HHS Public Access

Author manuscript

Wiley Interdiscip Rev Nanomed Nanobiotechnol. Author manuscript; available in PMC
2017 July 01.

Published in final edited form as:

Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2016 July ; 8(4): 535–553. doi:10.1002/wnan.1380.

Intraoperative mapping of SLN metastases using a clinically-translated ultrasmall silica nanoparticle

Michelle S. Bradbury,

Department of Radiology, Sloan Kettering Institute for Cancer Research, 1275 York Avenue, New York, NY 10065, USA. bradburm@mskcc.org

Mohan Pauliah,

Sloan Kettering Institute for Cancer Research, 1275 York Avenue, New York, NY 10065, USA

Pat Zanzonico,

Sloan Kettering Institute for Cancer Research, 1275 York Avenue, New York, NY 10065, USA

Ulrich Wiesner, and

Department of Materials Science & Engineering, 330 Bard Hall, Cornell University, Ithaca, NY 14853, USA

Snehal Patel

Department of Surgery, Sloan Kettering Institute for Cancer Research, New York, NY 10065, USA

Abstract

The management of regional lymph nodes in patients with melanoma has undergone a significant paradigm shift over the past several decades, transitioning from the use of more aggressive surgical approaches, such as lymph node basin dissection, to the application of minimally invasive sentinel lymph node (SLN) biopsy methods to detect the presence of nodal micrometastases. SLN biopsy has enabled reliable, highly accurate, and low-morbidity staging of regional lymph nodes in early stage melanoma as a means of guiding treatment decisions and improving patient outcomes. The accurate identification and staging of lymph nodes by imaging is an important prognostic factor, identifying those patients for whom the expected benefits of nodal resection outweigh attendant surgical risks. However, currently used standard-of-care technologies for SLN detection are associated with significant limitations. This has fueled the development of clinically promising platforms that can serve as intraoperative visualization tools to aid accurate and specific determination of tumor-bearing lymph nodes, map cancer-promoting biological properties at the cellular/molecular levels, and delineate nodes from adjacent critical structures. Among a number of promising cancer-imaging probes that might facilitate achievement of these ends is a first-in-kind ultrasmall tumor-targeting inorganic (silica) nanoparticle, designed to overcome translational challenges. The rationale driving these considerations and the application of this platform as an

Further Reading/Resources

[Ross MI, Gershenwald JE. Sentinel lymph node biopsy for melanoma: A critical update for dermatologists after two decades of experience. *Clinics in Dermatology* 2013, 31:298–310.

Hadjipanayis CG, Jiang H, Roberts DW, Yang L. Current and Future Clinical Applications for Optical Imaging of Cancer: From Intraoperative Surgical Guidance to Cancer Screening. *Seminars in Oncology* 2011, 38:109–118.]

intraoperative treatment tool for guiding resection of cancerous lymph nodes is discussed and presented within the context of alternative imaging technologies.

Introduction

Micrometastases to regional lymph nodes are known to be a vital prognostic predictor in early-stage melanoma¹. Malignant melanoma is one of the fastest rising cancers in the US. The American Cancer Society estimated that there were 76,100 new melanoma cases diagnosed in 2014 in the US, resulting in 9,710 deaths². In the United States, at present, melanoma ranks as the fifth most common cancer in males and sixth most common cancer in females³. Earlier diagnosis and treatment of melanoma are essential to minimizing morbidity and mortality. Thus, technologies that can reliably identify metastases by sentinel lymph node (SLN) mapping are essential, and have important implications for disease staging, prognosis, and treatment outcomes.

The current technology applied to melanoma utilizes radionuclide-based SLN detection that is often combined with a separate free dye injection during the surgical procedure. This paradigm is associated with a number of drawbacks, and has underscored the need to develop newer-generation, state-of-the-art molecular imaging platforms that can reliably visualize disease with higher sensitivity and specificity. While many classes of cancer-imaging probes have been developed for intraoperative use and are described herein, this review undertakes a detailed exposition of the translation of one such technology as a case study of targeted nodal imaging based on its favourable physicochemical, photophysical, and in vivo biological properties - an ultrasmall, dual-modality, silica nanoparticle. Tuned to sizes enabling renal clearance and enhanced target retention, this particle serves as an effective image-guided tool, offering the surgeon real-time visual intraoperative cues for localizing and treating SLNs harboring micrometastases identified preoperatively by scintigraphic detection. Such tools may lead to the adoption of new standard-of-care minimally-invasive procedures as well as to the use of one or more combined therapeutic options for selectively harvesting and treating melanoma-bearing nodes while reducing surgical risks that typically accompany more extensive nodal dissections.

Conventional Imaging Tools for Assessing Lymphadenopathy and Lymphangiogenesis

Standard imaging techniques

In pre-operative settings, conventional imaging techniques such as CT, MRI, single photon emission computed tomography (SPECT), positron emission tomography (PET), or combinations thereof, are used to screen for abnormally enlarged nodes (CT, MRI) or enhanced metabolic activity (PET-, SPECT-CT) that raise suspicion for metastatic nodal disease. Such assessments are prone to error. There are a number of non-cancerous causes of lymph node enlargement that can result in false-positive findings, including the presence of infectious or inflammatory processes. In addition, small metastases can exist in lymph nodes without enlargement, which thus may not be detected⁴. In addition to the inherent problems of relying on size to determine metastatic status and the relatively low sensitivity of these

approaches, such methods are unable to locate the SLN, and are not able to assess lymphatic flow. Nonetheless, given their widespread availability and ease of use, CT and MRI remain the most commonly used methods used to diagnose lymph node metastases. Finally, the direct mapping of metastatic disease sites identified on pre-operative planning studies (i.e., SPECT-, PET-CT) and its translation into three-dimensional (3D) locations in the intraoperative setting is challenging, and can limit the operating surgeon's ability to detect locoregional nodal disease within an exposed nodal basin. *By utilizing state-of-the-art combined pre-operative-intraoperative imaging paradigms alongside newer multimodality targeted platforms, complementary information may be obtained that improves localization of cancer-bearing nodes while at the same time speeding clinical throughput.*

Tumor Targeting Agents

A variety of tumor targeting agents – both metabolic and/or functional – have been developed with the aim of better characterizing the tumor lymphatics by recognizing specific biomarkers, pathways, or characteristics of the tumor microenvironment in order to maximize target to background signal (i.e., contrast). Coupled with metabolic imaging (i.e., PET-CT) methods, for instance, the use of fluorine-18 labeled 2-deoxy-2-[¹⁸F]fluoro-D-glucose (¹⁸F-FDG), a glucose mimetic, preferentially accumulates at sites of enhanced glycolytic activity (i.e., malignant cells^{5, 6}). This method offers advantages over standard anatomic modalities to diagnose malignancy and stage cancer-bearing nodes adjacent to tumors^{7, 8}, but a detailed discussion is beyond the scope of this overview. It is noteworthy that although different tumor types may demonstrate enhanced glucose metabolism and overexpression of glucose transporters (GLUTs) after ¹⁸F-FDG administration^{5, 9, 10}, this radiotracer is not an optimally sensitive or specific agent¹¹, as increased uptake can be seen in relation to other metabolic processes and these may, in fact, co-exist with dispersed cancerous cells. Furthermore, nodes with low FDG uptake or dimensions less than 1.5 cm may harbor micrometastases not be evident by traditional ¹⁸F-FDG PET-CT. Alternative PET molecular imaging agents may be used under such conditions for detecting nodal metastases and/or tumor recurrence, as has been the case for prostate cancer, in which radiolabeled choline¹², acetate¹³, or PSMA¹⁴ ligands have been administered.

Integrins, trans-membrane proteins that mediate cell-cell adhesion and attachment to the extracellular matrix (ECM)^{15, 16}, have also been intensively investigated as targets for angiogenesis imaging and therapy of various types of tumors; these include, in particular, integrin $\alpha_v\beta_3$, given its role in modulating key cancer-driven events, such as survival, proliferation, and angiogenesis^{16–18}. For clinical detection of nodal metastases^{19, 20}, imaging probes have largely been peptide ligands of integrin $\alpha_v\beta_3$, comprised of arginine-glycine-aspartic acid (RGD), and formulated as tracers. In addition, as described below, a newer-generation ultrasmall silica particle – a first-in-kind cancer-targeted platform and the focus of this review – has also been functionalized with this peptide²¹.

Although various radiolabeled molecular probes have been used to detect lymphatic/nodal metastases in conjunction with dual-modality imaging systems, there remains a critical need to develop novel cancer-targeting visualization tools that combine high sensitivity,

specificity and superior image contrast for improving detection of microscopic nodal tumor burden.

Lymphatic-specific targeting agents

Tumor-induced growth of the lymphatic vasculature, term lymphangiogenesis, along with enhanced expression of lymphangiogenic growth factors, precedes metastatic disease spread to locoregional nodes and is generally associated with a poor prognosis^{22–25}. Studies of lymphatic metastasis via this route have been hampered by the lack of molecular markers that can reliably distinguish lymphatic vessels from tumor-associated blood vessels²⁶. Although a number of lymphatic-specific markers such as podoplanin, Prox-1, LYVE-1, and VEGFR-3 have been previously identified^{26–28}, along with other mediators that promote lymphangiogenesis in tumors and other conditions^{29–31}, these continue to evolve. Targeted lymphatic imaging agents have been tested against specific markers^{32, 33} for mapping metastatic nodes using emerging in vivo imaging technologies³⁴. However, lymphangiogenesis can also occur under inflammatory conditions due to the high levels of lymphangiogenic factors produced by macrophages and granulocytes in inflamed tissue³⁵. Thus, specific differentiation of inflammation- from tumor-induced lymphangiogenesis may not be possible, and may limit the utilization of such technologies in the clinic³⁶.

