENTS OF THE MARKETING AUTHORISATION

- **Periodic Safety Update Reports**

The marketing authorisation holder shall submit periodic safety update reports for this product in accordance with the requirements set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and published on the European medicines web-portal.

D. CONDITIONS OR RESTRICTIONS WITH REGARD TO THE SAFE AND EFFECTIVE USE OF THE MEDICINAL PRODUCT

- **Risk Management Plan (RMP)**

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing Authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

If the dates for submission of a PSUR and the update of a RMP coincide, they can be submitted at the same time.

An updated RMP shall be submitted within 60 days of an important (pharmacovigilance or risk minimisation) milestone being reached.

- **Additional risk minimisation measures**

Gliolan should be used only by neurosurgeons who have attended a training course in accordance with the standards detailed below:

The Marketing Authorisation Holder in agreement with the competent authorities in the Member States shall implement, prior to launch:

- A training course for neurosurgeons which is aimed at risk minimisation and to support safe and effective use for the medicinal product. The training course will take place at qualified training centres using qualified trainers. This course shall consist of measures aiming to minimise adverse events associated with the Gliolan-fluorescence-guided surgery (in particular neurological serious adverse events) through adequate education about:
 - a) Theory and core principles of Gliolan-fluorescence-guided surgery and malignant glioma resection, including methods of eloquent sites identification;
 - b) On-site instructions on the use of the fluorescence-microscope, including pitfalls and recognition of problems;
 - c) Differentiation of fluorescence intensity, maintaining safety distances from eloquent areas;
 - d) The practice of Gliolan-fluorescence-guided surgery (including participation in at least one case using Gliolan-fluorescence-guided surgery in the operating room with on-site instructions on the use of the microscope or demonstration of a fluorescence-guided resection by video);
 - e) The current understanding of the benefits and risks of cytoreductive surgery in the management of patients with malignant gliomas;
 - f) The theoretical base for porphyrin accumulation in malignant gliomas;
 - g) The technical principles behind fluorescence-guided resections using Gliolan;
 - h) How to identify suitable candidates for fluorescence-guided resections using Gliolan;
 - i) How to apply Gliolan in the correct dose and timing regimen, and to understand the importance of concurrent corticosteroids;
 - j) How to identify patients at risk for neurological deficits using fluorescence-guided resections with Gliolan with special focus on aphasias and other critical focal deficits;
 - k) Techniques for intraoperative risk reduction (microsurgical technique, neurophysiological monitoring, choice of approach) and how to implement them;
 - l) How to identify fluorescence for resection through using the operating microscope in a hands-on setting in the operating room;
 - m) The benefits and risks of fluorescence-guided resections using Gliolan.

Minimum requirements for a qualified trainer are:

- Board-certification as neurosurgeon according to local, national requirements;
- Previous successful participation at a training course, or equivalent course during the phase III trial;
- Experience with Gliolan-fluorescence-guided surgery in at least 20 cases.

Minimum requirements for a qualified training centre are:

- Microscope modified for fluorescence-guided resection;
- Sufficient case load (at least 10 patients per year) of malignant gliomas (WHO grade III and IV);
- Neurophysiological monitoring techniques for surgery in eloquent brain regions.

ANNEX III
LABELLING AND PACKAGE LEAFLET

A. LABELLING

9. SPECIAL STORAGE CONDITIONS

Keep the vial in the outer carton in order to protect from light.

10. SPECIAL PRECAUTIONS FOR DISPOSAL OF UNUSED MEDICINAL PRODUCTS OR WASTE MATERIALS DERIVED FROM SUCH MEDICINAL PRODUCTS, IF APPROPRIATE

Single use vial – discard any content remaining after first use.

11. NAME AND ADDRESS OF THE MARKETING AUTHORISATION HOLDER

medac GmbH
Theaterstr. 6
22880 Wedel
Germany

12. MARKETING AUTHORISATION NUMBER(S)

EU/1/07/413/001
EU/1/07/413/002
EU/1/07/413/003

13. BATCH NUMBER

Batch

14. GENERAL CLASSIFICATION FOR SUPPLY

Medicinal product subject to medical prescription.

15. INSTRUCTIONS ON USE**16. INFORMATION IN BRAILLE**

Justification for not including Braille accepted

PARTICULARS TO APPEAR ON THE IMMEDIATE PACKAGING**Vial label****1. NAME OF THE MEDICINAL PRODUCT**

Gliolan 30 mg/ml powder for oral solution
5-aminolevulinic acid hydrochloride

2. STATEMENT OF ACTIVE SUBSTANCE(S)

One vial contains 1.17 g of 5-aminolevulinic acid, corresponding to 1.5 g 5-aminolevulinic acid hydrochloride (5-ALA HCl).

