Published in final edited form as:

Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2016 July ; 8(4): 498–511. doi:10.1002/wnan.1381.

Image-guided tumor surgery: will there be a role for fluorescent nanoparticles?

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Abstract

Image-guided surgery (IGS) using fluorescent nanoparticles (NPs) has the potential to substantially impact patient treatment. The use of fluorescence imaging provides surgeons with real-time feedback on the location of diseased tissue using safe, low-cost imaging agents and instrumentation. Fluorescent NPs are likely to play a role as they are capable of taking advantage of the enhanced permeability and retention (EPR) effect and can be modified to avoid clearance, increase circulation time, and specifically target tumors. Clinical trials of IGS using the FDA-approved fluorophores indocyanine green and methylene blue have already shown preliminary successes, and incorporation of fluorescent NPs will likely improve detection by providing higher signal to background ratio and reducing false-positive rates through active targeting. Preclinical development of fluorescent NP formulations is advancing rapidly, with strategies ranging from passive targeting to active targeting of cell surface receptors, creating pH-responsive NPs, and increasing cell uptake through cleavable proteins. This collective effort could lead to clinical trials using fluorescent NPs in the near future.

INTRODUCTION

The development of nanoparticles (NPs) for cancer therapeutics and imaging has shown significant growth over the past decade and a half. Clinical trials for NP formulations have increased significantly, but the number of clinically approved NPs is still limited.¹ Increasingly, investigations that use NPs for improved cancer imaging have appeared, primarily for pre-operative modalities such as magnetic resonance imaging (MRI).^{2,3} Image-guided surgery (IGS) is a relatively new field that seeks to identify the location of diseased tissue during the course of surgical resection, often in real time. A primary direction of research in this field is the use of fluorescent molecules to highlight tumors for resection in combination with dedicated imaging systems. Preclinical formulations examined to date have included both small molecules and NPs; clinical trials that utilize certain small molecule fluorophores have recently been initiated.^{4–7}

Fluorescent NPs have several distinct advantages over their small molecule counterparts. Compared to small molecules, NPs can, for example, reduce the rate of renal filtration and

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Conflict of interest: AMM is a co-inventor of image-guided surgery technology licensed to Spectropath, Inc. (Atlanta, GA).

improve retention both in the vasculature and in the tumor due to larger diameter and mass; have amphiphilicity for the delivery of hydrophobic molecules; and possess a high surface area-to-volume ratio to allow for conjugation of stealth and active targeting moieties, in addition to unique materials properties.^{8–10} In this article, we explore the rationale to support the opinion that NPs will have a role in fluorescence IGS. To that end, fluorescence, as opposed to modalities such as CT or MRI, is more cost efficient, mobile, requires less dedicated space for operation, and is in many ways safer for both patients and caregivers. Additionally, fluorescence imaging has the potential for high-resolution, real-time imaging that has minimal disruption to the surgical procedure. The unique and controllable fluorescence properties of NPs in combination with state-of-the-art intraoperative image systems have the potential to benefit surgical outcomes.

The Clinical Need for IGS

Surgery is one of the most common and effective forms of treatment for solid tumors.¹¹ It is commonly understood that the ultimate cause of death is not the primary tumor but rather the metastases that develop in critical areas and proceed to shut down physiological functions. It is therefore paramount that the primary tumor, local metastases, and metastatic lymph nodes are removed prior to distant metastasis. For example, 63–98% of either lung, breast, bladder, or colorectal cancer patients will receive surgery, depending on type, grade, and age at diagnosis.¹¹ In general, 6–57% of prostate cancer patients will receive surgical intervention depending on age, with younger patients more likely to receive surgery. Surgery is the only curative option for colorectal metastases to the liver.¹² However, much of the intraoperative detection of tumors relies on visual and tactile detection of malignant versus benign or healthy tissue, which in some cases result in clinically significant levels of residual cancer cells and undetected metastases that could be left in patients.

