Asian Journal of Urology 10 (2023) 1–8

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/ajur

Review

The application of indocyanine green in guiding prostate cancer treatment

Donghua Xie, Di Gu, Ming Lei, Cong Cai, Wen Zhong, Defeng Qi, Wenqi Wu, Guohua Zeng, Yongda Liu*

Department of Urology, Minimally Invasive Surgery Center, The First Affiliated Hospital of Guangzhou Medical University, Guangdong Key Laboratory of Urology, Guangzhou, China

Received 10 August 2020; received in revised form 31 May 2021; accepted 13 July 2021
Available online 22 April 2022

KEYWORDS
Indocyanine green;
Intraoperative imaging;
Prostate cancer;
Sentinel lymph node dissection;
Neurovascular bundle

Abstract *Objective:* Indocyanine green (ICG) with near-infrared fluorescence absorption is approved by the United States Food and Drug Administration for clinical applications in angiography, blood flow evaluation, and liver function assessment. It has strong optical absorption in the near-infrared region, where light can penetrate deepest into biological tissue. We sought to review its value in guiding prostate cancer treatment.
Methods: All related literature at PubMed from January 2000 to December 2020 were reviewed.
Results: Multiple preclinical studies have demonstrated the usefulness of ICG in identifying prostate cancer by using different engineering techniques. Clinical studies have demonstrated the usefulness of ICG in guiding sentinel node dissection during radical prostatectomy, and possible better preservation of neurovascular bundle by identifying landmark prostatic arteries. New techniques such as adding fluorescein in additional to ICG were tested in a limited number of patients with encouraging result. In addition, the use of the ICG was shown to be safe. Even though there are encouraging results, it does not carry sufficient sensitivity and specificity in replacing extended pelvic lymph node dissection during radical prostatectomy.
Conclusion: Multiple preclinical and clinical studies have shown the usefulness of ICG in identifying and guiding treatment for prostate cancer. Larger randomized prospective studies are warranted to further test its usefulness and find new modified approaches.

* Corresponding author.
E-mail address: 13719007083@163.com (Y. Liu).
Peer review under responsibility of Tongji University.

https://doi.org/10.1016/j.ajur.2021.07.004

1. Introduction

Optical fluorescence imaging is increasingly used to monitor biological functions of specific targets in small animals and humans. Near-infrared (NIR) fluorescence (700—1000 nm) detection avoids the natural background fluorescence interference of biomolecules, providing a high contrast between target and background tissue. NIR fluorescence imaging is a noninvasive alternative to radionuclide imaging in small animals or with probes in close proximity to the target in humans. Indocyanine green (ICG), with NIR fluorescence absorption at 780 nm and emission at 820 nm, is one of the few optical imaging agents approved by the United States Food and Drug Administration for clinical applications in angiography, blood flow evaluation, and liver function assessment [1].

Fluorescence guided surgery is used to detect fluorescently labeled structures during surgery, with a purpose to provide the surgeon of real-time visualization of the operating field and guide the surgery. The investigation of application of ICG on prostate cancer can trace back to 20 years ago. In a study using rat tumor model, ICG was found to be located in two of the tumor cell lines examined, which include a prostate cancer cell line R3327-H [2]. One study reported surface-enhanced Raman scattering (SERS) studies on ICG on colloidal silver and gold. A novel optical probe was demonstrated for applications in living cells. In addition to its own detection by the characteristic ICG SERS signatures, the ICG gold nanoprobe delivers spatially localized chemical information from its biological environment by employing SERS in the local optical fields of the gold nanoparticles. The probe therefore offers the potential to increase the spectral specificity and selectivity [3].

With the potential to better identification and preservation of neurovascular bundle (NVB), ICG holds promise for improving post-prostatectomy urinary continence and erectile function. In addition, ICG-guided sentinel lymph node (SLN) harvest has been reported in robot-assisted laparoscopic radical prostatectomy (RARP) to determine metastatic nodal status [4]. In patients with prostate cancer, a positive surgical margin is associated with an increased risk of cancer recurrence and poorer outcome. However, margin status cannot be determined during the surgery [5]. The potential utility of ICG for differentiation of oncological tissue from normal tissue remains encouraging [4,6].

2. Preclinical studies

Preclinical studies using prostate cancer cell lines and xenografts in rodents have been encouraging, either use ICG alone, or adding radiolabeling, or adding cancer selective ligands [1,4,7—11]. In a study using primary culture cell line, ICG had a favorable viability profile at almost all of the concentrations and time tested [12].

