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Image guided surgery: clinical validation of lesion identification technologies and exploration of nerve sparing approaches

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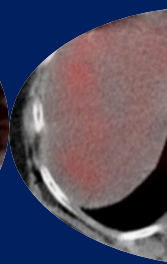
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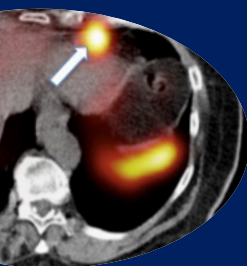
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Chapter 2





2

Multimodal imaging in radioguided surgery

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ABSTRACT

Radioguided surgery (RGS) utilizes radiolabeled tracers that accumulate in the lesion of interest following local or systemic administration. RGS is an expanding surgical guidance technology, but unfortunately most currently available radiotracers are unable to provide intraoperative optical information regarding the location of the lesion. In 2011, a new hybrid tracer (both radioactive and fluorescent) was introduced for sentinel lymph node detection. This hybrid tracer (indocyanine green-^{99m}Tc-nanocolloid) has helped to generate a hybrid surgical guidance concept that builds on traditional RGS. Based on its radioactive signature a hybrid tracer is able to preserve the “traditional” role for RGS. Furthermore, as an additional tool in the operating room, a hybrid tracer provides optical information via fluorescence guidance.

This review discusses the most common RGS approaches. Different clinically used radioactive tracers and their potential hybrid derivatives are also discussed. Furthermore, the various imaging devices designed for radioactivity-based detection are reviewed in the context of generating hybrid-imaging modalities.

INTRODUCTION

The goal of radioguided surgery (RGS) is to take advantage of local accumulation of a preoperatively injected radioactive tracer to achieve effective intraoperative localization of a lesion of interest. RGS, allows more complete resections, reducing the need for reoperations (Figure 1). Both gamma- and beta-emitting radioisotopes can be used for RGS, e.g., fluorine-18 (^{18}F), gallium-67 (^{67}Ga), technetium-99m ($^{99\text{m}}\text{Tc}$), indium-111 (^{111}In) and iodine-125 or iodine-131 (^{125}I , ^{131}I) [1]. With the radiation penetrating deeply through tissue, the influence of tissue attenuation on the detection sensitivity is limited. This beneficial feature also allows the radioactive signal to be used for the acquisition of preoperative (3D) images by means of, for example, single photon emission computed tomography (SPECT) or positron emission tomography (PET). The value of SPECT or PET data further improves when these modalities are supplemented with anatomical detail provided by computed tomography (CT) or magnetic resonance imaging (MRI). To this end, SPECT/CT and PET/CT were introduced, followed more recently by PET/MRI.

The information provided by these combined modalities, depicting the radioactive target lesion(s) within the patient's body, constitutes a useful roadmap for surgeons. While the radioactive signal allows the surgeon to sensitively locate the area of interest prior to incision, during the operation itself, the limited spatial resolution of the current nuclear modalities may impair resection accuracy. It must be noted that while good spatial resolution can be obtained with SPECT in the preoperative setting (0.25 mm), this resolution cannot be obtained intraoperatively with the currently available modalities [1, 2]. Furthermore, 'signal bleeding' can make it difficult to accurately locate the origin of the radioactive signal during surgery. To compensate for these limitations, there is an increasing need, alongside RGS, for approaches that provide high-resolution (optical) information.

Optical information plays a key role in real-time assessment and surgical decision-making. For example, it allows movement of anatomical structures to be monitored by the surgeon, while dyes can be used to mark an area of interest. The surgical identification of lymph vessels and lymph nodes using vital blue dye (e.g., patent or methylene blue) during radioguided sentinel lymph node (SLN) biopsy is perhaps the best known example of the clinical application of dyes [3, 4]. The use of Cerenkov-emitting PET tracers and/or (near-infrared) fluorescent dyes [e.g., fluorescein, indocyanine green (ICG) or 5-aminolevulinic acid] can further improve the optical detection of lesions [5, 6]. Since the tissue penetration

of a fluorescent dye is dependent on the excitation and emission wavelength of the dye, an increased penetration depth can be achieved by applying near-infrared dyes; ICG, for example, allows optical detection of lesions embedded in up to 1 cm of tissue. Clearly, the tissue penetration of these optical signals remains limited in comparison to the penetration depth of the above-mentioned radioactive tracers, rendering the nuclear approach indispensable for 3D localization. Preclinical validation was recently followed by the clinical introduction of multimodal (hybrid) tracers that contain both a radioactive and a fluorescent moiety [7, 8], and the potential of these hybrid tracers to enhance conventional RGS technologies with the beneficial properties of optical guidance has already been demonstrated [9, 10]. This review discusses the most common RGS approaches.

Different clinically used radioactive tracers and their potential hybrid derivatives are also discussed. Furthermore, the various imaging devices designed for radioactivity-based detection are reviewed in the context of generating hybrid-imaging modalities.

