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-\(^{99m}\)Tc-nanocolloid hybrid tracer versus \(^{99m}\)Tc-nanocolloid

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Abstract

Purpose There is some controversy about the value of sentinel lymph node excision (SLNE) in patients with head and neck malignancies. The gold standard for detection and targeted extirpation of the SLN is lymphoscintigraphy with \(^{99m}\)Tc-nanocolloid. The purpose of this prospective randomized study was to analyse the feasibility and clinical benefit of a hybrid tracer comprising the near-infrared (NIR) fluorescent indocyanine green (ICG) and \(^{99m}\)Tc-nanocolloid (ICG-\(^{99m}\)Tc-nanocolloid) in direct comparison with standard \(^{99m}\)Tc-nanocolloid for guiding SLNE in patients with head and neck cutaneous malignancies.

Methods We analysed the data from 40 clinically lymph node-negative patients with melanoma, high-risk cutaneous squamous cell carcinoma, Merkel cell carcinoma or sweat gland carcinoma who underwent SLNE with ICG-\(^{99m}\)Tc-nanocolloid (cohort A) or with the standard \(^{99m}\)Tc-nanocolloid (cohort B).

Results Overall SLNs were identified preoperatively in all 20 patients (100 %) in cohort A and in 18 of 20 patients (90 %) in cohort B. The SLN basin was detected preoperatively in 18 patients (90 %) in cohort A and also in 18 patients (90 %) in cohort B. SLNs were identified intraoperatively in all 20 patients (100 %) in cohort A and in 19 patients (95 %) in cohort B (\(p=0.487\)). Metastatic SLNs were detected in 9 patients (22.5 %), 3 (15.0 %) in cohort A and 6 (30.0 %) in cohort B (\(p=0.228\)).

Conclusion The hybrid tracer ICG-\(^{99m}\)Tc-nanocolloid is an innovative imaging tracer, reliably and readily providing additional information for the detection and excision of SLN in the head and neck region. Therefore, SLNE with combined radioactive and NIR fluorescence guidance is an attractive option for improving the SLN detection rate in patients with cutaneous head and neck malignancies.

Keywords Malignant melanoma · Head and neck cancer · Sentinel lymph node · Hybrid tracer · Indocyanine green

Introduction

Cutaneous malignancies including malignant melanoma and squamous cell carcinoma (SCC), depending on tumour thickness, can metastasize early into regional lymph nodes [1]. The histological status of the sentinel lymph node (SLN) is the most relevant predictive factor for overall survival of melanoma patients, independent of the primary tumour thickness [2]. Critics argue that SLN excision (SLNE) is a cost-intensive
surgical intervention with potential morbidity, which does not offer patients any advantage in terms of overall survival [3, 4]. In particular, SLNE in patients with cutaneous head and neck malignancies is controversial because of the complexity of the lymphatic drainage in that region and potential high morbidity. The head and neck region contains a large number of closely grouped lymphatic basins with up to 300 lymph nodes [5, 6]. Because of its close proximity to the primary cutaneous tumour, the SLN in this region might be masked by the radioactive injection site [7] so that it cannot be recognized and excised [8].

Currently, different colloidal agents labelled with $^{99m}$Tc are used for detection and targeted extirpation of the SLN. The reported false-negative rate is very high and varies between 5.7 % and 32.0 % for SLNE [9, 10]. Imaging modalities currently used for the detection of radioactive agents are indeed limited by their poor spatial resolution in solid tumour types in which the SLN are in close proximity to the primary tumour. Nonetheless, sufficient grounds exist to search for alternative methods, not only to improve the SLN detection rate but also to reduce the rate of false-negative SLN. For radiologically guided SLN identification in the preoperative setting, SPECT/CT has been shown to help place SLN in their anatomical context, enabling better planning of the surgical procedure [11].

There have been several reports on the use of near-infrared (NIR) fluorescent indocyanine green (ICG) for SLNE in breast cancer, skin cancer, gastric cancer, colorectal cancer and non-small-cell lung cancer [12, 13], but the penetration depth of NIR fluorescence (<1.5 cm) still prevents preoperative SLN mapping [14]. The combined use of radioactive tracer and NIR fluorescence in the form of the hybrid tracer ICG-$^{99m}$Tc-nanocolloid has already been described [15, 16]. Fluorescent ICG and radioactive $^{99m}$Tc are added to the same nanocolloid particle. This hybrid tracer is both radioactive and fluorescent, and therefore provides real-time optical localization using a NIR fluorescence camera. The feasibility and validity of this tracer has been shown in various tumour types. Although its potential use has been discussed in many reports, its effectiveness and limitations have not been evaluated in prospective randomized trials for SLNE in patients with cutaneous head and neck malignancies. The present prospective randomized trial sought to analyse the feasibility and medical benefit of the ICG-$^{99m}$Tc-nanocolloid hybrid tracer in direct comparison with standard $^{99m}$Tc-nanocolloid.