3. LIST OF EXCIPIENTS**4. PHARMACEUTICAL FORM AND CONTENTS**

Powder for oral solution

5. METHOD AND ROUTE(S) OF ADMINISTRATION

For oral use after reconstitution.
Read the package leaflet before use.

6. SPECIAL WARNING THAT THE MEDICINAL PRODUCT MUST BE STORED OUT OF THE SIGHT AND REACH OF CHILDREN

Keep out of the sight and reach of children.

7. OTHER SPECIAL WARNING(S), IF NECESSARY**8. EXPIRY DATE**

EXP

9. SPECIAL STORAGE CONDITIONS

Keep the vial in the outer carton in order to protect from light.

10. SPECIAL PRECAUTIONS FOR DISPOSAL OF UNUSED MEDICINAL PRODUCTS OR WASTE MATERIALS DERIVED FROM SUCH MEDICINAL PRODUCTS, IF APPROPRIATE

Single use vial – discard any content remaining after first use.

11. NAME AND ADDRESS OF THE MARKETING AUTHORISATION HOLDER

medac GmbH

12. MARKETING AUTHORISATION NUMBER(S)**13. BATCH NUMBER**

Batch

14. GENERAL CLASSIFICATION FOR SUPPLY

Medicinal product subject to medical prescription.

15. INSTRUCTIONS ON USE**16. INFORMATION IN BRAILLE**

B. PACKAGE LEAFLET

Package leaflet: Information for the user**Gliolan 30 mg/ml powder for oral solution**
5-aminolevulinic acid hydrochloride

Read all of this leaflet carefully before you start taking this medicine because it contains important information for you.

- Keep this leaflet. You may need to read it again.
- If you have any further questions, ask your doctor or pharmacist.
- If you get any side effects, talk to your doctor or pharmacist. This includes any possible side effects not listed in this leaflet. See section 4.

What is in this leaflet

1. What Gliolan is and what it is used for
2. What you need to know before you take Gliolan
3. How to take Gliolan
4. Possible side effects
5. How to store Gliolan
6. Contents of the pack and other information

1. What Gliolan is and what it is used for

Gliolan is used for the visualisation of certain brain tumours (called malignant glioma) during tumour surgery.

Gliolan contains a substance called aminolevulinic acid (5-ALA). 5-ALA accumulates preferably in tumour cells where it is transformed into another similar substance. If the tumour is then exposed to blue light, this new substance emits a red-violet light which helps to better see what is normal tissue and what is tumour tissue. This helps the surgeon to remove the tumour while sparing healthy tissue.

2. What you need to know before you take Gliolan**Do not take Gliolan**

- if you are allergic to 5-ALA or porphyrins.
- in case of known or suspected acute or chronic types of porphyria (i.e. inherited or acquired disorders of certain enzymes in the synthesis pathway of red blood pigment).
- in case of known or suspected pregnancy.

Warnings and precautions

Talk to your doctor or pharmacist before taking Gliolan.

- For 24 hours after administration of this medicine, **protect your eyes and skin from strong light** (for example direct sunlight or brightly focused indoor light).
- If you have a **heart disease** or had heart disease in the past, you should tell your doctor. In this case, this medicine should be used with caution because your blood pressure may be decreased.

Patients with renal or hepatic impairment

No trials have been performed in patients with poor liver or kidney function. Therefore, this medicine should be used with caution in such patients.

Elderly patients

There are no special instructions for use in elderly patients with normal organ function.

Children and adolescents (< 18 years)

There is no experience with Gliolan in children and adolescents. Therefore this medicine is not recommended in this age group.

Other medicines and Gliolan

Tell your doctor or pharmacist if you are taking, have recently taken or might take any other medicines, particularly medicines that may cause skin problems when the skin comes under strong light (for example some types of medicines called antibiotics), but also medicines obtained without prescription (for example hypericin or Saint John's wort extracts). One case of severe sunburn lasting for 5 days has been reported in a patient after having taken this medicine and a hypericin extract. You should not take any such products up to 2 weeks after you have taken Gliolan.

Within 24 hours after having taken Gliolan, avoid any other medicines that may harm the liver.