FIGURES

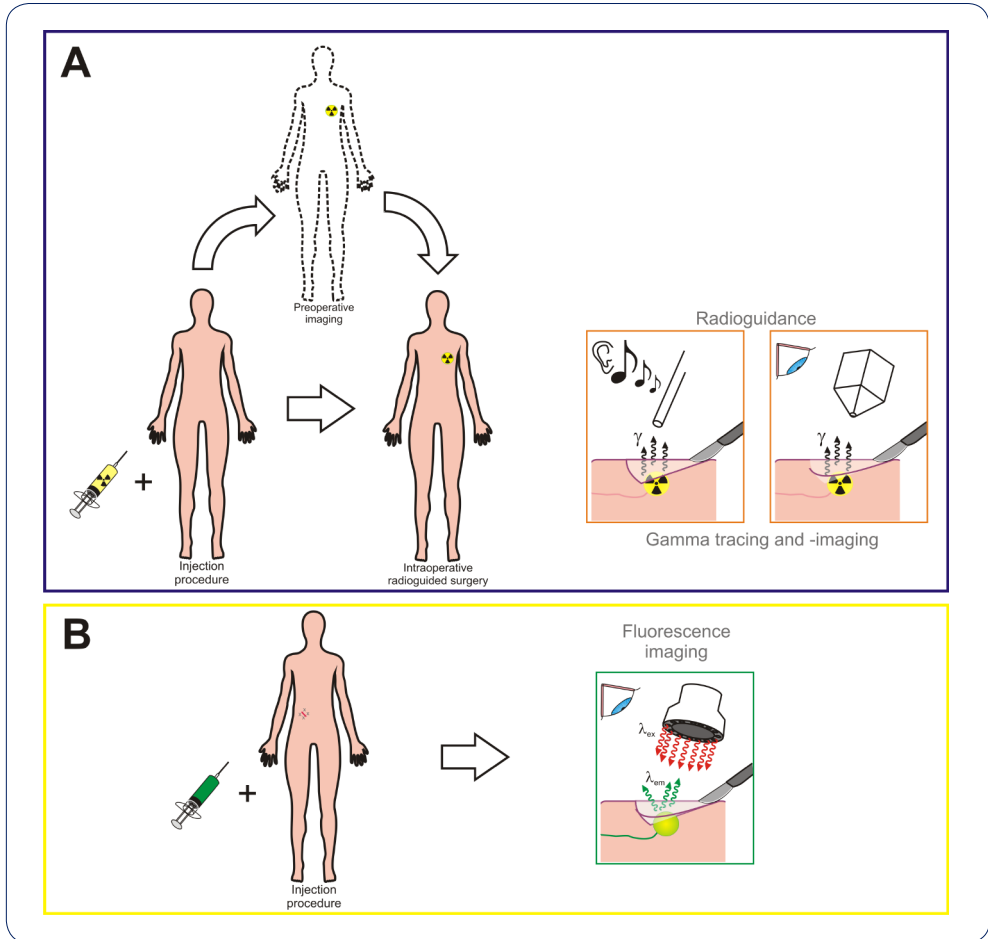


Figure 1. Schematic overview of the addition of optical guidance to the conventional radioguided surgery approach.

A Radioguided surgery approach. Following radiotracer injection, preoperative imaging can be performed to localize the lesion of interest. Intraoperatively, the lesion of interest can then be approached/ localized with a gamma/PET probe and/or via gamma imaging using a portable gamma camera.

B With the injection of a fluorescent tracer, e.g., incorporated in a hybrid tracer, intraoperative fluorescence imaging allows visualization of the lesion of interest.

RADIOGUIDED SURGERY TECHNIQUES

RADIOGUIDED SURGERY TECHNIQUES BASED ON INTRAVENOUS RADIOTRACER ADMINISTRATION

Radioguided intraoperative margin evaluation (RIME)

In RIME, radioactivity is used to check whether the resection margins are negative. Depending on the tumor targeting capabilities of the radiolabeled tracer, accumulation in the tumor, or on its surface, can, during the operation, be detected with a gamma-ray detection probe (hereafter referred to as gamma probe). The acoustic readout of the gamma probe helps to detect the extent of the tumor, and therefore allows more complete surgical removal [11]. If any residual activity is encountered in the resection area, this tissue can then also be excised. Radio-immuno-guided surgery (RIGS) is a technique applied in tumor localization. It involves the intravenous injection of radiolabeled monoclonal antibodies, or antibody fragments, that target antigens expressed on tumor cells or in the tumor stroma. These radiotracers can be used for both diagnostic imaging and, in a similar way, in RIME, for surgical localization of tumors [12]. For example, carcinoembryonic antigen-specific monoclonal antibody (anti-CEA MoAB) can be used to locate specific tumors, e.g., rectal cancer [13].

RADIOGUIDED SURGERY TECHNIQUES BASED ON LOCAL INJECTION OF A RADIOTRACER

Sentinel lymph node biopsy

The most commonly applied RGS procedure is SLN biopsy [1]. This approach involves local injection of a radiotracer, whose accumulation is used to detect occult lymph node (micro-) metastases at an early stage by means of selective identification, and biopsy of the lymph node(s) directly draining from the primary tumor (the SLN) [4].

The SLN approach was introduced by Morton et al. [3] and became the clinical standard for the staging of clinically lymph node-negative patients. Later, lymphoscintigraphy following a radiocolloid injection was combined with the use of optical blue dye for SLN identification [14]. Most commonly applied for breast cancer and melanoma, this technique has been found to be valuable for lymphatic staging in a variety of cancers [1].

Radioguided seed localization (RSL)

Radioguided seed localization with a radioactive ^{125}I -seed has been introduced as an alternative to the wire-guided localization (WGL) technology. The ^{125}I -seed is placed in the

tumor center stereotactically or under ultrasound (US) guidance. During surgery, the seed can be localized with a dual-isotope gamma probe, without interfering with, for example, ^{99m}Tc -based SLN biopsy.

