Melanoma and nuclear medicine

Andrés Perissinotti¹, Sergi Vidal-Sicart¹, Omgo Nieweg² & Renato Valdés Olmos^{*,3,4}

Practice points

- Implementing sentinel node biopsy (SNB) lymphoscintigraphy on the basis of dynamic study, as well as early and delayed planar static images, provides a roadmap for the surgeon.
- In addition to lymphoscintigraphy, the more recently introduced SPECT/CT technique has led to an improvement in the detection and anatomical localization of sentinel nodes.
- The American Joint Committee on Cancer and the Union International Contre le Cancer have included SNB in their staging system, as the tumor status of this node is the single most important prognostic factor for patients with intermediate-thickness melanoma.
- In patients with intermediate-thickness melanoma, SNB coupled with immediate complete lymphadenectomy improves survival among patients with a tumor-positive sentinel node.
- In patients with thinner or thicker lesions, SNB can be contemplated for staging and prognostic information.
- Innovative technological developments enable better delineation of the tumor's lymphatic drainage pathway and accurate real-time intraoperative identification of sentinel nodes.
- Routine ¹⁸F-fluorodeoxyglucose (FDG)–PET cannot be recommended for staging patients with positive SNB, but could be justified in patients with palpable lymph nodes before resection due to its potential impact on management decisions.
- FDG–PET is an excellent technique for whole-body staging in patients with advanced-stage melanoma and recurrence.
- With the advent of effective systemic drugs, FDG–PET is emerging as a tool for the assessment of therapeutic response.

SUMMARY Supported by a large body of published work, the contribution of nuclear medicine technologies to the assessment of melanoma has been increasing in recent years. Lymphoscintigraphy-assisted sentinel lymph node biopsy and PET are in continuous evolution with the aid of technological imaging advances, making it possible to fuse functional and anatomic images (e.g., with SPECT/CT, PET/CT and 3D rendering systems). The development of hybrid fluorescent–radioactive tracers that enable high-quality preoperative lymphoscintigraphy and SPECT/CT, and the optimization of modern intraoperative portable imaging technologies, such as free-hand SPECT and portable γ -cameras, are important innovations that have improved sentinel lymph node identification in complex anatomical

¹Nuclear Medicine Department, Hospital Clinic, C/Villarroel 170, 08036 Barcelona, Spain

²Melanoma Institute Australia, 40 Rocklands Road, North Sydney, NSW 2060, Australia

³Nuclear Medicine Department, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

⁴Interventional Molecular Imaging Laboratory & Nuclear Medicine Section, Department of Radiology, Leiden University Medical Hospital, Albinusdreef 2, PO Box 9600, 2300 RC, Leiden, The Netherlands

*Author for correspondence: rvaldes@nki.nl

SUMMARY (CONT.) areas, such as the pelvis and head and neck. Concurrently, ^{18}F -fluorodeoxyglucose–PET has proved its usefulness in the clinical staging and treatment decision-making process, and there is also emerging evidence regarding its utility in the evaluation of therapeutic response. The potential uses of other novel PET radiotracers could open up a new field of use for this technique. In this article, we review the current and future role of nuclear medicine in the management of melanoma.

KEYWORDS

• fluorescence • lymph node biopsy • melanoma • PET/CT • portable imaging technologies • radio-guided surgery

According to the WHO, the global number of new melanoma patients is currently in excess of 130,000 per year [1]. Melanoma is the disease showing the fastest increase in incidence of all cancers. In recent years, nuclear medicine has played a steadily increasing role in the assessment of this widespread malignancy. The initial diagnosis is made using histopathological analysis. The prognosis for a patient depends on the stage of the disease. For early melanomas, simple wide excision is sufficient and there is no need for further staging. Accurate staging is important in cases of a more advanced primary tumor, as this will provide prognostic information and will facilitate the choice of the appropriate treatment [2]. Sentinel node biopsy (SNB) and PET/CT substantially improve the accuracy of staging. In 2001, the American Joint Committee on Cancer (AJCC) and the Union International Contre le Cancer included SNB in their staging classification [3,4]. The tumor status of lymph nodes is the most important prognostic factor in patients with intermediate-thickness melanoma (AJCC stage I or II) [2–3,5]. Other prognostic factors include tumor thickness, presence of ulceration and the recently added mitotic rate [6]. The Clark level of invasion was omitted from the recent AJCC classification [3].

Melanoma can disseminate to lymph nodes and visceral organs, but it also has a peculiar tendency to disseminate through the regional lymph vessels as satellite or in-transit metastases. Accurate assessment of the various dissemination patterns improves the decision-making process, and PET/CT with ^{18}F -fluorodeoxyglucose (FDG–PET/CT) is emerging as a valuable tool in the evaluation of advanced and recurrent disease.

Nuclear medicine techniques are in continuous evolution with the aid of technological imaging advances. Sentinel node localization can sometimes be challenging, especially in patients with high BMI or in complex anatomical areas, such as the pelvis or head and neck. With the aim of finding more efficient methods to improve these techniques, hybrid

fluorescent–radioactive tracers that enable high-quality preoperative lymphoscintigraphy and SPECT/CT have been developed. In addition, the optimization of modern intraoperative portable imaging technologies (PITs), such as free-hand SPECT and portable γ -cameras, are important innovations that have enhanced the sentinel lymph node identification process. Furthermore, the technological advances in PET imaging have enhanced its capability to detect occult metastatic disease, making FDG–PET a useful tool in the clinical staging and treatment decision process. Moreover, there is emerging evidence regarding PET utility in the evaluation of therapeutic response and the potential use of other novel PET radiotracers that could open up a new field of use for this technique.

Nuclear medicine & SNB

• Rationale

The regional lymph nodes are the most common sites of melanoma dissemination [7]. A total of 20% of patients with a melanoma with a Breslow thickness of >1 mm have regional lymph node metastasis. Randomized studies showed no clear advantages when performing prophylactic regional lymphadenectomy [8]. There was a need for a diagnostic technique to identify patients with early lymph node metastases so that they could undergo a node dissection at an early stage. SNB was developed in order to fulfill this role. The procedure is based on the hypothesis of the stepwise dissemination of melanoma through the lymphatic system, and the sentinel node is defined as a node on a direct lymphatic drainage pathway [9].

Morton *et al.* developed the SNB technique as a minimally invasive surgical alternative for nodal staging [10], identifying clinically occult nodal metastasis in the patients who could benefit from complete lymphadenectomy [11]. Although SNB was initially used only in melanoma, its use soon expanded to other types of solid malignancies, and it is now part of the routine staging of various neoplastic diseases [12–16]. A complete regional lymphadenectomy is only

performed if histopathological analysis of the sentinel node reveals metastasis. When this specific lymph node is free of disease, the remaining nodes in the basin may be assumed to be disease free, and these patients can be spared the therapeutic procedure and associated morbidity.

• Results

The sentinel node is found in close to 100% of melanoma patients. The probability of finding metastatic sentinel nodes increases in line with the T stage of the tumor [17]. Long-term postoperative complications occur in 18%, the most notable being slight edema of the leg after SNB in the groin [18]. The false-negative rates (node-positive patients in whom the sentinel node is missed) range up to 38% [5–6,19–20]. The false-negative rates vary with the complexity of the drainage region. In the head and neck and the posterior trunk, they tend to be higher than elsewhere [21]. The risk of nodal recurrence after a negative SNB has been described as 5% or less [22].

Although some authors question whether all microscopic metastases will develop into clinical nodal disease [23], there is an important body of publications supporting the notion that, when left to observation, clinically occult lymph node metastases tend to grow and spread to other nodes and distant sites, decreasing the chances of long-term survival [2,11,24–27].

Interim analysis of the MSLT-1 trial, which examined the impact of SNB in patients with melanoma, showed the important contribution of SNB regarding staging information and prognostic value. Furthermore, a 20% survival benefit was seen in sentinel node-positive patients who underwent immediate lymphadenectomy compared with those in which delayed lymphadenectomy was performed due to nodal recurrence being detected after clinical observation [11]. Despite the fact that the MSLT-1 study did not provide a definitive answer to whether SNB offers a survival advantage, some authors believe that this trial did not have sufficient power to detect the survival benefits of the technique and that greater survival differences may emerge with longer follow-up [27]. The procedure is now considered to be standard care in various guidelines [2–5,11,28–30].