2.1. Uses ICG alone

Intravenously administered ICG was found to accumulate in prostate cancer cells in a murine model [7]. Multiple *in vitro* and xenograft studies using prostate cell lines have achieved success in identifying cancer using ICG biodegradable and biocompatible nanoparticles [10,11,13—16], even in poorly vascularized prostate cancer [11].

2.2. Radiolabeling of ICG

To improve surgical guidance toward prostate draining lymph nodes (LNs), one study investigated the potential of intraoperative fluorescence imaging and combined pre- and intra-operative multimodality imaging approaches. Transgenic mice with spontaneous prostate tumors were injected intratumorally with different regimens. They found that at 60-min postinjection, ICG significantly improved the detection of the LNs compared to a cocktail of patent blue (53% *vs.* 7%). Moreover, a cocktail of ICG and ^{99m}Tc-Nano-Coll® improved the fluorescent detection rate to 86% [8]. Antibody fragments including diabodies had more desirable pharmacokinetic characteristics than whole antibodies. An activatable optical imaging probe based on a cys-diabody targeting prostate-specific membrane antigen (PSMA) conjugated with ICG was designed such that it can only be activated when bound to the tumor, leading to high signal-to-background ratios. A biodistribution study using ^{125}I-labeled cys-diabody-ICG showed immediate uptake in the kidneys followed by a rapid decrease, while gastric activity increased due to released radioiodine during rapid cys-diabody-ICG catabolism in the kidneys. To achieve high tumor-specific detection, it would be preferable to use antibody fragments large enough not to be filtered through glomerulus or to conjugate the fragments with fluorescent dyes that are readily excreted into urine when cleaved from the cys-diabody [14].

2.3. Hybridization of ICG with cancer selective ligands

Technological advances in cancer biomarkers and immunology have prompted the hybridization of ICG with cancer selective ligands, to localize tumor by fluorescence. An *in vivo* optical imaging probe that could identify the tumor margins during surgery could result in improved outcomes. Most of studies have been focusing on developing agent with hybridization of ICG with PSMA [1,2,4,5,15—17], while one study chose human epidermal growth factor receptor 2 as the cancer selective ligand and found that it had high contrast and high efficiency for binding to prostate cancer cells [9]. Another study developed L-methyl-methionine—ICG-Der-02 demonstrating excellent cellular uptake of L-methyl-methionine—ICG-Der-02 on cancer cell lines including prostate cancer cell line PC3 without cytotoxicity [18].

PSMA is upregulated in prostate cancer by 100- to 1000-fold. It is a unique transmembrane-bound glycoprotein that is overexpressed on prostate tumor cells and in the neovasculature of most solid prostate tumors but not in the vasculature of normal tissue. This unique expression of PSMA makes it an important biomarker as well as a large extracellular target of imaging agents. Notably the J591 antibody has been delivered in several human clinical trials at high doses with a favorable safety profile [1,4,5]. Prior to binding to PSMA and cellular internalization, the conjugate J591 antibody yielded little light; however, after binding an

18-fold activation was observed permitting the specific detection of PSMA-positive tumors up to 10 days after injection of a low dose (0.25 mg/kg) of the reagent [5]. To further reduce background signal, short polyethylene glycol linkers were employed to improve the covalent bonding ratio of ICG in one study. New minibody against PSMAs conjugated with bifunctional ICG derivatives specifically visualized PSMA-positive tumor xenografts in mice bearing both PSMA-positive and -negative tumors within 6 h after injection. The addition of short polyethylene glycol linkers significantly improved target-to-background ratios; however, it did not significantly alter the biodistribution [19].

2.4. New imaging techniques

In one study, triple-modal imaging magnetic nanocapsules, encapsulating hydrophobic superparamagnetic iron oxide nanoparticles were used to magnetically target tumors after intravenous administration in tumor-bearing mice. The engineered magnetic polymeric nanocapsules with multiple imaging probes (*e.g.* ICG, superparamagnetic iron oxide nanoparticles and indium-111) were capable of achieving triple-modal imaging (fluorescence, magnetic resonance, and nuclear imaging) *in vivo*, overcoming the limitations of single modality imaging, and providing complementary information on the spatial distribution of the nanocarrier within the tumor [20].

In another study, a multicolor fluorescence guidance approach was able to discriminate between prostate draining LNs and lower limb draining LNs. In five porcine models, multispectral-fluorescence guidance was performed using da Vinci Si- and Xi-robot consoles. They received fluorescein into the lower limb(s) and ICG-nanocolloid into the prostate. Fluorescein was detected in 29 LNs and ICG-nanocolloid visualized in 12 LNs. Signal intensities appeared equal for both dyes and no visual overlap in the lymphatic drainage patterns was observed. Moreover, fluorescein supported both the identification of leakage from damaged lymphatic structures and the identification of ureters [21].

