Title: Feasibility of fluorescence lymph node imaging in colon cancer: FLICC

Short Title: FLICC Trial

Mr. Manish Chand, Department of Surgery and Interventional Sciences, University College London Hospitals, NHS Foundation Trusts, GENIE Centre, University College London, London, UK

Dr. Deborah S Keller, Department of Surgery and Interventional Sciences, University College London Hospitals, NHS Foundation Trusts, GENIE Centre, University College London, London, UK

Mr. Heman M Joshi, Department of Surgery and Interventional Sciences, University College London Hospitals, NHS Foundation Trusts, London, UK

Mr. Laurence Devoto, Department of Surgery and Interventional Sciences, University College London Hospitals, NHS Foundation Trusts, GENIE Centre, University College London, London, UK

Dr. Manuel Rodriguez-Justo, Department of Pathology, University College London Hospitals, NHS Foundation Trusts, London, UK

Mr. Richard Cohen, Department of Surgery and Interventional Sciences, University College London Hospitals, NHS Foundation Trusts, GENIE Centre, London, UK

Abstract count: 250/250

Word Count: 2551

Correspondence and Reprints:
Mr. Manish Chand, MBA FRCS PhD
Consultant Colorectal Surgeon and Senior Lecturer
Department of Surgery and Interventional Sciences, University College London Hospitals, NHS Trusts, GENIE Centre, University College London
235 Euston Rd, London, United Kingdom, NW1 2BU
Tel: 020 3447 5879
Fax: 020 3447 9218
m.chand@ucl.ac.uk

Abstract

Background: In colon cancer, appropriate tumour excision and associated lymphadenectomy directly impact recurrence and survival outcomes. Currently, there is no standard for mesenteric lymphadenectomy, with a lymph node yield of 12 acting as a surrogate quality marker. Our goal was to determine the safety and feasibility of Indocyanine green (ICG) fluorescence imaging to demonstrate lymphatic drainage in colon cancer in a dose-escalation study.

*Methods***:** A prospective pilot study of colon cancer patients undergoing curative laparoscopic resection was performed. At surgery, peritumoural subserosal ICG injection was done to demonstrate lymphatic drainage of the tumour. A specialized fluorescence system excited the ICG and assessed lymphatics in real-time. The primary outcome was the feasibility of ICG fluorescent lymphangiography for lymphatic drainage in colon cancer. Secondary outcomes were the optimal protocol for dose, injection site, and ICG lymphatic mapping timing.

Results: Ten consecutive patients were evaluated (6 males, mean age 69.5 years). In all, lymphatic channels were seen around the tumour to a varying extent. Eight (80%) had drainage to the sentinel node. In all cases where the lymphatic map was seen, there was no further spread 10 minutes after injection. Factors contributing to reduced lymphatic visualisation were inadequate ICG concentrations, excess India ink blocking drainage, and inflammation from tattoo placement.

*Conclusions***:** ICG can be safely injected into the peritumoural subserosa and demonstrate lymphatic drainage in colon cancer. This proof of concept and proposed standards for the procedure can lead to future studies to optimize the application of image-guided precision surgery in colon cancer.

Keywords

Immunofluorescence; Indocyanine green (ICG); Fluorescence lymphangiography; Fluorescence imaging; Colon cancer; Complete mesocolic excision (CME)

Introduction

Surgical management of colon cancer must address the risk of both local and distant disease recurrence. Currently, there is no standard of care for mesenteric lymphadenectomy, which directly influences the risk of distant failure and adjuvant treatment decisions. Lymph node yield acts as a surrogate marker for appropriate oncological surgery and procuring a minimum of 12 nodes is recommended by several international guidelines [1,2]. The number of nodes retrieved from the surgical specimen improves accuracy of prognosis and influences survival outcomes in patients with colon cancer [3–5].

Complete mesocolic excision (CME) has been advocated to increase the nodal yield and consequently improve survival outcomes [3–5]. However this approach is controversial and while the potential to improve cancer outcomes is stimulating, there is potential serious morbidity associated with the radical mesocolic excision, and insufficient consistent, high quality evidence to date to recommend widespread adoption of CME or resecting more nodes, in general [6–8]. Performing a more precise lymphadenectomy and resection may be a better option for overall patient outcomes.