• Indications

SNB should be offered to patients with a clinically localized melanoma and a substantial risk of nodal involvement, depending on patient factors

and the histopathologic characteristics of the primary lesion [3,29,31]. In cases where the primary lesion thickness is ≤ 0.75 mm, SNB is generally not recommended, as the risk of metastasis in the lymph nodes that are draining the melanoma is approximately 1%. When thickness ranges from 0.75 to 1 mm, some authors systematically recommend SNB, since the risk of regional lymph node metastases is approximately 5% [32], while others reserve it for patients with primary lesion ulcerations or high mitotic rates [3,33], in whom the risk of nodal metastasis exceeds 10% [3,6]. The need for staging and prognostic information in this type of patient is a valid indication. SNB should be offered to all patients with primary melanoma lesion thicknesses of 1–4 mm, as the risk of nodal metastasis increases from 8 to 30% in these patients, and early treatment improves their survival rates [34]. Patients with melanoma lesions thicker than 4 mm have a higher risk of distant metastasis and the risk of nodal metastasis is approximately 40%; however, metastatic lymph nodes are usually not clinically palpable, so SNB can provide important prognostic information and prevent regional recurrence [35].

• Technique

SNB is a multidisciplinary procedure that requires close cooperation between nuclear medicine physicians, surgeons and pathologists for accurate lymphatic mapping, excision and histopathological analysis [21]. In order to determine which lymph nodes are to be classified as sentinel nodes, the injection of a tracer and a subsequent pre- or intra-operative protocol of sentinel node identification are needed.

• The ‘open & see’ approach

The ‘open and see’ approach was used in the early days of this field based on the application of vital dyes, as presurgical imaging of the lymphatic drainage was still not incorporated into the procedure. This approach, which was described by Morton, together with the introduction of the modern sentinel node concept [10], recommended the search of sentinel nodes in the expected areas of lymphatic drainage. After skin incision, surgeons identified blue-stained lymphatic ducts and lymph nodes draining directly from the tumor site.

Blue dyes

Isosulfan Blue (LymphazurinTM, Covidien, Dublin, Ireland) and Patent Blue V, two

chemically related triphenylmethane dyes, have traditionally been the dyes of choice for SNB. The blue dye is injected into the dermis 10–20 min prior to surgical exploration in a volume of 0.5–1 ml around the primary tumor or scar. It travels through the lymphatic vessels and into the sentinel nodes without being trapped. The washout time is approximately 45 min [36]. The blue lymph vessel is identified in the nodal basin and dissected downstream of the sentinel node [29,37]. The main disadvantage of this tracer is that it does not allow preoperative lymphoscintigraphy. As blue dye is not visible from the skin surface, today it is mainly combined with radiocolloid tracers.

Fluorescent tracers

The recent introduction of near-infrared (NIR) tracers permits real-time transcutaneous and intraoperative visualization (up to depths of 1 cm) of lymphatic channels and subsequent detection of the sentinel nodes, potentially reducing surgery time and improving the localization of the lymphatic nodes so that a smaller incision can be made [38,39]. This type of tracer mimics the behavior of colloids: after injection, the tracer travels via the lymphatic vessels and is then incorporated into macrophages in the nodes by phagocytosis. Indocyanine Green (ICG; Pulsion Medical System SE, Germany) and fluorescein are the most commonly used fluorescent tracers for SNB in a wide range of cancers, such as breast, colon or gynecological tumors [38,40–43].

NIR fluorescent light emitted from the fluorescent tracer is essentially invisible to the human eye, and so special intraoperative imaging devices are required in order to excite the NIR tracer in the lymphatic system, collect the subsequently emitted photons and project the acquired information onto a screen, together with the video image of the surgical field [39–40,44]. The majority of clinical studies published so far have used the Photodynamic Eye™ (Hamamatsu Photonics, Japan) for this, but there is a wide range of other systems available as well, although not all of these can fuse the white light from the fluorescence camera with the color video images.

The published results on fluorescent tracers in dermatologic tumors are promising [45–47], although the use of this type of tracer has limitations, such as the need to perform part of the surgery in a low-light environment, the challenging identification of deep sentinel nodes

due to limited tissue penetration, a short diagnostic window due to the diffusion of the agent and the limited ability to perform preoperative lymphatic imaging for surgical planning.

• The ‘see & open’ approach

The introduction of radiotracers in the SNB technique enabled visualization of the physiological process of lymph drainage using γ -cameras. Based on the contribution of lymphoscintigraphy, the original ‘open and see’ paradigm evolved into a ‘see and open’ approach, because this enabled the visualization of the tumor’s lymphatic drainage pattern, facilitating the planning of the operation. Where possible, lymphatic mapping should be carried out before wide local excision of the primary melanoma, as excision disrupts lymph drainage pathways and causes the tracer fluids to travel to other lymph nodes that do not receive drainage from the tumor [48].

Radioactive tracers

The radiopharmaceuticals used for lymphatic mapping are mainly ^{99m}Tc -colloids that are phagocytized by lymph node macrophages. The choice of radiopharmaceutical varies according to geographic region and is usually guided by local legislation [29]. ^{99m}Tc -human serum albumin colloid is a common radiotracer in Europe, while ^{99m}Tc -sulfide colloid is common in North America and ^{99m}Tc -antimony trisulfide colloid in common Australia [32].

The radiopharmaceutical is injected intradermally around the primary lesion or the biopsy wound (two to four injections). Early dynamic imaging is recommended, as it enables the visualization of the radiotracer’s transit within the lymphatic channels and its accumulation in the sentinel nodes. Subsequently, early and late planar static images of all potential receiving sites are acquired for 3–5 min. During data acquisition, a ^{57}Co or ^{99m}Tc flood source or point source can be used to delineate the body contours. Finally, the location of each sentinel node is indicated on the skin using a pointer and an indelible ink marker [29].

Handheld γ -probe

The γ -ray detection probe collects the radioactive rays emitted by the radiocolloid tracer. The count rate is usually presented as an audible pitch or by volume variation, along with the visual display, adding an acoustic element to the ‘open and see’ approach. The handheld

probe contains a radiation detector surrounded by metal shielding as collimation in order to give a restricted field of view, which provides direction and precise spatial discrimination between the different hotspots [29].

Together with the information provided by the lymphoscintigrams and the skin mark, the ability of a handheld γ -probe to detect sentinel node radiation activity from the skin surface allows the surgeon to precisely place the incision. The probe is used to guide the dissection until the radioactive sentinel node is identified. The *ex vivo* count rate activity of the sentinel nodes is documented and the surgical bed is examined in order to confirm complete removal of all sentinel nodes. Although some authors believe that node resection is recommended until residual surgical bed activity is less than 10% of the hottest sentinel node [49], this statement is not meant to be taken as a rigid rule when an adequate use of preoperative lymphoscintigraphy and, eventually, SPECT/CT is applied for the identification of sentinel nodes. Virtually all metastatic nodes can be identified if the sentinel node concept is clearly understood and a strict technique is followed [37]. Mastering this technique involves a learning curve and is operator dependent.

SPECT/CT anatomic & functional imaging fusion

Localization of sentinel nodes on conventional planar lymphoscintigrams is sometimes challenging, especially when lymphatic drainage is ambiguous or the sentinel nodes are deep under the surface or close to the injection site. γ -cameras incorporating SPECT/CT can fuse the 3D functional SPECT images with the anatomical data from CT [50]. These devices enhance the 'see and open' approach, offering scatter- and attenuation-corrected nuclear images with a superior contrast and higher resolution. They improve anatomical localization and can modify the assessment of planar images in terms of number and localization of sentinel nodes. Moreover, SPECT/CT is able to discriminate closely placed nodes that can be depicted as a single hot node on planar images. The additional value of SPECT/CT has been proved in other neoplastic diseases, such as breast cancer, head and neck malignancies, gynecological tumors and prostate cancer [50–57]. This multimodal imaging technique can distinguish between lymph nodes that are close to one

another and sentinel nodes in close proximity to the injection site, where scatter activity often makes visualization difficult. SPECT/CT also facilitates the recognition of non-nodal tracer accumulation and helps to clarify the drainage pattern in case of inconclusive conventional images (e.g., nonvisualization or unclear location of the nodes). SPECT/CT adds valuable information when conventional planar images cannot distinguish an interval sentinel node from a lymphangioma, lymphatic lake or skin contamination [50–51,55,58–61].

