# 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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**Declared competing interests of authors:** David Jayne is a member of the Medical Research Council/ Efficacy and Mechanism Evaluation (EME) Strategy Group. This group has responsibility for setting EME programme funding policy. This position does not have an impact on the performance or results of this study. Nicholas West received grants outside this submitted work from Yorkshire Cancer Research, The Pathological Society of Great Britain and Ireland as well as the Academy of Medical Sciences in the last 3 years. Philip Quirke also received grants outside this submitted work from Yorkshire Cancer Research during the 36 months prior to submission of this report.

Published August 2016
DOI: 10.3310/eme03060

## Scientific summary

### GLiSten

Efficacy and Mechanism Evaluation 2016; Vol. 3: No. 6
DOI: 10.3310/eme03060

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# 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 ≈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/m$^2$ (range 19.1–37.8 kg/m$^2$) 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.

# Efficacy and Mechanism Evaluation

ISSN 2050-4365 (Print)

ISSN 2050-4373 (Online)

This journal is a member of and subscribes to the principles of the Committee on Publication Ethics (COPE) (www.publicationethics.org/).

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### This report

The research reported in this issue of the journal was funded by the EME programme as project number 11/100/24. The contractual start date was in October 2013. The final report began editorial review in December 2015 and was accepted for publication in May 2016. The authors have been wholly responsible for all data collection, analysis and interpretation, and for writing up their work. The EME editors and production house have tried to ensure the accuracy of the authors' report and would like to thank the reviewers for their constructive comments on the final report document. However, they do not accept liability for damages or losses arising from material published in this report.

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