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Title Page**Title: A feasibility study of indocyanine green fluorescence mapping for sentinel lymph node detection in cutaneous melanoma**

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Summary

Objectives: Sentinel lymph node biopsy (SLNB) is standard of care for staging regional LN in AJCC stage IB-IIc melanoma; using dual localization with radiolabelled colloid and blue dye. Combining these gives optimal accuracy; drawbacks include cumulative radiation exposure for healthcare workers, coordination between disciplines and anaphylaxis. An alternative tracer agent is indocyanine green (ICG); an optical enhancer that fluoresces in the near infrared range. This prospective cohort study assesses the feasibility of using ICG as a tracer agent to detect SLN in cutaneous melanoma.

Methods: Primary melanoma patients diagnosed with pT1b-pT4b tumours undergoing SLNB were recruited over a 6-month period at a tertiary referral centre. All underwent standard preoperative lymphoscintigraphy (LSG) using 20-40MBq of Tc^{99m} radiolabelled nanocolloid plus intraoperative Patent Blue dye (PBD). ICG was administered as a third tracer agent intraoperatively.

Results: 62 patients (33M/29F) were recruited; median age was 61 years. Median melanoma Breslow thickness was 1.6mm. 144 specimens containing 135 SLN were excised. Concordance rate for all 3 tracer agents was 88.1%(119/135 LN); that for radioisotope/PBD was 88.2%(95%CI:82.2,93.7). There were no discordance pairs between radioisotope/PBD compared to radioisotope/PBD/ICG. Radioisotope/ICG significantly increased the sensitivity of detecting SLN to 98.5%(95%CI:94.8,99.8); $p < 0.00001$ compared to radioisotope/PBD. Concordance rate of intraoperative ICG drainage pattern with LSG was 22.6%.

Conclusion: ICG utilization showed comparable sensitivity with gold standard. Technical challenges e.g. ICG leakage into biopsy field, poor concordance with LSG limits its efficacy in melanoma SLNB. We therefore do not recommend replacing current practice with ICG alone or by using a combination with TC^{99m}.

Keywords: cutaneous melanoma; sentinel lymph node biopsy; indocyanine green

Sentinel lymph node biopsy (SLNB) is the standard of care for staging regional lymph nodes (LN) in stage AJCC IB-IIC melanoma¹. The technique utilises dual localisation with a radiolabelled colloid and a blue dye such as Patent Blue (PB). Combining these give an excellent sensitivity and specificity^{2,3}

Potential drawbacks associated with the use of radioisotopes as tracer agents for localisation of SLN. include cumulative radiation exposure for healthcare workers, problems with surgical waste disposal and potential repeat of the 2009 European shortage of radioisotopes. Furthermore, co-ordinated efforts between different disciplines is essential.⁴ Adverse events associated with the blue dye include prolonged skin-staining and anaphylaxis⁵⁻⁷. Localisation techniques using another non-radioactive tracer which has comparable accuracy to PB and Tc⁹⁹ therefore warrants investigation.

A potential alternative is indocyanine green (ICG). Potential benefits include the absence of serious adverse reactions, (allergic reactions are rare⁸). ICG is utilised as an optical enhancer and fluoresces light in the near-infrared range. It also emits fluorescence when it makes contact with plasma proteins in the lymphovascular systems of the extracellular compartment.

The principle of fluorescence imaging has been applied to SLN mapping in early breast cancer. The advantage of using ICG in these cancer types is that their lymphatic drainage is relatively predictable, often obviating the need for pre-operative LSG. In contrast, lymphatic drainage of cutaneous melanoma is notoriously unpredictable¹⁵ necessitating the need for LSG to determine SLN location and potential nodal fields. Preliminary work with melanoma have been published, but with only a limited number of patients¹⁶⁻¹⁸.

The primary aim of this study was to assess the feasibility of utilising ICG to detect SLN in cutaneous melanoma; we aimed to achieve this by determining the sensitivity of ICG fluorescence imaging in SLN identification when combined with Tc⁹⁹ and PB. Secondary endpoints included evaluation the accuracy of percutaneous visualisation of lymphatic channels and nodal fields intra-operatively compared to pre-operative LSG. Standard patient demographics and tumour characteristics were also collected.