Alvarez Paez *et al.* and The Netherlands Cancer Institute group concluded that SPECT/CT hybrid fused images add clinically relevant data in 50% of patients and visualize additional sentinel nodes in 23% of patients with a melanoma of the trunk [58]. The presence of metastases in sentinel nodes that are depicted only by SPECT/CT changed the surgical approach in 35% of such patients [58]. Stoffels *et al.* demonstrated that SPECT/CT improved the identification of sentinel nodes in the head and neck area and in overweight patients with melanoma compared with only planar images [62]. Furthermore, this study showed that the use of SPECT/CT-aided SNB compared with SNB alone was associated with a higher frequency of metastatic involvement and a higher rate of disease-free survival.

SPECT/CT images allow for the correlation of hotspots with anatomic structures, which facilitates the appraisal of the number of sentinel nodes and their size. Using volumetric rendering techniques in 3D vision, different colors are assigned to different anatomical structures, providing better anatomical landmarks and schematic information about the sentinel node site. This not only makes lymphoscintigrams easier to read, but also helps with the correct interpretation of complex planar images and with planning the location and size of the surgical incision, so as to facilitate the sentinel node resection [58].

In spite of all these virtues of SPECT/CT, this modality should be considered as complementary to planar lymphoscintigraphy and of particular value in difficult cases. SPECT alone cannot replace the vital information obtained during dynamic lymphoscintigraphy. The identification of the individual lymphatic collectors reaching the sentinel node on such images is an accurate method of distinguishing a sentinel node from a radioactive second-tier node [61].

• **The 'see, open & see again' approach**

The technological imaging advances in modern PITs and the development of new hybrid radio-active–fluorescent tracers has led to a gradual transformation of the 'see and open' paradigm into a 'see, open and see again' approach. Principally in complex anatomical areas or in cases of malignancies with deep lymphatic drainage, the role of blue dye 'to see again' during the surgical act has been replaced with the use of PIT devices and hybrid tracers.

The combination of SPECT/CT with PIT enhances the reliability of the γ -probe, especially in complex cases, such as difficult lymphatic drainage areas or sentinel nodes close to the injection site. These devices need to satisfy certain conditions if they are to be useful during the operation. They should have a stable design, be portable, be small enough for handling in the operation room, provide an adequate spatial resolution, allow fast real-time imaging generation and also offer the possibility of changing and

adjusting their position in order to acquire special angle projections.

Portable γ -cameras

Most current systems consist of an articulated arm that holds 2D arrays of scintillation crystals or, more recently, solid-state semiconductor photodetectors connected to photomultipliers and equipped with collimators that allow for accurate detection and visual representation of the γ -rays emitted from the ^{99m}Tc -colloids. Some available equipment can also distinguish between two different energy peaks and display these separately on the screen, enabling the additional feature of using ^{153}Gd , ^{133}Ba or ^{125}I external pointers. Systems such as eZ-SCOPE (Anzai Medical, Japan), Sentinella S102 (Oncovision-GEM Imaging, Spain) and LumaGEM® (Gamma Medica Ideas, CA, USA) are especially useful when presurgical lymphoscintigraphy is not available and if presurgical evaluation indicates a problematic case [63,64]. In the operating room,

Figure 1. Patient with a melanoma in the right lumbar region. (A) Delayed (2 h) planar image showing drainage to both sides of the groin. **(B)** Hybrid portable γ -camera equipped with an optical device. **(C)** Fused image obtained with the hybrid γ -camera showing the same distribution as in **(A)**, but with an anatomical overlay. **(D)** Sentinel node excised from the right groin, showing a black metastatic deposit.

the detector arm of the portable γ -camera can be placed above the previously marked skin locations with the aid of a cross-shaped laser beam projected onto the patient's skin and displayed on the equipment screen. The external pointer can be used as a spatial reference. The matching of these two signals shows the exact location of a sentinel node. An example is shown in [Figure 1](#).

Free-hand SPECT

An innovative device, declipse®SPECT (SurgicEye, Germany), combines the readings supplied by the handheld γ -probe in counts per second with the spatial movement of the probe provided by a tracking system. The positioning system, based on infrared technology, tracks two fixed, detachable pointing devices: one placed on the patient's body and the other on the probe. Subsequently, all data are passed onto

a reconstruction algorithm that generates a 3D image coregistered with the image of the surgical field captured by a video camera. The surgical team can easily check the location and depth of the scanned radiolabeled tissues on the screen. This novel piece of equipment is able to generate intraoperative 3D images and also offers the possibility of loading and fusing the images in real time with previously acquired SPECT/CT studies. All of this information can be visually superimposed onto a patient's body following the concept of mixed reality with incorporated augmented reality devices. The use of this type of device can ameliorate sentinel node localization and resection and can extend the potential use of radio-guided surgery to other fields that are distinct from SNB technique, such as parathyroid resection and non-palpable lesion localization [65,66]. An example of this type of device is shown in [Figure 2](#).

Figure 2. Detection of sentinel nodes using free-hand SPECT navigation. (A) Planar lymphoscintigraphy in a patient with a right malar melanoma showing two sentinel nodes in the cervical area. **(B)** 3D volume-rendered image based on SPECT/CT data showing these sentinel nodes to be in cervical levels Ib and IIa. **(C)** Intraoperative free-hand SPECT navigation. **(D)** Excision of one of the two sentinel nodes.

Figure 3. Nanocolloid and Indocyanine Green hybrid radiotracer approach. (A & B) After the addition of Indocyanine Green to nanocolloid, different doses are prepared for administration in a similar way to the radiotracer-only approach. (C) Preoperative lymphoscintigraphy is performed as with conventional radiotracer-only approaches. (D) Intraoperative imaging with a dedicated fluorescence camera shows a high-resolution image of a sentinel node on the screen.

Hybrid tracers

Radiolabeled nanocolloids have been combined with ICG (ICG- ^{99m}Tc -nanocolloid; this is obtained by adding ICG to the product Nanocoll. Nanocoll® is manufactured by GE Healthcare, Eindhoven, The Netherlands) in order to aid in the identification of deep sentinel nodes, such as pelvic nodes from prostate cancer, or nodes in complex locations, such as the neck. Bimodal imaging agents that are both radioactive and fluorescent provide the best of both worlds, overcoming the limitations of the fluorescent tracers by allowing simultaneous standard radio-guided detection [67]. Some studies have confirmed that hybrid tracers can reproduce the same lymphatic drainage patterns as ^{99m}Tc -colloid radioactive tracers, enabling the combined use of lymphoscintigraphic presurgical

planning and intraoperative NIR optical detection (Figure 3) [66,68–71]. This approach could be of especial interest in complex cases where sentinel nodes are located in close proximity to the injection site, such as in the head and neck region. While the radioactive component of the hybrid tracer enables preoperative lymphoscintigraphy, with SPECT/CT if necessary, the fluorescent component is not hampered by the background signal coming from the injection site, enabling visual identification of the sentinel node, which stands out among a cluster of lymph nodes and the surrounding structures [72].

Although excellent results have been described using blue dyes or a radioactive tracer alone, it is preferable to use a combination of available detection techniques [28]. The recent addition of the fluorescent–radioactive hybrid tracers to

the nuclear medicine repertoire could refine the SNB technique in melanoma. However, further regulatory approval and financial evaluation in hospitals are needed before these hybrid tracers can become available for general clinical use.

Recently, the guided intraoperative scintigraphic tumor targeting concept was introduced with the aim of encompassing the whole spectrum of basic and advanced nuclear medicine procedures that are needed to provide a surgery-optimizing roadmap (Figure 4) [73]. As lymphatic skin drainage patterns are highly variable [48,74], predicting the region of lymphatic drainage without a prior lymphoscintigraphy can be erroneous in up to 30% of cases [75]. Guided intraoperative scintigraphic tumor targeting techniques offer excellent accuracy and reproducibility of lymphatic mapping [76,77] and serve several purposes: to point out the draining lymph node field that is at risk for metastatic disease; to indicate

the number of sentinel nodes; to help distinguish first-tier nodes from secondary nodes; to detect sentinel nodes in unpredictable locations [48,74]; to mark the location of the sentinel node on the skin; and to facilitate intraoperative identification and resection (Figure 5).

Although often the case, the sentinel node is not necessarily the most active or the nearest node [9,10]. A precise characterization of the lymphatic tumor drainage is mandatory, as sometimes sentinel nodes in unexpected areas of drainage (also known as in-transit or aberrant nodes) are the only metastatic nodes. The incidence rate of metastatic in-transit nodes has been reported to be up to 19% [59,78–79].

