Intraoperative Fluorescence Imaging for Sentinel Lymph Node Detection

Prospective Clinical Trial to Compare the Usefulness of Indocyanine Green vs Technetium Tc 99m for Identification of Sentinel Lymph Nodes

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ABSTRACT

Importance
The metastatic status of regional lymph nodes is the most relevant prognostic factor in breast cancer, melanoma, and other solid organ tumors with a lymphatic spread. The current gold standard for detection and targeted excision of the sentinel lymph node is preoperative lymphoscintigraphy with technetium Tc 99m. Because of the worldwide shortage of technetium Tc 99m, physicians are looking for nonradioactive dyes for sentinel lymph node labeling. Based on several retrospective studies, the fluorescent dye indocyanine green is considered a possible alternative to technetium Tc 99m.

Objective
To analyze the feasibility and clinical benefit of intraoperative near infrared fluorescence sentinel lymph node excision (SLNE) compared with standard technetium Tc 99m–guided SLNE using malignant melanoma in which SLNE is firmly established.

Design, Setting, and Participants
Analysis of a prospective clinical trial at the Skin Cancer Center, University Hospital Essen. Eighty patients with malignant melanoma on the trunk or extremities (upper and lower) who were scheduled to undergo SLNE were included in this study from January 1, 2013, to June 27, 2014.

Main Outcomes and Measures
Concordance of preoperative and intraoperative sentinel lymph node detection rates.

Results
During the study period, 80 patients were operated on with an additional intraoperative application of a near infrared fluorescent dye. In these 80 surgical procedures, 147 SLNs were excised. Detection of a technetium Tc 99m–marked SLN before surgery was possible in all cases. Intraoperative visualization of the SLN by indocyanine green before skin incision was successful in only 17 of 80 patients (21%). The number of SLNs identified using the near infrared fluorescence technique in the operative site after skin incision and initial tissue preparation was 141 of 147 (96%).

Conclusions and Relevance
Among patients in whom the lymph node basin cannot be predicted correctly (eg, in cutaneous melanoma on the trunk), the use of indocyanine green for SLN detection is severely limited compared with SLNE using standard technique guided by technetium Tc 99m. Therefore, SLNE with
the use of radiocolloid, followed if possible by single-photon emission computed tomography, remains the gold standard.

**Trial Registration**
German Clinical Trials Register identifier [DRKS00004619](https://drks.de/study/DRKS00004619)

**Introduction**

Sentinel lymph node excision (SLNE) was introduced as a staging procedure for penile carcinoma in 1977 by Cabanas. Since then, SLNE has been performed in various tumors such as malignant melanoma, breast cancer, vulvar cancer, penile cancer, Merkel cell carcinoma, squamous cell carcinoma, sweat gland carcinoma, and colorectal carcinoma. The metastatic status of regional lymph nodes is the most relevant prognostic factor in tumors with a lymphatic spread such as breast cancer, malignant melanoma, gastric and colorectal cancer, prostate cancer, and lung cancer. Investigations have shown that the status of the SLN is an accurate indicator of the status of the second and subsequent-tier nodes. Detection of the SLN has become the standard of care for malignant melanoma, breast cancer, Merkel cell carcinoma, and sweat gland carcinoma in patients with non-clinically detectable metastases. The current gold standard for detection and targeted excision of the SLN is preoperative lymphoscintigraphy with technetium Tc 99m. Using lymphoscintigraphy, the dynamic behavior of radiocolloid in the lymphatic vessels, as well as subsequently the accumulation in the first draining lymph node, can be visualized. The reported false-negative rate is high and ranges between 5.7% and 32.0% for SLNE. However, the prognostic significance of detection of small metastases remains controversial. In addition, clinical application of the SLN concept to other type of cancers (eg, squamous cell carcinoma, gastrointestinal, penile, prostate, and lung) is limited, and the clinical relevance is controversial. One of the main factors preventing its application to other cancer types is the technological challenge in correctly identifying the SLN. Imaging modalities currently used for detection of radioactive agents are limited by their poor spatial resolution in solid tumor types in which the SLN is in proximity to the primary tumor. Furthermore, when blue dyes are used, visualization is not always possible, as is the case for cancer located in internal organs (eg, lung or esophagus) or where tissue penetration is poor.