Patients and methods

Study design and patients

This prospective randomized clinical trial was approved by the institutional review board (12-4972-BO) and registered with the German Clinical Trials Register (DRKS00004622). SLNE was performed as a standard procedure according to the guidelines of the Deutsche Dermatologische Gesellschaft (DDG, German Association of Dermatology).

Forty patients fulfilling the inclusion criteria with stage Ib and II (AJCC 2009) melanoma, SCC, Merkel cell carcinoma or sweat gland carcinoma in the head and neck region, aged >18 years and with clinically negative lymph nodes as assessed by ultrasonography were included in this prospective randomized trial from November 2012 to May 2014. Exclusion criteria included age <18 years, pregnancy, lactation and allergy to iodine or known intolerance to ICG. All patients gave written informed consent. Due to our own previous results we expected a limited concordance of 65 % between the two methods [14] mainly because of the challenging identification of SLN due to the limited tissue penetration depth (up to 1 cm) of the fluorescent signal and due to difficult lymphatic mapping in the head and neck with its rich abundant interlacing lymphatic drainage patterns, which can lead to unusual and unexpected drainage patterns. We calculated that 34 patients needed to be examined in the two cohorts to give a 95 % power and a type I error (alpha) of 5 % (two-sided test). To take into account possible exclusions, we decided to include 40 patients.

Patients in cohort A received the ICG-$^{99m}$Tc-nanocolloid hybrid tracer and patients in cohort B were given standard $^{99m}$Tc-nanocolloid. The primary study endpoints were the number of excised SLN and the duration of the surgical intervention. Baseline characteristics are summarized in Table 1.

Tracer preparation, sentinel node scintigraphic technique and SPECT/CT

ICG-$^{99m}$Tc-nanocolloid was prepared via noncovalent self-assembly of $^{99m}$Tc-labelled nanosized human albumin particles and ICG. $^{99m}$Tc-labelled nanocolloid was prepared by adding sodium pertechnetate (approximately 1,000 MBq) in 2 ml saline to a vial containing 0.5 mg nanosized human albumin colloidal particles (Nanotop®; ROTOP Pharmaka AG, Dresden, Germany). After 30 min of incubation at room temperature, 50 μl 6.4 mmol/l (0.25 mg) ICG (Pulsion Medical Systems, Munich, Germany) was dissolved in sterilized water to obtain ICG-$^{99m}$Tc-nanocolloid at a final ICG concentration of 160 μmol/l. To obtain a twofold higher concentration of ICG-nanocolloid, $^{99m}$Tc-labelled nanocolloid was prepared by adding pertechnetate (approximately 500 MBq) in 1 ml saline to 0.5 mg nanocolloid, while the amount of ICG (0.25 mg) was kept the same, resulting in a final ICG concentration of 320 μmol/l. The hybrid tracer was administered by injection from one syringe.

Dynamic images of the corresponding anatomical region and their adjacent lymphatic basins were acquired at 30 s per frame for 5 min with a total of ten frames. Afterwards,
anterior, lateral, and oblique projections were acquired for 5 min each, using a dual-detector gamma camera with a mounted two-row multidetector CT scanner (Symbia T®; Siemens Healthcare, Erlangen, Germany). Further details are as described previously [11].

Intraoperative NIR fluorescence imaging

Intraoperative NIR imaging was performed using a Fluobeam® system (Fluoptics, Grenoble, France) as previously described [14]. To allow fluorescence navigation under operating room light conditions, a modified surgical headlight with a LED light source (LED DayLite Twin Beam®; Designs for Vision, New York, NY) with a low-pass filter (that cut radiation under 800 nm) was used during SLNE. NIR imaging was performed to determine whether the lymphatic pathways and SLN could be visualized through the intact skin. If the NIR signal was not visible, a hand-held gamma probe was used to determine the point of incision and further preparation. The gamma probe was then used in conjunction with the NIR imaging system for SLN detection and excision. Excised SLN were imaged ex vivo for NIR fluorescence and for radioactivity. The procedures were performed by two experienced surgeons who also had previous experience in the use of ICG for the detection of SLN.