The combined use of multiple portable devices, such as γ -probes, γ -cameras and NIR cameras, means that surgical exploration can be guided by fused real-time images. These technological advances complement the ‘see, open and

Figure 4. Patient with a melanoma on the dorsum of the right foot. (A) The planar posterior view shows two lymphatic channels. One goes up to the groin and the other to the popliteal fossa where two nodes are depicted, while there is a hint of a third node downstream. **(B)** The 3D volume-rendered reconstructed image provides the anatomical habitat of the two nodes and confirms the presence of the faint third node. **(C)** The intraoperative image with a portable γ -camera shows the first two popliteal nodes. SPECT/CT is a very useful tool for planning the surgical approach. **(D)** In this case, a very deep popliteal node is demonstrated in the axial slice, requiring the surgeon to make a long incision.

Figure 5. Sentinel nodes in unexpected areas of lymphatic drainage. (A) Anterior planar image of a patient with a melanoma in the right flank showing not only drainage to the right groin, but also to the medial area of the trunk (arrow). Note that the sentinel node is clearly defined on (B & C) 3D imaging and (D) transversal SPECT/CT in the paravertebral muscle on the right. (E) In another patient with a melanoma of the back, posterior planar imaging shows drainage to both axillae and the left supraclavicular region (arrow). This sentinel node is anatomically indicated on (F) 3D imaging and transversal (G) SPECT/CT and corresponds on (H) CT with a slightly enlarged node (circle), which contained metastases at histopathology. (I) In another patient with a melanoma of the left underarm, anterior planar imaging shows drainage to the left axilla and to the area of the left elbow (arrow). (J–L) On SPECT/CT, this sentinel node is seen as a dorsal subcutaneous node.

see again' approach in a context of augmented reality, upgrading the concept to an evolved 'see and navigate' approach. A major effort is currently being made to combine all of these features into one hybrid camera that can then present the information via intuitive devices, such as spectacles or other portable screens, which could display virtual elements onto the field of view.

The intraoperative use of systemically administered FDG in combination with γ -detection probe technology has also been proposed and described in the clinical management of selected cases of metastatic melanoma. Although the concept was well received within the surgical community, further development and evaluation of the technique will be needed [80]. A more practical approach is the intralesional administration of a radioactive or hybrid tracer in order to enable resection of isolated melanoma metastases (Figure 6).

Irrespective of the chosen method for surgical sentinel node identification, the surgeon should always palpate the nodal basins and search for suspicious nodes that the tracer may not have reached due to blockage by a metastatic tumor.

PET

PET can demonstrate the metabolic changes that precede discernible anatomic alterations. FDG is the most commonly used radiotracer in oncology thanks to its capacity to depict hypermetabolic, glucose-consuming, neoplastic lesions. Melanoma lesions are highly avid for FDG and the use of FDG–PET/CT is appropriate in selected cases. In this section, the role of FDG–PET/CT will be discussed, as well as the development of novel PET tracers that offer new potential applications in this technique.

- **FDG–PET/CT in early-stage melanoma**

The vast majority of patients with clinically localized melanomas have no metastases, and if they do, these are typically of minimal size. In contrast to the aforementioned SNB, which benefits from microscopic examination, the yield of FDG–PET/CT in such patients is hampered by its limited spatial resolution [81,82]. The lack of value of FDG–PET/CT for metastasis detection in cases of a clinically localized melanoma has been reported [83,84]. Nevertheless, some authors consider this technique to be of some use in individual clinical situations where the risk of metastasis is high (e.g., trunk and upper extremity melanomas, Breslow thickness of >4 mm, presence of ulceration and high mitotic index) [82].

Substantial effort is currently being made in the pursuit of enhancing PET's ability to detect microscopic metastatic disease. Although there has been a considerable spatial resolution increase from the first PET devices, we believe that the role of PET/CT in the staging and surgical management of melanoma will be refined thanks to the development of new PET tracers that could improve the sensitivity and specificity

of the technique. The potential role of specific melanoma radiotracers will be discussed in more detail below.

- **FDG–PET/CT in advanced-stage melanoma**

Excision of the metastases is recommended in stage III melanoma patients. This is also the best option in cases of a limited number of distant metastases. For this reason, accurate identification of all of the sites of metastatic spread is essential before an operation [85]. Some authors state that complete resection of metastases in M1a disease (skin, subcutaneous or distant lymph node metastases) can be achieved in 70–80% of cases [86], and one study showed that over half of patients with stage IV recurrence were candidates for surgical resection [87]. However, there is still some controversy regarding patient selection criteria and the standardization of surgical procedures [88], which could lead to a lower number of patient candidates for metastasectomy in some centers.

FDG–PET/CT has proven to be more sensitive and at least as specific compared with other imaging techniques, such as ultrasound, CT and MRI. Multimodal imaging through the addition

Figure 6. Radio-guided surgery of isolated melanoma metastases. (A) In a melanoma patient, PET/CT showed a solitary ^{18}F -fluorodeoxyglucose-avid lesion in the right breast. (B) After ultrasound-guided injection of a radioactive tracer, the lesion was indicated by (C & D) SPECT/CT and subsequently (E) resected by the surgeon under guidance of a portable γ -ray device.

Figure 7. Widespread disease. (A & B) PET and PET/CT fused images showing extensive ^{18}F -fluorodeoxyglucose deposits throughout the body, especially in the liver and bone. (C) PET/CT offers an overview of the lesions in the 3D reconstruction display.

of CT to FDG–PET has substantially improved the detection of lung metastases, and recent data indicate that PET/CT may be the most accurate test for the diagnosis of bone metastases, surpassing bone scintigraphy [4,89]. Although small liver, lung or brain lesions can sometimes be missed, FDG–PET/CT allows for a whole-body patient evaluation (Figure 7), adding the advantage of detecting incidental secondary tumors [90]. The inclusion of the head and lower extremities in FDG–PET/CT images increases the acquisition time by only 15 min, but Niederkoher *et al.* found no justification for the inclusion of these regions in terms of changes to clinical management for patients with no known or suspected primary or metastatic melanomas in these parts of the body [91].

A systematic review of 28 studies involving 2905 patients suggested that FDG–PET/CT was associated with a disease management change rate of 33% (range: 15–64%) [89]. Although FDG–PET cannot be recommended as a routine investigation for staging patients with positive SNB [92], the technique could be justified in patients with palpable lymph nodes

before resection, due to its potential impact on management decisions [93,94].

• FDG–PET in follow-up & melanoma recurrence

Based on the available evidence, there are not enough data to support the notion that regional control, survival and quality of life improve through surveillance of melanoma patients. Few patients who have metastases are identified by routine imaging studies or blood testing, and even fewer survive because they underwent these tests. It is far more likely that false-positive results will occur, which invariably cause unnecessary concern, lead to additional unnecessary testing and may even result in needless surgery. Therefore, FDG–PET/CT is not a suitable tool for routine follow-up; however, it is an excellent tool for the evaluation of recurrence [93]. In a single whole-body evaluation session, this multimodal imaging technique can provide assessment of the extent of disease with a higher sensitivity than conventional imaging [95]. FDG–PET/CT can be justified in a specific subset of patients where valuable additional information

can influence management decisions, namely with regards to confirming suspicious lesions that have been spotted by other imaging studies in patients suspected or at high risk of nodal or distant metastatic disease, in patients coursing with increased serum S-100B during follow-up [96] and for possible restaging of patients prior to surgical metastasectomy [82,97].

• FDG–PET/CT in the assessment of therapeutic response

Based on the concept that FDG uptake is directly related to viable tumor cell numbers, a reduction in FDG activity should reflect the rate of destruction of tumor cells. However, it should be remembered that the inflammatory reaction associated with tumor necrosis could sometimes increase the standard uptake value. A mean reduction in standard uptake value is to be expected after effective chemotherapy in responding patients, with no significant uptake reduction changes in nonresponders and uptake increments when disease progression [98]. With the definition of the Positron Emission Tomography Response Criteria in Solid Tumors (PERCIST) [99], the value of FDG–PET/CT for the assessment of therapeutic response has seen an increase.

Melanoma is relatively insensitive to chemotherapy. Only the high doses that can be administered during isolated regional perfusion produce substantial remissions. PET has proven to be successful in the monitoring of the effect of such therapy, although minimal remaining tumor nests cannot be discerned [100]. It has not been until recently that two forms of effective systemic therapy have become available. Targeted therapy with vemurafenib induces a response in half of the patients who have the required BRAF mutation [101]. Responses occur very quickly, usually within 2 weeks. Unfortunately, most patients recur after an average of 6 months. Immunotherapy with ipilimumab works more slowly and has a response rate of 20%, but responses last much longer than with vemurafenib [102]. Both drugs have substantial morbidity. Developments in targeted therapy and immunotherapy have been very quick, and new and even more promising drugs are being introduced. We believe that FDG–PET/CT may have an important role in the evaluation of the response to these innovative treatment regimens. PET could be a suitable tool for identifying responders at an early stage and also

patients who recur, so that those for whom the therapy is not effective anymore can be spared its morbidity [98,103–105]. While early reports are encouraging, not all studies support its use [106], and there is no doubt that a considerable body of additional research will be required.