Radioguided seed localization is also useful in the neoadjuvant setting. Because of the long half-life of the ^{125}I -seed (59 days), it can be placed prior to the start of the neoadjuvant chemotherapy while remaining traceable after completion of the chemotherapy regimen [15]. Migration of the seed is rare and high rates of radical excision have been reported both in primary surgery and after neoadjuvant chemotherapy [15, 16].

Radioguided occult lesion localization (ROLL)

Radioguided occult lesion localization, similar to RSL, also provides an alternative to WGL. With this technique, injection of ^{99m}Tc -labeled radiocolloid into the tumor is followed by a local excision, guided by the gamma probe. In the neoadjuvant setting, a twist marker is inserted into the tumor before the start of neoadjuvant chemotherapy. After the chemotherapy and prior to surgery, the ^{99m}Tc is inserted next to the marker. High rates of radical resections have been reported with ROLL [17]. ^{99m}Tc has a short half life (6 h) and, for this reason, its application in the neoadjuvant setting requires, for ROLL, two interventions before surgery (i.e., insertion of the marker and injection of the radiocolloid); for RSL, on the other hand, only insertion of the ^{125}I seed is required [18]. An advantage of ROLL over RSL is that ROLL can be directly combined with SLN biopsy, resulting in so-called sentinel node occult lesion localization (SNOLL) [19, 20].

Radioguided ultrasound-guided lymph node localization (RULL)

In RULL, radiocolloid injection is followed by conventional preoperative SLN mapping. This is then combined with US examination of the SLNs to ascertain whether there are metastases present. The positive lymph nodes are marked and then surgically excised. RULL constitutes a possible refinement of the “traditional” SLN biopsy procedure [21].

RADIOTRACERS

RADIOTRACERS FOR INTRAVENOUS ADMINISTRATION

Antibodies

In RIGS, the overexpression of specific biomarkers on tumor cells (or in their stroma) is used to identify areas of disease. Targeting is accomplished using radiolabeled monoclonal antibodies or antibody fragments [12]. In colorectal cancer patients, targeting with anti-TAG-72 antibodies was used to detect residual tumor tissue; a poorer survival outcome was found in patients with residual hot spots residual hot spots after resection [22]. This finding suggests that the technique may help optimize radical surgery. Povoski et al. [23] recently applied RIGS in the resection of renal cell carcinoma; the imaging and detection approach used by these authors was based on ^{124}I -cG250 (^{124}I -girentuximab), which targets carbonic anhydrase IX.

In addition to complete antibodies, ^{111}In -labeled antibody fragments of trastuzumab have also been used in human epidermal growth factor 2-positive carcinoma of the breast [24]. Unfortunately, in a pilot study of six patients this tracer did not aid in intraoperative tumor localization; the main reason for the detection failure was believed to be the low dose of activity administered (74 MBq).

Although antibodies have a high affinity and specificity for their target antigen, a disadvantage of applying antibodies for RGS is their long circulation time. To obtain sufficient tumor to-background ratios, the unbound antibodies must be allowed to clear [13]. With antibody fragments, this unbound antibody clearance should be faster, making it possible to reduce the time elapsing between injection and surgery.

^{123}I -iodide and ^{131}I -iodide

The standard therapy for thyroid cancer is complete thyroidectomy followed by ^{131}I -iodide ablation therapy. The two most important radionuclides for localization of thyroid tissue are ^{123}I -iodide and ^{131}I -iodide. When performing a complete thyroidectomy, tracing the radioactive iodine may be used to confirm complete resection; iodine is taken up by thyroid tissue and, on the basis of the radioactive signal, can be used to locate residual thyroid tissue after resection [25]. However, a study by Tunca et al. [26] concluded that the RGS approach was not superior to conventional complete thyroidectomy (Figure 2).

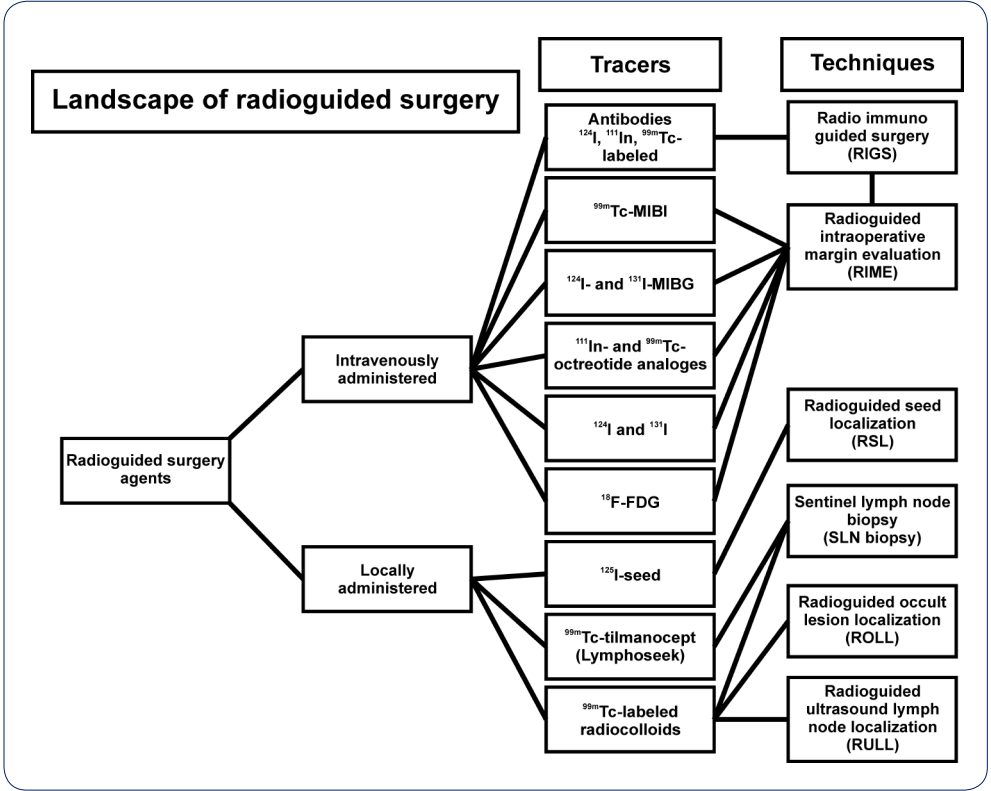


Figure 2. The landscape of radioguided surgery.