Positive margins (PMs) are residual cancer deposits found at the borders of surgically removed tissue, and pose a significant health risk. Approximately 30% of breast cancer patients will experience recurrence of disease locally or systemically,^{13,14} and rates of PM occurrence are estimated at 20–40% of patients receiving breast-conserving surgery.¹⁵ For hepatic resection of colorectal metastases, the intrahepatic recurrence rate is 11–37.5%, and overall recurrence is as high as 40–50%, with PMs observed in 2–5% of patients.^{16,17} Importantly, in patients receiving both chemotherapy and surgical treatment for colorectal metastases to the liver, PMs have been identified as the only risk factor for patient survival that is associated with the surgery itself.^{18,19} PMs also occur in 11–38% of patients receiving radical prostatectomy and are a strong negative predictor of recurrence and patient survival.^{20–22} Traditional imaging modalities such as radiography, MRI, PET, and single photon emission computed tomography typically involve instrumentation that is too cumbersome or dangerous to use in the surgical theater, while ultrasound (US) is often incapable of providing tumor-specific information with contrast and resolution high enough to identify PMs and local metastases.²³ To combat these limitations, a number of image-guided modalities are currently under investigation, among them is fluorescence imaging of both endogenous and exogenous fluorophores.^{24–26}

Fluorescence Imaging in Cancer

Fluorescence IGS has seen a number of preliminary successes.^{5,27,28} Biomedical fluorescence imaging is concerned with wavelengths in the visible spectrum (400–700 nm), and the near infrared (NIR) spectrum (700–900 nm). Commercially available fluorophores are available for a large number of excitation and emission peaks. For research purposes, most are found in the visible spectrum. However, due to tissue autofluorescence and high absorbance in the visible spectrum, NIR fluorophores are better suited for *in vivo* imaging. Wavelengths below 700 nm are strongly absorbed by endogenous molecules, such as hemoglobin and myoglobin, while water and lipids absorb strongly above 900 nm.^{29,30} This prevents practical imaging using these wavelengths beyond more than 1 mm into tissue. The ‘NIR window,’ from 700 to 900 nm is the result of minimized absorbance and a predominance of scattering in tissues, allowing for imaging and detection up to several millimeters deep.^{29,30}

There are currently two FDA-approved NIR fluorophores: indocyanine green (ICG) (ex. 780 nm; em. 820 nm) and methylene blue (ex. 665 nm; em. 700 nm). ICG is of greater interest than methylene blue as the excitation and emission wavelengths show less tissue absorption and it has a greater quantum yield.^{26,31} ICG is used clinically outside of IGS to measure cardiac output, hepatic function, and retinal angiography.²⁶ Within IGS, ICG has been used for multiple oncological indications, including sentinel lymph node (SLN) mapping and identification of solid tumors.^{26,27} A number of additional contrast agents are currently being investigated, including several other cyanines such as Cy5.5, Cy7, Cy7.5, IR-dyes (LICOR, Lincoln, NE.), NP formulations, and visible spectrum dyes.³²

SLN mapping and biopsy is often used in an effort to detect metastatic disease, but proper identification of lymph nodes draining from the tumor must be achieved with high precision. Current methods utilize the chromophore Patent Blue or gamma probes, or a combination of both.³³ The use of ICG in SLN mapping is recent, but becoming well established. Use of ICG fluorescence has resulted in detection of nearly 99% of SLN in breast cancer patients and 98% in melanoma patients.^{26,34} A number of clinical studies have also shown success in SLN mapping with ICG in cervical, vulvar, and endometrial cancers.²⁵ Similar success rates of SLN detection occur for skin and gastric cancers; however, gastric and colorectal cancers may exhibit higher false-negative rates when ICG is used, depending on tumor grade and location.^{26,34–36} Studies that compare ICG fluorescence to the current clinical practice of radioisotope identification show that ICG fluorescence alone is comparable to radioisotope identification, and is just as effective when used in combination with radioisotope identification.^{34,36}