Histology

The dissected tissue containing the SLN was placed in 4 % formalin solution. Analysis of frozen slides was not performed. The SLN were separated from surrounding adipose tissue, lamellated and embedded. Consecutive serial sections were made. Conventional staining was performed with haematoxylin and eosin (H&E) staining as well as immunohistological staining with antibodies to S100, MelanA and Human Melanoma Black 45 (HMB 45) for melanoma [17, 18], CK-20 and pancytokeratin for Merkel cell carcinoma and cytokeratin antibody MNF 116 for the high-risk cutaneous SCC and sebaceous gland carcinoma.

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total (n=40)</th>
<th>Cohort A, hybrid nanocolloid (n=20)</th>
<th>Cohort B, standard nanocolloid (n=20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean (standard deviation)</td>
<td>67.7 (17.4)</td>
<td>67.4 (19.2)</td>
<td>68.1 (15.8)</td>
<td>0.850</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>29 (72.5)</td>
<td>15 (75)</td>
<td>14 (70)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>11 (27.5)</td>
<td>5 (25)</td>
<td>6 (30)</td>
<td></td>
</tr>
<tr>
<td>Tumour entity, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>20 (50.0)</td>
<td>10 (50)</td>
<td>10 (50)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>16 (40.0)</td>
<td>9 (45)</td>
<td>7 (35)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Merkel cell carcinoma</td>
<td>2 (5.0)</td>
<td>–</td>
<td>2 (10)</td>
<td></td>
</tr>
<tr>
<td>Sweat gland carcinoma</td>
<td>2 (5.0)</td>
<td>1 (5)</td>
<td>1 (5)</td>
<td></td>
</tr>
<tr>
<td>Location of SLN, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical region</td>
<td>27 (67.5)</td>
<td>14 (70)</td>
<td>13 (65)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Preauricular region</td>
<td>9 (22.5)</td>
<td>6 (30)</td>
<td>3 (15)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Submandibular region</td>
<td>3 (7.5)</td>
<td>–</td>
<td>3 (15)</td>
<td></td>
</tr>
<tr>
<td>Supraclavicular region</td>
<td>1 (2.5)</td>
<td>–</td>
<td>1 (5)</td>
<td></td>
</tr>
</tbody>
</table>

Randomization

Patients were randomly allocated via sealed opaque envelopes containing an identifier to cohort A (n=20) or cohort B (n=20).

Sample size calculation and statistical evaluation

The power calculation was based on previous data showing a concordance of 65 % during SLN detection by 99mTc and ICG [14]. These data revealed that 20 patients per cohort were needed to achieve at least 85 % power using a two-tailed t test (α=0.05). Continuous data are presented as medians (range). Normally distributed data (age and tumour size) were tested using the independent-samples t test, and the Mann-Whitney U test was used for analysis of variables with a nonnormal distribution. To compare categorical characteristics between the two groups, Fisher’s exact test was used for binary data and the χ² test for nonbinary data. P<0.050 was considered significant. SPSS statistical software version 20.0 (IBM, Armonk, New York, NY) was used for statistical analysis.

Results

A total of 40 patients (20 in cohort A and 20 in cohort B) undergoing SLN biopsy were included in this prospective randomized trial. The demographic characteristics of the 40
enrolled patients who satisfied the inclusion and exclusion criteria are given in Table 1. All patients were Caucasian, 11 women and 29 men, ranging in age from 19 to 91 years (mean 67.7 years, median 72.5 years). Malignant melanoma with a Breslow index of >1.0 mm (n=20) was included. Tumour thickness varied from 1.2 to 9.1 mm (mean 2.92 mm, median 2.65 mm). Of the 20 patients with melanoma, 6 (30 %) showed ulceration of the primary tumour. In addition, 16 patients with a primary cutaneous SCC and a tumour thickness of >4.0 mm in the head and neck area were included. Tumour thickness of the high-risk cutaneous SCC varied between 4.0 and 9.0 mm (mean 5.49 mm, median 4.80 mm). Two patients with Merkel cell carcinoma and two with sweat gland carcinoma were also included.