¹²⁴I iodine-124, ¹²⁵I iodine-125, ¹³¹I iodine-131, ¹¹¹In indium-111, ^{99m}Tc technetium-99m, ¹⁸F-FDG fluorine-18-fluorodeoxyglucose, MIBG meta-iodobenzylguanidine

^{99m}Tc-sestamibi (^{99m}Tc-MIBI)

Parathyroidectomy with bilateral lymphatic neck dissection is the standard procedure for primary hyperparathyroidism patients [27]. This procedure has very high success rates, but a single residual adenoma can lead to recurrent hyperparathyroidism. Due to its retention in mitochondria, ^{99m}Tc-MIBI can be used for preoperative parathyroid identification using scintigraphy and SPECT/CT imaging (Figure 3) [28]. During surgery, a gamma probe can then be used to localize the ^{99m}Tc-MIBI accumulated in adenomas. Similarly, ^{99m}Tc-MIBI can also be used for recurrent parathyroid adenomas [29].

^{99m}Tc-MIBI has also been used for RGS in iodine negative recurrent differentiated and medullary thyroid cancer [30]. This agent allowed gamma probe localization and subsequent resection of thyroid tissue that had not shown uptake of ¹²³I-iodide or ¹³¹I-iodide.

^{99m}Tc-MIBI scintigraphy can also be used for the detection of breast carcinoma. Its use in a clinical study assessing the RIME technique in breast cancer patients showed a 82.6 % success rate, with a mean tumor free margin of 4.8 mm [11].

Meta-iodobenzylguanidine (MIBG)

¹²³I- and ¹³¹I-MIBG (Adreview®) can be used for the detection/treatment of neuroendocrine tumors (NETs). MIBG mimics the structure of noradrenaline and, therefore, targets the noradrenaline transporter. A systematic review by van Hulsteijn et al. [31] discussed the application of MIBG for the intraoperative detection and resection of NETs. They concluded that in specific cases, like small non-palpable tumors or tumors located in adhesive scar tissue, MIBG can be useful for intraoperative detection.

However, in some instances somatostatin analogs like ¹¹¹In-pentetreotide may have higher sensitivity than MIBG labeled with iodine for targeting NETs [31]. ¹¹¹In- and ^{99m}Tc-octreotate analogs ¹¹¹In-diethylenetriamine penta-acetic (DTPA)-octreotide (Octreoscan®), ^{99m}Tc-depreotide (Neotect®) and ¹¹¹In-pentetreotide can be used for the detection of tumors overexpressing the somatostatin receptor [e.g. NETs, (non) small cell lung cancer] (Figure 4) [32–34]. The highly specific uptake of these compounds in the tumor tissue allowed radioguidance towards the tumor. For example, complete resection of ACTH-secreting bronchial carcinoids was achieved with RGS after injection of ¹¹¹In-pentetreotide [34]. With this tracer, even millimeter-sized tumor nodules and lymph node metastases could be resected. A big advantage of these receptor-targeted peptides is their good biodistribution and rapid unbound tracer clearance, which results in a good tumor-to-background ratio in a relatively short time (1–24h). However, their short *in vivo* half-life does impose strict timing of injection, preoperative imaging, surgical planning and the RGS procedure.