Fluorescence imaging (FI) is a tool that that could be used to guide lymphatic mapping and a more precise, oncologic resection. Pairing peritumoural injection of ICG with FI lymphangiography could highlight drainage from the primary tumour within the mesentery, demonstrating the precise margins and involved nodes. The purpose of this pilot study was to determine the safety and feasibility of indocyanine green (ICG) fluorescence imaging for intraoperative localization of the primary tumour, lymphatic drainage and sentinel node mapping in colon cancer. Our hypothesis was that ICG

fluorescence imaging would be safe, feasible, and successfully demonstrate the intraoperative localization of the primary tumour, its lymphatic drainage, and sentinel node map in colon cancer.

Materials and methods

Study design and patient population

This dose-escalation study received approval from the Joint Research Office, University College London Hospital. For this pilot study, 10 consecutive patients with colon cancer were prospectively identified from the colorectal cancer multidisciplinary team (MDT) conference at a single tertiary referral. Patients were eligible for enrollment if they over 18 years of age; diagnosed with primary colon cancer proximal to the rectosigmoid junction (confirmed by lower endoscopy and computed tomography (CT) imaging); able undergo routine staging with intravenous contract enhanced CT scan and tolerate mechanical bowel preparation for surgery; and scheduled for curative surgical resection of the cancer. Patients were excluded if, under 18, or presenting with a bowel obstruction; undergoing an emergent/ urgent resection or surgery for palliative intent; if they had distant metastases; an allergy or history of adverse reaction to ICG, iodine or iodine dyes; hepatic dysfunction with a MELD Score > 10; or renal dysfunction with a Creatinine level over 2.0mg/dL. All patients had a preoperative work-up per standard of care, including a colonoscopy with tissue biopsy confirming adenocarcinoma of the colon, staging for distant metastatic disease with a CT scan of the chest, abdomen and pelvis, and presentation at the multidisciplinary team (MDT) meeting to guide management.

Technique

Tumours were marked with India ink at the time of the index colonoscopy to assist intraoperative identification. Surgical resection was performed within 4 weeks of diagnosis. All cases were performed by a single colorectal surgeon through a multiport laparoscopic approach. The laparoscopic procedure was carried out as per routine. Following ligation of the main vascular pedicle (the ileocolic artery or inferior mesenteric/ascending left colic artery) and mobilization of the colon, the specimen was extracorporalized through a small midline incision using a wound retractor. Intraoperative ICG injection was then administered as the study intervention. A 1mL subserosal injection of ICG was placed in 4 sites around the tumour (4mL in total) (Figure 1). Great care was taken not to spill ICG during this process, as it would ruin the surgical field when it was made to fluoresce. An alcohol swab was used to wipe the surface of the bowel following each injection. The concentration of ICG was varied from 5mg/10mL, 5mg/5mL, and 5mg/3mL. Using commercial laparoscopic fluorescence camera systems (Pinpoint™, Novadaq, Mississauga, Ontario, Canada and AIM 1588™, Stryker, Kalamazoo, Michigan, USA), the ICG was excited by light in the near infrared (NIR) spectrum, for image comparison in standard white light and NIR, and real-time visualisation of the lymphatic drainage. The main outcome measure was the feasibility of ICG to identify lymph node drainage. Secondary outcomes included defining the optimal protocol for dose, injection site, and timing for ICG lymphatic mapping.

Patient and tumour demographics, operative, and lymphangiographic procedural details were assessed. Data points evaluated included the age, gender, preoperative staging,

procedure performed, level of anastomosis, if the tumour was localised with ICG, if the ICG localization was consistent with the preoperative imaging, if there was visualisation of the lymphatic drainage, the time to central lymph node visualisation, the presence of lymph nodes outside the proposed mesenteric resection, the pathologic stage, the number of lymph nodes procured, the number of positive lymph nodes, and the presence of positive resection margins. Descriptive statistics were used to describe the outcomes, including means (with standard deviation), medians (with range), and values (with percentage).