• Other PET radiotracers

Although FDG is currently the only PET tracer that is approved for clinical practice, it does not provide sufficient sensitivity to identify regional node micrometastases and it is not specific enough to discriminate between a metastatic or inflammatory lymph node. Major efforts are currently being made in order to develop a specific radiotracer with a selective affinity for melanoma tissue that could enhance the technique, particularly in terms of early detection and treatment response assessment. The ideal radiotracer should bind to a distinctive target that is exclusively found in melanoma cells, offer optimal contrast between malignant and normal tissue and should not be limited to centers that are equipped with a cyclotron. For example, ^{18}F -labeled radiotracers have a 110 min half-life that enables their regional distribution to other sites that only have a PET scanning facility.

Melanin pigment is an attractive potential target for melanoma imaging, and several groups have succeeded in synthesizing ^{18}F -labeled probes for melanin-containing tumors, claiming superiority in specificity and target-to-background ratios. These findings suggest potential use in diagnosis, follow-up and treatment monitoring [107–109]. Denoyer *et al.* presented a new ^{18}F -benzamine-based radiotracer that was used in mice to detect regional lymph node metastasis after subcutaneous administration at the primary tumor site [110]. Another focus of investigation is the radiolabelling of α -MSH analogs, compounds that bind to the overexpressed MC1 receptors in human melanoma cells [111–113]. The high specific activity found in these studies shows promise for melanoma diagnosis at an early stage. Maissonial *et al.* recently introduced a novel approach [114]. They successfully synthesized a new $^{18}\text{F}/^{125}\text{I}$ -labeled matched-pair melanin radiotracer that could enable combined PET/SPECT imaging and also targeted radionuclide therapy. Other studies have developed tracers to target the neoangiogenesis and hypoxia phenomena that are associated with melanoma lesions [115,116]. It should be remembered that the literature in this field is still limited, and

most published works concern a preclinical phase. However, the promising results seen so far are encouraging further research in potential clinical applications.

Conclusion

Nuclear medicine has been closely involved in the management of melanoma in recent decades. SNB is now routinely used for lymph node status evaluation in patients with clinically localized melanoma and lymphoscintigraphy with SPECT/CT is a key element in the procedure. The role of nuclear medicine technology in identifying the sentinel node in the operating room is quickly expanding. In patients with more advanced disease, FDG–PET/CT is increasingly being used for staging and therapeutic response assessment. The evolution of nuclear medicine techniques has been one of continuous refinement through ongoing technological advances and the interaction with other medical disciplines. Naturally, there is a need for further improvement. We see the potential for innovative progress, and the preliminary steps are certainly encouraging.

Future perspective

Nuclear medicine has been involved in many aspects of the management of melanoma over the last 20 years. The addition of SPECT/CT to conventional images for preoperative sentinel node visualization has resulted in better disease-free survival for patients. Currently available technologies, such as portable γ -cameras, have been increasingly incorporated into the intraoperative sentinel node procedure. Furthermore,

hybrid tracers combining radioactivity and fluorescence are gaining acceptance as a strategy for identifying sentinel nodes in the operating room in melanomas draining to lymphatic basins in areas of more complex anatomy. In the coming years, the use of these new technologies will refine the process of intraoperative sentinel node search, leading to a gradual transformation of the ancient ‘see and open’ paradigm into a ‘see, open and see again’ approach, due to the opportunity to visualize intraoperative navigation.

Moreover, in advanced-stage melanoma patients, FDG–PET/CT and new target molecules will see an increase in their relevance to staging and therapeutic response assessment. Novel insights into the molecular mechanisms of metastasis are in continuous development, and PET/CT technology offers an unparalleled opportunity to study these mechanisms. Certainly, further improvement is still required; the above considerations highlight the need to combine such techniques in a way that provides a positive impact on management while ensuring high-quality assessment of melanoma patients at reasonable costs.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
• of interest

- WHO. Ultraviolet radiation and the INTERSUN Programme. <http://www.who.int/uv/faq/skincancer/en/index1.html>
- Balch CM, Soong SJ, Gershenwald JE *et al.* Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *J. Clin. Oncol.* 19(16), 3622–3634 (2001).
- Balch CM, Gershenwald JE, Soong S-J *et al.* Final version of 2009 AJCC melanoma staging and classification. *J. Clin. Oncol.* 27(36), 6199–6206 (2009).
- Mohr P, Eggermont AMM, Hauschild A, Buzaid A. Staging of cutaneous melanoma. *Ann. Oncol.* 20(Suppl. 6), vi14–vi21 (2009).
- Testori A, De Salvo GL, Montesco MC *et al.* Clinical considerations on sentinel node biopsy in melanoma from an Italian multicentric study on 1,313 patients (SOLISM-IMI). *Ann. Surg. Oncol.* 16(7), 2018–2027 (2009).
- Kesmodel SB, Karakousis GC, Botbyl JD *et al.* Mitotic rate as a predictor of sentinel lymph node positivity in patients with thin melanomas. *Ann. Surg. Oncol.* 12(6), 449–458 (2005).
- Reintgen D, Cruse CW, Wells K *et al.* The orderly progression of melanoma nodal metastases. *Ann. Surg.* 220(6), 759–767 (1994).
- Balch CM, Soong SJ, Bartolucci AA *et al.* Efficacy of an elective regional lymph node dissection of 1 to 4 mm thick melanomas for patients 60 years of age and younger. *Ann. Surg.* 224(3), 255–263 (1996).
- Nieweg OE, Tanis PJ, Kroon BB. The definition of a sentinel node. *Ann. Surg. Oncol.* 8(6), 538–541 (2001).
- Morton DL, Wen DR, Wong JH *et al.* Technical details of intraoperative lymphatic mapping for early stage melanoma. *Arch. Surg.* 127(4), 392–399 (1992).
- Morton DL, Thompson JF, Cochran AJ *et al.* Sentinel-node biopsy or nodal observation in melanoma. *N. Engl. J. Med.* 355(13), 1307–1317 (2006).