Gliolan with food and drink

This medicine is generally used once only, namely 2-4 hours before anaesthesia for surgery for certain brain tumours called glioma. You should not drink or eat for at least 6 hours before anaesthesia.

Pregnancy and breast-feeding*Pregnancy*

It is not known whether Gliolan will harm an unborn baby. Do not use this medicine if you are pregnant.

Breast-feeding

It is not known whether this medicine enters breast milk. Breast-feeding mothers should not breast-feed for 24 hours after treatment with this medicine.

Driving and using machines

This medicine itself has no influence on the ability to drive and use machines.

3. How to take Gliolan

This medicine is a powder that must be first mixed with drinking water before use. This is always done by a pharmacist or a nurse and not by yourself. The usual dose is 20 mg 5-ALA HCl per kilogram body weight. The pharmacist or nurse will calculate the exact dose you need. You have to drink the prepared solution 2-4 hours before anaesthesia.

If the anaesthesia/surgery is delayed by some hours, additional doses of this medicine must not be given. If the surgery is delayed by one or more days, another dose of this medicine can be taken 2 – 4 hours before anaesthesia.

If you take more Gliolan than you should

If you have taken more Gliolan than you should, your doctor will decide on any necessary measures to avoid any problems, including sufficient protection from strong light (for example direct sunlight).

If you forget to take Gliolan

This medicine is given once only at the day of surgery, 2 – 4 hours before start of anaesthesia. If you have forgotten to take this medicine during this time period, it is not advisable to take it just before start of anaesthesia. In this case, anaesthesia and surgery must be postponed for at least 2 hours, if possible.

If you have any further questions on the use of this medicine, ask your doctor or pharmacist.

4. Possible side effects

Like all medicines, this medicine can cause side effects, although not everybody gets them.

Most serious side effects include mild alterations of blood cell counts (red and white cells, platelets), neurological disorders (disorders that affect the nervous system like hemiparesis [partial paralysis of one side of the body] and thromboembolism (blood clots that may obstruct blood vessels). Further frequently observed side effects are vomiting (sickness), nausea (feeling sick) and slight increase of some enzymes (transaminases, γ -GT, amylase) or bilirubin (a bile pigment produced in the liver by breakdown of red blood pigment) in the blood.

Tell your doctor immediately if you experience any complaints.

Side effects are divided into the following two categories:

- immediate side effects after having taken Gliolan and before anaesthesia
- combined side effects of Gliolan, anaesthesia, and tumour resection.

After having taken Gliolan and before start of anaesthesia, the following side effects may occur:

Uncommon side effects (may affect up to 1 in 100 people):

Nausea (feeling sick), decrease of blood pressure, skin reactions (for example rash, looking like sunburn).

In combination with anaesthesia and tumour resection further side effects may occur:

Very common side effects (may affect more than 1 in 10 people):

Mild alterations of blood cell counts (red and white cells, platelets), and slight increase of some enzymes (transaminases, γ -GT, amylase) or bilirubin (a bile pigment produced in the liver by breakdown of red blood pigment) in the blood. These changes peak between 7 and 14 days after surgery. The changes will completely resolve within a few weeks. Usually you will not experience any symptoms when these changes occur.

Common side effects (may affect up to 1 in 10 people):

Nausea (feeling sick), vomiting (sickness), neurological disorders (disorders that affect the nervous system like hemiparesis [partial paralysis of one side of the body], aphasia [total or partial loss of ability to use or understand language], convulsions [seizures] and hemianopsia [blindness for half the field of vision in one or both eyes]), and thromboembolism (blood clots that may obstruct blood vessels).

Uncommon side effects (may affect up to 1 in 100 people):

Decrease of blood pressure, brain oedema (brain swelling).

Very rare side effects (may affect up to 1 in 10,000 people) or not known (frequency cannot be estimated from the available data):

Hypaesthesia (decrease of your sense of touch), and diarrhoea (loose or watery stools).

Reporting of side effects

If you get any side effects, talk to your doctor or pharmacist. This includes any possible side effects not listed in this leaflet. You can also report side effects directly via [the national reporting system](#) listed in [Appendix V](#). By reporting side effects you can help provide more information on the safety of this medicine.

5. How to store Gliolan

Keep this medicine out of the sight and reach of children.

Do not use this medicine after the expiry date which is stated on the carton. The expiry date refers to the last day of that month.

Keep the vial in the outer carton in order to protect from light.

The reconstituted solution is physically-chemically stable for 24 hours at 25 °C.

Do not throw away any medicines via wastewater or household waste. Ask your pharmacist how to throw away medicines you no longer use. These measures will help protect the environment.