RESULTS

Ten consecutive patients with a diagnosis of colon cancer undergoing curative resection between March 2017 and June 2017 at University College London Hospitals were enrolled in the study and evaluated. The mean patient age was 69.5 years (SD ±7.13). There were 6 males and 4 females. The tumour distribution in the colon was 7 (70%) ascending, 2 (20%) sigmoid, and 1 (10%) descending. All patients had biopsy-proven adenocarcinoma, of which 70% were preoperatively staged as T3 (30% were T1 or T2) and 10% as N1/N2 (the remainder were N0). All patients had a preoperative computed tomography (CT) scan for staging. No patient had CT-demonstrable distant metastases, and none received neoadjuvant chemotherapy. The demographic data and tumour stage is presented in Table 1.

Procedural details are listed in Table 2. The procedures performed were a right hemicolectomy (n=7, 70%), a sigmoid colectomy (n=2, 20%) and a left hemicolectomy

(n=1,10%). All procedures were performed laparoscopically as curative, restorative resections, with 7 ileocolic anastomoses and 3 colo-colonic anastomoses. There were no intraoperative conversions to open laparotomy.

The ICG was injected into the subserosal layer after extracorporealisation, as described in the methods. The optimal concentration of ICG was 5mg/10mL. The primary tumour was localised in all patients within 60 seconds (Figure 2). The ICG localization was consistent with the preoperative imaging in 8 of 10 patients (80%). Lymphatic drainage was variably demonstrated in all 10 patients (100%) (Figure 3). In 7 of 10 (70%), there was complete drainage visible - a vibrant and clearly visible map from the tumor across the mesenteric lymphatics to the nodes. In the remaining 3 (30%) patients, there was partial demonstration- an incomplete map of the mesenteric lymphatics between the tumor and the nodes of lymphatic drainage, which was not sufficient to guide a lymphadenectomy. Eight patients (80%) had ICG drainage to the sentinel node demonstrated. The mean time to central lymph node visualisation was 4.32 minutes (SD±1.45). The optimal timing to demonstrate lymphatic drainage was 10 minutes, after which no further lymphatics were visualised. In 2 patients (20%), additional or aberrant lymph nodes located outside of the proposed resection margins were demonstrated. These nodes would not have been visualised without ICG FI; here, the ICG was preferentially held in these aberrant nodes and showed significant central intensity of signal. Furthermore, these nodes would have been outside the standard resection margins one would expect to follow for each case and not included in the planned resection or routine lymphadenectomy. In one patient the node was located in the mesentery adjacent to the Hemolok clip on the ileocolic artery.

This node was not readily palpable but clearly visible on all 3 modes of imaging. In this case the mesenteric resection was extended to safely include the node and there was no need to perform a more proximal ligation of the vessel. As the mesenteric lymphadenectomy had not been completed it was possible to make this change without needing to perform a more complex dissection. In the second case, the visible node was found to be outside the planned resection margin but was safely included in the resection by taking additional mesenteric tissue at this margin. In both cases, there was no need to perform a more radical dissection(Figure 4). In both patients, these nodes were positive on histopathology.

Overall, the median lymph node yield was 22 (range, 14-49 nodes), and the median positive lymph nodes were 2 (range, 0-8) nodes. Final pathological staging was 30% Stage II (n=3), 30% Stage III (n=3), and 40% Stage IV (n=4). There were no positive resection margins. No intraoperative or injection-related adverse effects occurred with 30-day follow-up. Procedural and pathologic details are listed in Table 3.

Factors noted that may have contributed to lack of lymphatic identification were inadequate concentrations of ICG, excess India ink (from the endoscopic tattoo) blocking lymphatic drainage, and inflammation around the tumour from tattoo placement. No intraoperative or injection-related adverse effects occurred with 30-day follow-up.