The median time between injection of ICG-99mTc-nanocolloid and surgery was 23 h (19 – 27 h) and 21 h (20 – 24 h) for the standard nanocolloid respectively. Overall, SLNs were identified preoperatively in all patients in cohort A (20/20, 100 %) and 90 % (18/20) in cohort B. In one patient in cohort B the SLN was detected after excision of the safety margins and thereby the tracer depot was removed. The SLN basins were clearly visualized by ICG before skin incision in only four patients in cohort A (20.0 %). The SLN basin was detected preoperatively by 99mTc-nanocolloid in 90 % (18/20) of the patients in cohort A. The two patients in cohort A (2/20; 10.0 %) mentioned above without a lymph node identified preoperatively by 99mTc-nanocolloid had a total of three SLN (3/36; 8.3 %), which were only extirpated on the basis of ICG-99mTc-nanocolloid marking. The number of SLNs ranged from none to three (average 1.65) in patients in the cohort B and from one to six (average 1.8) in patients in cohort A (Table 2). The intraoperative SLN identification rate was higher in cohort A than in cohort B (100 % vs. 95.0 %; p=0.487).

The time for SLNE was not different between cohort A and cohort B (36.0 min, SD 15.0 min vs. 45.0 min, SD 22.0 min; p=0.23; Table 2). Metastatic SLNs were detected in 9 of the 40 patients (22.5 %); 3 (15.0 %) in cohort A and 6 (30.0 %; p=0.228) in cohort B (Table 2). Similar to the use of ICG, no adverse reactions were associated with the use of ICG-99mTc-nanocolloid. All patients underwent the surgical procedure under local anaesthesia.

**Discussion**

It is highly relevant clinically to perform accurate tumour staging in patients with cutaneous melanoma and carcinoma with a high risk of lymph node metastases. Lymph node micrometastases are of high prognostic value, and such a finding is part of the classification system as well as a stratification factor in numerous clinical trials. The introduction of SLNE as a diagnostic procedure in patients with cutaneous malignancies has proven to be of tremendous advantage for the detection of subclinical lymph node metastases [19]. The technique of SLNE with the primary tumour on the trunk or the extremities has become well standardized for the identification of a patient subpopulation with subclinical metastasis [20].

Detection of SLN in the head and neck region is much more difficult than on the trunk or extremities because of the complexity of the lymphatic drainage [6]. The head and neck region contains a very large number of closely grouped lymphatic basins with up to 300 lymph nodes [5]. Because of its close proximity to the primary cutaneous tumour, the SLN might be masked by the radionuclide injection site [4]. These factors might contribute to higher false-negative rates in SLNE in this region [8, 21]. Also lymphatic escape routes following previous surgery may exist and possible involvement of the contralateral lymph nodes at the primary tumour site has also been reported [22].

In several studies, ICG identified SLN in addition to those identified by 99mTc-nanocolloid, indicating that this technique could potentially reduce the SLN false-negative rate [23]. In the last 2 years many studies of ICG-guided SLNE have been published in the field of breast cancer suggesting that this technique would be a feasible method for SLN mapping. A large prospective study by Ballardini et al. have validated the ICG method by demonstrating that it is statistically non-inferior to 99mTc-nanocolloid [24] and therefore concluded that the ICG method could be used as a reliable and safe alternative to the radiotracer method. The question remains as to whether these results are transferable to malignancies in which the correct lymph node basins are unpredictable, such as cutaneous malignancies of the head and neck area.

To our knowledge, this is the first prospective randomized trial performed to study the potential benefit of the ICG-99mTc-nanocolloid hybrid tracer compared with standard 99mTc-nanocolloid in patients with cutaneous malignancies in the head and neck area. Optical imaging using NIR fluorescence alone has been tested extensively for SLN detection in breast cancer and in other cancers, such as melanoma [14, 25–27]. The fluorescent tracer ICG is used for detection of SLNs at tissue depths up to several millimetres [12]. This tracer has outperformed blue dye staining for SLN identification in multiple clinical trials [28–30]. Nevertheless, radioactive colloids are still considered the standard of care for preoperative planning. Several studies have confirmed the benefit of preoperative SPECT/CT, especially in the head and neck area (Figs. 1 and 2) [11, 20, 31]. Radioactive colloids are essential for localization of more deeply located SLNs, for example in patients with a SLN covered by a muscle or in patients with a higher body mass index. The hybrid tracer ICG-99mTc-nanocolloid combining radioactive and NIR fluorescence qualities in one tool has already been described [16]. In this hybrid tracer the fluorescent label ICG and the...
radioactive label $^{99m}$Tc are integrated into the same colloidal particle.