2.5. New contrast agent

A new contrast agent, LipImage™ 815 was designed and compared to previously described ICG-loaded lipid nanoparticles (ICG-lipidots®) in one study. While ICG-lipidots® displays a relatively short plasma lifetime, LipImage™ 815 circulates in blood for longer time, allowing the efficient uptake of fluorescence signal in human prostate cancer cells implanted in mice. Prolonged tumor labeling is observed for more than 21 days [22]. In another study, a series of NIR fluorescent ligands targeting the PSMA were synthesized and each compound was tested for its ability to image PSMA-positive tissue in experimental prostate cancer models. The agents were prepared by conjugating commercially available active esters of NIR dyes. The highest tumor uptake was observed with IRDye800CW employing a poly(ethylene glycol) or lysine-suberate linker, as in 800CW-2 and 800CW-3, while the highest tumor to nontarget tissue ratios were obtained for Cy7 with these same linkers, as in Cy7-2 and Cy7-3 [23].

2.6. Cancer cell killing effect

ICG can be used as a photosensitizer to kill cancer cells selectively, which has been demonstrated in different prostate cancer and other cancer cell lines [9,24,25]. This effect is more significant in prostate cancer than normal prostate cells, possibly due to stronger ICG uptake by the cancer cells. However, ICG does not act as a radiosensitizer if used with ionizing radiation. The combined treatment of photodynamic therapy and radiotherapy produces an additive effect which does not depend on the sequence of the two treatments [25].

Laser immunotherapy is a novel approach that aims at the tumor-directed stimulation of the host immune system. It involves an intratumor administration of a laser-absorbing dye and an immunoadjuvant, followed by noninvasive laser irradiation. Using glycated chitosan (GC) as immunoadjuvant and ICG as laser-absorbing dye in the treatment of metastatic prostate tumors, both the laser-ICG and laser-ICG-GC treatments significantly reduced the growth of primary tumors and lung metastases. In their preliminary studies, long-term survival of the rats bearing the prostate tumors was also observed after the laser immunotherapy treatment [26].

3. Clinical studies

3.1. The application of ICG on SLN dissection

Most clinical studies have been focusing on using ICG on SLN dissection. SLN detection techniques have the potential to change the standard of surgical care for patients with prostate cancer [27,28].

3.1.1. Most frequent location of positive LNs

There are different outcomes regarding where is the dominant area for metastatic LN [30,31] with one showing that internal iliac artery area is the dominant site [29] while another study showing that external iliac artery is the dominant site [30]. In a study with 14 patients who were candidates for radical retropubic prostatectomy and pelvic lymph node dissection (PLND), under *in vivo* and *ex vivo* probing, the fluorescence intensity of internal iliac nodes was greater than that of external iliac or obturator nodes. The major lymphatic pathway involved in the spreading of prostate cancer appears to relate to internal iliac LNs, which means that the standard PLND covering external iliac and obturator regions would not keep the cancer from spreading [30]. In another retrospective study, 20 patients received transperineal prostatic injections of ICG. The external iliac group was the most common site of fluorescence in 27.2% of patients, followed by the common iliac (21.3%), obturator (20.3%), internal iliac (18.5%), and node of Cloquet (7.7%) [29]. In another study with 42 patients who received systematic or specific ICG injections to prostate, they found that (1) external and internal iliac regions contain the majority of SLNs; (2) common iliac regions contain up to 22% of all SLNs; (3) a prostatic lobe could drain into the contralateral group of pelvic LNs; and (4) the fossa of Marcille also receives significant drainage [31]. In another study with 66 consecutive patients, SLNs