DISCUSSION

The results of the present study show that FI using ICG can variably demonstrate draining lymphatic vessels in colon cancer. Factors including tumour reaction and inflammation, excess India ink used to localise the tumour and inadequate ICG concentration may contribute to suboptimal visualisation. This novel approach to demonstrate tumour lymphatics has the potential to guide mesenteric lymphadenectomy in colon cancer.

Currently, there is no standard of care for resection of colon cancer, with the bone of contention being the extent of the mesenteric lymphadenectomy. CME attempts to translate the concept of the intact 'package' of the tumour and its main lymphatic drainage proposed by Heald into total mesorectal excision (TME) for optimal rectal cancer surgery [9,10]. With the increased mesocolon and number of nodes resected, proponents of the technique claim improved oncologic outcomes compared with conventional colon resection [3]. However, there is additional morbidity associated with CME, and no conclusive evidence for its oncologic benefit [6–8].

Performing a more precise, patient-specific lymphadenectomy and tumour resection is an alternative to CME. Lymphatic mapping allows a focused review of the lymph nodes, helping to identify those most likely to harbour metastases, which can improve the staging and stratification for adjuvant treatment [11]. ICG FI has the potential to guide resection by identifying the tumour, lymphatic map, and involved nodes in real time, possibly allowing the surgeon to adjust the resection margins and ensure all oncologically

relevant tissue is removed with precise mesocolic margins, instead of performing a radical CME. Intraoperative lymphangiography could also help establish the lymphatic drainage for tumours with a variable course or in cases of reoperation where lymphatic-bearing tissue has been excised [12,13].

ICG has many different uses in gastrointestinal surgery [14], and specifically, has been shown to effectively demonstrate perfusion within the bowel when injected intravenously [15–19]. Fluorescence lymphangiography has been previously described for sentinel lymph node detection in other cancers. The technique has been proven successful in breast, melanoma, penile, vulvar, oesophageal, gastric, and oral squamous cell cancers [20–27]. There only limited data available on the use of FI to demonstrate sentinel nodes in colorectal cancer [11,28–30]. The published studies found value for identifying sentinel and aberrant mesocolic lymph nodes; however, no standardized technique has been described and variable rates of accuracy, sensitivity, and specificity are reported. In our study, 80% of patients had drainage to the sentinel node demonstrated, with a mean time to central lymph node visualisation of 4.32 minutes. This rate of identification was similar to published results of 89% to 98% detection, with a false negative rate of 18-67% [31]. A recent systematic review of 12 studies including 248 patients reported pooled sensitivity and specificity rates were 71% and 84.6%, with median sensitivity, specificity, and accuracy rates of 73.7, 100, and 75.7% [32]. However the review included both colon and rectal cancer, and demonstrated the heterogeneity in reported data. The authors concluded that ICG is a promising technique for detecting sentinel nodes in colorectal cancer although its oncological utility remains unknown.

While the prognostic value of the sentinel node in colon cancer is still under debate, the lymphatic drainage may be a better marker for optimal resection, as this can help determine the ideal mesocolic resection margin, as well as change the operative course and recommendations for adjuvant therapy postoperatively. In the present study, we had visualisation of the lymphatic drainage in all patients. Earlier studies also described the safety, feasibility, and high success rate of ICG FI in this application [30,33,34]**.** In our sample, the lymphatic drainage was partially demonstrated in 3 patients, insufficient to guide the lymphadenectomy. From this, we established what variables likely impacted the success of the lymphatic mapping, including inadequate concentrations of ICG, excess India ink blocking lymphatic drainage, and inflammation around the tumour from tattoo placement. To our knowledge, no study to date has described factors likely to block lymphatic uptake. One study by van der Pas et. al., described factors which may contribute to false-negative findings, including large tumour size, drainage to adjacent lymphatic vessels, and the use of a rigid needle [29]. These pearls may help others through roadblocks in fluorescence lymphangiography.