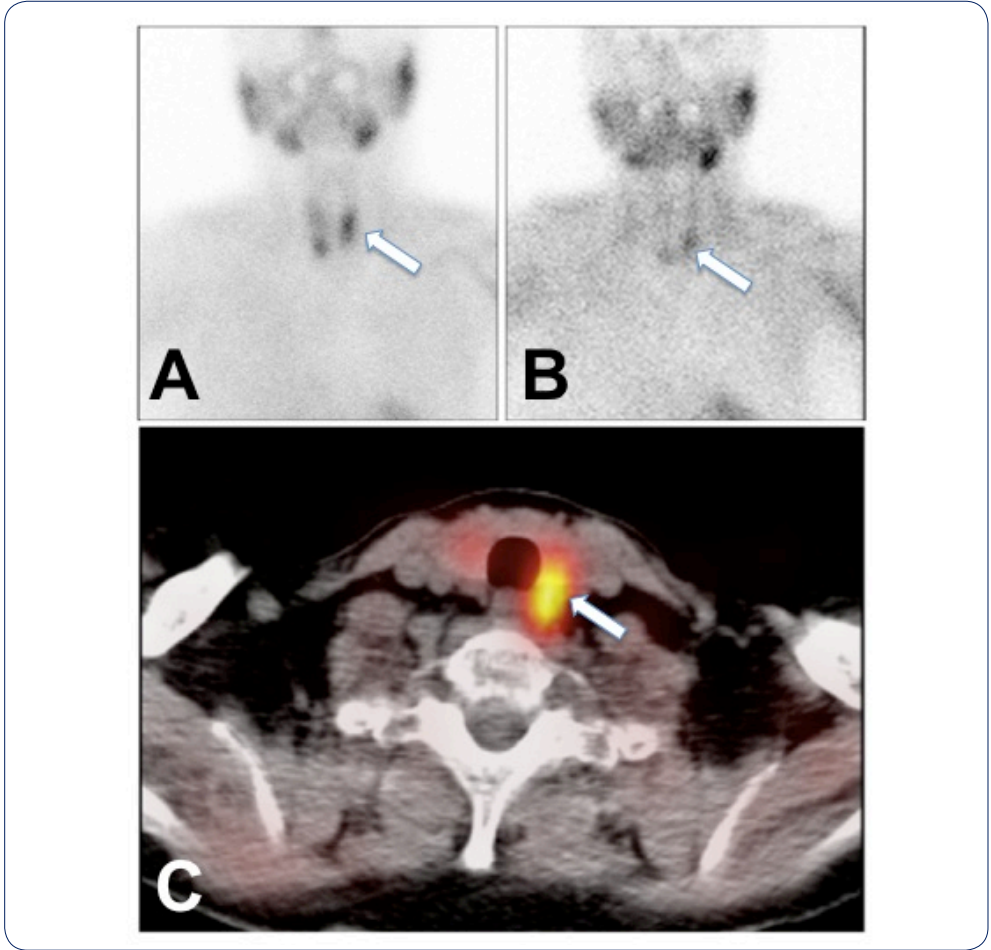


Figure 3. ^{99m}Tc-MIBI for parathyroid adenoma localization.

A ^{99m}Tc- MIBI scintigram showing heterogenous thyroid uptake on the early image with **B** retention of the tracer in the left lobe on the 3 h postinjection image. **C** SPECT/CT clarifies the abnormal uptake seen in the dorsal area of the left thyroid lobe. During radioguided surgery a parathyroid adenoma was resected

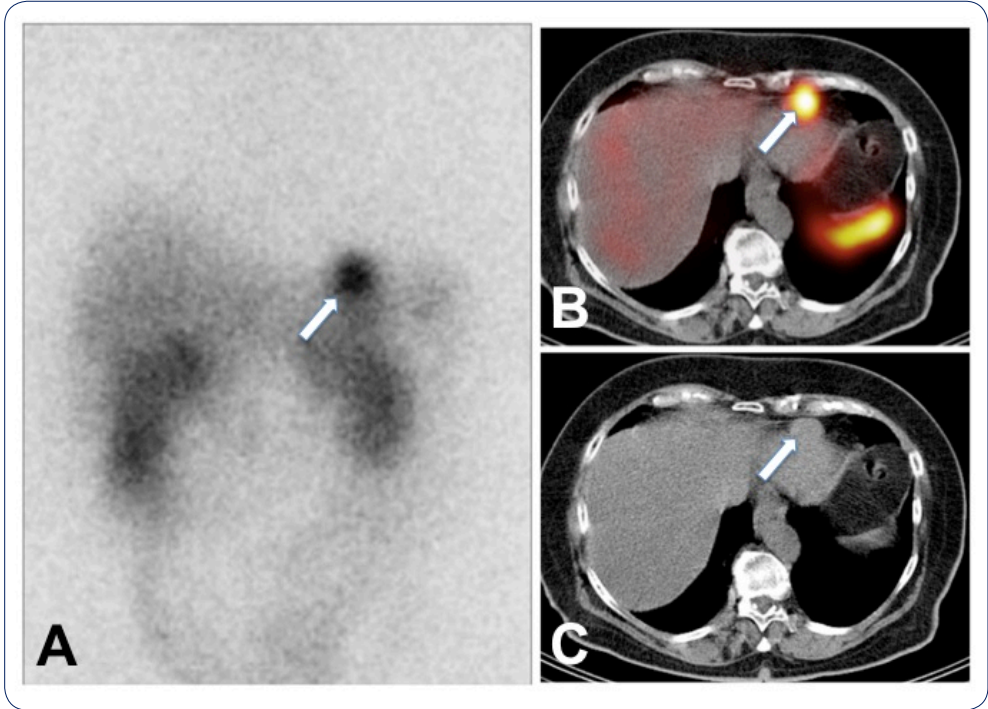


Figure 4. ^{111}In -DTPA octreotide for carcinoid tumor localization.

A Following an intravenous injection of ^{111}In -DTPA octreotide, accumulation in the left side of the liver was seen on the scintigram (arrow).

B Fused SPECT/CT and **c** CT image showing radiotracer uptake in a prehepatic lymph node.

¹⁸F-FDG

Generally, malignant tumors have an accelerated glucose metabolism, which results in higher uptake of the PET tracer ¹⁸F-FDG. Due to the high signal-to-background ratios obtained, ¹⁸F-FDG is considered valuable for imaging tumor diffusion throughout the body in a variety of malignancies [35]. The ability to detect different types of malignancies makes this tracer a possible generic tracer for RGS-based tumor resection [36].