- 12 Giuliano AE, Kirgan DM, Guenther JM, Morton DL. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. *Ann. Surg.* 220(3), 391–398 (1994).
- 13 Broglie MA, Stoeckli SJ. Relevance of sentinel node procedures in head and neck squamous cell carcinoma. *Q. J. Nucl. Med. Mol. Imaging* 55(5), 509–520 (2011).
- 14 De Hullu JA, Doting E, Piers DA *et al.* Sentinel lymph node identification with technetium-99m-labeled nanocolloid in squamous cell cancer of the vulva. *J. Nucl. Med.* 39(8), 1381–1385 (1998).
- 15 Torné A, Pahisa J, Vidal-Sicart S *et al.* Transvaginal ultrasound-guided myometrial injection of radiotracer (TUMIR): a new method for sentinel lymph node detection in endometrial cancer. *Gynecol. Oncol.* 128(1), 88–94 (2013).
- 16 Horenblas S, Jansen L, Meinhardt W, Hoefnagel CA, de Jong D, Nieweg OE. Detection of occult metastasis in squamous cell carcinoma of the penis using a dynamic sentinel node procedure. *J. Urol.* 163(1), 100–104 (2000).
- 17 Rousseau DL, Ross MI, Johnson MM *et al.* Revised American Joint Committee on Cancer staging criteria accurately predict sentinel lymph node positivity in clinically node-negative melanoma patients. *Ann. Surg. Oncol.* 10(5), 569–574 (2003).
- 18 Estourgie SH, Nieweg OE, Valdés Olmos RA, Hoefnagel CA, Kroon BBR. Review and evaluation of sentinel node procedures in 250 melanoma patients with a median follow-up of 6 years. *Ann. Surg. Oncol.* 10(6), 681–688 (2003).
- 19 Van Akkooi ACJ, Voit CA, Verhoef C, Eggermont AMM. New developments in sentinel node staging in melanoma: controversies and alternatives. *Curr. Opin. Oncol.* 22(3), 169–177 (2010).
- 20 Nieweg OE. False-negative sentinel node biopsy. *Ann. Surg. Oncol.* 16(8), 2089–2091 (2009).
- 21 Bagaria S. Sentinel node biopsy in melanoma: technical considerations of the procedure as performed at the John Wayne Cancer Institute. *J. Surg. Oncol.* 101(8), 669–676 (2010).
- 22 Valsecchi ME, Silbermins D, de Rosa N, Wong SL, Lyman GH. Lymphatic mapping and sentinel lymph node biopsy in patients with melanoma: a meta-analysis. *J. Clin. Oncol.* 29(11), 1479–1487 (2011).
- 23 Van Akkooi ACJ, Bouwhuis MG, de Wilt JHW, Kliffen M, Schmitz PIM, Eggermont AMM. Multivariable analysis comparing outcome after sentinel node biopsy or therapeutic lymph node dissection in patients with melanoma. *Br. J. Surg.* 94(10), 1293–1299 (2007).
- 24 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.* 242(3), 302–311 (2005).
- 25 Kretschmer L, Hilgers R, Möhrle M *et al.* Patients with lymphatic metastasis of cutaneous malignant melanoma benefit from sentinel lymphonodectomy and early excision of their nodal disease. *Eur. J. Cancer* 40(2), 212–218 (2004).
- 26 Cascinelli N, Morabito A, Santinami M, MacKie RM, Belli F. Immediate or delayed dissection of regional nodes in patients with melanoma of the trunk: a randomised trial. WHO Melanoma Programme. *Lancet* 351(9105), 793–796 (1998).
- 27 Ross MI, Gershenwald JE. How should we view the results of the Multicenter Selective Lymphadenectomy Trial-1 (MSLT-1)? *Ann. Surg. Oncol.* 15(3), 670–673 (2008).
- 28 Cochran AJ, Balda BR, Starz H *et al.* The Augsburg Consensus. Techniques of lymphatic mapping, sentinel lymphadenectomy, and completion lymphadenectomy in cutaneous malignancies. *Cancer* 89(2), 236–241 (2000).
- 29 Chakera AH, Hesse B, Burak Z *et al.* EANM–EORTC general recommendations for sentinel node diagnostics in melanoma. *Eur. J. Nucl. Med. Mol. Imaging* 36(10), 1713–1742 (2009).
- **Very complete revision and current guideline for sentinel node in Europe.**
- 30 Crippa F, Leutner M, Belli F *et al.* Which kinds of lymph node metastases can FDG PET detect? A clinical study in melanoma. *J. Nucl. Med.* 41(9), 1491–1494 (2000).
- 31 Ross MI. Early-stage melanoma: staging criteria and prognostic modeling. *Clin. Cancer Res.* 12(7 Pt 2), 2312s–2319s (2006).
- 32 Ortega Candil A, Rodríguez Rey C, Carreras Delgado JL. Malignant melanoma. *ISRN Dermatol.* 2012, 308279 (2012).
- 33 Wright BE, Scheri RP, Ye X *et al.* Importance of sentinel lymph node biopsy in patients with thin melanoma. *Arch. Surg.* 143(9), 892–899 (2008).
- 34 Mays MP, Martin RCG, Burton A *et al.* Should all patients with melanoma between 1 and 2 mm Breslow thickness undergo sentinel lymph node biopsy? *Cancer.* 116(6), 1535–1544 (2010).
- 35 Gajdos C, Griffith KA, Wong SL *et al.* Is there a benefit to sentinel lymph node biopsy in patients with T4 melanoma? *Cancer* 115(24), 5752–5760 (2009).
- 36 Rietbergen DD, van den Berg NS, van Leeuwen FW, Valdés Olmos RA. Hybrid techniques for intraoperative sentinel lymph node imaging: early experiences and future prospects. *Imaging Med.* 5(2), 147–159 (2013).
- 37 Nieweg OE, Jansen L, Kroon BB. Technique of lymphatic mapping and sentinel node biopsy for melanoma. *Eur. J. Surg. Oncol.* 24(6), 520–524 (1998).
- 38 Schaafsma BE, Mieog JSD, Hutteman M *et al.* The clinical use of indocyanine green as a near-infrared fluorescent contrast agent for image-guided oncologic surgery. *J. Surg. Oncol.* 104(3), 323–332 (2011).
- **Introduces near-infrared imaging and its application in sentinel node mapping and tumor imaging.**
- 39 Gioux S, Choi H, Frangioni J. Image-guided surgery using invisible near-infrared light: fundamentals of clinical translation. *Mol. Imaging* 9(5), 237–255 (2010).
- 40 Crane LMA, Themelis G, Pleijhuis RG *et al.* Intraoperative multispectral fluorescence imaging for the detection of the sentinel lymph node in cervical cancer: a novel concept. *Mol. Imaging Biol.* 13(5), 1043–1049 (2011).
- 41 Rossi EC, Ivanova A, Boggess JF. Robotically assisted fluorescence-guided lymph node mapping with ICG for gynecologic malignancies: a feasibility study. *Gynecol. Oncol.* 124(1), 78–82 (2012).
- 42 Hirche C, Mohr Z, Kneif S *et al.* Ultrastaging of colon cancer by sentinel node biopsy using fluorescence navigation with indocyanine green. *Int. J. Colorectal Dis.* 27(3), 319–324 (2012).
- 43 Yuasa Y, Seike J, Yoshida T *et al.* Sentinel lymph node biopsy using intraoperative indocyanine green fluorescence imaging navigated with preoperative CT lymphography for superficial esophageal cancer. *Ann. Surg. Oncol.* 19(2), 486–493 (2012).
- 44 Troyan SL, Kianzad V, Gibbs-Strauss SL *et al.* The FLARE intraoperative near-infrared fluorescence imaging system: a first-in-human clinical trial in breast cancer sentinel lymph node mapping. *Ann. Surg. Oncol.* 16(10), 2943–2952 (2009).
- 45 Van der Vorst JR, Schaafsma BE, Verbeek FPR *et al.* Dose optimization for near-infrared fluorescence sentinel lymph node mapping in