A further benefit of real-time intraoperative ICG is identification of aberrant lymph nodes. In the present study, 2 patients had aberrant notes identified in NIR mode, and we changed the course of our mesocolic resection to include these nodes. In both cases, these nodes were positive on pathologic assessment; without the ICG lymphangiography, these positive nodes would not have been included in the standard resection as they were not

immediately palpable, which could have impacted the oncologic outcomes of the patients. ICG lympangiography helps provide a real-time anatomical model for the lymphatics which may lead to more precise oncological resections. Prior work has supported the value of ICG FI to identify aberrant nodes [28,30,35–37]. In a series of 18 patients, Cahill et. al. found 4 patients had fluorescing sentinel nodes outside the planned resection field [34]. In 21 patients undergoing laparoscopic resection for colorectal cancer, Nishigori et. al. reported ICG FI lymph node mapping led to 23.5% of patients requiring modifications in the extent of lymphadenectomy, and 16.7 % required a change in the intestinal resection plan [30]. Thus, the improved visualisation of involved lymph nodes in real-time afforded by the ICG FI compared to white light could improve the oncologic resection and outcomes.

This work adds unique aspects to the literature in that the technique is detailed to standardize the protocol for future work, including the ideal site for injection, dosage, and concentration. In this study, we had successful identification of the primary tumour from peritumoural injection into the subserosal space using 1mL of ICG solution (5mg/10mL) in all patients. The ICG localization was consistent with pre-operative imaging in 8 of our 10 patients, showing potential value for localization, especially in laparoscopic cases, where there is no tactile sense. Published work has wide variations in the ICG concentrations (0.5, 2.5, 5 mg/mL), dosage (0.2-5 mL), site of ICG injection (submucosal, subserosal, both submucosal and subserosal, or intravenous) and the timing of the injection (preoperative, intraoperative, or both pre- and intraoperative) [22,26,29,30,33,34,38–41]. We have previously reported our technique of subserosal

injection to visualize lymphatic drainage in a colon cancer patient [12]. The choice of the subserosal injection site was supported by van der Pas et. al., who found it was more effective, especially with a flexible tip needle. Watanabe et. al. used a submucosal injection site, which was associated with lower rates of tumour identification [42]. The authors described 93.8% visibility of the tumour intraoperatively after a submucosal injection of 0.5 mL of ICG (2.5 mg/mL) during colonoscopy, with significantly better visibility after an interval of < 7 days between injection and surgery than after ≥10 days [42]. In a previously published work we injected the Establishing best practice can help reduce variability in future work.

We recognize some limitations of this study. The study design was a pilot for safety and feasibility. Thus, a small sample was used with no comparison group. In addition, a single surgeon at a single centre performed all cases, which could limit the generalizability of the results. However, demonstrating the ideal protocol, safety and feasibility, as well as potential benefits supports further work on ICG lymphatic mapping for defining the resection margins and its impact on pathological staging.

Conclusions

Our results indicate that ICG fluorescence lymphangiography is safe and feasible for identifying the primary tumour, its lymphatic drainage, and potentially malignant nodes in colon cancer. Most importantly, ICG fluorescent lymphangiography had benefit in identifying additional and aberrant positive lymph nodes outside of the standard resection

margins, which changed our operative plan. There is ongoing work looking at the utility of ICG to highlight malignant nodes, as this could have great implications for oncologic outcomes and guiding the surgical course. Further studies are needed to determine the value of this technique for more precise, oncologic resections in colon cancer.

Conflict of interest: Mr. Chand reports speaking fees for Novadaq, Inc. outside of the scope of this work

Dr Keller, Dr Joshi, Dr Devoto, Dr Rodriguez-Justo and Mr Cohen declare that they have no conflict of interest