SLN Biopsy Management of Melanoma: Intraoperative Practice Guidelines

More than 85% of individuals newly diagnosed with cutaneous melanoma have disease clinically localized to the primary site (American Joint Committee on Cancer stage I and II)³⁷. Definitive strategies for the management of the majority of stage I and II patients who have excellent prognoses evaluate both the primary tumor site and nodal beds and include: (1) wide local excision of the primary cutaneous tumor site with adjuvant radiation for specific indications and (2) management of regional lymph node basins, ranging from observation to less invasive SLN mapping procedures or to more invasive lymph node basin dissection. The evaluation of lymph nodes as part of this strategy is based on the fact that regional metastases are typically the first site of relapse in melanoma patients after treatment of the primary tumor. As a key prognostic predictor, metastatic disease in regional nodes has been shown to occur in approximately 20% of patients with intermediate-thickness cutaneous melanomas (i.e., Breslow thickness, 1 to 4 mm) involving any anatomic site^{38, 39}.

At the time of primary diagnosis, a significant percentage of patients with melanoma, predicted by increasing thickness of the primary lesion, ulceration, or other aggressive histologic features of the primary tumor² such as mitotic rate³⁸, harbor occult nodal metastases (i.e., micrometastases) which may not be clinically, radiographically, and/or microscopically detectable. In most patients, these cancerous foci will evolve into palpable (macroscopic) nodal disease if left untreated^{40, 41}, rendering a guarded and less favorable prognosis³⁸. At this stage of clinical nodal involvement, it is generally more difficult to achieve regional disease control and long-term survival following lymph node dissection, as compared with surgical approaches aimed at treating microscopic nodal burden. In the former case, high rates of distant metastatic disease (at least 50%^{2, 39}) and relapse (15–50%^{42–44}) have been found in the treated nodal basin.

As an alternative approach, in principle, if lymph nodes were removed at the stage of microscopic nodal involvement, clinically apparent lymph node disease would not develop, thereby abrogating distant metastatic spread in a significant fraction of patients. Moreover, durable regional disease control could be achieved, along with reductions in recurrence rates that would otherwise occur in the treated basin. Resistance to this approach, however, has focused on the fact that as microscopic nodal disease is infrequently detected at diagnosis, most patients would not benefit, but would simply incur the morbidity of a costly and unnecessary procedure⁴⁵. Furthermore, in prospective randomized trials comparing outcomes of early-stage (i.e., stage I, II) melanoma patients that underwent either lymph node dissection or nodal observation, an overall survival advantage could not be identified^{46–48}.

In response to this controversy, routine lymph node basin dissection is no longer offered to patients with clinically and radiographically uninvolved regional nodes because of the associated morbidity. Lymphatic mapping and SLN biopsy, a less invasive procedure, has instead become standard-of-care for assessment of the status of regional lymph node basins. The status of the SLNs is then used to risk-stratify patients, particularly higher-risk patients with nodal micrometastases (occult or stage III disease)¹, for additional treatment that may consist of formal lymph node dissection and/or systemic therapy feasible only in a clinical-trials setting. Implementation of this strategy ensures that more aggressive surgical approaches (e.g., lymph node dissection) and systemic therapies are used more selectively, with the intent of maximizing functional outcomes of higher-risk patient subsets, in addition to limiting treatment-related morbidity.

SLN Biopsy for Melanoma: Technical Considerations for Lymphatic Mapping

SLN mapping techniques specifically identify the node(s) at highest risk of tumor metastases⁴⁹ by defining afferent lymphatic drainage pathways from the primary tumor site to the regional lymph node basin. The accuracy of the SLN biopsy procedure in determining the presence or absence of nodal micrometastases has been established by early proof-of-concept studies⁴⁵ following intradermal injection of blue dyes (e.g., isosulfan blue, lymphazurin 1%) at the primary tumor site; these dyes are currently used in clinical practice as an *intraoperative* adjunct for localizing SLNs. SLNs accumulate these dyes following peritumoral injection, enabling visualization of a “blue” node within the regional nodal basin and leading to identification rates of 80% to 85%^{49–52}. A primary limitation of this method is its lack of pre-operative SLN localization, thereby necessitating the use of a non-invasive imaging test, such as lymphoscintigraphy. In addition, this is an invasive procedure requiring surgical expertise, and nodes need to be superficially exposed within the operative field of view to be seen after a skin incision close to the site of dye injection.

Significant improvements in SLN identification rates (i.e., about 95%) were found by implementing cutaneous lymphoscintigraphy^{49, 51, 52} (see below); this method relies upon the accumulation of radiocolloid in SLNs after intradermal injection about the primary tumor in order to localize nodes with an intraoperative hand-held detection device^{53, 54}

Utilizing a combined multimodality approach (radiocolloid plus blue dye), SLN identification increased to 99%, as compared to less than 85% with blue dye alone^{53, 55}. In addition to localizing the sentinel node within the formal nodal basin, SLNs outside or proximal to this nodal basin^{56, 57}, termed in transit, interval, or ectopic nodes, may also be identified using preoperative lymphoscintigraphy and an intraoperative gamma detection device. These latter nodes, found with a frequency ranging from 5% to 10%, demonstrate microscopic disease similar to that of SLNs typically harvested from formal nodal basins (e.g., neck)⁵⁸. Failure to identify and detect tumor within these or other SLNs may lead to understaging of the disease, and may serve as potential sources of clinical recurrences^{57, 59, 60}.

Sentinel Lymph Node Mapping: Current Clinical Practice

The identification of lymph node metastases utilizing SLN mapping approaches such as lymphoscintigraphy is a reliable predictor of the presence of tumor. A positive SLN biopsy suggests that metastases are likely to be present in nodes (and/or other tissues) that are remote from the primary site, while a negative biopsy precludes nodal resection and its attendant risks.

Pre-operative standard-of-care SLN mapping techniques, such as lymphoscintigraphy, rely on the uptake of conventional radiotracers such as filtered 99m-technetium (^{99m}Tc) sulfur colloid or newer molecular imaging tracer, such as Lymphoseek (^{99m}Tc-tilmanocept)⁶¹ to yield structural and functional information of lymphatic drainage for preoperative staging of the regional nodal basin in early melanoma. Lymphoscintigraphy is a more versatile method than that used with isosulfan blue dye. The physicochemical properties of the agents employed (e.g., size) largely dictate the efficiency of the procedure. After pre-operative injection of an agent, such as ^{99m}Tc-sulfur colloid, about the primary tumor site, it is taken up by the lymphatic vessels and transported to SLNs (see Fig. 1a). Its anatomic localization within the tumor lymphatics and nodes can be visualized with a gamma camera. Importantly, adequate localization of these agents in regional lymph nodes⁶², typically via resident macrophages, is governed by a number of factors, including patency and function of the tumor lymphatics, integrity of lymph nodes, other biologically driven factors such as phagocytic activity of macrophages^{62, 63} and post-treatment changes involving sites of lymphatic drainage. Using a hand-held intraoperative gamma probe, surgeons may more precisely localize the SLN for excision by measuring radioactive emissions in the region of the draining tumor lymphatics.

The current SLN mapping technique suffers from a number of significant drawbacks. First, it has coarse spatial resolution, and the visualization of the nodal bed may be partially obscured by background signal from radiotracer activity remaining at the injection site⁶⁴, precluding direct mapping of locoregional nodal distributions. In approximately 10% of cases, there was a failure to identify any drainage pattern or to localize small (i.e., 4–5 mm in size) nodes within the head and neck, an anatomically complex region⁶⁵. Second, there is no real-time or detailed anatomic visualization of nodes and lymphatic channels within the operative field. Although the SLN may be radioactive, the operating surgeon principally depends on an abnormal visual appearance and palpation to reliably discriminate it from the

other adjoining tissues. Third, the foregoing classes of agents are not tumor-specific. Rather, these tracers identify SLNs, *whether they are cancer-bearing or not*, presumably via the phagocytic activity of macrophages. Finally, the risk of injury to adjacent vital soft tissues (i.e., neurovascular structures) may occur, and subsequently alter normal function, such as speech and swallowing, as well as cosmetic appearance. These limitations have hampered intraoperative melanoma staging, particularly in the head and neck, given unpredictable patterns of metastatic disease spread, difficult-to-detect nodes, and differentiation of small nodes from vital structures. *The foregoing limitations associated with standard-of-care SLN mapping techniques have spurred innovative preoperative and/or intraoperative lymphatic imaging strategies that incorporate novel targeted probes, multimodality imaging tools, and portable intraoperative device technologies for localizing and harvesting SLNs.*

Optical Image-Guided Surgical Navigation for SLN mapping

A significant volume of intraoperative work is based on utilizing near-infrared (NIR) fluorescent dyes or dye-incorporated agents^{66–68}, including indocyanine green (ICG), a commonly used FDA-approved fluorophore for selected clinical applications⁶⁹, for imaging lymphatic flow and SLNs^{70–72}. These NIR dyes offer improved depth resolution and target-to-background ratios over dyes with emissions below the NIR wavelength range of 650–900 nm. Optical imaging tools are relatively inexpensive, easy to employ, do not use ionizing radiation, and can be used for real-time fluorescence multiplexed fluorescence detection of different nodal basins^{73–75} after administering multiple color NIR dyes or dye-containing nanoformulations. More specifically, as NIR dyes or dye-containing platforms can each be conjugated to a different targeting ligands, the expectation would be that multiple cancer markers could be detected simultaneously by a multi-channel fluorescence camera system. This may lead to increased sensitivity and specificity of human tumor cell detection by such optically-driven approaches. Thus, real-time optical tools to stage and treat cancer, evaluate therapeutic response, and provide functional tissue assessments offer many important benefits, and may potentially lead to a paradigm shift in the treatment of cancer.

However, intraoperative optical imaging approaches have traditionally been hampered by a number of limitations⁷⁶, including (1) the small number of imaging agents available with emissions in the near-infrared (NIR) range, (2) high background autofluorescence that restricts useful imaging penetration depths and overall detection sensitivity, (3) large spectral overlap among optical agents that can result in destructive spectral interference and prevent concurrent detection of multiple targets (i.e., multispectral imaging applications)⁷⁷, and (4) rapid photobleaching that reduces brightness and imaging duration. Moreover, the lack of tumor-selective targeting found with these agents limits their utility for many applications aimed at specific detection of cancer-bearing tissues.