Nonetheless, sufficient grounds exist to search for alternative methods, not only to improve the SLN detection rate and thus decrease the rate of false-negative SLNs but also to reduce known disadvantages of the use of technetium Tc 99m. The use of technetium Tc 99m can represent a radioactive burden for patients and surgical personnel. A further problem is the shortage of technetium Tc 99m faced by physicians worldwide. The radioisotope technetium Tc 99m can be ordered from only 5 research reactors. Therefore, new nonradioactive dyes must be developed and tested for SLN identification.

A potential dye for SLN labeling and detection is indocyanine green, which is a nonradioactive fluorescent dye of small molecular weight. There have been several studies on the use of indocyanine green fluorescence for SLNE in breast cancer, skin cancer, gastric cancer, colorectal cancer, and non–small cell lung cancer. Although its potential use has been discussed in many reports, the effectiveness and limitations have not been evaluated to date in prospective trials for SLNE. The SLN concept is firmly established in malignant melanoma, and the SLN status is incorporated in the American Joint Committee on Cancer’s staging classification. If a melanoma is localized on the extremities (eg, breast or vulvar cancer), lymphatic drainage is predictable. However,
if a melanoma is localized on the trunk (eg, prostate or colorectal carcinoma), lymphatic drainage is unpredictable. Therefore, we selected malignant melanoma as a tumor entity to analyze the feasibility and potential medical benefit of intraoperative near infrared fluorescence SLN detection with indocyanine green compared with standard technetium Tc 99m–guided SLNE in this prospective study.

**Methods**

**Study Design and Patients**

This prospective clinical trial was approved by the institutional review board at University Hospital Essen (12-4973-BO) and was registered at the German Clinical Trials Register (DRKS00004619). Eighty patients fulfilling the inclusion criteria with stages Ib and II (per the American Joint Committee on Cancer’s 2009 Cancer Staging Manual) melanoma on the trunk or extremities (upper and lower) and who were 18 years or older who were scheduled to undergo SLNE were included in this prospective, nonrandomized study from January 1, 2013, to June 27, 2014. Patients with SLNE in the head and neck area were included in a parallel prospective study.

Based on our preliminary results, we expected limited concordance of 65% between the 2 methods. We anticipated this low concordance mainly because of the challenging identification of deep SLNs due to limited tissue penetration (up to 1-cm depth). We calculated that 121 SLNs needed to be examined to demonstrate concordance of 65% between the 2 methods with 95% power and a type I error (α level) of 5%.

All patients received both technetium Tc 99m and indocyanine green in parallel. Exclusion criteria included age younger than 18 years, pregnancy, lactation, and allergy to iodine or known intolerance to indocyanine green. All patients provided written informed consent to proceed with both lymphoscintigraphy and near infrared fluorescence imaging using indocyanine green. The primary study end point was per lymph node concordance of indocyanine green and technetium Tc 99m. Baseline characteristics are summarized in Table 1.
Table 1. Characteristics of 80 Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (N = 80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>55.51 (17.65)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>57.36</td>
</tr>
<tr>
<td>Median</td>
<td></td>
</tr>
<tr>
<td>Sex, No. (%)</td>
<td>52 (65)</td>
</tr>
<tr>
<td>Male</td>
<td>28 (35)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>27.9 (5.7)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>26.4</td>
</tr>
<tr>
<td>Median</td>
<td></td>
</tr>
<tr>
<td>Range, No. (%)</td>
<td>25 (31)</td>
</tr>
<tr>
<td>18.5-24.9</td>
<td>37 (46)</td>
</tr>
<tr>
<td>25.0-29.9</td>
<td>12 (15)</td>
</tr>
<tr>
<td>30.0-34.9</td>
<td>0</td>
</tr>
<tr>
<td>35.0-39.9</td>
<td>6 (8)</td>
</tr>
<tr>
<td>Tumor depth, mm</td>
<td>2.12 (1.60)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>1.50</td>
</tr>
<tr>
<td>Median</td>
<td></td>
</tr>
<tr>
<td>Ulceration, No. (%)</td>
<td>19 (24)</td>
</tr>
<tr>
<td>Localization of primary, No. (%)</td>
<td>40 (50)</td>
</tr>
<tr>
<td>Trunk</td>
<td>12 (15)</td>
</tr>
<tr>
<td>Upper limb</td>
<td>28 (35)</td>
</tr>
<tr>
<td>Lower limb</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared).