Primary cutaneous melanoma patients over the age of 18 years diagnosed with pT1b-pT4b tumours, clinically N0, undergoing wide local excision and SLNB were

identified at specialist skin MDT and recruited in a prospective and consecutive manner over a 6-month period at a tertiary melanoma referral centre. Exclusion criteria included prior LN surgery, previous history of cutaneous melanoma, failed pre-operative LSG and contraindication to ICG such as pregnancy, lactating patients and those with thyroid problems.

All patients underwent standard pre-operative LSG using 20-40 MBq of Tc-99m radiolabelled nanocolloid (ELUMATIC III) in addition to intra-operative PB (Bleu Patente®; Guerbet)¹⁸. ICG was administered as a third tracer agent intra-operatively.

This study protocol was approved by the Research Ethics Committee and MHRA (Eudra CT number: 2012-002244-25).

62 patients (33M:29F) were recruited with a median age of 61 years (31-78 years). The distribution of melanoma and LN drainage characteristics are described in table 1. Median melanoma Breslow thickness was 1.6mm. None of the patients experienced anaphylaxis.

144 specimens were excised, average 2.3 specimens per patient. 135 SLN were included in the histological analysis. The concordance rate for all 3 tracer agents was 88.1% (119 of 135 LNs - table 2). The concordance rate between radio-isotope and PB was 88.2% (95% CI: 82.2%-93.7%). There were no discordant pairs between radio-isotope/PB compared to radio-isotope/PB/ICG fluorescence. One extra LN demonstrated fluorescence and blue staining only. All positive SLN were radioactive, blue and fluorescent (n=19).

Radio-isotope and ICG fluorescence significantly increased the sensitivity of detecting SLN to 98.5% (95% CI: 94.8%-99.8%; $p < 0.00001$) compared to radio-isotope or PB alone.

In 98.4% (61/62) of patients, lymphatic channels were visualised prior to incision using ICG. In 38 patients (62.3%), percutaneous visualisation with PDE showed greater detail or extra channels when compared against pre-operative LSG. However in 10 cases (16.1%), less details or less channels were visualised with PDE ;this

would have translated to missing a lymphatic drainage basin in 3 cases. Percutaneous LN visualisation was variably dependent on the depth of LN beneath the skin, however this was not consistent when compared to different LN drainage sites.

According to our data, sensitivity of ICG with PB was 88.9%, which is lower than gold standard^{3,19}. Moreover, there is a level of inaccuracy when interpreting ICG; only 22.6% of patients had exact corresponding lymphatic channel imaging when comparing PDE and pre-operative LSG. This is likely due to the high variability of lymphatic drainage in melanoma^{15,20}, and thus a challenge when using ICG.

Of our cohort, only 14.5% (n=9) patients presented with a head and neck (H&N) primary melanoma; however this translated to a quarter of the LN drainage basin. It is difficult to conclude whether ICG would contribute a better operative achievement in H&N melanoma.

PB alone only identified 120 of 135 SLNs (88.9% sensitivity) which correlates with data on development of dual technique^{2,3}. Although the data suggests PB adds very little to the SLNB technique combined with Tc⁹⁹ or ICG, practically it may aid the surgeon in SLN visualisation intra-operatively. Interestingly, radio-sensitive and positive fluorescence alone identified all but one SLN with a sensitivity of 98.5%; however utilisation of ICG was associated with some technical problems. Poor visualisation of lymphatic channels using ICG pre-operatively in obese patients makes it challenging to plan incisions. This is in keeping with studies that have found that when SLNs located deeper, such as the axilla or inguinal region, there is unreliable tracking percutaneously using fluorescence^{21,22}.

Other tracer agents such as Tc-99m Tilmanocept (Lymphoseek®; Navidea) have been trialled in two near-identical non-randomised phase III trials that compared it to blue dye. Results have shown that it identifies 98.7% of blue nodes²³. This shows promising results for an alternate tracer agent. Other studies have investigated the use of hybrid radioactive and fluorescent tracer (ICG-^{99m}Tc-nanocolloid in SLNB for H&N malignancies with favourable results^{24,25}; however further studies are required to establish its use in melanoma outside the H&N region.