Using ICG to delineate tumors in humans has shown a number of preliminary successes.²⁷ Gotoh et al. used intravenous injections of ICG between 1 and 8 days prior to surgery and found that ICG fluorescence successfully identified 100% of primary tumors and in 40% of the cases also identified additional, small (3–6 mm) hepatocellular carcinomas that went undetected either preoperatively or by intraoperative US.³⁷ More recently, studies have examined ICG for use in image-guided resection of multiple cancer types, including lung and chest masses, and ovarian cancer.^{6,38} Tummers et al. showed successful imaging of 100% of malignant ovarian lesions; however there was no difference in fluorescence level of

malignant and nonmalignant lesions, causing a false-positive rate of 62%.³⁸ Holt et al. showed successful imaging of human lung and chest tumors, as well as canine tumors, with an average signal to background ratio of 6.7, which assisted in tumor resection.⁶ However, surgeons had difficulty distinguishing inflamed from diseased tissue based on NIR fluorescence. The use of other fluorophores in IGS for additional cancers has also been met with clinical success.

Additional fluorophores have also been implemented in humans. A phase III multicenter clinical trial investigating the use of fluorescence IGS for glioma patients showed significantly higher levels of complete tumor resection (90 versus 36%) and 6-month progression-free survival (41 versus 21%) when compared to conventional surgery conducted only with white light.³⁹ In another study, folate-FITC was used as a targeted contrast agent for ovarian cancer, and resulted in significantly more tumors being identified than using white light alone.⁵ Additionally, fluorescence intensity qualitatively correlated with folate receptor- α expression and tumor grade, indicating successful active targeting. These studies show the exceptional ability of fluorescence IGS to assist surgeons in diseased tissue identification. Another study utilized the FDA-approved NIR fluorophore, methylene blue, to assist in identification and resection of breast cancer, resulting in an 83% identification rate.⁴ Importantly, this study also indicated the potential of NIR IGS to identify PMs because fluorescent signal occurred in 2/4 patients with PMs identified histologically.

Fluorescence IGS Instrumentation

A number of IGS systems are currently available, in clinical trials, or under investigation.^{25,40,41} These instruments are composed of an excitation source, generally either a laser or LED system, for excitation of fluorophores, and light collection system that preferably provides both white and NIR image capture for overlay of the fluorescent signal onto the anatomical field, as shown in Figure 1. Additionally, the use of a handheld excitation source may also allow for spectral collection, providing a secondary means of signal detection. By detecting fluorescence emission through a handheld excitation source, which may be placed closer to the tissue of interest, the surgeon may rely on an additional criterion for determining diseased tissue.

Excitation light sources and wavelengths can be tuned to the contrast agents, which highlights the interdependency of the contrast agents and instrumentation used. The light source must be powerful enough to provide significant fluence to excite the fluorophore in the surgical field, while maintaining a working distance for the surgery itself.⁴² LED excitation sources are capable of illuminating the entire field of view, but heat dissipation and adequate fluence are a concern. Laser excitation is another option, which allows for greater surgical control but requires personal protective goggles and there are concerns regarding patient exposure.³² The field of view must be sufficient and adjustable for the surgeon to adequately capture the surgical field, and must be illuminated by a light source that does not overlap with the fluorescence signal. The collection optics and camera system are important, as signal loss by cameras due to low quantum efficiency and resolution loss will significantly affect the imaging potential.³² The display typically uses overhead

monitors, but dedicated goggles are also being investigated.⁴³ Currently there is no standard of care in NIR fluorescence imaging, and further studies are required to determine optimal system requirements and contrast agents.