6. Contents of the pack and other information

What Gliolan contains

The active substance is 5-aminolevulinic acid hydrochloride (5-ALA HCl). One vial contains 1.17 g of 5-aminolevulinic acid (5-ALA), corresponding to 1.5 g 5-ALA HCl.

One ml of reconstituted solution contains 23.4 mg of 5-ALA, corresponding to 30 mg 5-ALA HCl.

What Gliolan looks like and contents of the pack

This medicine is a powder for oral solution. The powder is a white to off-white cake. The reconstituted solution is a clear and colourless to slightly yellowish fluid.

Gliolan is provided in a glass vial and presented in packs of 1, 2 and 10 vials. Not all pack sizes may be marketed.

Marketing Authorisation Holder

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Gesellschaft für klinische
Spezialpräparate mbH
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22880 Wedel
Germany
Tel. +49 4103 8006-0
Fax: +49 4103 8006-100

Manufacturer

medac
Gesellschaft für klinische
Spezialpräparate mbH
Theaterstr. 6
22880 Wedel
Germany

This leaflet was last revised in

Detailed information on this medicine is available on the European Medicines Agency web site:

<http://www.ema.europa.eu>.

ANNEX

**CONDITIONS OR RESTRICTIONS WITH REGARD TO THE SAFE AND EFFECTIVE USE
OF THE MEDICINAL PRODUCT TO BE IMPLEMENTED BY THE MEMBER STATES**

CONDITIONS OR RESTRICTIONS WITH REGARD TO THE SAFE AND EFFECTIVE USE OF THE MEDICINAL PRODUCT TO BE IMPLEMENTED BY THE MEMBER STATES

The Member States should ensure that all conditions or restrictions with regard to the safe and effective use of the medicinal product described below are implemented:

Gliolan should be used only by neurosurgeons who have attended a training course in accordance with the standards detailed below:

The Member States shall agree with the Marketing Authorisation Holder, prior to launch, measures for the implementation of the following:

- A training course for neurosurgeons which is aimed at risk minimisation and to support safe and effective use for the medicinal product. The training course will take place at qualified training centres using qualified trainers. This course shall consist of measures aiming to minimise adverse events associated with the Gliolan-fluorescence-guided surgery (in particular neurological serious adverse events) through adequate education about:
 - a) Theory and core principles of Gliolan-fluorescence-guided surgery and malignant glioma resection, including methods of eloquent sites identification;
 - b) On-site instructions on the use of the fluorescence-microscope, including pitfalls and recognition of problems;
 - c) Differentiation of fluorescence intensity, maintaining safety distances from eloquent areas;
 - d) The practice of Gliolan-fluorescence-guided surgery (including participation in at least one case using Gliolan-fluorescence-guided surgery in the operating room with on-site instructions on the use of the microscope or demonstration of a fluorescence-guided resection by video);
 - e) The current understanding of the benefits and risks of cytoreductive surgery in the management of patients with malignant gliomas;
 - f) The theoretical base for porphyrin accumulation in malignant gliomas;
 - g) The technical principles behind fluorescence-guided resections using Gliolan;
 - h) How to identify suitable candidates for fluorescence-guided resections using Gliolan;
 - i) How to apply Gliolan in the correct dose and timing regimen, and to understand the importance of concurrent corticosteroids;
 - j) How to identify patients at risk for neurological deficits using fluorescence-guided resections with Gliolan with special focus on aphasias and other critical focal deficits;
 - k) Techniques for intraoperative risk reduction (microsurgical technique, neurophysiological monitoring, choice of approach) and how to implement them;
 - l) How to identify fluorescence for resection through using the operating microscope in a hands-on setting in the operating room;
 - m) The benefits and risks of fluorescence-guided resections using Gliolan.

Minimum requirements for a qualified trainer are:

- Board-certification as neurosurgeon according to local, national requirements;
- Previous successful participation at a training course, or equivalent course during the phase III trial;
- Experience with Gliolan-fluorescence-guided surgery in at least 20 cases.

Minimum requirements for a qualified training centre are:

- Microscope modified for fluorescence-guided resection;
- Sufficient case load (at least 10 patients per year) of malignant gliomas (WHO grade III and IV);
- Neurophysiological monitoring techniques for surgery in eloquent brain regions.