RADIOTRACERS FOR LOCAL ADMINISTRATION

Radiocolloids

The most frequently used radiotracers for RGS, especially for SLN biopsy, are ^{99m}Tc-sulfur colloid, ^{99m}Tc-colloidal human albumin (^{99m}Tc-nanocolloid), ^{99m}Tc antimony trisulfide colloid, and ^{99m}Tc-tin colloid [1, 37]. ^{99m}Tc-labeled radio colloids have been applied mainly in SLN biopsy and ROLL/SNOLL procedures [4, 17]. Particle sizes and the amount/volume of radiocolloid injected influence the lymphatic flow and the overflow of these tracers into higher echelon nodes; accumulation of all these SLN tracers is based on uptake in the macrophages [38]. Recently, a PET derivative was introduced in the form of 89-zirconium (⁸⁹Zr)-labeled nanocolloid [39].

Tilmanocept (Lymphoseek®)

^{99m}Tc-tilmanocept was introduced as an alternative to the above-mentioned radiocolloids [40, 41]. ^{99m}Tc-tilmanocept is smaller than most radiocolloids (mean molecular diameter of 7 vs. 10–600 nm); it is based on a dextran backbone substituted with multiple mannose subunits and chelates to allow radiolabeling with ^{99m}Tc [42]. It has been suggested that ^{99m}Tc-tilmanocept accumulates in the SLNs by binding to mannose receptors on reticulo-endocytes [43].

INTRODUCTION OF (FLUORESCENT) DYE GUIDANCE TO EXTEND RADIOGUIDED SURGERY

Radioguided surgery is an excellent technique for the preoperative localization of a lesion of interest. However, as mentioned above, in the intraoperative setting the limited spatial resolution of this modality is suboptimal for precise (last millimeter) localization. For this part of the resection, an additional tool based on real-time and high-resolution imaging (e.g., fluorescence) can be of great benefit.

Blue dye is commonly used to visualize the lymph vessels and the SLN during the surgical procedure. Motomura et al. [44] were the first to describe the use of the near infrared dye ICG for SLN biopsy in breast cancer; ICG was used in a manner similar to blue dye. Thereafter, many different research groups adapted this fluorescence-based approach with ICG for SLN biopsy in different types of cancer, e.g., in melanoma and breast cancer [45, 46]. Fluorescent dyes have a higher tissue penetration compared to conventional blue dye, but unfortunately, the tissue penetration remains limited to approximately 1 cm [46]. This limitation can be overcome by combining ICG with the high tissue penetration of radioactive agents [9, 47, 48].

Hybrid-imaging agents

In 2011, we introduced the clinical application of a hybrid radiolabeled colloid (ICG non-covalently bound to ^{99m}Tc -nanocolloid) in prostate cancer patients [49]. With a single injection of this hybrid tracer, we were able to visualize the SLNs both preoperatively, using lymphoscintigraphy and SPECT/CT, and intraoperatively via fluorescence imaging (Figure 5). The same hybrid tracer has now been used for SLN biopsy in various types of malignancies, e.g., melanoma and penile cancer [10, 47, 50]. This success should encourage further extension of the hybrid approach to surgical areas where there is room for improvement of the RGS technique.

To provide a hybrid derivative for the detection of parathyroid disease, methylene blue has been iodinated with ^{123}I [51]. However, in a clinical study, this tracer was not found to be effective enough to replace the standard tracer ^{99m}Tc -MIBI [52]. Methylene blue labeled with ^{125}I has instead been evaluated for SLN biopsy in a clinical trial in breast cancer [10]. The solution of ^{125}I -methylene blue are injected locally in or around the tumor, after which it was transported by the lymphatic system to the SLN. With a hand-held gamma camera, the gamma rays were detected and the blue signature of methylene blue was used to optically identify the SLN. Rapid lymphatic drainage of the hybrid tracer is a possible downside of this approach.

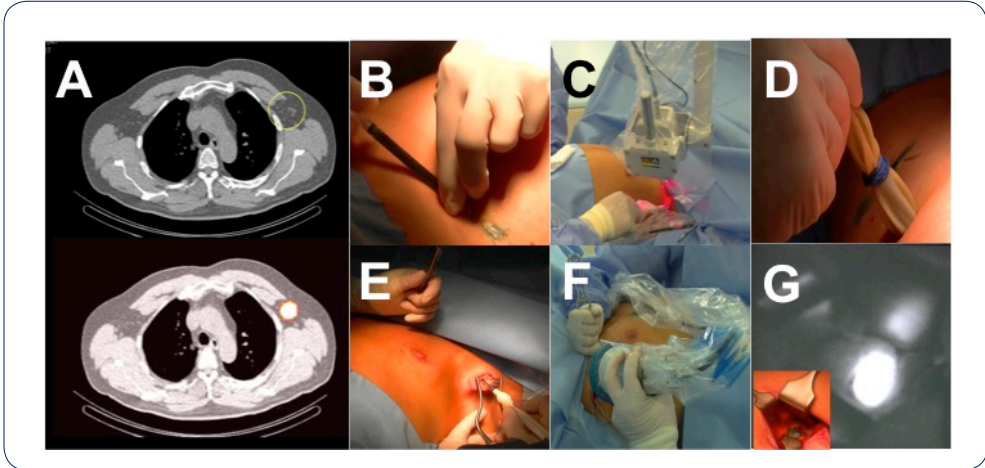


Figure 5. Multimodal surgical guidance towards the sentinel lymph node.

- A** Following ICG- ^{99m}Tc -nanocolloid injection, preoperative lymphoscintigraphy and SPECT/CT imaging are performed to locate the SLN (circle).
- B** Prior to the start of the operation patent blue dye is injected.
- C** Prior to incision, an image with gamma camera is obtained to confirm the localization of the SLN.
- D** Pre-incision gamma tracing with the gamma probe allows localization of the SLN.
- E** Incision.
- F** Camera used to detect the fluorescent signal in the SLN. **G** Fluorescence signal detected in the SLN. SLN sentinel lymph node.