Cost and space are additional advantages of fluorescence imaging over traditional CT and MRI instruments. Currently, new MRI instruments cost several million dollars and CT scanners may cost several hundred thousand dollars. Both require a significant area of dedicated space for installation and operation, which limit the availability of such systems. Fluorescence imaging systems on the other hand have the potential to be far less expensive, smaller, and more mobile. One cost estimate of the (fluorescence-assisted resection and exploration) FLARE system is \$120,000 USD and \$40,000 USD for the mini-FLARE.⁴⁴ These attributes could help to mitigate the cost of implementation as well as provide imaging to multiple operating rooms with a single instrument. Fluorescence imaging also has advantages over intraoperative US, as resolution can be substantially higher using fluorescence.²³ The breadth of fluorophore wavelengths and biologically relevant ligands to which they can be attached also far outweighs what is possible with microbubbles used in US because microbubbles are generally restricted to the vasculature due to their large size.²³

Advantages of Fluorescent NPs Over Small Molecules

Tumor Structure and the Enhanced Permeability and Retention Effect—For those NPs that reach the tumor, penetration into the interstitium and delivery to cells provides an additional barrier. Tumor biology is complex and varies between tumor type, location, and patient. For these reasons, the vascular and stromal structures of any one tumor will be unique, and will thus result in different NP distributions. The extent of NP penetration into and retention within tumors depends on the vascular endothelial discontinuities, density and composition of the extracellular matrix, pressure, and lymphatic drainage. These features are likely to be so variable that different NP formulations may be required for different tumor types. Nonetheless, similarities exist between all tumors exhibiting enhanced permeability and retention.

The EPR effect results from the poor vascular formation in solid tumors, causing preferential retention of macromolecules and NPs in solid tumors, as shown in Figure 2.^{8,45–48} When tumors reach a sufficient size, oxygen and nutrients cannot diffuse rapidly enough to support cells in the interior of the tumor.⁴⁹ When this occurs, growth factors are released that trigger angiogenesis in the surrounding tissue, forming new blood vessels. The vasculature formed, however, is not well structured. Endothelial cell linings are not as close as in healthy vasculature and there is often a poorly formed or complete lack of smooth muscle cells lining the basement membrane. These properties lead to the increased extravasation of all molecules that fit between the discontinuities in the endothelial layer, ranging from 200 to 2000 nm. After extravasation, larger particles are preferentially retained in the tumor due to the lack of appropriate lymphatic drainage and slower rates of diffusion. This is advantageous as many first-generation NPs are nontargeted, and NPs loaded with FDA-approved fluorophores may be readily delivered to tumors associated with the EPR effect. However, due to variability in the structure and extent of vasculature, tumor stroma, and

lymphatic drainage, the EPR effect varies by tumor type and location, making it a useful but nonuniversal targeting method.

NP Size—The effect of size on NP delivery is multifaceted. Nanomaterials are by definition on the order of nanometers in at least one dimension, and in the case of NPs for biomedical application, three dimensions. These scales are chosen for the unique physical and chemical properties that arise from particles at this scale, one of which is delivery and accumulation within tumors. NPs are advantageous because particles above 10 nm will avoid renal clearance, while those smaller than the vascular discontinuity size of tumors will penetrate into the tumor interstitium.⁸ This prevents a primary mode of clearance experienced by small molecules and can thus allow for much longer circulations times.^{50,51} However, a further hindrance to delivery is the 50–100 nm fenestrations in the liver and interaction with hepatocytes and Kupffer cells that results in RES uptake.⁵² Additionally, what is optimal for avoiding RES uptake may not be optimal for tumor uptake.⁵³ Complicating the matter more, what is optimal for tumor uptake may not be optimal for tumor retention.⁵⁴ Experiments performed strictly examining the MW on the effect of tumor penetration and retention found that total tumor accumulation was highest for 40–70 kDa molecules, as higher MWs resulted in lower penetration but longer circulation time, while lower MW resulted in higher penetration but rapid diffusion back into circulation.⁵⁵ Thus, a balance must be struck with NP size to maximize circulation time and thus uptake into tumors. However, the increased retention within tumors resulting from increased size, as well as prevention of RES uptake by surface modification, are substantial advantages of NP formulations over free small molecule delivery.