REFERENCES

1. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Rectal Cancer, Version 3.2017. Accessed October 2017. Available online at: https://www.nccn.org/professionals/physician_gls/PDF/rectal.pdf
2. National Institute for Health and Care Excellence (NICE); Colorectal cancer: diagnosis and management. Clinical guideline [CG131] December 2014. Accessed October 2017. Available online at: https://www.nice.org.uk/guidance/cg131
3. Hohenberger W, Weber K, Matzel K, Papadopoulos T, Merkel S (2009) Standardized surgery for colonic cancer: complete mesocolic excision and central ligation--technical notes and outcome. Colorectal Dis 11: 354-64; discussion 364.
4. West NP, Hohenberger W, Weber K, Perrakis A, Finan PJ, Quirke P (2010) Complete mesocolic excision with central vascular ligation produces an oncologically superior specimen compared with standard surgery for carcinoma of the colon. J Clin Oncol 28: 272-278.
5. Zheng MH, Zhang S, Feng B (2016) Complete mesocolic excision: Lessons from anatomy translating to better oncologic outcome. World J Gastrointest Oncol 8: 235-239.
6. Bertelsen CA, Neuenschwander AU, Jansen JE, et al. (2016) Short-term outcomes after complete mesocolic excision compared with 'conventional' colonic cancer surgery. Br J Surg 103: 581-589.
7. Emmanuel A, Haji A (2016) Complete mesocolic excision and extended (D3) lymphadenectomy for colonic cancer: is it worth that extra effort? A review of the literature. Int J Colorectal Dis 31: 797-804.
8. Bertelsen CA, Kirkegaard-Klitbo A, Nielsen M, Leotta SM, Daisuke F, Gögenur I (2016) Pattern of Colon Cancer Lymph Node Metastases in Patients Undergoing Central Mesocolic Lymph Node Excision: A Systematic Review. Dis Colon Rectum 59: 1209-1221.
9. Heald RJ (1988) The 'Holy Plane' of rectal surgery. J R Soc Med 81: 503-508.
10. Heald RJ, Moran BJ, Ryall RD, Sexton R, MacFarlane JK (1998) Rectal cancer: the Basingstoke experience of total mesorectal excision, 1978-1997. Arch Surg 133: 894-899.
11. Tuech JJ, Pessaux P, Regenet N, Bergamaschi R, Colson A (2004) Sentinel lymph node mapping in colon cancer. Surg Endosc 18: 1721-1729.
12. Keller DS, Joshi HM, Rodriguez-Justo M, Walsh D, Coffey JC, Chand M (2017) Using fluorescence lymphangiography to define the ileocolic mesentery: proof of concept for the watershed area using real-time imaging. Tech Coloproctol 21: 757-760.
13. Joshi HM, Keller DS, Chand M (2017) Utilization of Indocyanine green to demonstrate lymphatic mapping in colon cancer. J Surg Oncol 116: 1005-1007.
14. Keller DS, Ishizawa T, Cohen R, Chand M (2017) Indocyanine green fluorescence imaging in colorectal surgery: overview, applications, and future directions. Lancet Gastroenterol Hepatol 2: 757-766.
15. Ris F, Hompes R, Cunningham C, Lindsey I,et al. (2014) Near-infrared (NIR) perfusion angiography in minimally invasive colorectal surgery. Surg Endosc 28: 2221-2226.
16. Cahill RA, Ris F, Mortensen NJ (2011) Near-infrared laparoscopy for real-time