- patients with melanoma. *Br. J. Dermatol.* 168(1), 93–98 (2013).
- 46 Stoffels I, von der Stüick H, Boy C *et al.* Indocyanine green fluorescence-guided sentinel lymph node biopsy in dermatology. *J. Dtsch Dermatol. Ges.* 10(1), 51–57 (2012).
- 47 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. Aesthet. Surg.* 62(10), e373–e378 (2009).
- 48 Uren RF, Howman-Giles R, Thompson JF. Patterns of lymphatic drainage from the skin in patients with melanoma. *J. Nucl. Med.* 44(4), 570–582 (2003).
- 49 McMasters KM, Reintgen DS, Ross MI *et al.* Sentinel lymph node biopsy for melanoma: how many radioactive nodes should be removed? *Ann. Surg. Oncol.* 8(3), 192–197 (2001).
- 50 Even-Sapir E, Lerman H, Lievshitz G *et al.* Lymphoscintigraphy for sentinel node mapping using a hybrid SPECT/CT system. *J. Nucl. Med.* 44(9), 1413–1420 (2003).
- 51 Van der Ploeg IMC, Nieweg OE, Kroon BBR *et al.* The yield of SPECT/CT for anatomical lymphatic mapping in patients with breast cancer. *Eur. J. Nucl. Med. Mol. Imaging* 36(6), 903–909 (2009).
- 52 Khafif A, Schneebaum S, Fliss DM *et al.* Lymphoscintigraphy for sentinel node mapping using a hybrid single photon emission CT (SPECT)/CT system in oral cavity squamous cell carcinoma. *Head Neck* 28(10), 874–879 (2006).
- 53 Vidal-Sicart S, Brouwer OR, Valdés-Olmos R. Evaluation of the sentinel lymph node combining SPECT/CT with the planar image and its importance for the surgical act. *Rev. Esp. Med. Nucl.* 30(5), 331–337 (2011).
- 54 Vermeeren L, Meinhardt W, Bex A *et al.* Para-aortic sentinel lymph nodes: toward optimal detection and intraoperative localization using SPECT/CT and intraoperative real-time imaging. *J. Nucl. Med.* 51(3), 376–382 (2010).
- 55 Vermeeren L, Valdés Olmos RA, Meinhardt W *et al.* Value of SPECT/CT for detection and anatomic localization of sentinel lymph nodes before laparoscopic sentinel node lymphadenectomy in prostate carcinoma. *J. Nucl. Med.* 50(6), 865–870 (2009).
- 56 Perissinotti A, Paredes P, Vidal-Sicart S *et al.* Use of SPECT/CT for improved sentinel lymph node localization in endometrial cancer. *Gynecol. Oncol.* 129(1), 42–48 (2013).
- 57 Martínez A, Zerdoud S, Mery E, Bouissou E, Ferron G, Querleu D. Hybrid imaging by SPECT/CT for sentinel lymph node detection in patients with cancer of the uterine cervix. *Gynecol. Oncol.* 119(3), 431–435 (2010).
- 58 Alvarez Paez AM, Brouwer OR, Veenstra HJ *et al.* Decisive role of SPECT/CT in localization of unusual periscapular sentinel nodes in patients with posterior trunk melanoma. *Melanoma Res.* 22(3), 278–283 (2012).
- 59 Ortín-Pérez J, Vidal-Sicart S, Doménech B, Rubí S, Lafuente S, Pons F. In-transit sentinel lymph nodes in malignant melanoma. What is their importance? *Rev. Esp. Med. Nucl.* 27(6), 424–429 (2008).
- 60 Veenstra HJ, Vermeeren L, Olmos RAV, Nieweg OE. The additional value of lymphatic mapping with routine SPECT/CT in unselected patients with clinically localized melanoma. *Ann. Surg. Oncol.* 19(3), 1018–1023 (2012).
- Provides evidence in favour of the routine use of SPECT/CT in addition to conventional lymphoscintigraphy for melanoma patients undergoing lymphatic mapping.
- 61 Uren RF. SPECT/CT lymphoscintigraphy to locate the sentinel lymph node in patients with melanoma. *Ann. Surg. Oncol.* 16(6), 1459–1460 (2009).
- 62 Stoffels I, Boy C, Pöppel T, Kuhn J. Association between sentinel lymph node excision with or without preoperative SPECT/CT and metastatic node detection and disease-free survival in melanoma. *JAMA* 308(10), 1007–1014 (2012).
- High-ranking publication that compares the use of conventional lymphoscintigraphy with SPECT/CT in melanoma patients analysing the incidence of metastatic nodes and disease-free survival.
- 63 Vidal-Sicart S, Paredes P, Zanón G *et al.* Added value of intraoperative real-time imaging in searches for difficult-to-locate sentinel nodes. *J. Nucl. Med.* 51(8), 1219–1225 (2010).
- 64 Stoffels I, Poepel T, Boy C *et al.* Radio-guided surgery: advantages of a new portable γ -camera (Sentinella) for intraoperative real time imaging and detection of sentinel lymph nodes in cutaneous malignancies. *J. Eur. Acad. Dermatol. Venereol.* 26(3), 308–313 (2012).
- Introduces the portable γ -camera as an innovative imaging technique to improve the detection of SLN in cutaneous malignancies and could help to reduce false negative SLN results.
- 65 Wendler T, Hartl A, Lasser T *et al.* Towards intra-operative 3D nuclear imaging: reconstruction of 3D radioactive distributions using tracked gamma probes. *Med. Image Comput. Assist. Interv.* 10(Pt 2), 909–917 (2007).
- Presents the potential benefits to advance standard procedures towards intra-operative 3D nuclear imaging offering a novel approach for localization of functional information to facilitate less invasive, image-guided surgery.
- 66 Brouwer OR, Buckle T, Bunschoten A *et al.* Image navigation as a means to expand the boundaries of fluorescence-guided surgery. *Phys. Med. Biol.* 57(10), 3123–3136 (2012).
- 67 Chin PTK, Buckle T, Aguirre de Miguel A, Meskers SCJ, Janssen RAJ, van Leeuwen FWB. Dual-emissive quantum dots for multispectral intraoperative fluorescence imaging. *Biomaterials* 31(26), 6823–6832 (2010).
- 68 Brouwer OR, Buckle T, Vermeeren L *et al.* Comparing the hybrid fluorescent-radioactive tracer indocyanine green- ^{99m}Tc -nanocolloid with ^{99m}Tc -nanocolloid for sentinel node identification: a validation study using lymphoscintigraphy and SPECT/CT. *J. Nucl. Med.* 53(7), 1034–1040 (2012).
- Validates the hybrid tracer use of indocyanine green- ^{99m}Tc -nanocolloid for sentinel node identification by a comparison study with the conventional ^{99m}Tc -nanocolloid tracer.
- 69 Van der Poel HG, Buckle T, Brouwer OR, Valdés Olmos RA, van Leeuwen FWB. Intraoperative laparoscopic fluorescence guidance to the sentinel lymph node in prostate cancer patients: clinical proof of concept of an integrated functional imaging approach using a multimodal tracer. *Eur. Urol.* 60(4), 826–833 (2011).
- 70 Buckle T, van Leeuwen AC, Chin PTK *et al.* A self-assembled multimodal complex for combined pre- and intraoperative imaging of the sentinel lymph node. *Nanotechnology* 21(35), 355101 (2010).
- 71 Frontado LM, Brouwer OR, van den Berg NS *et al.* Added value of the hybrid tracer indocyanine green- ^{99m}Tc -nanocolloid for sentinel node biopsy in a series of patients with different lymphatic drainage patterns. *Rev. Esp. Med. Nucl. Imagen Mol.* 32(4), 227–233 (2013).
- 72 Brouwer OR, Klop WMC, Buckle T *et al.* Feasibility of sentinel node biopsy in head and