Surface Modification—A substantial advantage of fluorescent NPs over small molecules is the ability to modify the surface by a number of synthetic methods. For example, adding stealth and/or targeting moieties to the surface can efficiently increase the circulation time and targeting ability of fluorophores contained within the NP. Macrophages and other immune cells are major contributors to the clearance of NPs from circulation. The most common methodology for avoiding this uptake is by making ‘stealth’ NPs using PEGylation.⁵⁶ PEGylation works because PEG is uncharged, nonspecific, and has low protein association. This provides a physical buffer that reduces protein interaction with the underlying material, which reduces binding of opsonins, decreases uptake by immune cells, and lowers interaction with the extracellular matrix. Studies have shown that PEGylated HLA conjugated to Cy5.5 improve circulation time and tumor signal over non-PEGylated NPs.^{57,58} However, the strategy of PEGylation is not without issues. Treatment with PEGylated NPs may result in development of hypersensitivity to PEG, which can lead to decreased efficacy of future treatments. Judge et al. found that treatment of mice with PEGylated plasmid-containing liposomes resulted in the production of an anti-PEG antibody that decreased delivery to tumors after repeated exposure.⁵⁹ In addition to adaptive immune response, PEG may also trigger a response of the innate immune system through complement activation.⁶⁰ The quantity and molecular weight of PEG also influences biodistribution, and in micellar and liposomal formulations the total quantity of PEG may also affect the stability of the NPs themselves.⁶¹ It has been reported that adding a sheddable layer of PEG could provide the benefits of increased circulation time while allowing for

reduced toxicity and sensitivity.⁶¹ Additional methods of making stealth NPs have been investigated as well, including alternative polymers⁶² or conjugation of ‘self’ peptides that cause immune recognition of NPs as belonging to the host.⁶³ Regardless of the specific chemical composition, it is clearly possible to improve the circulation time and delivery of NPs beyond that achievable by administration of free small molecules.

Active targeting refers to the strategy of linking proteins, peptides, antibodies, or any molecule that is specific to some aspect of tumor biology in order to improve accumulation within tumors or uptake by cancer cells. Active targeting is possible using small molecules,⁵ but active targeting with NPs is advantageous since no chemical modification of the payload is necessary, the targeting moiety: drug/fluorophore ratio is lower, and active targeting using NPs may provide additional tumor accumulation and internalization beyond what is possible with small molecule delivery or passively targeted NPs. Several targeted NP formulations are currently in clinical trials.^{64,65} Numerous targeting moieties including folate, hyaluronan, various peptides, aptamers, and antibodies, have been investigated for targeting of cancer cells expressing folate receptor, CD44, various integrins, EGFR, HER2, and numerous other targets.^{64,65} Increased uptake of actively targeted NPs has been observed both *in vitro* and *in vivo*. Complications include determining the optimal surface ligand density and occurrence of off-target accumulation.^{64,65} While active targeting does not appear to influence the physical factors determining NP deposition within tumors, evidence is building that it does affect receptor-mediated endocytosis and thus serves as a complementary strategy in tumor accumulation.⁸ The large number of FDA-approved immunoconjugated drugs and imaging agents suggests that there is indeed a basis for active targeting with NPs and methodologies of drug-loaded NPs could be easily extended to fluorescent NPs.⁶⁶ A summary of select targeted and non-targeted NPs is shown in Table 1.

Specific Activation and Release—NPs are advantageous over small molecule counterparts in their ability to contain multiple chemical entities, and thus multiple activities, in a single package. In addition to active targeting of ligands, NPs may be synthesized which respond directly to the tumor microenvironment or uptake into tumor cells. Olson et al. designed NPs which were linked to activatable cell penetrating peptides, which are cleaved in the presence of MMPs commonly found in tumor stroma.⁸² Cleavage of these peptides then activated uptake by tumor cells and substantially increased fluorescence and deposition of Gd in the tumors. Another strategy is the use of pH-sensitive NPs for specific activatable fluorescence. Wang et al. utilized pH sensitive nanoprobes for activation within lysosomes or the acidic tumor microenvironment.⁸¹ This improved the tumor signal up to 628 times that of blood. The specificity of these formulations has substantial potential to improve tumor imaging over nontargeted fluorophores such as ICG and methylene blue. Additionally, as benign lesions may not exhibit the same acidity or express the same receptors, active targeting of NPs to tumors could both increase the signal-to-background ratio and potentially decrease occurrence of false positives. Examples of pH- and enzyme-sensitive NPs are shown in Figure 3a and c, as well as ICG-loaded formulations utilizing iron oxide NPs (B) and hyaluronic acid NPs (D).