In this successful feasibility study, we have demonstrated that ICG, in combination with Tc⁹⁹ radio-labelled nanocolloid has excellent sensitivity (98.5%) in detecting SLN in melanoma. However, in practice, the requirement for additional equipment, the relatively impracticality of being unable to follow the fluorescent imaging whilst using standard operating lights severely limits its application. Whilst we have identified some positive aspects to the technique, replacement of Tc⁹⁹ or PB with ICG in SLNB for cutaneous melanoma is not recommended and we suggest that further developmental work is required.

Conflict of interest statement

Conflicts of interest: none declared

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References

1. National Institute for Health and Care Excellence. *Melanoma: assessment and management*. NICE guidelines [NG14]. London” National Institute for Health

and Care Excellence; July 2015. Available from

<https://www.nice.org.uk/guidance/ng14>

2. Morton DL, Wen DR, Wong JH, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. *Arch Surg*. 1992;**127**(4):392-9.
3. Morton DL, Cochran AJ, Thompson JF et al. Sentinel node biopsy for early-stage melanoma: accuracy and morbidity in MSLT-I, an international multicenter trial. *Ann Surg*. 2005;**242**(3):302-13.
4. Chakera AH, Hesse B, Burak Z, Ballinger JR, et al. European Association of Nuclear Medicine-European Organisation for Research and EANM-EORTC general recommendations for sentinel node diagnostics in melanoma. *Eur J Nucl Med Mol Imaging*. 2009;**36**:1713-42.
5. Scherer K, Studer W, Figueiredo V, Bircher AJ. Anaphylaxis to isosulfan blue and cross-reactivity to patent blue V: case report and review of the nomenclature of vital blue dyes. *Ann Allergy Asthma Immuno*. 2006;**96**(3):497-500.
6. Van Zuuren E, Polderman MC, Kuijken I. Anaphylaxis to patent blue during sentinel lymph node identification. *Contact Dermatitis*. 2005;**53**(3):171-.
7. Tripathy S, Nair PV. Adverse drug reaction, patent blue V dye and anaesthesia. *Indian J Anaesth..* 2012;**56**(6):563.
8. Motomura K, Inaji H, Komoike Y, Kasugai T, Noguchi S, Koyama H. Sentinel node biopsy guided by indocyanin green dye in breast cancer patients. *Jpn J Clin Oncol*. 1999;**29**(12):604-7.
9. Kitai T, Inomoto T, Miwa M, Shikayama T. Fluorescence navigation with indocyanine green for detecting sentinel lymph nodes in breast cancer. *Breast Cancer*. 2005;**12**(3):211-5.
10. Wishart GC, Loh SW, Jones L, Benson JR. A feasibility study (ICG-10) of indocyanine green (ICG) fluorescence mapping for sentinel lymph node detection in early breast cancer. *Eur J Surg Oncol*. 2012;**38**(8):651-6.
11. Sugie T, Sawada T, Tagaya N, et al. Comparison of the indocyanine green fluorescence and blue dye methods in detection of sentinel lymph nodes in early-stage breast cancer. *Ann Surg Oncol*. 2013;**20**(7):2213-8.
12. Tajima Y, Yamazaki K, Masuda Y, et al. Sentinel node mapping guided by indocyanine green fluorescence imaging in gastric cancer. *Ann Surg*. 2009;**249**(1):58-62.