intra-operative arterial and lymphatic perfusion imaging. Colorectal Dis 13 Suppl 7: 12-17.
17. Jafari MD, Lee KH, Halabi WJ, et al. (2013) The use of indocyanine green fluorescence to assess anastomotic perfusion during robotic assisted laparoscopic rectal surgery. Surg Endosc 27: 3003-3008.
18. Jafari MD, Wexner SD, Martz JE, et al. (2015) Perfusion assessment in laparoscopic left-sided/anterior resection (PILLAR II): a multi-institutional study. J Am Coll Surg 220: 82-92.e1.
19. Boni L, Fingerhut A, Marzorati A, Rausei S, Dionigi G, Cassinotti E (2017) Indocyanine green fluorescence angiography during laparoscopic low anterior resection: results of a case-matched study. Surg Endosc 31: 1836-1840.
20. Hojo T, Nagao T, Kikuyama M, Akashi S, Kinoshita T (2010) Evaluation of sentinel node biopsy by combined fluorescent and dye method and lymph flow for breast cancer. Breast 19: 210-213.
21. Wishart GC, Loh SW, Jones L, Benson JR (2012) A feasibility study (ICG-10) of indocyanine green (ICG) fluorescence mapping for sentinel lymph node detection in early breast cancer. Eur J Surg Oncol 38: 651-656.
22. Hirche C, Engel H, Hirche Z, et al. (2014) Real-time lymphography by indocyanine green fluorescence: improved navigation for regional lymph node staging. Ann Plast Surg 73: 701-705.
23. Bjurlin MA, Zhao LC, Kenigsberg AP, Mass AY, Taneja SS, Huang WC (2017) Novel Use of Fluorescence Lymphangiography During Robotic Groin Dissection for Penile Cancer. Urology 107: 267.
24. Verbeek FP, Tummers QR, Rietbergen DD, et al. (2015) Sentinel Lymph Node Biopsy in Vulvar Cancer Using Combined Radioactive and Fluorescence Guidance. Int J Gynecol Cancer 25: 1086-1093.
25. Yuasa Y, Seike J, Yoshida T, et al. (2012) Sentinel lymph node biopsy using intraoperative indocyanine green fluorescence imaging navigated with preoperative CT lymphography for superficial esophageal cancer. Ann Surg Oncol 19: 486-493.
26. Kusano M, Tajima Y, Yamazaki K, Kato M, Watanabe M, Miwa M (2008) Sentinel node mapping guided by indocyanine green fluorescence imaging: a new method for sentinel node navigation surgery in gastrointestinal cancer. Dig Surg 25: 103-108.
27. KleinJan GH, Karakullukçu B, Klop WMC, Engelen T, van den Berg NS, van Leeuwen FWB (2017) Introducing navigation during melanoma-related sentinel lymph node procedures in the head-and-neck region. EJNMMI Res 7: 65.
28. Hirche C, Mohr Z, Kneif S, et al. (2012) Ultrastaging of colon cancer by sentinel node biopsy using fluorescence navigation with indocyanine green. Int J Colorectal Dis 27: 319-324.
29. van der Pas MH, Ankersmit M, Stockmann HB, et al. (2013) Laparoscopic sentinel lymph node identification in patients with colon carcinoma using a near-infrared dye: description of a new technique and feasibility study. J Laparoendosc Adv Surg Tech A 23: 367-371.
30. Nishigori N, Koyama F, Nakagawa T, et al. (2016) Visualization of Lymph/Blood Flow in Laparoscopic Colorectal Cancer Surgery by ICG Fluorescence Imaging (Lap-IGFI). Ann Surg Oncol 23 Suppl 2: S266-74.