Challenges to Implementation

A number of barriers to the clinical implementation of NPs for IGS remain to be surmounted. Currently there is a significant gap in outcomes between preclinical cancer models and clinical cancer treatment due to the high variability in the biology and physical environment that naturally occurring human tumors develop.⁸⁹ This results in intrinsic limitations of preclinical studies that must be subsequently investigated in expensive clinical trials. A primary issue evident in published clinical trials is the high false-positive rate of fluorescence signal. Studies have shown difficulty in separating healthy versus diseased tissues by fluorescence intensity using nonspecific fluorophores, which could lead to increased morbidity from unnecessary surgical resection.^{6,38} However, this could be overcome by the use of targeting and specific activation upon delivery of fluorophores. Clinical implementation will also involve training of surgeons to use fluorescence IGS in addition to visual and tactile feedback.⁹⁰ Ultimately the potential for surgery guided by fluorescent NPs will be determined from its ability to save and improve the quality of the lives of patients by elimination of diseased tissue. Continuing clinical trials and development of next generation imaging agents will help to clarify the role of fluorescent NPs in IGS in the years to come (Box 1).

BOX 1

NPs FOR THERANOSTIC IGS AND PHOTOTHERMAL THERAPY

NPs for combination imaging and therapy is showing rapid preclinical development. An important point to be noted is that NPs for IGS are intended to be used only once per surgical treatment, and thus the therapeutic aspect of these NPs must be effective with a single dose. Photothermal therapy is one such possible application that could be combined with fluorescent imaging.⁹¹ Previous research has shown the potential for active targeting of NIR fluorescing gold nanorods which could target EGFR-expressing cancer cells for photothermal therapy.⁹¹ More recently, NIR-absorbing graphene oxide nanosheets were conjugated to Cy5 and an RGD-targeting peptide was shown to target U87MG cells *in vitro*, combining active targeting, fluorescence imaging, and photothermal therapy in a single formulation.⁹² Results utilizing a combined ICG-loaded superparamagnetic iron oxide NPs also showed improved fluorescent tumor signal over free ICG in mice, as well as tumor ablation using photothermal therapy.⁸⁶ While still experimental, photothermal therapy could be an effective adjuvant treatment during surgical resection by providing additional insurance against PMs and allowing for specific tissue ablation over large areas or in difficult to resect regions.

CONCLUSION

The use of both fluorescence imaging and NP-mediated delivery of fluorophores will likely play a significant role in future image-guided tumor resection. Image-guided tumor resection has the ability to substantially change patient management during the course of the surgery by removal of PMs and local metastases that would otherwise go unnoticed.^{4,5} Fluorescence imaging has the potential to occur in real time, provide detection up to several millimeters

deep into tissue, is safer for both patients and operating room staff, and can be more cost effective than other imaging modalities.²⁷ NPs can affect delivery of fluorophores and therefore tumor contrast in a number of ways: (1) increasing retention time within tumors due to the EPR effect, (2) prolonging circulation time by evading immune detection and decreasing renal and hepatic filtration, (3) actively targeting tumor-specific receptors, and (4) specifically activating fluorescence when delivered to tumors through degradation by MMPs or within lysosomes.^{8,82,93,94} A number of barriers remain to be overcome before clinical implementation, which include further clinical studies, physician training, and scientific development. However, NP-mediated fluorescence IGS will likely play an important role in future tumor resections.

Acknowledgments

This work was supported by grants from the National Institutes of Health (P30 CA036727, R01 EB019449, and R00 CA153916).

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FIGURE 1.