were found to be located in the obturator fossa, internal, and external iliac regions but rarely in the common iliac and presacral regions [32]. In a prospective cohort of 50 consecutive patients with intermediate- to high-risk localized prostate cancer who had undergone laparoscopic radical prostatectomy, SLN biopsies by fluorescence detection using intraoperative imaging with ICG and backup extended pelvic lymph node dissection (ePLND) were conducted prior to prostatectomy. Over 90% of positive SLNs were identified at two predominant sites. Priority should be given to the removal of these SLNs, which are located closer to the prostate, in PLND [33]. In another report, 100 ICG-guided ePLNDs were carried out in patients with localized intermediate- and high-risk prostate cancer. ICG was injected transrectally using ultrasound sonography before each surgery. Lymphatic drainage routes were successfully determined in 91 right-sided and 90 left-sided cases. Five main lymphatic pathways and sites were identified: (i) an internal route (57%), (ii) a lateral route (50%), (iii) a presacral route (20%), (iv) a paravesical artery site (20%), and (v) a pre-prostatic site (5%). LN metastasis was positive in 15 patients, with 44 pathologically confirmed metastatic LNs. Metastatic fluorescent LNs (FLNs) were found in 15 out of 44 (34.1%) LNs. Although the direct role of FLNs in SLN identification appears to be limited, the identification of lymphatic pathways could contribute to high-quality ePLND during RARP [34]. In another study, ICG was injected into the prostate under transrectal ultrasound guidance just before surgery for 66 consecutive patients with clinically localized prostate cancer who underwent open radical prostatectomy. Lymphatic vessels were successfully visualized in 65 (98%) patients and SLNs in 64 (97%) patients. SLNs were found to be located in the obturator fossa, internal and external iliac regions, and rarely in the common iliac and presacral regions. A median of four SLNs per patient was detected and three lymphatic pathways including the paravesical, internal, and lateral routes were identified. All pathologically positive LNs were detected [35].

3.1.2. Sensitivity and specificity

Most of the studies reported relatively low sensitivity of ICG in detecting metastatic LN [31,36] but high negative predictive value [26,32]. Its high negative predictive value could allow us to avoid ePLND if we had an accurate intraoperative lymph fluorescent analysis [36]. There are also a few studies showing its high sensitivity [7,33,37]. In one study with 38 consecutive men with intermediate- or high-risk prostate cancer who underwent fluorescence targeted PLND during laparoscopic radical prostatectomy, super-ePLND was added as the control. Fluorescence stained nodes were found on each side in all except one patient. A total of 700 LNs were removed, of which 531 (75.9%) were fluorescence stained. LN metastases were found in 15 (39.5%) patients. Two (5.3%) patients had a solitary micro-metastasis and 3 (7.9%) had nodes containing isolated tumor cells. Metastases were found outside the ePLND template in five of 15 (33.3%) patients. Fluorescence targeted PLND showed superior sensitivity and negative predictive value compared to ePLND and super-ePLND to detect LN metastasis [32]. For SLN identification, in the study with the 100 ICG-guided ePLNDs, 34.0% sensitivity and 64.8% specificity rates were detected in regard to identification of LN metastasis [34].

In the other two studies, low specificity of ICG in detecting positive LN was noted [37,38]. In a meta-analysis with 10 clinical trials included, they found that SLN mapping in bladder and prostate cancer is a method with a high detection rate, although its specificity to predict LN invasion remains poor [38].

3.1.3. Hybrid imaging with radiolabeling

The use of a hybrid tracer such as ICG-^{99m}Tc-nanocolloid has become possible to determine the accumulation of tracer and correlate this to intraoperative fluorescence-based rates of identification [39,40]. Integration of molecular imaging and in particular intraoperative image guidance is expected to improve the surgical accuracy of laparoscopic LN dissection [41—44]. In one study with 11 patients with an increased risk of nodal metastasis, fluorescence particularly improved surgical guidance in areas with a high radioactive background signal such as the injection site. *Ex vivo* analysis revealed a strong correlation between the radioactive and fluorescent content in the excised LNs. Radio guidance to the areas of interest is still desirable since fluorescence detection is limited by the severe tissue attenuation of the signal [41]. In a prospective trial conducted with 501 procedures in a cohort of 495 patients with different malignancies including prostate cancer, a total of 1327 SLN-related hotspots were identified on 501 preoperative single-photon emission computed tomography scans. Intraoperatively, a total number of 1643 SLNs were identified based on the combination of gamma-tracing and fluorescence-guidance. In patients wherein blue dye was used fluorescence-based SLN detection was superior over visual blue dye-based detection. With ICG-^{99m}Tc-nanocolloid, the SLN biopsy procedure has become more accurate and independent of the use of blue dye [45].