31. Marano A, Priora F, Lenti LM, Ravazzoni F, Quarati R, Spinoglio G (2013) Application of fluorescence in robotic general surgery: review of the literature and state of the art. World J Surg 37: 2800-2811.
32. Emile SH, Elfeki H, Shalaby M, et al. (2017) Sensitivity and specificity of indocyanine green near-infrared fluorescence imaging in detection of metastatic lymph nodes in colorectal cancer: Systematic review and meta-analysis. J Surg Oncol 116:730-740.
33. Watanabe J, Ota M, Suwa Y, Ishibe A, Masui H, Nagahori K (2016) Real-Time Indocyanine Green Fluorescence Imaging-Guided Complete Mesocolic Excision in Laparoscopic Flexural Colon Cancer Surgery. Dis Colon Rectum 59: 701-705.
34. Cahill RA, Anderson M, Wang LM, Lindsey I, Cunningham C, Mortensen NJ (2012) Near-infrared (NIR) laparoscopy for intraoperative lymphatic road-mapping and sentinel node identification during definitive surgical resection of early-stage colorectal neoplasia. Surg Endosc 26: 197-204.
35. Boni L, David G, Mangano A, et al. (2015) Clinical applications of indocyanine green (ICG) enhanced fluorescence in laparoscopic surgery. Surg Endosc 29: 2046-2055.
36. Handgraaf HJ, Boogerd LS, Verbeek FP, et al. (2016) Intraoperative fluorescence imaging to localize tumors and sentinel lymph nodes in rectal cancer. Minim Invasive Ther Allied Technol 25: 48-53.
37. Kazanowski M, Al Furajii H, Cahill RA (2015) Near-infrared laparoscopic fluorescence for pelvic side wall delta mapping in patients with rectal cancer--'PINPOINT' nodal assessment. Colorectal Dis 17 Suppl 3: 32-35.
38. Liberale G, Vankerckhove S, Galdon MG, Donckier V, Larsimont D, Bourgeois P (2015) Fluorescence imaging after intraoperative intravenous injection of indocyanine green for detection of lymph node metastases in colorectal cancer. Eur J Surg Oncol 41: 1256-1260.
39. Liberale G, Galdon MG, Moreau M, et al. (2016) Ex vivo detection of tumoral lymph nodes of colorectal origin with fluorescence imaging after intraoperative intravenous injection of indocyanine green. J Surg Oncol 114: 348-353.
40. Liberale G, Vankerckhove S, Caldon MG, et al. (2016) Fluorescence Imaging After Indocyanine Green Injection for Detection of Peritoneal Metastases in Patients Undergoing Cytoreductive Surgery for Peritoneal Carcinomatosis From Colorectal Cancer: A Pilot Study. Ann Surg 264: 1110-1115.
41. Nagata J, Fukunaga Y, Akiyoshi T, et al. (2016) Colonic Marking With Near-Infrared, Light-Emitting, Diode-Activated Indocyanine Green for Laparoscopic Colorectal Surgery. Dis Colon Rectum 59: e14-8.
42. Watanabe M, Murakami M, Ozawa Y, Yoshizawa S, Matsui N, Aoki T (2017) Intraoperative Identification of Colonic Tumor Sites Using a Near-Infrared Fluorescence Endoscopic Imaging System and Indocyanine Green. Dig Surg 34:495-501.

Table 1: Patient and tumour details

Variable	n
Age, years, (mean,±SD)	69.50 (±7.13)
Male/ Female (n,%)	4 (40%)/ 6 (60%)
Tumour location (n,%)	
Ascending colon	7 (70%)
Sigmoid colon	2 (20%)
Descending colon	1 (10%)

Table 2: Procedural details

Variable	n (%)
Procedure performed	
Laparoscopic Right hemicolectomy	5 (50%)
Laparoscopic Left hemicolectomy	3 (30%)
Laparoscopic Sigmoid colectomy	2 (20%)
Stoma created?	1 (10%)
Level of anastomosis	
Ileocolic	7 (70%)
Colorectal	3 (30%)

Table 3: Protocol and pathologic details

Variable	n
Tumour localised with ICG? (n,%)	10 (100%)
ICG localization consistent with preoperative imaging? (n,%)	8 (80%)
Visualisation of lymphatic drainage?(n,%)	10 (100%)
Time to central lymph node visualisation, minutes (mean ±SD)	4.32 (±1.45)
Patients with lymph nodes identified with ICG outside of the proposed mesenteric resection margin? (n,%)	2 (20%)
Lymph node yield (median, range)	22 (14-49)
Number of positive lymph nodes (median, range)	2 (0-8)
Positive resection margins (n,%)	0 (0%)
Surgical Pathology (pTNM)	
Patient 1	T4b N2 M1
Patient 2	T4 N2 M0
Patient 3	T4b N2 M1
Patient 4	T3 N0 Mx
Patient 5	T1 N1 Mx
Patient 6	T3 N0 Mx
Patient 7	T3 N1 M1
Patient 8	T4b N0 Mx
Patient 9	T4b N1 M0
Patient 10	T4b N1 M1

ICG= Indocyanine green.

Figure legends

Figure 1- Subserosal peritumoural injection of Indocyanine green

Figure 2 – Demonstration of the primary colon tumour with indocyanine green fluorescence imaging

Figure 3 – Demonstration of the lymphatic channels associated with a colon tumour using indocyanine green fluorescence imagingI

Figure 4- Localization of an aberrant lymph node with indocyanine green fluorescence imaging; the node was outside of the standard resection margin, but was mesocolic excision was amended to include this node in the specimen. The node was positive on pathological assessment.