Fluorescence IGS instrumentation requires three main optical components: an excitation source, a collection source for both fluorescence and white light, and a display for overlaying the fluorescence signal onto the white channel for anatomical localization. Processing and algorithm components are also necessary for overlay and display. Fluorescence light sources are typically either laser or LED based, and a number of systems are currently available with both of these options. Display is commonly performed with overhead monitors, but research into goggle display for surgeons has also been performed. Fluorescence is then used in conjunction with visual and tactile feedback to improve tumor resection to reduce incidence of PMs and local metastases.

FIGURE 2.

NPs are injected systemically where they circulate to tumors and extravasate across the vascular endothelium. These discontinuities may range in size from 200 to 2000 nm, allowing for extravasation of NPs. Once NPs have extravasated they are retained in the tumor stroma due to their size. NPs may then be broken down by extracellular enzymes or taken up into lysosomes where they may be specifically activated. NPs may also be constitutively active, or may activate upon transferring fluorophores to serum proteins, causing increased tumor signal through passive targeting. NPs delivered in this way rely solely on the EPR effect for delivery, whereas targeted NPs rely on both the EPR effect as well as specific proteins or conditions found in tumors such as matrix metalloproteinases (MMPs) or low pH. Clearance of NPs from systemic circulation and other tissues may or may not be necessary, depending on the fluorescence activation and concentration in other

tissues at the time of surgery. Formulations will require additional testing to determine optimal surgical timing after injection.

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FIGURE 3.

(a) Ultra pH-sensitive nanoprobes (UPS_i) were targeted to $\alpha_v\beta_3$ -expressing tumors using cyclic-arginine-glycine-aspartate (cRGD) linkers. This shows specifically activated fluorescence in the tumor of cRGD-UPS_i, and the prevention of specific uptake of cRGD-UPS_i by blocking with the cRGD ligand. (b) ICG-loaded SPIO NPs (left) show increased fluorescence signal compared to free ICG-treated mice (right) 24 h after tail vein injection. (c) Delivery of MMP activatable cell-penetrating peptides bound to dendrimeric NPs and Cy5 (ACPPD-Cy5) improved tumor to skin and tumor to muscle signal ratios over commercially available MMP probes ProSense and MMPSense. (d) ICG-loaded hyaluronic acid-derived NPs improved tumor contrast over mice injected compared to mice injected with free ICG. These results demonstrate the potential for multiple active targeting avenues, multimodality imaging, and use of existing FDA-approved fluorophores with biocompatible materials to improve tumor resection. (a. Reprinted with permission from Ref 84. Copyright 2014 Macmillan Publishers Ltd). A nanoparticle-based strategy for the imaging of a broad range of tumours by nonlinear amplification of microenvironment signals. (b. Reprinted with permission from Ref 89. Copyright 2013 Elsevier). Indocyanine green-loaded SPIO nanoparticles with phospholipid-PEG coating for dual-modal imaging and photothermal therapy, 7711. (c. Reprinted with permission from Ref 85. Copyright 2010 National Academy of Sciences). Activatable cell-penetrating peptides linked to nanoparticles as dual probes for in vivo fluorescence and MR imaging of proteases. (d. Reprinted with permission from Ref 7. Copyright 2015 American Chemical Society). Indocyanine green-loaded nanoparticles for image-guided tumor surgery.

TABLE 1

Selected Dyes and NP Formulations Relevant to Image-Guided Surgery

NP type	Examples	Ex./Em. (nm)	References
Free dye	ICG	780/820	26,67,68
	Methylene blue	665/700	4,69
Embedded Dye	NanoICG	780/820	7
	Liposome-embedded ICG	780/820	70–73
	Doped silica-ICG	780/820	74,75
Conjugated Dye	HA-Cy5.5	673/707	76–78
	HA-ICG-OSU	780/820	79
	cRGD-MeO-PEG-b-PDPA/MeO-PEG-b-PC7A-Cy5.5	673/707	80,81
	Polyamidoamine-cell penetrating peptide-Cy5	616/646	82
Hard NP	QD	775/840 (varies)	83–85
	SPIO-phospholipid-PEG-ICG	780/820	86
Upconversion NP	Ln ³⁺ -NaYF ₄	980/(420–700) (varies)	87,88