However, significant progress continues to be made on a number of fronts. Fueled by the emergence of an increasing number of new, diverse, and clinically promising NIR fluorescence-based probes, including particle-based agents, that can enhance soft tissue contrast, detection sensitivity, and depth penetration, some of these key drawbacks are being addressed. These probes require an intraoperative optical imaging system with clinical-grade accuracy. A number of tumor-targeted molecular products, including dye-bound antibodies

and peptides, are enhancing intraoperative visualization during image-guided procedures to improve examination of tumor borders or localize tumor deposits by binding to upregulated cancer receptors^{78–80}. Although not yet reaching full potential in surgical practice, potential benefits of optical imaging have been already been demonstrated in clinical studies utilizing targeted molecular probes^{66, 81}.

Newer generation intraoperative imaging probe technologies for SLN biopsy

A number of newer-generation molecular and particle-based agents, including non-targeted activatable^{82–84} and targeted organic fluorophores^{85–87}, gadolinium-labeled dendrimers^{88, 89}, ultrasound microbubbles^{90, 91}, liposomes^{92–94}, and other nanocarriers^{95–98} as well as macromolecular probes^{77, 99} have been developed for imaging tumor lymphatics and SLNs. The more recent introduction of multimodal particle probes^{21, 100, 101} for use with at least two imaging modalities, such as PET and optical imaging, can potentially improve lymph node resection efforts by aiding pre-operative planning and intraoperative guidance with a single platform technology. Radionuclide imaging, for example, yields increased depth penetration and absolute quantification, whereas NIR optical imaging with increasingly sensitive and higher-resolution portable optical imaging devices permits real-time adjustments to image quality, and enables image-guided treatment under the direct control of the operating surgeon.

Many of these agents are near-ideal in size for efficient lymphatic uptake and retention - small enough (i.e., with dimensions less than about 12 nm) to rapidly enter the tumor lymphatics and flow with the lymph fluid, yet large enough to remain confined to the lymphatic system without leakage into capillary vessels¹⁰². By contrast, larger size agents (i.e., with dimensions greater than about 12 nm) will result in prolonged diffusion from the interstitial space into the lymphatic channels and slower rates of accumulation within SLNs. Further, while providing a longer imaging window for nodal detection, they may also allow a substantially increased time to nodal resection. An extensive discussion of each of these newer classes of agents is beyond the scope of this article; a number of excellent reviews^{102–108} detailing this subject are available.

Below we present one such class of nanomaterials, an ultras-small organic-inorganic (silica) hybrid nanoparticles, termed Cornell dots (C dots)^{21, 109}, which incorporate a number of key design features (see below) that have facilitated its translation to the clinic (Fig 1b). This platform has previously detected and localized cancer-infiltrated local/regional nodes within clinically relevant models. Its selective detection of and subsequent retention within cancer-bearing nodes differentiates this type of particle platform from non-specific probes that identify the SLN itself, whether or not it contains cancerous cells. Newer-generation cancer-selective C dots have been FDA-IND approved for use as either a single- (i.e., optical) or dual-modality (optical-PET) platform, exhibit extended blood residence half-times (i.e., hours), and demonstrate bulk renal clearance. Their unique physicochemical and photophysical features have been exploited for use in a variety of surgically driven and/or minimally invasive surgical applications. Coupling with a real-time handheld fluorescence

camera system has resulted in a clinically approved combined technology platform for visualizing and molecularly characterizing regional tumor-draining lymph nodes within the nodal basin of melanoma models.

Cancer-Targeted Ultrasmall Hybrid C dots for Image-Directed Mapping of Metastatic Nodal Disease: Design Considerations

The preferential delivery and accumulation of molecularly targeted delivery vehicles at sites of cancerous spread are necessary in maximizing target-to-background ratios while reducing (or abrogating) possible dose-limiting toxicity. The design and surface chemistry of particle platforms, such as the one described here, need to be precisely tailored to achieve this end as well as to ensure other favorable in vivo biological properties. Recent advances in imaging probe developments for nanomedicine, and the hurdles associated with their subsequent clinical translation, highlight this challenge. In addition to the foregoing considerations, additional critical properties of these probes need to be considered, including their size, charge, shape, chemical stability, colloidal stability against aggregation, toxicity, surface coat uniformity, and the type/number of particle surface components (i.e., targeting and/or contrast-producing ligands).

To advance a highly engineered clinically translatable platform for molecular cancer imaging, C dots are tuned to sub-10 nm sizes, covalently core-encapsulate reactive NIR dye molecules, are surface-coated with polyethylene glycol (PEG), and surface-adapted with integrin-targeting cyclic arginine-glycine-aspartic acid peptides (cRGDY), enabling attachment of longer-lived radiolabels (i.e., iodine-124, ^{124}I ; half-life, 4 days) and yielding ^{124}I -cRGDY-PEG-C dots²¹. These FDA-IND approved silica nanoparticles constitute the first fluorescent hybrid inorganic platform of its class and properties to be approved for clinical use as either a PET-optical¹¹⁰ or an optical probe. A human SLN mapping trial is currently underway in melanoma patients to assess the feasibility of utilizing combined optical imaging technologies—FDA, IND-approved NIR fluorescent cRGDY-conjugated C dots and a handheld fluorescence camera system—for detecting cancer-bearing lymph nodes in the intraoperative setting.

Prior preclinical studies leading to an approved IND for SLN mapping utilized the targeted particle tracer and PET-CT imaging in a well-established spontaneous melanoma miniswine model (Sinclair miniature swine, Sinclair Research Center)¹¹¹. The results of these studies suggested that ^{124}I -cRGDY-PEG-C dots specifically localized and accumulated in metastatic lymph nodes, in contrast to findings with the standard-of-care radiotracer, ^{18}F -FDG^{21, 112}. In addition, targeted C dots permitted discrimination of nodal tumor burden among cancer-bearing nodes (see discussion below)²¹. Improved target-to-background ratios were also observed with the non-radiolabeled cRGDY-conjugated platform relative to those of free dye¹¹². A recently completed first-in-human clinical trial in a cohort of metastatic melanoma patients demonstrated the safety of the particle tracer after systemic injection¹¹⁰ and reproducible human pharmacokinetic (PK) signatures defined by renal excretion. Findings also suggested that the particle did not preferentially accumulate in the major organs/tissues of the RES (i.e., did not behave as an RES agent). Preferential targeted accumulation of the

particle tracer was seen in several tumors despite a very low particle dose per patient (< 10 nmol)¹¹⁰.

Importantly, the following key design features were introduced to create an optimum, highly-functionalized diagnostic platform for surgical navigation^{109, 113} – one which is versatile, non-toxic, exquisitely bright, and renally excreted – for use in intraoperative applications:

- Highly integrated ultrasmall platform with tunable sizes ranging from ~5.0 to ~10 nm diameter to produce favorable pharmacokinetic, including clearance, profile;
- Silica encapsulation of deep red/NIR fluorophores (e.g., Cy5, emission maxima, ~680 nm; Cy5.5, emission maxima, ~710 nm) to enhance photophysical features (i.e., brightness, photostability) and thereby improve signal tissue penetration and in vivo detection sensitivity;
- Presence of cancer-directed targeting ligands to enhance preferential accumulation and retention of this platform in melanoma-infiltrated nodes²¹;
- Neutrally-charged platform due to PEG-coated particle surface, which decrease non-specific uptake by the reticuloendothelial system (RES);
- Ease of surface adaptation with different molecular components (i.e., targeting ligand, contrast label, drugs) to create a highly functionalized diagnostic imaging vehicle.
- *Target-or-clear* paradigm: Sub-10 nm diameter C dots are an ideal diagnostic platform, as they either target disease or clear the body (i.e., whole body clearance half-times of ¹²⁴I-cRGDY-PEG-C dots range from 13– 21 hours in humans).

The hydrodynamic diameter of particles modulates their in vivo biological properties, including circulation half-times (or residence times), PK, and clearance profiles. For diagnostic studies, it is crucial that peptide-conjugated platforms exhibit relatively rapid clearance, preferably via a renal route to limit prolonged whole-body particle exposure. Although targeting may increase with particle size¹⁰⁴, earlier studies have shown that for larger particles sizes (>10 nm), slower physiologic transport within cancer-infiltrated tissues may hinder a more uniform diffusion of particles throughout the interstitium. In addition, Cho¹¹⁴ suggested that size cutoffs of 10 nm or less are desirable for bulk renal glomerular filtration, as prolonged exposure to particle loads may lead to unwanted toxicity or adverse effects.

While rapid renal clearance minimizes adverse effects, longer particle probe circulation half-times (i.e., hours) are desirable for increasing bioavailability and target tissue penetration, selectivity and preferential uptake and retention of nanoparticle-based agents at the target site will primarily depend upon the enhanced permeability and retention (EPR) effect^{104, 115}. The EPR effect is based on known heterogeneous alterations in the permeability of tumor neovasculature within and across tumors^{116, 117}. By utilizing probes (i.e., peptides, antibodies and nanoparticles) targeting highly expressed critical cancer targets, such as

integrins²¹ or cathepsins^{104, 117, 118}, improved accumulation and retention times have been observed, thus permitting delineation of tumor metastases⁸¹ or tumor-infiltrated nodal tissue²¹ from normal tissue^{21, 81} or other disease processes (inflammation, infection) that may similarly manifest with nodal enlargement.

The transport properties of particles across the vasculature and within the interstitium may be influenced by its net surface charge. Particles with a net surface charge may be opsonized by serum proteins^{109, 119}, which effectively increases probe size and thereby prevents renal excretion. By coating the particle surface with neutrally-charged PEG chains, the surface is rendered more chemically inert which, in turn, should enhance its diffusion and homogeneous distribution within the interstitial space of cancer-infiltrated tissues.