13. Nimura H, Narimiya N, Mitsumori N, et al. Infrared ray electronic endoscopy combined with indocyanine green injection for detection of sentinel nodes of patients with gastric cancer. *Br J Surg*. 2004;**91**(5):575-9.
14. Ito N, Fukuta M, Tokushima T, et al. Sentinel node navigation surgery using indocyanine green in patients with lung cancer. *Surg Today*. 2004;**34**(7):581-5.
15. Reynolds HM, Walker CG, Dunbar P, et al. Functional anatomy of the lymphatics draining the skin: a detailed statistical analysis. *J Anat*. 2010;**216**(3):344-55.
16. Polom K, Murawa D, Rho YS, et al. Skin melanoma sentinel lymph node biopsy using real-time fluorescence navigation with indocyanine green and indocyanine green with human serum albumin. *Br J Dermatol*. 2012;**166**(3):682-3.
17. Cloyd JM, Wapnir IL, Read BM, et al. Indocyanine green and fluorescence lymphangiography for sentinel lymph node identification in cutaneous melanoma. *J Surg Oncol*. 2014;**110**(7):888-92.
18. Fujiwara M, Mizukami T, Suzuki A, Fukamizu H. Sentinel lymph node detection in skin cancer patients using real-time fluorescence navigation with indocyanine green: preliminary experience. *J Plast, Reconstr Aesth Surg*. 2009;**62**(10):e373-8.
19. Wong SL, Balch CM, Hurley P, et al. Sentinel lymph node biopsy for melanoma: American Society of Clinical Oncology and Society of Surgical Oncology joint clinical practice guideline. *J Clin Oncol*. 2012;**30**(23):2912-8.
20. Uren RF, Howman-Giles R, Thompson JF. Patterns of lymphatic drainage from the skin in patients with melanoma. *J Nucl Med*. 2003;**44**(4):570-
21. Van der Vorst JR, Schaafsma BE, Verbeek FP, Swijnenburg RJ, Hutteman M, Liefers GJ, van de Velde CJ, Frangioni JV, Vahrmeijer AL. Dose optimization for near-infrared fluorescence sentinel lymph node mapping in patients with melanoma. *Br J Dermatol*. 2013;**168**(1):93-8.
22. Gilmore DM, Khullar OV, Gioux S, et al. Effective low-dose escalation of indocyanine green for near-infrared fluorescent sentinel lymph node mapping in melanoma. *Ann Surg Oncol*. 2013;**20**(7):2357-63.
23. Sondak VK, King DW, Zager JS, et al. Combined analysis of phase III trials evaluating [^{99m}Tc] tilmanocept and vital blue dye for identification of

- sentinel lymph nodes in clinically node-negative cutaneous melanoma. *Ann Surg Oncol.* 2013;**20**(2):680-8.
24. Brouwer OR, Klop WM, Buckle T, et al. Feasibility of sentinel node biopsy in head and neck melanoma using a hybrid radioactive and fluorescent tracer. *Ann Surg Oncol.* 2012;**19**(6):1988-94.
25. Stoffels I, Leyh J, Pöppel T, et al. Evaluation of a radioactive and fluorescent hybrid tracer for sentinel lymph node biopsy in head and neck malignancies: prospective randomized clinical trial to compare ICG-99mTc-nanocolloid hybrid tracer versus 99mTc-nanocolloid. *Eur J Nucl Med Mol Imaging.* 2015;**42**(11):1631-8.

Table 1: Melanoma and lymph node characteristics of cohort

	Number (n)	Percentage (%)
Melanoma staging		
pT1b	14	22.6
pT2a	24	38.7
pT2b	3	4.9
pT3b	7	11.3

pT4a	4	6.5
pT4b	3	3.9
Unknown	1	1.6
Site of primary tumour		
Head and neck	9	14.5
Trunk	28	45.2
Limb	25	40.3
Ulceration		
Present	15	24.2
Absent	46	74.2
Unknown	1	1.6
Perineural invasion		
Present	1	1.6
Absent	60	96.8
Unknown	1	1.6
Lymphovascular invasion		
Present	1	1.6
Absent	56	90.3
Possible	4	6.5
Unknown	1	1.6
Lymph node drainage basin		
Axilla	70	50
Neck	37	26.4
Groin	26	18.6
Interval	7	5
Number of drainage basins		
1	44	71

2	15	24.2
3	2	3.2
4	1	1.6
Number of lymph nodes		
1	17	27.4
2	22	35.4
3	16	25.8
4	4	6.5
5	3	4.8

Table 2: Sensitivity of tracer agents in the detection of SLN. 135 SLN excised in total.

Tracer agent	SLN detected	Sensitivity	95% CI	Significance: McNemar's test (vs. radioisotope/ICG)
Radio/PB/ICG	119	88.2%	(82.2% - 93.7%)	p=1.0000
Radio/PB	119	88.2%	(82.2% - 93.7%)	Reference
ICG/PB	120	88.9%	(82.3% - 93.7%)	p=1.0000
Radio/ICG	133	98.5%	(94.8 % - 99.8%)	p<0.0001
Radio	134	99.3%	(95.9% - 100%)	p<0.0001
PB	134	99.3%	(95.9% - 100%)	p<0.0001
ICG	120	88.9%	(82.3% - 93.7%)	p=1.0000

SLN – sentinel lymph node, Radio – radioisotope, PB – Patent Blue, ICG – indocyanine green,
CI – confidence interval