Table 1. Characteristics of 80 Patients

SLN Scintigraphic Technique
Preoperative lymphoscintigraphy was performed according to a 2-day protocol with 80 MBq of technetium Tc 99m nanocolloid. The colloid was injected in a total volume of 0.4 mL in 4 intradermal depots of 0.1 mL as previously described. 17

SLN Excision
Sentinel lymph node excision is performed as a standard procedure by the Department of Dermatology, Venereology and Allergology at University Hospital Essen according to guidelines of the Deutsche Dermatologische Gesellschaft. 18 The procedure has been previously described. 17

Indocyanine Green
Indocyanine green is a member of the chemical group of cyanines, has a dark green color, and is a powderlike solid substance at 25°C. The absorption maximum is 697 nm in water at a concentration of 0.5 g/L and 779 nm at 0.0005 g/L. After intravenous injection, indocyanine green binds almost completely to globulins (preferentially to α-lipoproteins) within 1 to 2 seconds. Its use in detection of SLNs in stomach, 8 breast, 19 20 ovarian, 21 and cutaneous 15 22 malignant neoplasms has been
reported. In malignant melanoma, no prospective comparison has been performed to date between the standard technique with technetium Tc 99m and indocyanine green.

**Intraoperative Near Infrared Fluorescence Imaging**

Intraoperative near infrared imaging was performed using a handheld device (Fluobeam; Fluoptics). This device includes a camera and an integrated near infrared light source intended to image a fluorescent moiety having a maximum absorption between 750 and 800 nm and a maximum emission between 780 and 850 nm. The excitation was provided by a class 1 expanded laser source at 780 nm. The irradiance on the imaging field was 7 mW/cm$^2$. The fluorescence signal is collected by a charge-coupled device through a high-pass filter with a high transmittance for wavelengths exceeding 800 nm. To allow fluorescence navigation under operating room light conditions, a modified surgical headlight with an LED (light-emitting diode) light source (LED DayLite Twin Beam; Designs for Vision) was applied with a filter (low-pass filter that cuts radiation <800 nm) and used during SLNE.

**Histology**

The dissected tissue containing the SLN was placed in 4% formalin solution. Frozen-slide analysis was not performed. The SLN was separated from surrounding adipose tissue, lamellated, and embedded. Subsequent serial sections were made. Conventional staining was performed with hematoxylin-eosin staining, as well as immunohistological staining with antibodies to S-100, MelanA, and human melanoma black 45 for melanoma.\(^{23,24}\)

**Statistical Analysis**

The statistical plan defined the population for assessing concordance as all enrolled patients who received both technetium Tc 99m and indocyanine green and who had at least 1 technetium Tc 99m–marked histologically confirmed lymph node. Drug safety was analyzed in all enrolled patients who received indocyanine green. The primary efficacy end point (per lymph node concordance of indocyanine green and technetium Tc 99m) was defined as (1) the number of preoperative technetium Tc 99m–marked lymph node basins that was detected by indocyanine green divided by the number of technetium Tc 99m–marked lymph node basins, and (2) the number of intraoperative technetium Tc 99m–marked nodes that were detected by indocyanine green divided by the number of technetium Tc 99m–marked lymph nodes. Concordance was tested in a pooled analysis with the alternative hypothesis that indocyanine green would be considered concordant to technetium Tc 99m if 90% of technetium Tc 99m–marked lymph nodes were fluorescent per the above definition. A secondary end point was the per patient concordance rate, defined as the percentage of patients for whom all nodes detected by technetium Tc 99m were detected by indocyanine green. Another secondary end point (reverse concordance) evaluated the proportion of all nodes detected by indocyanine green that were also technetium Tc 99m positive. The proportions of pathologically positive lymph nodes that were technetium Tc 99m positive or fluorescent were compared using the McNemar test. Statistical analysis of the data was performed with a software program (SPSS, version 20; SPSS Inc). The $\chi^2$ test was used to evaluate relationships between categorical variables. Differences were considered significant at $P < .05$.\(^{23,24}\)
Results

Eighty patients with melanoma were enrolled in this comparative trial. The demographic characteristics of the 80 enrolled patients who satisfied the inclusion and exclusion criteria are listed in Table 1. There was a predominance of male patients (65%). All patients underwent the surgical procedure using local anesthesia.