Newer-generation fluorescence probes (e.g., organic dyes, fluorescent proteins, dye-bound proteins/macromolecules, dye-containing nanoparticles) that emit in the deep-red/NIR region of the optical spectrum (650–900 nm) will exhibit decreased tissue attenuation and autofluorescence from non-target tissues. By covalently incorporating organic dyes (i.e., Cy5.5) into the silica matrix of our particle probe to prevent dye leaching^{109, 120}, notable photophysical enhancements over the free dye have been observed. For example, dye-encapsulated C dots were found to exhibit significantly increased brightness (200–300%) and photostability (2–3 fold increases) by fluorescence correlation spectroscopy, as compared with the free dye (Table 1)¹⁰⁹. Higher penetration depths of up to 2 cm were also found in our in vivo SLN mapping studies using a handheld multichannel fluorescence camera system (*vide infra*)¹¹².

Image-Guided Fluorescent Camera Systems

About a decade ago, the FDA launched a Critical Path Initiative (<http://www.fda.gov/ScienceResearch/SpecialTopics/CriticalPathInitiative/ucm076689.htm>) to accelerate the development of technological advances leading to cost-effective, innovative medical products, including advanced imaging systems, which can improve data acquisition, feature extraction, and standardization in clinical practice. The opportunity to combine novel multimodal and multi-parametric image-driven metrics and informatics tools with these evolving systems is expected to play a key role in ultimately improving outcome measures and clinical radiology practice and will serve to accelerate device developments in the field of nanomedicine. An imaging modality that meets these objectives, although in the relatively early phases of clinical implementation in the surgical setting, NIR fluorescence optical imaging has emerged as a highly sensitive and inexpensive molecular imaging technology that offers high resolution, high-speed data acquisition, real-time visualization, and multiplexing capabilities for identifying critical cancer targets without radiation exposure (Fig 2).

For intraoperative applications designed to capture real-time biological events, optical signal detection from cancerous or other soft tissues of interest needs to meet established criteria for achieving superior contrast-to-noise ratios at the lowest detectable doses administered. These requirements will directly impact the ability of these combined systems to interrogate biological structures at the tissue and cellular levels with high sensitivity, thus enabling

differentiation of cancer-bearing nodes from normal tissue structures and assessing small-volume or microscopic nodal disease.

Several excellent comprehensive reviews highlight a variety of NIR fluorescence imaging devices that have evolved to address a range of commercial and investigational clinical applications in conjunction with ICG.^{121–123} Importantly, a major breakthrough in the design of these fluorescence imaging devices has been their miniaturization¹²⁴ for difficult-to-navigate anatomical cavities and organs. One such state-of-the-art intraoperative imaging device, the ArteMISTM Spectrum, is a hand-held NIR fluorescence system (Fig. 3a) which has been adapted for interrogating tissues close-up by utilizing a minimally invasive laparoscope (Figs. 3b, 3c) or open-lens configuration (Fig 3c). A distinct advantage of this handheld system is that it can be maneuvered into anatomic locations that are typically difficult to navigate; signal detection can thus be made within several millimeters of the tissue surface. This high-resolution camera system simultaneously detects and spectrally de-mixes finely tuned optical signals arising from different fluorescent channels after excitation of multiple NIR dye-containing probes. Optical fluorescence images are acquired in video mode (15 frames/sec and higher), enabling real-time detection and/or monitoring of (1) flow within tumor lymphatic channels and nodes, (2) tumor margins, (3) residual sites of disease post-resection, and/or (4) response during or after therapeutic intervention. The resulting 4-panel display shows all individual channels as well as composite images, the latter an overlay of color (RGB) and NIR fluorescent images. A handheld gamma probe for radiodetection (Fig 3d), calibrated for I-124, was utilized to measure detected gamma emissions prior to lymph node to confirm particle localization.

Real time image-guided SLN Mapping

Image-guided metastatic disease detection, staging, and the assessment of differential tumor burden in SLNs have been evaluated in a melanoma miniswine model^{111, 125, 126}. To screen for metastatic disease in miniswine, dynamic ¹⁸F-FDG PET scanning following systemic injection of ¹⁸F-FDG 48 hours prior to local administration of ¹²⁴I-cRGDY-PEG-C dots. PET-avid nodes were confirmed intraoperatively within the exposed surgical bed by visual inspection and gamma counting using hand-held devices prior to excision for histopathological correlation (Fig 4). In most of the cases, baseline activity measurements, made over the primary tumor and SLN sites using the portable gamma probe, showed a 20-fold increase in activity within the SLN relative to background.

In a separate cohort of miniswine the draining of the tumor lymphatics and nodal metastases was further investigated real-time optical imaging using the ArteMISTM fluorescence camera system after a subdermal percutaneous injection of ¹²⁴I-cRGDY-PEG-C dots was performed near the tumor site. Two-channel NIR optical imaging of the exposed nodal basin after local injection of Cy5.5-encapsulating C dots is displayed in the RGB color (green, Fig. 5a) and NIR fluorescent channels (white, Fig. 5b) flowing from the injection site (see Fig. 5c) into the main proximal (Figs. 5c and d), mid (Fig 5e) and distal (Fig. 5f) lymphatic branches towards the SLN (see Fig 5f) are visualized. In addition, smaller-caliber lymphatic channels are seen (see Figs. 5d and e). Fluorescence signal seen within *in situ* (Fig. 5g) and *ex-vivo* (Fig 5h) nodal specimens was corroborated using the gamma probe and found to be

consistent with melanoma and further confirmed by HMB45 expression on low-power (Fig 5i) and high-power views (Fig 5j).

^{124}I -cRGDY-PEG-C dots enabled superior detection sensitivity and discrimination between metastatic tumor infiltration and inflammatory processes was demonstrated in about one-third of the miniswine; ^{18}F -FDG in many instances failed to accurately stage cancer spread. On the other hand, in about one-third of cases, ^{18}F -FDG identified sites of inflammation found to be additionally present in some of these animals, while the particle tracer did not, as confirmed by histopathologic analysis (Fig 6). This result is best seen when comparing 3D integrated PET-CT ^{18}F -FDG images (Fig 7a) and ^{124}I -cRGDY-PEG-C dot images (Figs 7b, 7c). PET-CT fused maximum-intensity projection (MIP) images generated from dynamic PET imaging data reveals the bilateral metastatic neck nodes only after injection of the particle tracer. While ^{18}F -FDG did not visualize nodal metastases, diffusely increased activity is seen within metabolically-active bony structures in these young animals, along with draining lymphatic channels. These findings suggest mechanistic differences at the cellular/subcellular levels related to the nature of these agents – one is a strictly metabolic probe (^{18}F -FDG), while the other is a non-metabolic, integrin-targeting probe. These results highlight the potential utility of ^{124}I -cRGDY-PEG-C dots to selectively target, localize, and stage metastatic disease.

Conclusion

SLN biopsy accurately stages the regional lymph node basins in stage-I and -II melanoma patients with minimal morbidity, promoting the more restrictive use of formal lymph node dissection. In addition to maximizing functional outcomes, the utilization of SLN biopsy procedures enables treatment of SLN-positive patients to be delivered at earlier stages, when regional nodal tumor burden is microscopic. In turn, this increases the likelihood of long-term survival and durable regional control. On the basis of such an assessment, SLN-positive patients will then be offered standard adjuvant therapy or be allowed to participate in prospective clinical trials that assess the potential benefit of novel therapies. Importantly, low-risk patients can be spared the unnecessary morbidity of additional surgery and adjuvant therapy. However, present SLN mapping strategies suffer from a number of significant drawbacks that hamper intraoperative melanoma staging, particularly in the head and neck. This, in turn, has accelerated the development of new lymphatic imaging strategies, including multiplexing, that might utilize one or more novel cancer-targeting probes, along with portable intraoperative imaging technology, to specifically identify metastatic nodal disease. However, the clinical translation of newer-generation cancer-directed imaging probes poses considerable challenges in a constantly evolving regulatory environment. By introducing a number of key design features that are tailored to specific clinical applications and/or biological questions of interest, it is possible to overcome technical hurdles that may impede translation. Such molecularly-targeted imaging approaches may more efficiently stratify patients to appropriate treatment arms in a more timely fashion, potentially improving their quality of life and prolonging survival. Further, as lymph node metastases are a powerful predictor of outcome for melanoma, earlier detection of micrometastases in regional lymph nodes using real-time optical visualization tools may offer a distinct advantage over radioactivity-based identification of SLN/s, particularly in anatomically

complex areas of the body. Whether such early identification of SLN metastases will truly offer a survival benefit will need to be addressed in future clinical trials. Further, even given limitations in current optical imaging strategies, such methods may eventually lead to changes in treatment management and standard-of-care surgical practice. The ability to further probe critical cancer targets in a broader range of cancer types may also elucidate important insights into the cellular and molecular processes that govern metastatic disease spread. Finally, the opportunity to combine these optical technologies with novel imaging-driven metrics and informatics tools may improve patient outcome measures and ultimately define the scope of clinical radiology practice.

Acknowledgments

This work was supported by the National Cancer Institute and National Institutes of Health with Grant Numbers: U54 CA199081 and R01 CA161280.