In the preclinical setting, a great number of hybrid alternatives to existing RGS tracers are currently being developed and evaluated (Table 1). For example, ^{99m}Tc -tilmanocept has been covalently labeled with Cy7 for fluorescence imaging. In a mouse model it was shown that the popliteal SLN could be visualized with fluorescence imaging following a footpad injection with Cy7- ^{99m}Tc -tilmanocept [53]. Multimodal marker seeds have been shown to allow hybrid RSL towards a mouse prostate [54]. Here, the ^{99m}Tc signature allowed preoperative SPECT imaging, whereas intraoperative fluorescence detection provided optical information about the exact location of the seed [54].

Targeted hybrid-imaging agents

Research efforts are currently focusing on the development of targeted hybrid tracers allowing accurate tumor identification. Despite these efforts, which have been reviewed by Kuil et al. [8] and Azhdarinia et al. [55], to date no such tracers have been clinically implemented. Hybrid-labeled derivatives of the clinically applied octreotide have been extensively evaluated

preclinically, yielding fluorescent and radioactively labeled imaging agents with high affinity for the somatostatin receptor [8, 56]. Alongside these hybrid counterparts of clinically used peptide-based tracers, several other interesting hybrid tracers are being developed, based, for example, on tumor targeting peptides targeting the chemokine receptor 4 and the gastric releasing peptide receptor [8]. A hybrid tracer targeting the prostate-specific membrane antigen (PSMA), which is frequently overexpressed in prostate cancer, was recently developed by Banerjee et al. [57] by combining the near-infrared dye CW800 and ^{111}In -1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracarboxylic acid (DOTA) with a PSMA-inhibitor.

Multiple hybrid tracers have been generated for avb3- integrin, which is overexpressed by activated endothelial cells during angiogenesis. These hybrid tracers contain a Cy5- or Cy7-derivative as fluorescent dye and a DTPA, deferoxamine or DOTA chelate to introduce ^{111}In , ^{64}Cu or ^{68}Ga , respectively [8]. Liu et al. [58] recently introduced a tracer that combines the fluorescent dye BODIPY, containing ^{18}F , with the cyclic arginine–glycine–aspartate (RGD) peptide targeting the avb3-integrin for combined PET and optical imaging.

In addition, various hybrid-labeled antibodies have been tested in mouse models [59–62]. ^{64}Cu -1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA)-bevacizumab-800CW was applied to detect human glioblastoma tumors by both nuclear and optical imaging [63]. The anti-CD105 antibody TRC105 was labeled with ^{64}Cu or ^{89}Zr for PET imaging and 800CW for intraoperative optical fluorescence-based detection in a mouse model of breast cancer-derived lung metastases [64]. Recently, Cohen et al. [65] described a protocol for the labeling of monoclonal antibodies with 800CW and ^{89}Zr for hybrid imaging. Although not yet used for clinical studies these antibody-based hybrid tracers seem promising for future applications.

MODALITIES USED DURING HYBRID-RADIOGUIDED SURGERY

Different modalities can be used for the intraoperative localization of the preoperatively injected radiotracers. Devices to locate both gamma- and positron-emitting radioisotopes, as well as devices that can excite fluorescent dyes and collect their emission light, are discussed below. Ideally, the introduction of a hybrid approach also drives the development of hybrid-imaging devices.


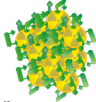
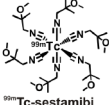
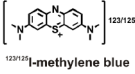
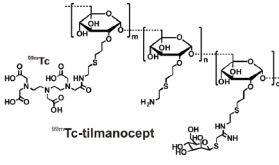
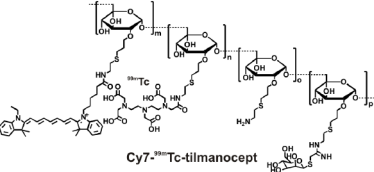

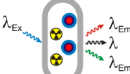
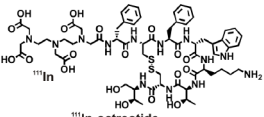
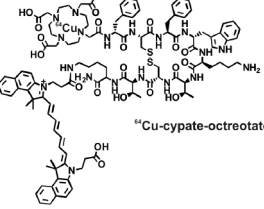

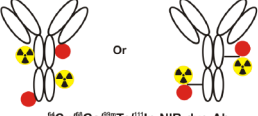


Radiotracers for RGS	Hybrid tracers for combined RGS / Fluorescence guided surgery	Hybrid tracers in clinical use	Ref
 <p>^{99m}Tc-nanocolloid</p>	 <p>^{99m}Tc-nanocolloid</p>	Yes	49
 <p>^{99m}Tc-sestamibi</p>	 <p>[^{123I/125I}] ^{123I/125I}-methylene blue</p>	Yes	10, 51
 <p>^{99m}Tc-tilmanocept</p>	 <p>Cy7-^{99m}Tc-tilmanocept</p>	No (Preclinical)	53
 <p>¹²⁵I marker seed</p>	 <p>Hybrid marker seed</p>	No (Preclinical)	54
 <p>¹¹¹In-octreotide</p>	 <p>⁶⁴Cu-cypate-octreotate</p>	No (Preclinical)	56
 <p>⁶⁴Cu/⁶⁷Ga/^{99m}Tc/¹¹¹In-Ab</p>	 <p>Or ⁶⁴Cu/⁶⁷Ga/^{99m}Tc/¹¹¹In-NIR-dye-Ab</p>	No (Preclinical)	59-62
 <p>¹²³I-MIBG</p>	Not available	Not applicable	-
 <p>¹⁸F-FDG</p>	Not available	Not applicable	-