Intraoperative Node Identification

In all patients, one SLN basin was marked before surgery by technetium Tc 99m. Visualization of the SLN basins by indocyanine green before skin incision was clearly observable in only 17 of 80 patients (21%). The initial lymphatic route extending from the primary tumor site was detectable by indocyanine green in 58 patients (73%). After skin incision and initial tissue preparation, the number of SLNs identified was 147 (100%) using technetium Tc 99m and 141 of 147 (96%) using the near infrared fluorescence technique ($P > .05$). Of these, 45 (56%) were in the axilla, 31 (39%) in the groin, 3 (4%) in the popliteal region, and 1 (1%) in the pectoral region (Table 2).

### Table 2. Detection Rate of 147 Sentinel Lymph Nodes (SLNs)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value (n = 147)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of SLNs identified per patient, mean (SD)</td>
<td>1.81 (0.81)</td>
<td>NA</td>
</tr>
<tr>
<td>Transcutaneous detection of SLN basins before surgery</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Technetium Tc 99m, No. (%)</td>
<td>147 (100)</td>
<td></td>
</tr>
<tr>
<td>Near infrared fluorescence, No./total No. (%)</td>
<td>17/80 (21)</td>
<td></td>
</tr>
<tr>
<td>Detection in situ, No. (%)</td>
<td>147 (100)</td>
<td>.16</td>
</tr>
<tr>
<td>Technetium Tc 99m</td>
<td>141 (96)</td>
<td></td>
</tr>
<tr>
<td>Near infrared fluorescence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localization of SLNs, No. (%)</td>
<td>(n = 80)</td>
<td>NA</td>
</tr>
<tr>
<td>Axilla</td>
<td>45 (56)</td>
<td></td>
</tr>
<tr>
<td>Groin</td>
<td>31 (39)</td>
<td></td>
</tr>
<tr>
<td>Pectoral</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>Popliteal</td>
<td>3 (4)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: NA, not applicable.

Concordance of Indocyanine Green and Technetium Tc 99m

The primary study end point was defined as (1) the number of preoperative technetium Tc 99m–marked lymph node basins that was detected by indocyanine green and (2) the number of intraoperative technetium Tc 99m–marked nodes that was detected by indocyanine green. Before surgery, 80 SLN basins (100%) were detected by technetium Tc 99m and 17 SLN basins (21%) by
The rate of preincision per lymph node concordance was not statistically significant ($P > .05$).

There were 147 technetium Tc 99m–marked nodes identified during surgery, and 141 of these (96%) were also fluorescent (Table 3). This rate of per lymph node concordance was statistically significant ($P < .001$), rejecting the null hypothesis of less than 90% concordance.

Table 3. Comparison of the Detection Rates of 2 Techniques at 3 Time Points

<table>
<thead>
<tr>
<th>Variable</th>
<th>Technetium Tc 99m</th>
<th>ICG Fluorescence Transcutaneous Detection Before Surgery</th>
<th>ICG Fluorescence Detection In Situ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with detection of SLN basins, No. (%) (n = 80)</td>
<td>80 (100)</td>
<td>17 (21)</td>
<td>78 (98)</td>
</tr>
<tr>
<td>No. of SLNs identified per patient (n = 147)</td>
<td>147 (100)</td>
<td>29 (20)</td>
<td>141 (96)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>1.81 (0.81)</td>
<td>1.70 (0.23)</td>
<td>1.76 (0.81)</td>
</tr>
<tr>
<td>Median</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Positive SLNs, No./Total No. (%)</td>
<td>27/147 (18)</td>
<td>7/29 (24)</td>
<td>27/141 (19)</td>
</tr>
<tr>
<td>Positive patients, No./Total No. (%)</td>
<td>24/80 (30)</td>
<td>NA</td>
<td>24/78 (31)</td>
</tr>
</tbody>
</table>

Abbreviations: ICG, indocyanine green; NA, not applicable; SLN, sentinel lymph node.