The present prospective randomized trial showed that the use of ICG-$^{99m}$Tc-nanocolloid for SLN biopsy is feasible in patients with head and neck cancer. This tracer permits preoperative imaging with lymphoscintigraphy and SPECT/CT for, for example, intraoperative guidance with a NIR camera and a hand-held gamma probe. By combining NIR fluorescence and radioactivity in a single tracer, discrepancies between the two imaging modalities used for SLN localization are less likely to occur. In accordance with findings of Brouwer et al., this prospective randomized trial confirmed the better resolution of ICG that can be obtained compared with gamma-tracing modalities enhancing the visual identification of the SLN [32]. This advantage has proved to be especially helpful when $^{99m}$Tc-nanocolloid background signals using the hand-held gamma probe are impeded by the SLN, e.g. in the head and neck region. In these patients fluorescence imaging proved to be the most accurate technology for identification of these SLNs during surgery.

In this study we were also able to demonstrate, regarding reliability and effectiveness, that ICG imaging alone cannot replace $^{99m}$Tc-nanocolloid for detecting SLN in patients in whom the correct lymph node basin is unpredictable. SLN basins were detected percutaneously prior to surgery in 18 patients (90.0 %) in cohort B with the standard technique using $^{99m}$Tc-nanocolloid, but the SLN were detected in only 4 patients (20.0 %) in cohort A by combining the information from ICG and $^{99m}$Tc-nanocolloid.

There were some limitations to this study. Following our own preliminary results and those of other studies, we expected a limited concordance between the two methods because of the challenging identification of deep SLN due to the limited tissue penetration of the fluorescence signal [14, 33, 34]. The expected differences between the cohorts were not reached, so the trial was underpowered because of the small number of patients. In addition, identification of SLN with ICG-$^{99m}$Tc-nanocolloid leads to the removal of a larger number of nodes, with possible side effects. It must be assumed that ICG does not bind completely and permanently to the nanocolloid and therefore free ICG can drain into additional SLN. This also corresponds to the observations of van der Vorst et al. [35] who showed that a fluorescent tracer (indocyanine green adsorbed on human serum albumin) quickly migrates beyond the SLN to higher-tier nodes, which can be additionally stressed by a 2-day protocol. Furthermore, in that study no stratification was performed with respect to the primary tumour and size or depth of the lesion.

The poor preoperative visualization rate of ICG fluorescence could be explained by several factors such as the location of the nodes and less intervening subcutaneous fatty tissue in the preauricular region as compared with SLN in the deep cervical region and, unlike in breast cancer, the primary sites of cutaneous malignancies in the head and neck region vary considerably; thus, the sites of the identified SLNs may also vary considerably [36]. At the operative site, on the other

### Table 2 Detection rates in the two cohorts at different time points

<table>
<thead>
<tr>
<th></th>
<th>Total ($n=40$)</th>
<th>Cohort A, hybrid nanocolloid ($n=20$)</th>
<th>Cohort B, standard nanocolloid ($n=20$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative percutaneous SLN identification, $n$ (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall identification rate of SLN basin</td>
<td>40/40</td>
<td>20 (100)</td>
<td>18 (90.0)</td>
<td>0.487</td>
</tr>
<tr>
<td>$^{99m}$Tc detection*</td>
<td>36 (90)</td>
<td>18 (90)</td>
<td>18 (90.0)</td>
<td></td>
</tr>
<tr>
<td>Fluorescence detection</td>
<td>4 (10)</td>
<td>4 (20.0)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Intraoperative SLN identification</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Identification rate, $n$ (%)</td>
<td>39/40 (97.5)</td>
<td>20/20 (100)</td>
<td>19/20 (95.0)</td>
<td>1</td>
</tr>
<tr>
<td>Number of SLN detected per patient, mean; median (range)</td>
<td>1.7; 1.5 (0 – 4)</td>
<td>1.8; 1 (1 – 4)</td>
<td>1.65; 2 (0 – 3)</td>
<td>0.93</td>
</tr>
<tr>
<td>Total number of SLN removed</td>
<td>70</td>
<td>36</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Method of intraoperative detection, no. of SLN (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{99m}$Tc</td>
<td>64 (91.3)</td>
<td>30 (83.3)</td>
<td>34 (100)</td>
<td></td>
</tr>
<tr>
<td>Fluorescence</td>
<td>6 (8.7)</td>
<td>36 (100)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Sentinel lymph node histology, $n$ (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>61 (87.1)</td>
<td>33 (91.7)</td>
<td>28 (82.4)</td>
<td>0.288</td>
</tr>
<tr>
<td>Positive</td>
<td>9 (12.9)</td>
<td>3 (8.3)</td>
<td>6 (17.6)</td>
<td></td>
</tr>
<tr>
<td>Patients with positive SLN, $n$ (%)</td>
<td>9/40 (22.5)</td>
<td>3/20 (15.0)</td>
<td>6/20 (30.0)</td>
<td>0.451</td>
</tr>
<tr>
<td>Time for SLNE (min), mean (SD)</td>
<td>40.0 (19.0)</td>
<td>36.0 (15.0)</td>
<td>45.0 (22.0)</td>
<td>0.228</td>
</tr>
</tbody>
</table>
| $^*$ Depends on the lymphoscintigraphy and cutaneous marking
hand, the detection rate of SLNs was 100%. This finding may be one of the reasons why this issue of the preoperative identification of SLNs has not been discussed in relation to breast cancer, in which the node fields are almost fixed in the axilla. We must conclude that the maximum penetration depth of the fluorescence signal is actually limited to 1 – 1.5 cm. Perhaps this can be improved in the future by changes in NIR camera technology or by the use of other fluorescent dyes.