Appendix 5 End-of-trial documentation

Declaration of the End of Trial Form (cf. Section 4.2.1 of the *Detailed guidance on the request to the competent authorities for authorisation of a clinical trial on a medicinal product for human use, the notification of substantial amendments and the declaration of the end of the trial*¹)

NOTIFICATION OF THE END OF A CLINICAL TRIAL OF A MEDICINE FOR HUMAN USE TO THE COMPETENT AUTHORITY AND THE ETHICS COMMITTEE

For official use

Date of receipt :	Competent authority registration number: MREC ref 13/LO/0214 Ethics committee registration number: ISRCTN79949827
-------------------	--

To be filled in by the applicant

A MEMBER STATE IN WHICH THE DECLARATION IS BEING MADE :

B TRIAL IDENTIFICATION

B.1 EudraCT number: 2012-002623-15
B.2 Sponsor's protocol code number: University of Leeds (GS11/9681)
B.3 Full title of the trial: Next Generation intraoperative Lymph node staging for Stratified colon cancer surgery

C APPLICANT IDENTIFICATION (please tick the appropriate box)

C.1 DECLARATION FOR THE COMPETENT AUTHORITY	<input type="checkbox"/>
C.1.1 Sponsor	<input type="checkbox"/>
C.1.2 Legal representative of the sponsor	<input type="checkbox"/>
C.1.3 Person or organisation authorised by the sponsor to make the application.	<input checked="" type="checkbox"/>
C.1.4 Complete below:	
C.1.4.1 Organisation: St James's University Hospital	
C.1.4.2 Name of person to contact: Miss Helen Andrew	
C.1.4.3 Address: Rm 7.2 Clinical Sciences Building, St James's University Hospital, LS9 7TF	
C.1.4.4 Telephone number: +44 (0)7966087652	
C.1.4.5 Fax number:	
C.1.4.6 E-mail: h.andrew@leeds.ac.uk	

C.2 DECLARATION FOR THE ETHICS COMMITTEE	<input type="checkbox"/>
C.2.1 Sponsor	<input type="checkbox"/>
C.2.2 Legal representative of the sponsor	<input type="checkbox"/>
C.2.3 Person or organisation authorised by the sponsor to make the application.	<input checked="" type="checkbox"/>
C.2.4 Investigator in charge of the application if applicable ² :	
• Co-ordinating investigator (for multicentre trial):	<input type="checkbox"/>
• Principal investigator (for single centre trial):	<input type="checkbox"/>
C.2.5 Complete below:	
C.2.5.1 Organisation: St James's University Hospital	
C.2.5.2 Name: Miss Helen Andrew	
C.2.5.3 Address: Rm 7.2 Clinical Sciences Building, St James's University Hospital, LS9 7TF	
C.2.5.4 Telephone number: [REDACTED]	
C.2.5.5 Fax number:	
C.2.5.6 E-mail: h.andrew@leeds.ac.uk	

D END OF TRIAL

D.1 Date of the end of the complete trial in all countries concerned by the trial?
D.1.1 (2015/08/07):
D.2 Is it an early termination?³ yes <input type="checkbox"/> no <input checked="" type="checkbox"/>

¹ OJ, C82, 30.3.2010, p. 1; hereinafter referred to as 'detailed guidance CT-1'.

² According to national legislation.

³ Cf. Section 4.2. of the detailed guidance CT-1.

D.2.1 If yes, give date (YYYY/MM/DD):
 D.2.2 Briefly describe in an annex (free text):
 D.2.2.1 The justification for early termination of the trial;
 D.2.2.2 Number of patients still receiving treatment at time of early termination in the MS concerned by the declaration and their proposed management;
 D.2.2.3 The consequences of early termination for the evaluation of the results and for overall risk benefit assessment of the investigational medicinal product.

E SIGNATURE OF THE APPLICANT IN THE MEMBER STATE




E.1 I hereby confirm that/confirm on behalf of the sponsor that (delete which is not applicable):

- The above information given on this declaration is correct; and
- That the clinical trial summary report will be submitted within the applicable deadlines in accordance with the applicable guidance by the Commission.⁴

E.2 APPLICANT TO THE COMPETENT AUTHORITY (as stated in C.1)

E.2.1 
 E.2.2 
 E.2.3 

E.3 APPLICANT TO THE ETHICS COMMITTEE (as stated in C.2) :

E.3.1 
 E.3.2 
 E.3.3 

⁴ Section 4.3. of the detailed guidance CT-1.

A decorative graphic consisting of numerous thin, parallel green lines that curve from the left side of the page towards the right, creating a sense of movement and depth.

**EME
HS&DR
HTA
PGfAR
PHR**

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