Pathological Findings

Among 80 patients with melanoma who received both indocyanine green and technetium Tc 99m, the SLN positivity rate was 18% (27 of 147). Technetium Tc 99m detected all of these tumor-positive lymph nodes. Indocyanine green identified 27 of 27 positive nodes during surgery. Of 63 SLN basins that were identified before surgery only by technetium Tc 99m and not by indocyanine green, 27% (17 of 63) showed histopathological tumor involvement.

Adverse Events

No adverse events related to the use of indocyanine green occurred in this study. No major surgical complications were noted, and there was no operative fatality. Three of eighty patients (4%) developed a postoperative lymphocele requiring treatment. One postoperative wound infection (1%) was observed.
Discussion

The recent introduction of near infrared tracers (eg, indocyanine green) permits real-time transcutaneous and intraoperative visualization of lymphatic ducts and SLNs (Figure). This could result in reduced operating time and potentially improve localization of the SLN, so that smaller incisions can be made. 25 26 A further advantage of indocyanine green is its small molecular weight of only 774 Da. 27 Indocyanine green binds rapidly to albumin. 28 These indocyanine green complexes (2.6 nm) are significantly smaller compared with the radiotracers in Europe (technetium Tc 99m nanocolloid), North America (technetium Tc 99m sulfide colloid), and Australia (technetium Tc 99m antimony trisulphide colloid). Therefore, it can be assumed that these smaller indocyanine green complexes also flow through partially obliterated lymphatic vessels (inflammation, tumor cells, scarring from previous surgery, etc). 29 30 In several retrospective investigations, indocyanine green identified additional SLNs compared with technetium Tc 99m, indicating that this technique could potentially reduce the false-negative rate in SLNs. 31 In the last 2 years, many studies of indocyanine green–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 32 validated the indocyanine green method by demonstrating that it is statistically noninferior to technetium Tc 99m. Those authors concluded that the indocyanine green method can be used as a reliable and safe alternative to the radiotracer method. It is unknown whether these results could also be transferred to malignant neoplasms in which the correct lymph node basins are unpredictable such as in patients with melanoma. Published results on fluorescent tracers in dermatologic tumors are promising, but these studies 31 33 were retrospectively designed.

Figure. Patient With Malignant Melanoma
A, Malignant melanoma on the right upper extremity. The asterisk shows a scar from the primary excision. B, Preoperative mapping of lymphatic drainage with indocyanine green showing 2 separate lymphatic vessels. The asterisk shows the primary region, and the arrowheads show the end of the visible indocyanine green. C, Preoperative sentinel lymph detection shown on the skin by indocyanine green (arrowheads) and technetium Tc 99m (plus sign). The asterisk shows the scar from the primary excision. D, Preoperative lymphoscintigraphy. The arrowhead and red line show the axilla, with an elevated upper extremity, and the plus sign shows a sentinel lymph node. The black dot shows a downstream lymph node containing technetium Tc 99m. E, Single-photon emission computed tomography in the sagittal plane of the axillary region with a sentinel lymph node (plus sign). F, Intraoperative site of the right axilla with fluorescent dye labeling of the sentinel lymph node (plus sign).
Regarding reliability and effectiveness, we demonstrated herein that intraoperative indocyanine green imaging cannot replace technetium Tc 99m for confirming SLNE in patients in whom the correct lymph node basins are unpredictable. With standard technique using technetium Tc 99m, we detected and excised 147 SLNs in 80 patients with melanoma. In only 21% of patients (17 of 80), the SLN was detectable by indocyanine green before skin incision. In 16 of 17 patients (94%), the SLN was located in the groin, and it was located in the axillary in only 1 patient (6%). This poor preoperative visualization rate can be explained by several factors. First is the more superficial location of the nodes and less intervening subcutaneous fatty tissue in the groin region compared with the axilla region in patients of normal weight. Second, unlike in breast cancer, the primary sites of cutaneous melanoma vary considerably, and the sites of the identified SLNs can also differ. 15 However, the detection rate of SLNs was high (141 of 147 [95%]) at the operative site. This finding may help explain why preoperative identification of SLNs has not been considered in breast cancer, in which the node fields are almost fixed in the axilla. Third, based on our observation, the superficial lymphatic drainage extending from the primary tumor site often alters the tissue level to deeper layers by several centimeters before draining into the SLN. An exact localization is then no longer possible (Figure). We conclude that the maximum penetration depth of the fluorescence signal is actually 1 to 1.5 cm. This may be improved in the future by altering the near infrared camera technology or by using other fluorescent dyes.