References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin*. 2014; 64:9–29. [PubMed: 24399786]
2. Balch CM, Soong SJ, Gershenwald JE, Thompson JF, Reintgen DS, Cascinelli N, Urist M, McMasters KM, Ross MI, Kirkwood JM, et al. Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2001; 19:3622–3634. [PubMed: 11504744]
3. ACS. Cancer Facts & Figures 2012. 2012:68.
4. Cserni G. Metastases in axillary sentinel lymph nodes in breast cancer as detected by intensive histopathological work up. *J Clin Pathol*. 1999; 52:922–924. [PubMed: 10711258]
5. Sorensen J. How Does the Patient Benefit from Clinical PET? *Theranostics*. 2012; 2:427–436. [PubMed: 22768023]
6. Kelloff GJ, Hoffman JM, Johnson B, Scher HI, Siegel BA, Cheng EY, Cheson BD, O'Shaughnessy J, Guyton KZ, Mankoff DA, et al. Progress and promise of FDG-PET imaging for cancer patient management and oncologic drug development. *Clin Cancer Res*. 2005; 11:2785–2808. [PubMed: 15837727]
7. Gambhir SS, Czernin J, Schwimmer J, Silverman DH, Coleman RE, Phelps ME. A tabulated summary of the FDG PET literature. *J Nucl Med*. 2001; 42:1S–93S. [PubMed: 11483694]
8. Conti PS, Lilien DL, Hawley K, Keppler J, Grafton ST, Bading JR. PET and [18F]-FDG in oncology: a clinical update. *Nucl Med Biol*. 1996; 23:717–735. [PubMed: 8940714]
9. Liu Y. Role of FDG PET-CT in evaluation of locoregional nodal disease for initial staging of breast cancer. *World J Clin Oncol*. 2014; 5:982–989. [PubMed: 25493234]
10. Yuan Y, Gu ZX, Tao XF, Liu SY. Computer tomography, magnetic resonance imaging, and positron emission tomography or positron emission tomography/computer tomography for detection of metastatic lymph nodes in patients with ovarian cancer: a meta-analysis. *Eur J Radiol*. 2012; 81:1002–1006. [PubMed: 21349672]
11. Veit-Haibach P, Vogt FM, Jablonka R, Kuehl H, Bockisch A, Beyer T, Dahmen G, Rosenbaum S, Antoch G. Diagnostic accuracy of contrast-enhanced FDG-PET/CT in primary staging of cutaneous malignant melanoma. *Eur J Nucl Med Mol Imaging*. 2009; 36:910–918. [PubMed: 19156409]
12. de Jong IJ, Pruim J, Elsinga PH, Vaalburg W, Mensink HJ. Preoperative staging of pelvic lymph nodes in prostate cancer by 11C-choline PET. *J Nucl Med*. 2003; 44:331–335. [PubMed: 12620996]

13. Fricke E, Machtens S, Hofmann M, van den Hoff J, Bergh S, Brunkhorst T, Meyer GJ, Karstens JH, Knapp WH, Boerner AR. Positron emission tomography with ¹¹C-acetate and ¹⁸F-FDG in prostate cancer patients. *Eur J Nucl Med Mol Imaging*. 2003; 30:607–611. [PubMed: 12589476]
14. Afshar-Oromieh A, Zechmann CM, Malcher A, Eder M, Eisenhut M, Linhart HG, Holland-Letz T, Hadaschik BA, Giesel FL, Debus J, et al. Comparison of PET imaging with a (68)Ga-labelled PSMA ligand and (18)F-choline-based PET/CT for the diagnosis of recurrent prostate cancer. *Eur J Nucl Med Mol Imaging*. 2014; 41:11–20. [PubMed: 24072344]
15. Caswell P, Norman J. Endocytic transport of integrins during cell migration and invasion. *Trends Cell Biol*. 2008; 18:257–263. [PubMed: 18456497]
16. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell*. 2002; 110:673–687. [PubMed: 12297042]
17. Hood JD, Cheresch DA. Role of integrins in cell invasion and migration. *Nat Rev Cancer*. 2002; 2:91–100. [PubMed: 12635172]
18. Benezra M, Phillips E, Overholtzer M, Zanzonico PB, Tuominen E, Wiesner U, Bradbury MS. Ultrasmall integrin-targeted silica nanoparticles modulate signaling events and cellular processes in a concentration-dependent manner. *Small*. 2015; 11:1721–1732. [PubMed: 25471698]
19. Beer AJ, Niemeyer M, Carlsen J, Sarbia M, Nahrig J, Watzlowik P, Wester HJ, Harbeck N, Schwaiger M. Patterns of alphavbeta3 expression in primary and metastatic human breast cancer as shown by ¹⁸F-Galacto-RGD PET. *J Nucl Med*. 2008; 49:255–259. [PubMed: 18199623]
20. Haubner R, Maschauer S, Prante O. PET radiopharmaceuticals for imaging integrin expression: tracers in clinical studies and recent developments. *Biomed Res Int*. 2014; 2014:871609. [PubMed: 25013808]
21. Benezra M, Penate-Medina O, Zanzonico PB, Schaer D, Ow H, Burns A, DeStanchina E, Longo V, Herz E, Iyer S, et al. Multimodal silica nanoparticles are effective cancer-targeted probes in a model of human melanoma. *The Journal of clinical investigation*. 2011; 121:2768–2780. [PubMed: 21670497]
22. Harrell MI, Iritani BM, Ruddell A. Tumor-induced sentinel lymph node lymphangiogenesis and increased lymph flow precede melanoma metastasis. *Am J Pathol*. 2007; 170:774–786. [PubMed: 17255343]
23. Ji RC. Lymph node lymphangiogenesis: a new concept for modulating tumor metastasis and inflammatory process. *Histol Histopathol*. 2009; 24:377–384. [PubMed: 19130407]
24. Rinderknecht, MDM. Molecular mechanisms of lymph node metastasis. In: Stacker, SA.; MG, A., editors. *Lymphangiogenesis In Cancer Metastasis*. Germany: Springer; 2009. p. 55–82.
25. Alitalo A, Detmar M. Interaction of tumor cells and lymphatic vessels in cancer progression. *Oncogene*. 2012; 31:4499–4508. [PubMed: 22179834]
26. Karaman S, Detmar M. Mechanisms of lymphatic metastasis. *J Clin Invest*. 2014; 124:922–928. [PubMed: 24590277]
27. Breiteneder-Geleff S, Soleiman A, Horvat R, Amann G, Kowalski H, Kerjaschki D. [Podoplanin--a specific marker for lymphatic endothelium expressed in angiosarcoma]. *Verh Dtsch Ges Pathol*. 1999; 83:270–275. [PubMed: 10714221]
28. Banerji S, Ni J, Wang SX, Clasper S, Su J, Tammi R, Jones M, Jackson DG. LYVE-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. *J Cell Biol*. 1999; 144:789–801. [PubMed: 10037799]
29. Holopainen T, Saharinen P, D'Amico G, Lampinen A, Eklund L, Sormunen R, Anisimov A, Zarkada G, Lohela M, Helotera H, et al. Effects of angiopoietin-2-blocking antibody on endothelial cell-cell junctions and lung metastasis. *J Natl Cancer Inst*. 2012; 104:461–475. [PubMed: 22343031]
30. Bjorndahl M, Cao R, Nissen LJ, Clasper S, Johnson LA, Xue Y, Zhou Z, Jackson D, Hansen AJ, Cao Y. Insulin-like growth factors 1 and 2 induce lymphangiogenesis in vivo. *Proc Natl Acad Sci U S A*. 2005; 102:15593–15598. [PubMed: 16230630]
31. Cao R, Bjorndahl MA, Religa P, Clasper S, Garvin S, Galter D, Meister B, Ikomi F, Tritsarlis K, Dissing S, et al. PDGF-BB induces intratumoral lymphangiogenesis and promotes lymphatic metastasis. *Cancer Cell*. 2004; 6:333–345. [PubMed: 15488757]

32. Licha K, Debus N, Emig-Vollmer S, Hofmann B, Hasbach M, Stibenz D, Sydow S, Schirner M, Ebert B, Petzelt D, et al. Optical molecular imaging of lymph nodes using a targeted vascular contrast agent. *J Biomed Opt.* 2005; 10:41205. [PubMed: 16178629]
33. Kishimoto TK, Jutila MA, Butcher EC. Identification of a human peripheral lymph node homing receptor: a rapidly down-regulated adhesion molecule. *Proc Natl Acad Sci U S A.* 1990; 87:2244–2248. [PubMed: 2179952]
34. Sevick-Muraca EM, Kwon S, Rasmussen JC. Emerging lymphatic imaging technologies for mouse and man. *J Clin Invest.* 2014; 124:905–914. [PubMed: 24590275]
35. Baluk P, Tammela T, Ator E, Lyubynska N, Achen MG, Hicklin DJ, Jeltsch M, Petrova TV, Pytowski B, Stacker SA, et al. Pathogenesis of persistent lymphatic vessel hyperplasia in chronic airway inflammation. *J Clin Invest.* 2005; 115:247–257. [PubMed: 15668734]
36. Niu G, Chen X. Lymphatic imaging: focus on imaging probes. *Theranostics.* 2015; 5:686–697. [PubMed: 25897334]
37. Wong SL, Balch CM, Hurley P, Agarwala SS, Akhurst TJ, Cochran A, Cormier JN, Gorman M, Kim TY, McMasters KM, et al. Sentinel lymph node biopsy for melanoma: American Society of Clinical Oncology and Society of Surgical Oncology joint clinical practice guideline. *J Clin Oncol.* 2012; 30:2912–2918. [PubMed: 22778321]
38. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, Buzaid AC, Cochran AJ, Coit DG, Ding S, et al. Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol.* 2009; 27:6199–6206. [PubMed: 19917835]
39. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Ding S, Byrd DR, Cascinelli N, Cochran AJ, Coit DG, Eggermont AM, et al. Multivariate analysis of prognostic factors among 2,313 patients with stage III melanoma: comparison of nodal micrometastases versus macrometastases. *J Clin Oncol.* 2010; 28:2452–2459. [PubMed: 20368546]
40. A'Hern RP. Sentinel-node biopsy in melanoma. *N Engl J Med.* 2007; 356:418. author reply 419–421. [PubMed: 17256189]
41. Morton DL, Thompson JF, Cochran AJ, Mozzillo N, Elashoff R, Essner R, Nieweg OE, Roses DF, Hoekstra HJ, Karakousis CP, et al. Sentinel-node biopsy or nodal observation in melanoma. *N Engl J Med.* 2006; 355:1307–1317. [PubMed: 17005948]
42. Slingluff CL Jr, Stidham KR, Ricci WM, Stanley WE, Seigler HF. Surgical management of regional lymph nodes in patients with melanoma. Experience with 4682 patients. *Ann Surg.* 1994; 219:120–130. [PubMed: 8129482]
43. Gershenwald JE, Berman RS, Porter G, Mansfield PF, Lee JE, Ross MI. Regional nodal basin control is not compromised by previous sentinel lymph node biopsy in patients with melanoma. *Ann Surg Oncol.* 2000; 7:226–231. [PubMed: 10791854]
44. Lee RJ, Gibbs JF, Proulx GM, Kollmorgen DR, Jia C, Kraybill WG. Nodal basin recurrence following lymph node dissection for melanoma: implications for adjuvant radiotherapy. *Int J Radiat Oncol Biol Phys.* 2000; 46:467–474. [PubMed: 10661355]
45. Ross MI, Gershenwald JE. Sentinel lymph node biopsy for melanoma: a critical update for dermatologists after two decades of experience. *Clin Dermatol.* 2013; 31:298–310. [PubMed: 23608449]
46. 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.* 1998; 351:793–796. [PubMed: 9519951]
47. Balch CM, Soong S, Ross MI, Urist MM, Karakousis CP, Temple WJ, Mihm MC, Barnhill RL, Jewell WR, Wanebo HJ, et al. Long-term results of a multi-institutional randomized trial comparing prognostic factors and surgical results for intermediate thickness melanomas (1.0 to 4.0 mm). *Intergroup Melanoma Surgical Trial. Ann Surg Oncol.* 2000; 7:87–97. [PubMed: 10761786]
48. Veronesi U, Adamus J, Bandiera DC, Brennhovd IO, Caceres E, Cascinelli N, Claudio F, Ikonopisov RL, Javorskj VV, Kirov S, et al. Inefficacy of immediate node dissection in stage I melanoma of the limbs. *N Engl J Med.* 1977; 297:627–630. [PubMed: 895764]
49. Reintgen D, Cruse CW, Wells K, Berman C, Fenske N, Glass F, Schroer K, Heller R, Ross M, Lyman G, et al. The orderly progression of melanoma nodal metastases. *Ann Surg.* 1994; 220:759–767. [PubMed: 7986143]