In summary, we consider that colloidal radiotracer (labelled with $^{99m}$Tc) is still of value when performing SLNE for cutaneous malignancies in the head and neck region. ICG alone is not an adequate substitute for $^{99m}$Tc-nanocolloid. The use of ICG together with the NIR camera has significant limitations, such as the challenging identification of deep SLN due to low tissue penetration of the fluorescence signal (1 – 1.5 cm), and the limited ability to perform preoperative lymphatic imaging.

Fig. 1 Patient with a malignant melanoma on the right cheek. a Preoperative lymphoscintigraphy image (2-day protocol, cohort A; red lines head and neck, arrow 1 nose, arrow 2 injection site). b SPECT/CT image in the axial plane of the head showing the injection site (asterisk). c SPECT/CT image in the axial plane of the head showing one SLN (plus symbol). d Preoperative image obtained with the NIR camera of the right cheek showing the ICG signal in the region of the primary tumour. e, f Preoperative SLN detection by ICG (crosses) in the preauricular region. Black marks on the skin were placed as indicated by the detection of $^{99m}$Tc radioactivity (subsequent SLN).

Fig. 2 Patient with a high risk squamous cell carcinoma on the left side of the nose. a SPECT/CT image in the axial plane of the head showing the injection site (asterisk). b SPECT/CT image in the axial plane of the mandibular region with a SLN (plus symbol). c SPECT/CT image in the axial plane of the upper neck with a subsequent SLN (plus symbol). d Preoperative image obtained with the NIR camera of the left mandibular region. A transcutaneous ICG signal cannot be detected. e Intraoperative site with low fluorescence labelling of the SLN. f Intraoperative site with fluorescence labelling of the SLN after further preparation of the fatty tissue.
using for example SPECT/CT for surgical planning. As reported previously, the use of SPECT/CT-aided SLNE compared with SLNE alone in melanoma patients is associated with a higher frequency of metastatic involvement and a higher rate of disease-free survival [11]. Therefore it seems reasonable to combine the qualities of ICG and 99mTc in a hybrid tracer [16, 32, 37]. In this study the hybrid tracer provided several advantages with no significant difference in detection of positive SLN from the use of the standard 99mTc-labelled radiotracer. The 99mTc-labelled colloidal radiotracer and ICG are injected by one physician at the same time and at the same point on the primary tumour area. By combining a fluorescence dye with a radioactive tracer, the accuracy of SLN detection can be further improved. The fluorescent dye allows lymphatic migration to be monitored visually whilst the radioactive signal of the nodes (which may be located too deep to be visible by fluorescence imaging) is detectable by the use of a hand-held gamma probe or gamma camera.

**Conclusion**

The hybrid tracer ICG-99mTc-nanocolloid was used successfully for image-guided SLN biopsy in patients with cutaneous malignancies in the head and neck area. The ICG-99mTc-nanocolloid provides fully integrated preoperative and intraoperative radioactive and NIR fluorescence guidance with no need for an injection immediately before surgery.

**Compliance with ethical standards**

**Conflicts of interest** Dr. Schadendorf reported receiving consultancy fees, having board membership, and receiving lecture fees from GlaxoSmithKline, Novartis, Amgen, Bristol-Myers Squibb, Roche, Genentech, Boehringer Ingelheim and MSD. The other authors declare that they have no conflicts of interest.

**Ethical approval** All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the principles of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

**Statement on the welfare of animals** This article does not describe any studies with animals performed by any of the authors.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

**References**