Table 1. Currently used radiotracers and preclinically and clinically used hybrid-imaging agents

PET detection probes

Positron emission tomography detection probes are capable of detecting the 511-KeV gamma rays that are emitted by the annihilation of a positron–electron pair. Unfortunately, the collimators and shielding used for detection make these probes less convenient during surgery; 511-keV gamma rays are highly penetrating [36, 66, 67]. Another PET tracer-detection method is direct detection of the β -particles (positrons). These particles have limited soft tissue penetration (max. 2.4 mm) [68]. As a result, direct positron detection is easily hindered by overlying/ surrounding tissue, similarly to what happens with the use of fluorescent dyes [6]. On the other hand, this property may provide increased resolution, making it possible to distinguish between tumor tissue and healthy tissue [66].

Cerenkov luminescence emitted by β -emitters can also potentially be used for intraoperative optical detection and might be an alternative to hybrid agents with a fluorescent dye [69]. That said, Chin et al. [6] concluded that with the current imaging modalities, the fluorescent signature of a hybrid tracer is superior to Cerenkov luminescence.

Gamma probes

Gamma probes can be used to locate different isotopes with low, medium and high energies. The technical features and capabilities of common gamma probes have been reviewed by Zanzonico and Heller [66, 70]. In these reviews they also discuss the capabilities of the gamma probes.

With gamma probes, it is sometimes difficult to locate areas of interest near the injection site, because the signal can be masked by background ‘signal bleeding’ [71]. As hybrid radioactive and fluorescent imaging agents emerge, a possible future development would be a gamma probe that is also capable of (acoustic) fluorescence detection.

Hybrid-imaging devices can be obtained either by modifying existing RGS devices or fluorescence cameras or by developing new imaging devices. Although it is not yet available for fluorescence imaging, there does exist a system that allows acoustic gamma tracing and acoustic blue dye tracing (EuroRad, Strasbourg, France). A gamma probe that can also detect fluorescence could be used to determine margins intraoperatively, overcoming the limited tissue penetration of near-infrared dyes.

Gamma cameras

While a gamma probe provides an acoustic signal when it detects gamma rays, a gamma camera allows the generation of 2D visual images of the location of the radioactive signal [36]. Compared to gamma probes, mobile gamma cameras show improved imaging

resolution [66]. Several hand-held and mobile gamma cameras are described in a review by Tsuchimochi and Hayama [36]. The main advantage of a mobile gamma camera is that it allows reproduction, in the operating room, of preoperatively acquired images. Moreover, by generating pre-incision and post-excision images, a comparison can be made to determine whether there is any residual activity at the excision site, which should also be removed.

A disadvantage of gamma cameras is the delay in visualization of hot spots, which results in long acquisition times in the presence of a weak radioactive signal in the lesion of interest. Moreover, since the current systems do not provide 'white light' options to visualize the surgical field, there is a lack of anatomical information of the area of interest. Furthermore, the current gamma camera systems do not usually provide depth information.

A promising development for gamma cameras may therefore be their integration with optical cameras to provide visualization of radioactive hot spots within the corresponding anatomical context. Blake et al. [72] published data relating to the application of a prototype mini-gamma camera integrated with an optical camera: the first results in patients injected with ^{99m}Tc -hydroxymethylene diphosphate for a standard bone scan showed accumulation of the activity in the thumb.

A next step may be the integration of fluorescence cameras. In combination with the right hybrid tracer, devices of this kind would be capable of hybrid surgical guidance using a single modality.

Intraoperative navigation devices

The simplest example of navigation uses a mobile gamma camera that allows dual-isotope detection, as shown by Vermeeren et al. [73] during laparoscopic SLN biopsy in prostate cancer. After a ^{125}I -seed had been attached to the tip of a laparoscopic gamma probe, its location could be detected on screen together with the signal coming from ^{99m}Tc in the SLN. By moving the gamma probe towards the SLN, the signal from the ^{125}I -seed could be followed on the gamma probe, providing on-screen 2D navigation to the SLN [74].

The introduction of freehand SPECT technology has made it possible for surgeons to intraoperatively acquire a SPECT scan of a region of interest. By tracking both the gamma probe and a positioning system fixed on the patient's body, this technique allows intraoperative 3D mixed reality-based navigation of the gamma probe to the lesion of interest, e.g., during SLN biopsy for breast cancer [75]. As well as allowing real-time acquisition of SPECT scans in the operating room, the system can also load preoperatively acquired SPECT/CT data. It has been shown that this approach can also be used to navigate tools other than the gamma probe, e.g., a fluorescence laparoscope, towards radioactive hotspots [76]. Fluorescence

imaging allows errors within the 1-cm range (due to organ movement or deformation for example) to be compensated for [76].

With the exception of a navigation system that uses virtual reality to visualize the radioactive hot spots in the anatomical context, the current RGS techniques do not provide anatomical information [77]. An additional tool that combines the beneficial properties of both modalities would be of great value in this field of surgery.

CONCLUSION

The applications of RGS are continuously expanding as new receptor-targeted imaging agents and intraoperative tools emerge. Since radio- and fluorescence-based surgical guidance modalities each have their own beneficial properties, the development of hybrid approaches, which combine advantageous features of both techniques, holds great promise for the further refinement of RGS.

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