50. Morton DL, Wen DR, Wong JH, Economou JS, Cagle LA, Storm FK, Foshag LJ, Cochran AJ. Technical details of intraoperative lymphatic mapping for early stage melanoma. *Arch Surg.* 1992; 127:392–399. [PubMed: 1558490]
51. Ross MI, Reintgen D, Balch CM. Selective lymphadenectomy: emerging role for lymphatic mapping and sentinel node biopsy in the management of early stage melanoma. *Semin Surg Oncol.* 1993; 9:219–223. [PubMed: 8516607]
52. Thompson JF, McCarthy WH, Bosch CM, O'Brien CJ, Quinn MJ, Paramaesvaran S, Crotty K, McCarthy SW, Uren RF, Howman-Giles R. Sentinel lymph node status as an indicator of the presence of metastatic melanoma in regional lymph nodes. *Melanoma Res.* 1995; 5:255–260. [PubMed: 7496161]
53. Gershenwald JE, Tseng CH, Thompson W, Mansfield PF, Lee JE, Bouvet M, Lee JJ, Ross MI. Improved sentinel lymph node localization in patients with primary melanoma with the use of radiolabeled colloid. *Surgery.* 1998; 124:203–210. [PubMed: 9706139]
54. Albertini JJ, Cruse CW, Rapaport D, Wells K, Ross M, DeConti R, Berman CG, Jared K, Messina J, Lyman G, et al. Intraoperative radio-lympho-scintigraphy improves sentinel lymph node identification for patients with melanoma. *Ann Surg.* 1996; 223:217–224. [PubMed: 8597518]
55. Kapteijn BA, Nieweg OE, Liem I, Mooi WJ, Balm AJ, Muller SH, Peterse JL, Valdes Olmos RA, Hoefnagel CA, Kroon BB. Localizing the sentinel node in cutaneous melanoma: gamma probe detection versus blue dye. *Ann Surg Oncol.* 1997; 4:156–160. [PubMed: 9084853]
56. Sumner WE 3rd, Ross MI, Mansfield PF, Lee JE, Prieto VG, Schacherer CW, Gershenwald JE. Implications of lymphatic drainage to unusual sentinel lymph node sites in patients with primary cutaneous melanoma. *Cancer.* 2002; 95:354–360. [PubMed: 12124836]
57. Uren RF, Howman-Giles R, Thompson JF, McCarthy WH, Quinn MJ, Roberts JM, Shaw HM. Interval nodes: the forgotten sentinel nodes in patients with melanoma. *Arch Surg.* 2000; 135:1168–1172. [PubMed: 11030873]
58. Thompson JF, Uren RF, Shaw HM, McCarthy WH, Quinn MJ, O'Brien CJ, Howman-Giles RB. Location of sentinel lymph nodes in patients with cutaneous melanoma: new insights into lymphatic anatomy. *J Am Coll Surg.* 1999; 189:195–204. [PubMed: 10437842]
59. Uren RF, Thompson JF, Howman-Giles R. Sentinel nodes. Interval nodes, lymphatic lakes, and accurate sentinel node identification. *Clin Nucl Med.* 2000; 25:234–236. [PubMed: 10698433]
60. Uren RF, Thompson JF, Howman-Giles R, Shaw HM. Melanoma metastases in triangular intermuscular space lymph nodes. *Ann Surg Oncol.* 1999; 6:811. [PubMed: 10622512]
61. Wallace AM, Hoh CK, Ellner SJ, Darrah DD, Schulteis G, Vera DR. Lymphoseek: a molecular imaging agent for melanoma sentinel lymph node mapping. *Ann Surg Oncol.* 2007; 14:913–921. [PubMed: 17146742]
62. Moghimi SM, Rajabi-Siahboomi R. Advanced colloid-based systems for efficient delivery of drugs and diagnostic agents to the lymphatic tissues. *Prog Biophys Mol Biol.* 1996; 65:221–249. [PubMed: 9062433]
63. Strand SE, Bergqvist L. Radiolabeled colloids and macromolecules in the lymphatic system. *Crit Rev Ther Drug Carrier Syst.* 1989; 6:211–238. [PubMed: 2692844]
64. Jain R, Dandekar P, Patravale V. Diagnostic nanocarriers for sentinel lymph node imaging. *J Control Release.* 2009; 138:90–102. [PubMed: 19445982]
65. Erman AB, Collar RM, Griffith KA, Lowe L, Sabel MS, Bichakjian CK, Wong SL, McLean SA, Rees RS, Johnson TM, et al. Sentinel lymph node biopsy is accurate and prognostic in head and neck melanoma. *Cancer.* 2012; 118:1040–1047. [PubMed: 21773971]
66. Rosenthal EL, Warram JM, de Boer E, Chung TK, Korb ML, Brandwein-Gensler M, Strong TV, Schmalbach CE, Morlandt AB, Agarwal G, et al. Safety and Tumor Specificity of Cetuximab-IRDye800 for Surgical Navigation in Head and Neck Cancer. *Clin Cancer Res.* 2015; 21:3658–3666. [PubMed: 25904751]
67. Hadjipanayis CG, Jiang H, Roberts DW, Yang L. Current and future clinical applications for optical imaging of cancer: from intraoperative surgical guidance to cancer screening. *Semin Oncol.* 2011; 38:109–118. [PubMed: 21362519]
68. Ntziachristos V, Yoo JS, van Dam GM. Current concepts and future perspectives on surgical optical imaging in cancer. *J Biomed Opt.* 2010; 15:066024. [PubMed: 21198198]

69. Schaafsma BE, Mieog JS, Hutteman M, van der Vorst JR, Kuppen PJ, Lowik CW, Frangioni JV, van de Velde CJ, Vahrmeijer AL. The clinical use of indocyanine green as a near-infrared fluorescent contrast agent for image-guided oncologic surgery. *J Surg Oncol*. 2011; 104:323–332. [PubMed: 21495033]
70. Sevick-Muraca EM, Sharma R, Rasmussen JC, Marshall MV, Wendt JA, Pham HQ, Bonefas E, Houston JP, Sampath L, Adams KE, et al. Imaging of lymph flow in breast cancer patients after microdose administration of a near-infrared fluorophore: feasibility study. *Radiology*. 2008; 246:734–741. [PubMed: 18223125]
71. Troyan SL, Kianzad V, Gibbs-Strauss SL, Gioux S, Matsui A, Oketokoun R, Ngo L, Khamene A, Azar F, Frangioni JV. The FLARE intraoperative near-infrared fluorescence imaging system: a first-in-human clinical trial in breast cancer sentinel lymph node mapping. *Ann Surg Oncol*. 2009; 16:2943–2952. [PubMed: 19582506]
72. Crane LM, Themelis G, Arts HJ, Buddingh KT, Brouwers AH, Ntziachristos V, van Dam GM, van der Zee AG. Intraoperative near-infrared fluorescence imaging for sentinel lymph node detection in vulvar cancer: first clinical results. *Gynecologic oncology*. 2011; 120:291–295. [PubMed: 21056907]
73. Kobayashi H, Hama Y, Koyama Y, Barrett T, Regino CA, Urano Y, Choyke PL. Simultaneous multicolor imaging of five different lymphatic basins using quantum dots. *Nano Lett*. 2007; 7:1711–1716. [PubMed: 17530812]
74. Kobayashi H, Longmire MR, Ogawa M, Choyke PL, Kawamoto S. Multiplexed imaging in cancer diagnosis: applications and future advances. *Lancet Oncol*. 2010; 11:589–595. [PubMed: 20338808]
75. Kobayashi H, Koyama Y, Barrett T, Hama Y, Regino CA, Shin IS, Jang BS, Le N, Paik CH, Choyke PL, et al. Multimodal nanoprobe for radionuclide and five-color near-infrared optical lymphatic imaging. *ACS Nano*. 2007; 1:258–264. [PubMed: 19079788]
76. Thakor AS, Gambhir SS. Nanooncology: the future of cancer diagnosis and therapy. *CA Cancer J Clin*. 2013; 63:395–418. [PubMed: 24114523]
77. Hama Y, Koyama Y, Urano Y, Choyke PL, Kobayashi H. Two-color lymphatic mapping using Ig-conjugated near infrared optical probes. *The Journal of investigative dermatology*. 2007; 127:2351–2356. [PubMed: 17522707]
78. Wu AM, Olafsen T. Antibodies for molecular imaging of cancer. *Cancer J*. 2008; 14:191–197. [PubMed: 18536559]
79. Weissleder R, Pittet MJ. Imaging in the era of molecular oncology. *Nature*. 2008; 452:580–589. [PubMed: 18385732]
80. Tsien RY. Building and breeding molecules to spy on cells and tumors. *FEBS Lett*. 2005; 579:927–932. [PubMed: 15680976]
81. van Dam GM, Themelis G, Crane LM, Harlaar NJ, Pleijhuis RG, Kelder W, Sarantopoulos A, de Jong JS, Arts HJ, van der Zee AG, et al. Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor- α targeting: first in-human results. *Nat Med*. 2011; 17:1315–1319. [PubMed: 21926976]
82. Wunderbaldinger P, Turetschek K, Bremer C. Near-infrared fluorescence imaging of lymph nodes using a new enzyme sensing activatable macromolecular optical probe. *European radiology*. 2003; 13:2206–2211. [PubMed: 12802615]
83. Mahmood U, Weissleder R. Near-infrared optical imaging of proteases in cancer. *Molecular cancer therapeutics*. 2003; 2:489–496. [PubMed: 12748311]
84. Keereweer S, Mieog JS, Mol IM, Van Driel PB, Snoeks TJ, Baatenburg de Jong RJ, Vahrmeijer AL, Kerrebijn JD, Lowik CW. Detection of oral squamous cell carcinoma and cervical lymph node metastasis using activatable near-infrared fluorescence agents. *Archives of otolaryngology--head & neck surgery*. 2011; 137:609–615. [PubMed: 21690514]
85. Withrow KP, Newman JR, Skipper JB, Gleysteen JP, Magnuson JS, Zinn K, Rosenthal EL. Assessment of bevacizumab conjugated to Cy5.5 for detection of head and neck cancer xenografts. *Technology in cancer research & treatment*. 2008; 7:61–66. [PubMed: 18198926]