A further challenge is obesity, with 46% (37 of 80) of our patients being overweight. As summarized in Table 1, a body mass index of 30.0 or higher was recorded in 23% (18 of 80). From our perspective, we cannot dispense with the use of technetium Tc 99m when performing SLNE for cutaneous melanoma at this time. Indocyanine green is not an adequate substitute for technetium Tc 99m. The use of indocyanine green has limitations, including the challenging identification of deep SLNs because of low tissue penetration (1-1.5 cm) and limited ability to perform preoperative lymphatic imaging such as single-photon emission computed tomography for surgical planning. In 2012, our group demonstrated that among patients with melanoma the use of single-photon emission computed tomography–aided SLNE compared with SLNE alone was associated with the findings of a higher frequency of metastatic involvement and a higher rate of disease-free survival. 17 Therefore, it seems reasonable to combine the 2 qualities of indocyanine green and technetium Tc 99m in a hybrid tracer. 34 35 36 This would offer the advantage that technetium and indocyanine green would not be injected by different personnel at different times and varying points of the primary tumor area. By combining fluorescent dyes with radioactive tracers, the accuracy of SLN detection may be further improved. This is because lymphatic migration can be monitored visually using the fluorescent dye, while the radioactive signal of the nodes (which may be located too deep to be visible via fluorescence imaging) is detectable by the use of a gamma handheld probe or gamma camera.

However, the indocyanine green fluorescence technique also has some advantages. Visualization of lymphatic pathways in real time may facilitate identification of SLNs by surgeons. Therefore, we anticipate future advances in the indocyanine green fluorescence technique. Jain et al 37 found that indocyanine green–based imaging is a powerful form of optical imaging compared with blue dyes. This is in part owing to the use of composite visible and near infrared cameras in the operating
theater, which increases the sensitivity, specificity, and accuracy of SLN detection over conventional dyes.

**Conclusions**

Among patients in whom the number of the lymph node basin cannot be predicted correctly (eg, cutaneous melanoma on the trunk), the use of near infrared fluorescence for SLN detection cannot be substituted for SLNE performed using the standard technique guided by technetium Tc 99m. Therefore, SLNE with the use of technetium Tc 99m radiocolloid, followed if possible by single-photon emission computed tomography, 17 remains the gold standard.

**Article Information**

**Accepted for Publication:** October 22, 2014.

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**Published Online:** May 27, 2015. doi:10.1001/jamasurg.2014.3502.

**Author Contributions:** Drs Stoffels and Klode had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

*Study conception and design:* Stoffels, Klode.

*Acquisition, analysis, or interpretation of data:* Stoffels, Pöppel, Schadendorf, Klode.

*Drafting of the manuscript:* Stoffels, Dissemond, Pöppel, Klode.

*Critical revision of the manuscript for important intellectual content:* Stoffels, Schadendorf, Klode.

*Statistical analysis:* Stoffels, Klode.

*Administrative, technical, or material support:* Stoffels, Pöppel, Klode.

*Study supervision:* Stoffels, Schadendorf, Klode.

**Conflict of Interest Disclosures:** Dr Schadendorf reported receiving consultancy fees, holding board membership, and obtaining lecture honoraria from GlaxoSmithKline, Novartis, Amgen, Bristol-Myers Squibb, Roche, Genentech, and MSD. No other disclosures were reported.
References