- neck melanoma using a hybrid radioactive and fluorescent tracer. *Ann. Surg. Oncol.* 19(6), 1988–1994 (2012).
- 73 Zaknun JJ, Giammarile F, Olmos RAV, Vidal-Sicart S, Mariani G. Changing paradigms in radio-guided surgery and intraoperative imaging: the GOSTT concept. *Eur. J. Nucl. Med. Mol. Imaging* 39(1), 1–3 (2012).
 - 74 Statius Muller MG, Hennipman FA, van Leeuwen PAM, Pijpers R, Vuylsteke RJ, Meijer S. Unpredictability of lymphatic drainage patterns in melanoma patients. *Eur. J. Nucl. Med. Mol. Imaging* 29(2), 255–261 (2002).
 - 75 Thompson JF, Uren RF. Lymphatic mapping in management of patients with primary cutaneous melanoma. *Lancet Oncol.* 6(11), 877–885 (2005).
 - 76 Vidal M, Vidal-Sicart S, Torrents A *et al.* Accuracy and reproducibility of lymphoscintigraphy for sentinel node detection in patients with cutaneous melanoma. *J. Nucl. Med.* 53(8), 1193–1199 (2012).
 - 77 Rettenbacher L, Koller J, Kässmann H, Holzmannhofer J, Rettenbacher T, Galvan G. Reproducibility of lymphoscintigraphy in cutaneous melanoma: can we accurately detect the sentinel lymph node by expanding the tracer injection distance from the tumor site? *J. Nucl. Med.* 42(3), 424–429 (2001).
 - 78 Vidal-Sicart S, Pons F, Fuentes S *et al.* Is the identification of in-transit sentinel lymph nodes in malignant melanoma patients really necessary? *Eur. J. Nucl. Med. Mol. Imaging* 31(7), 945–949 (2004).
 - 79 Uren RF, Howman-Giles R, Thompson JF *et al.* Interval nodes: the forgotten sentinel nodes in patients with melanoma. *Arch. Surg.* 135(10), 1168–1172 (2000).
 - 80 Povoski SP, Neff RL, Mojzisek CM *et al.* A comprehensive overview of radio-guided surgery using gamma detection probe technology. *World J. Surg. Oncol.* 7, 11 (2009).
 - 81 Jiménez-Requena F, Delgado-Bolton RC, Fernández-Pérez C *et al.* Meta-analysis of the performance of ¹⁸F-FDG PET in cutaneous melanoma. *Eur. J. Nucl. Med. Mol. Imaging* 37(2), 284–300 (2010).
 - 82 Belhocine T, Scott A. Role of nuclear medicine in the management of cutaneous malignant melanoma. *J. Nucl. Med.* 47(6), 957–967 (2006).
 - 83 Yancovitz M, Finelt N, Warycha MA *et al.* Role of radiologic imaging at the time of initial diagnosis of stage T1b–T3b melanoma. *Cancer* 110(5), 1107–1114 (2007).
 - 84 Clark PB, Soo V, Kraas J, Shen P, Levine EA. Futility of fluorodeoxyglucose F 18 positron emission tomography in initial evaluation of patients with T2 to T4 melanoma. *Arch. Surg.* 141(3), 284–288 (2006).
 - 85 Pfannenberger C, Aschoff P, Schanz S *et al.* Prospective comparison of ¹⁸F-fluorodeoxyglucose positron emission tomography/computed tomography and whole-body magnetic resonance imaging in staging of advanced malignant melanoma. *Eur. J. Cancer* 43(3), 557–564 (2007).
 - 86 Ollila DW, Gleisner AL, Hsueh EC. Rationale for complete metastasectomy in patients with stage IV metastatic melanoma. *J. Surg. Oncol.* 104(4), 420–424 (2011).
 - 87 Howard JH, Thompson JF, Mozzillo N *et al.* Metastasectomy for distant metastatic melanoma: analysis of data from the First Multicenter Selective Lymphadenectomy Trial (MSLT-I). *Ann. Surg. Oncol.* 19(8), 2547–2555 (2012).
 - 88 Ollila DW. Complete metastasectomy in patients with stage IV metastatic melanoma. *Lancet Oncol.* 7(11), 919–924 (2006).
 - 89 Krug B, Crott R, Lonneux M, Baurain J-F, Pirson A-S, Vander Borgh T. Role of PET in the initial staging of cutaneous malignant melanoma: systematic review. *Radiology* 249(3), 836–844 (2008).
 - 90 Ishimori T, Patel P, Wahl R. Detection of unexpected additional primary malignancies with PET/CT. *J. Nucl. Med.* 46(5), 752–757 (2005).
 - 91 Niederkoher RD, Rosenberg J, Shabo G, Quon A. Clinical value of including the head and lower extremities in ¹⁸F-FDG PET/CT imaging for patients with malignant melanoma. *Nucl. Med. Commun.* 28(9), 688–695 (2007).
 - 92 Horn J, Sjøstrand H, Lock-Andersen J, Loft A. [PET scanning for malignant melanoma and positive sentinel node diagnostics]. *Ugeskr. Laeger* 172(15), 1126–1130 (2010).
 - 93 Aukema TS, Valdés Olmos RA, Wouters MWJM *et al.* Utility of preoperative ¹⁸F-FDG PET/CT and brain MRI in melanoma patients with palpable lymph node metastases. *Ann. Surg. Oncol.* 17(10), 2773–2778 (2010).
 - 94 Bastiaannet E, Wobbes T, Hoekstra OS *et al.* Prospective comparison of [¹⁸F] fluorodeoxyglucose positron emission tomography and computed tomography in patients with melanoma with palpable lymph node metastases: diagnostic accuracy and impact on treatment. *J. Clin. Oncol.* 27(28), 4774–4780 (2009).
 - 95 Fuster D, Chiang S, Johnson G, Schuchter LM, Zhuang H, Alavi A. Is ¹⁸F-FDG PET more accurate than standard diagnostic procedures in the detection of suspected recurrent melanoma? *J. Nucl. Med.* 45(8), 1323–1327 (2004).
 - 96 Aukema TS, Olmos RAV, Korse CM *et al.* Utility of FDG PET/CT and brain MRI in melanoma patients with increased serum S-100B level during follow-up. *Ann. Surg. Oncol.* 17(6), 1657–1661 (2010).
 - 97 Reinhardt MJ, Joe AY, Jaeger U *et al.* Diagnostic performance of whole body dual modality ¹⁸F-FDG PET/CT imaging for N- and M-staging of malignant melanoma: experience with 250 consecutive patients. *J. Clin. Oncol.* 24(7), 1178–1187 (2006).
 - **Evaluates FDG–PET/CT diagnostic performance for N- and M-staging suggesting its use for wholebody tumor staging in melanoma patients, especially for detection or exclusion of distant metastases.**
 - 98 Engell-Noerregaard L, Hendel HW, Johannesen HH, Alslev L, Svane IM. FDG PET scans as evaluation of clinical response to dendritic cell vaccination in patients with malignant melanoma. *Cancer Immunol. Immunother.* 62(1), 17–25 (2013).
 - 99 Wahl RL, Jacene H, Kasamon Y, Lodge MA. From RECIST to PERCIST: evolving considerations for PET response criteria in solid tumors. *J. Nucl. Med.* 50(Suppl. 1), 122S–150S (2009).
 - 100 Van Ginkel RJ, Kole AC, Nieweg OE *et al.* L-[1-¹¹C]-tyrosine PET to evaluate response to hyperthermic isolated limb perfusion for locally advanced soft-tissue sarcoma and skin cancer. *J. Nucl. Med.* 40(2), 262–267 (1999).
 - 101 Flaherty KT, Puzanov I, Kim KB *et al.* Inhibition of mutated, activated BRAF in metastatic melanoma. *N. Engl. J. Med.* 363(9), 809–819 (2010).
 - 102 Hodi FS, O'Day SJ, McDermott DF *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* 363(8), 711–723 (2010).
 - 103 Strobel K, Skalsky J, Steinert HC *et al.* S-100B and FDG–PET/CT in therapy response assessment of melanoma patients. *Dermatology* 215(3), 192–201 (2007).
 - 104 Beasley GM, Parsons C, Broadwater G *et al.* A Multicenter prospective evaluation of the clinical utility of F-18 FDG–PET/CT in patients with AJCC stage IIIB or IIIC extremity melanoma. *Ann. Surg.* 256(2), 350–356 (2012).

- 105 Kraeber-Bodéré F, Carlier T, Naegelen VM *et al.* Differences in the biologic activity of 2 novel MEK inhibitors revealed by ¹⁸F-FDG PET: analysis of imaging data from 2 Phase I trials. *J. Nucl. Med.* 53(12), 1836–1846 (2012).
- 106 Kruijff S, Bastiaannet E, Speijers MJ, Kobold AC, Brouwers AH, Hoekstra HJ. The value of pre operative S-100B and SUV in clinically stage III melanoma patients undergoing therapeutic lymph node dissection. *Eur. J. Surg. Oncol.* 37(3), 225–232 (2011).
- 107 Aarntzen EH, Srinivas M, De Wilt JH *et al.* Early identification of antigen-specific immune responses *in vivo* by [¹⁸F]-labeled 3'-fluoro-3'-deoxy-thymidine ([¹⁸F]FLT) PET imaging. *Proc. Natl Acad. Sci. USA* 108(45), 18396–18399 (2011).
- 108 Liu H, Liu S, Miao Z *et al.* Development of ¹⁸F-labeled picolinamide probes for PET imaging of malignant melanoma. *J. Med. Chem.* 56(3), 895–901 (2013).
- 109 Rbah-Vidal L, Vidal A, Besse S. Early detection and longitudinal monitoring of experimental primary and disseminated melanoma using ICF01006, a highly promising melanoma PET tracer. *Eur. J. Nucl. Med. Mol. Imaging* 39(9), 1449–1461 (2012).
- 110 Denoyer D, Potdevin T, Roselt P *et al.* Improved detection of regional melanoma metastasis using ¹⁸F-6-fluoro-N-[2-(diethylamino)ethyl] pyridine-3-carboxamide, a melanin-specific PET probe, by perilesional administration. *J. Nucl. Med.* 52(1), 115–122 (2011).
- 111 Cheng Z, Xiong Z, Subbarayan M, Chen X, Gambhir SS. ⁶⁴Cu-labeled alpha-melanocyte-stimulating hormone analog for microPET imaging of melanocortin 1 receptor expression. *Bioconjug. Chem.* 18(3), 765–772 (2007).
- 112 Cantorias M V, Figueroa SD, Quinn TP *et al.* Development of high-specific-activity ⁶⁸Ga-labeled DOTA-rhenium-cyclized alpha-MSH peptide analog to target MC1 receptors overexpressed by melanoma tumors. *Nucl. Med. Biol.* 36(5), 505–513 (2009).
- 113 Ren G, Liu Z, Miao Z *et al.* PET of malignant melanoma using ¹⁸F-labeled metalloptides. *J. Nucl. Med.* 50(11), 1865–1872 (2009).
- 114 Maissonial A, Billaud EMF, Besse S *et al.* Synthesis, radioiodination and *in vivo* screening of novel potent iodinated and fluorinated radiotracers as melanoma imaging and therapeutic probes. *Eur. J. Med. Chem.* 63 840–853 (2013).
- 115 Wei L, Ye Y, Wadas TJ *et al.* ⁶⁴Cu-labeled CB-TE2A and diamsar-conjugated RGD peptide analogs for targeting angiogenesis: comparison of their biological activity. *Nucl. Med. Biol.* 36(3), 277–285 (2009).
- 116 Wyss MT, Honer M, Schubiger PA, Ametamey SM. NanoPET imaging of [¹⁸F] fluoromisonidazole uptake in experimental mouse tumours. *Eur. J. Nucl. Med. Mol. Imaging* 33(3), 311–318 (2006).