86. Lee SB, Hassan M, Fisher R, Chertov O, Chernomordik V, Kramer-Marek G, Gandjbakhche A, Capala J. Affibody molecules for in vivo characterization of HER2-positive tumors by near-infrared imaging. *Clinical Cancer Research*. 2008; 14:3840–3849. [PubMed: 18559604]
87. Gleysteen JP, Duncan RD, Magnuson JS, Skipper JB, Zinn K, Rosenthal EL. Fluorescently labeled cetuximab to evaluate head and neck cancer response to treatment. *Cancer biology & therapy*. 2007; 6:1181–1185. [PubMed: 17637562]
88. Kobayashi H, Kawamoto S, Bernardo M, Brechbiel MW, Knopp MV, Choyke PL. Delivery of gadolinium-labeled nanoparticles to the sentinel lymph node: comparison of the sentinel node visualization and estimations of intra-nodal gadolinium concentration by the magnetic resonance imaging. *Journal of controlled release*. 2006; 111:343–351. [PubMed: 16490277]
89. Koyama Y, Talanov VS, Bernardo M, Hama Y, Regino CA, Brechbiel MW, Choyke PL, Kobayashi H. A dendrimer-based nanosized contrast agent dual-labeled for magnetic resonance and optical fluorescence imaging to localize the sentinel lymph node in mice. *Journal of magnetic resonance imaging : JMRI*. 2007; 25:866–871. [PubMed: 17345640]
90. Wisner ER, Ferrara KW, Short RE, Ottoboni TB, Gabe JD, Patel D. Sentinel node detection using contrast-enhanced power Doppler ultrasound lymphography. *Invest Radiol*. 2003; 38:358–365. [PubMed: 12908703]
91. Mattrey RF, Kono Y, Baker K, Peterson T. Sentinel lymph node imaging with microbubble ultrasound contrast material. *Acad Radiol*. 2002; 9(Suppl 1):S231–S235. [PubMed: 12019877]
92. Plut EM, Hinkle GH, Guo W, Lee RJ. Kit formulation for the preparation of radioactive blue liposomes for sentinel node lymphoscintigraphy. *J Pharm Sci*. 2002; 91:1717–1732. [PubMed: 12115835]
93. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. *Nat Rev Drug Discov*. 2005; 4:145–160. [PubMed: 15688077]
94. Phillips WT, Klipper R, Goins B. Use of (99m)Tc-labeled liposomes encapsulating blue dye for identification of the sentinel lymph node. *J Nucl Med*. 2001; 42:446–451. [PubMed: 11337521]
95. Ballou B, Ernst LA, Andreko S, Harper T, Fitzpatrick JA, Waggoner AS, Bruchez MP. Sentinel lymph node imaging using quantum dots in mouse tumor models. *Bioconjug Chem*. 2007; 18:389–396. [PubMed: 17263568]
96. Surguladze RZ GB, T Tskitishvili. Novel method of sentinel lymph node detection in malignant tumors using preparation 'UNIMAG', *Breast Cancer Res*. 9 (2007) P15. *Breast Cancer Res*. 2007; 9:15.
97. Pan D, Pramanik M, Senpan A, Ghosh S, Wickline SA, Wang LV, Lanza GM. Near infrared photoacoustic detection of sentinel lymph nodes with gold nanobeacons. *Biomaterials*. 2010; 31:4088–4093. [PubMed: 20172607]
98. Harisinghani MG, Barentsz J, Hahn PF, Deserno WM, Tabatabaei S, van de Kaa CH, de la Rosette J, Weissleder R. Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. *N Engl J Med*. 2003; 348:2491–2499. [PubMed: 12815134]
99. Wallace AM, Hoh CK, Vera DR, Darrah DD, Schulteis G. Lymphoseek: a molecular radiopharmaceutical for sentinel node detection. *Annals of surgical oncology*. 2003; 10:531–538. [PubMed: 12794019]
100. Olson ES, Jiang T, Aguilera TA, Nguyen QT, Ellies LG, Scadeng M, Tsien RY. Activatable cell penetrating peptides linked to nanoparticles as dual probes for in vivo fluorescence and MR imaging of proteases. *Proc Natl Acad Sci U S A*. 2010; 107:4311–4316. [PubMed: 20160077]
101. Madru R, Kjellman P, Olsson F, Wingardh K, Ingvar C, Stahlberg F, Olsrud J, Latt J, Fredriksson S, Knutsson L, et al. 99mTc-labeled superparamagnetic iron oxide nanoparticles for multimodality SPECT/MRI of sentinel lymph nodes. *J Nucl Med*. 2012; 53:459–463. [PubMed: 22323777]
102. Lucarelli RT, Ogawa M, Kosaka N, Turkbey B, Kobayashi H, Choyke PL. New approaches to lymphatic imaging. *Lymphatic research and biology*. 2009; 7:205–214. [PubMed: 20143919]
103. Jain R, Dandekar P, Patravale V. Diagnostic nanocarriers for sentinel lymph node imaging. *Journal of controlled release : official journal of the Controlled Release Society*. 2009; 138:90–102. [PubMed: 19445982]

104. Jain RK, Stylianopoulos T. Delivering nanomedicine to solid tumors. *Nature reviews. Clinical oncology*. 2010; 7:653–664. [PubMed: 20838415]
105. Keereweer S, Kerrebijn JD, van Driel PB, Xie B, Kaijzel EL, Snoeks TJ, Que I, Hutteman M, van der Vorst JR, Mieog JS, et al. Optical image-guided surgery--where do we stand? *Molecular imaging and biology : MIB : the official publication of the Academy of Molecular Imaging*. 2011; 13:199–207. [PubMed: 20617389]
106. Schroeder A, Heller DA, Winslow MM, Dahlman JE, Pratt GW, Langer R, Jacks T, Anderson DG. Treating metastatic cancer with nanotechnology. *Nature reviews. Cancer*. 2012; 12:39–50. [PubMed: 22193407]
107. Yudd AP, Kempf JS, Goydos JS, Stahl TJ, Feinstein RS. Use of sentinel node lymphoscintigraphy in malignant melanoma. *RadioGraphics*. 1999; 19:343–353. [PubMed: 10194783]
108. Keereweer S, Van Driel PB, Snoeks TJ, Kerrebijn JD, Baatenburg de Jong RJ, Vahrmeijer AL, Sterenborg HJ, Lowik CW. Optical image-guided cancer surgery: challenges and limitations. *Clin Cancer Res*. 2013; 19:3745–3754. [PubMed: 23674494]
109. Burns AA, Vider J, Ow H, Herz E, Penate-Medina O, Baumgart M, Larson SM, Wiesner U, Bradbury M. Fluorescent silica nanoparticles with efficient urinary excretion for nanomedicine. *Nano letters*. 2009; 9:442–448. [PubMed: 19099455]
110. Phillips E, Penate-Medina O, Zanzonico PB, Carvajal RD, Mohan P, Ye Y, Humm J, Gonen M, Kalaigian H, Schoder H, et al. Clinical translation of an ultrasmall inorganic optical-PET imaging nanoparticle probe. *Sci Transl Med*. 2014; 6:260ra149.
111. Misfeldt ML, Grimm DR. Sinclair miniature swine: an animal model of human melanoma. *Veterinary immunology and immunopathology*. 1994; 43:167–175. [PubMed: 7856049]
112. Bradbury MS, Phillips E, Montero PH, Cheal SM, Stambuk H, Durack JC, Sofocleous CT, Meester RJ, Wiesner U, Patel S. Clinically-translated silica nanoparticles as dual-modality cancer-targeted probes for image-guided surgery and interventions. *Integr Biol (Camb)*. 2013; 5:74–86. [PubMed: 23138852]
113. Choi J, Burns AA, Williams RM, Zhou Z, Flesken-Nikitin A, Zipfel WR, Wiesner U, Nikitin AY. Core-shell silica nanoparticles as fluorescent labels for nanomedicine. *Journal of biomedical optics*. 2007; 12:064007. [PubMed: 18163823]
114. Choi CH, Zuckerman JE, Webster P, Davis ME. Targeting kidney mesangium by nanoparticles of defined size. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:6656–6661. [PubMed: 21464325]
115. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *Journal of controlled release : official journal of the Controlled Release Society*. 2000; 65:271–284. [PubMed: 10699287]
116. Iyer AK, Khaled G, Fang J, Maeda H. Exploiting the enhanced permeability and retention effect for tumor targeting. *Drug Discov Today*. 2006; 11:812–818. [PubMed: 16935749]
117. Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Girard OM, Hanahan D, Mattrey RF, Ruoslahti E. Tissue-Penetrating Delivery of Compounds and Nanoparticles into Tumors. *Cancer Cell*. 2009; 16:510–520. [PubMed: 19962669]
118. Karmali PP, Kotamraju VR, Kastantin M, Black M, Missirlis D, Tirrell M, Ruoslahti E. Targeting of albumin-embedded paclitaxel nanoparticles to tumors. *Nanomedicine*. 2009; 5:73–82. [PubMed: 18829396]
119. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: theory to practice. *Pharmacological reviews*. 2001; 53:283–318. [PubMed: 11356986]
120. Burns A, Ow H, Wiesner U. Fluorescent core-shell silica nanoparticles: towards "Lab on a Particle" architectures for nanobiotechnology. *Chemical Society reviews*. 2006; 35:1028–1042. [PubMed: 17057833]
121. Sevvick-Muraca EM, Rasmussen JC. Molecular imaging with optics: primer and case for near-infrared fluorescence techniques in personalized medicine. *J Biomed Opt*. 2008; 13:041303. [PubMed: 19021311]
122. Alander JT, Kaartinen I, Laakso A, Patila T, Spillmann T, Tuchin VV, Venermo M, Valisuo P. A review of indocyanine green fluorescent imaging in surgery. *Int J Biomed Imaging*. 2012; 2012:940585. [PubMed: 22577366]

123. Zhu B, Sevick-Muraca EM. A review of performance of near-infrared fluorescence imaging devices used in clinical studies. *Br J Radiol*. 2015; 88:20140547. [PubMed: 25410320]
124. Dorval, PAI.; Barabino, G.; Henry, M.; Keramidas, M.; Stenard, F.; Milet, C.; Righini, C.; Rizo, P.; Guillermet, S., et al. Miniaturized Clinical Imaging Device for Optical Surgery. In: Dip, F.; Ishizawa, T.; Kokudo, N.; R, R., editors. *Fluorescence Imaging for Surgeons*. Switzerland: Springer; 2015. p. 341-351.
125. Millikan LE, Boylon JL, Hook RR, Manning PJ. Melanoma in Sinclair swine: a new animal model. *The Journal of investigative dermatology*. 1974; 62:20–30. [PubMed: 4809019]
126. Oxenhandler RW, Adelstein EH, Haigh JP, Hook RR Jr, Clark WH Jr. Malignant melanoma in the Sinclair miniature swine: an autopsy study of 60 cases. *The American journal of pathology*. 1979; 96:707–720. [PubMed: 474716]

Fig. 1. Schematic of SLN mapping in the head and neck using ^{124}I -cRGDY-PEG-Cdots. (a) Injection of ^{124}I -cRGDY-PEG-C dots about an oral cavity lesion with drainage to preauricular and submandibular nodes. (b) ^{124}I -cRGDY-PEG-ylated core-shell silica nanoparticle with surface-bearing radiolabels and peptides and core-containing reactive dye molecules (insets). (Adapted from Integr. Biol., 2013, 5, 74–86).

Artemis™ Portable Fluorescence Image-Guided Camera System Technology

Device	CT	PET	Conventional MRI	Artemis Portable Optical Camera
Temporal Resolution	Relatively Fast Discrete Images (sec)	Slower than CT Discrete Images (sec - min)	Slower than CT Discrete Images (sec- min)	Video mode Real time (>15 frames/sec)
Radiation	X-rays	Gamma Rays	Radio waves/ no radiation	None
Depth	100%	100%	100%	1 – 20 mm
Spatial Resolution	50µm	< 1 cm	50µm	50µm
Device Cost	\$\$	\$\$\$	\$\$\$	\$

Fig. 2. Real-time Intraoperative Fluorescence Camera System Technology: Comparison with Conventional Imaging Modalities. Device parameters for CT, PET, and MRI versus those used for the Artemis™ handheld fluorescence camera system.

Fig. 3. Minimally invasive surgery utilizing a handheld fluorescence camera system. (a) ArteMIS™ handheld fluorescence camera system for open and laparoscopic procedures. (b) Minimally invasive surgery using laparoscopic tools. (c) System components (top to bottom): camera, laparoscope, and ring light. (d) Handheld gamma probe for radiodetection. (Adapted from *Integr. Biol.*, 2013, 5, 74–86).

Figure 4a

Figure 4b

Fig. 4.

Imaging of metastatic disease in a spontaneous melanoma miniswine model. (a) Whole-body ^{18}F -FDG PET-CT sagittal and axial views demonstrating primary tumor (green arrow) and single SLN (white arrow) posteriorly within the right (Rt) neck after i.v. injection. ant, anterior. (b) High-resolution PET-CT scan reveals bilateral nodes 1 hour after subdermal, 4-quadrant, peritumoral injection of ^{124}I -cRGDY-PEG-C dots (SLN, arrow; left-sided node, arrowhead). (c, d) Gross images of the cut surfaces of the black-pigmented SLN (asterisk, c)

and contralateral metastatic node (arrowhead, d) in the left posterior neck. (e) Low-power view of H&E-stained SLN demonstrating scattered melanomatous clusters (white arrowhead). (f) Corresponding high-power view of H&E-stained SLN, revealing melanoma cells (yellow arrowheads) and melanophages (white arrowhead). (g) Low-power image of a melanoma-specific marker, HMB-45 (white arrowhead), in representative SLN tissue. (h) High-power image of HMB-45-stained SLN tissue. (i) Low-power view of H&E-stained contralateral lymph node showing scattered melanomatous clusters (white arrowhead). (j) High-power image of contralateral node showing infiltration of melanomatous cells (yellow arrowheads). (k) Low-power image of representative normal porcine nodal tissue. (l) High-power image of representative normal porcine nodal tissue. Scale bars: 1 mm (e, g, i, k); 20 mm (f, h, j, l). (Adapted from *Integr. Biol.*, 2013, 5, 74–86).

Fig. 5.

Image-guided SLN mapping in a spontaneous melanoma miniswine model: Real-time intraoperative optical imaging with correlative histology. Intraoperative SLN mapping was performed on the animal shown in Fig. 5. (a–i) Two-channel NIR optical imaging of the exposed nodal basin. Local injection of Cy5.5-incorporated particles displayed in dual-channel model (a) RGB color (green) and (b) NIR fluorescent channels (white). (c–f) Draining lymphatics distal to the site of injection. Fluorescence signal within the main draining proximal (c, d), mid (e), and distal (f) lymphatic channels (yellow arrows) extending toward the SLN ('N'). Smaller caliber channels are also shown (arrowheads). Images of the SLN displayed in the (g) color and (h) NIR channels. (i) Color image of the exposed SLN. (j) Images of SLN in the color and NIR channels during excision. (Adapted from *Integr. Biol.*, 2013, 5, 74–86).

Fig. 6.

Discrimination of inflammation from metastatic disease: comparison of ^{18}F -FDG and ^{124}I -cRGDY-PEG-C dot tracers. (a–d) Imaging of inflammatory changes using ^{18}F -FDG-PET with tissue correlation. (a) Axial CT scan of the ^{18}F -FDG PET study shows calcification within the left posterior neck (yellow arrows). (b) Fused axial ^{18}F -FDG PET-CT reveals hypermetabolic activity at this same site (yellow arrows). Increased PET signal is also seen in metabolically active osseous structures (asterisks). (c) Low and (d) high-power views of H&E-stained calcified tissue demonstrate extensive infiltration of inflammatory cells. (e–k) Metastatic disease detection following injection of ^{124}I -cRGDY-PEG C dots about the tumor site. (e) Pre-injection axial CT scan of ^{124}I -cRGDY-PEG-C dots shows calcified soft tissues within the posterior neck (yellow arrows). (f) Co-registered PET-CT shows no evident activity corresponding to calcified areas (arrow), but demonstrates a PET-avid node on the right (arrowhead). (g) Axial CT at a more superior level shows nodes (arrowheads) bilaterally and a calcified focus (yellow arrow). (h) Fused PET-CT demonstrates PET-avid nodes (N) and lymphatic drainage (curved arrow). Calcification shows no activity (arrow). (i) Low- and (j) high-power views confirm the presence of nodal metastases. (k) Single frame from a three-dimensional (3D) PET image reconstruction shows multiple bilateral

metastatic nodes (arrowheads) and lymphatic channels (solid arrow) draining injection site (white asterisk). Bladder activity is seen (dashed arrow) with no significant tracer accumulation in the liver (black asterisk). Bladder activity is seen with no significant tracer accumulation in the liver. Scale bars: 500 mm (c, d); 100 mm (i, j). (Adapted from Integr. Biol., 2013, 5, 74–86).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 7. 3D integrated ^{18}F -FDG and ^{124}I -cRGDY-PEG-C dot PET-CT. (a–c) 3D volume rendered images were generated from CT and PET imaging data shown in Fig. 7. (a) PET-CT fusion image (coronal view) shows no evident nodal metastases (asterisks). Increased activity within bony structures is identified. (b, c) High-resolution PET-CT fusion images showing coronal (b) and superior views (c) of bilateral metastatic nodes (open arrows) and lymphatic channels (curved arrows) within the neck following local injection of ^{124}I -cRGDY-PEG-C dots. (Adapted from *Integr. Biol.*, 2013, 5, 74–86).

Table 1

Photophysical characterization of Surface Functionalized C dots using Fluorescence Correlation Spectroscopy

Specifications	Native Dye (Cy5)	Particle (cRGDY-PEG-C dots)
Brightness / Particle (kHz)	3.48	10.13
Concentration (mol/L)	5.37×10^{-4}	8.80×10^{-6}
Hydrodynamic Radius (nm)	0.67+/-0.008	3.40+/-0.04