To determine the diagnostic capabilities of combined PSMA positron emission tomography/computed tomography (PET/CT) and SLN biopsy in PSMA PET/CT negative patients for the primary LN staging in prostate cancer patients, one study was carried out with 53 consecutive patients with primary diagnosed intermediate- or high-risk prostate cancer who underwent a preoperative PSMA PET/CT followed by robot-assisted radical prostatectomy and ePLND included. All patients without suspected LN metastases on PSMA PET/CT were considered candidates for SLN biopsy with ICG-^{99m}Tc-nanocolloid or ^{99m}Tc-nanocolloid with free ICG as used tracers. The combined use of SLN biopsy and PSMA PET/CT identified all pN1 patients and performed correct nodal staging in 50 of 53 patients. SLN biopsy identified significantly smaller LN metastases than PSMA PET/CT. PSMA PET/CT and SLN biopsy rather than ePLND could be a preferred diagnostic approach for staging purposes of men prior to radiotherapy for prostate cancer [46].

3.1.4. Routes of administration of ICG

Most of the studies used intraprostatic injection of ICG either transrectally [31,42,46] or percutaneously with

robotic guidance [37], or transperineally [29]. There is no comparative study with regards to which approach is optimal but one study believed that percutaneous, robotic-guided ICG injection was proved superior to cystoscopy or transrectal delivery [37].

3.1.5. Timing of administration of ICG

ICG was usually administered shortly before the surgery, or intraoperatively [29,32,47], with one study administered 18 h before the surgery [43].

3.1.6. Dosing

There is no universal dosing for the studies so far. In the retrospective study with 20 patients who received transperineal prostatic injections of ICG, patients were cycled through five doses so that optimal ICG dosing could be discovered early. ICG injection was able to identify FLN packets in all 20 patients. Compared to the higher ICG doses, the 1.25 mg and 2.5 mg doses had fewer FLN packets and were abandoned after one dose each. The median numbers of FLN packets were 4.0, 6.0, and 4.5 for the respective doses of 3.75 mg, 5.0 mg, and 7.5 mg [30]. In a study with 40 penile and 20 prostate cancer patients scheduled for SLN biopsy using ICG-^{99m}Tc-nanocolloid, the ability to provide intraoperative fluorescence guidance is found to be dependent on the amount and concentration of the fluorescent dye accumulated in the lesion(s) of interest. The study revealed that intraoperative fluorescence detection with ICG is possible above a micromolar concentration [39].

3.1.7. Can ICG-guided LN dissection replace ePLND?

While most studies found that it was useful [7,3—34,41], most of the studies also concluded that it could not replace ePLND because of its low sensitivity and complex drainage pattern [29,31]. In the retrospective study with twenty patients who received transperineal prostatic injections of ICG, across all patients, ICG had 62% sensitivity, 50% specificity, 8% positive predictive value, and 95% negative predictive value in detecting LN metastases. The low sensitivity of ICG for the detection of LN metastases highlights why FLN dissection with ICG does not represent an alternative to ePLND [29]. In a prospective randomized study with 120 patients with intermediate- or high-risk prostate cancer, in the intervention group, ICG was injected transrectally into the prostate before docking of the robot. In both the control and the intervention groups, ePLND was performed including additional dissection of FLN in the ICG group. A total of 2609 LNs were found with significantly more LNs after ICG-supported ePLND with a median of 25 versus 17 LNs in control group. Nodal metastases were detected in six patients in control group (25 cancerous LNs) versus nine patients in intervention group (62 positive LNs) ($p=0.40$). In seven of nine patients, ICG-ePLND identified at least one cancer-positive LN (sensitivity 78%), and 27 of 62 cancerous LNs were fluorescent. After a median follow-up of 22.9 months, prostate-specific antigen levels were similar. While ICG-ePLND seems to be beneficial for a better understanding of the lymphatic drainage and a more meticulous diagnostic approach, the sensitivity is not sufficient to recommend stand-alone ICG LN dissection [48].

3.2. The application of ICG on NVB preservation

Robot-assisted radical prostatectomy may be supplemented by ICG imaging to identify the prostatic NVB, even though in literature the use of ICG for NVB preservation seems very limited and does not have a clinical impact to date. Kumar et al. [49] reported a case serial of 10 patients who underwent nerve sparing RARP with 20 NVBs examined. Prior to clamping the pedicle or dissection of the NVB, 0.75 mL of ICG was given. The Firefly® technology was engaged on the robotic console and a period of 20—40 s was allowed for the ICG to enter the vascular system. The landmark prostatic artery and its pathway could be identified in 17/20 (85%) NVBs. In the other three patients we were unable to visualize the artery as it was underneath some large veins. They concluded that the use of ICG and Firefly® technology during NS radical prostatectomy has the potential to more accurately and more frequently identify the landmark prostatic artery that runs along the NVB. There was a similar finding in another study with 26 patients [50].

3.3. New techniques

3.3.1. Fluorescein as a fluorescent dye

While most of the clinical studies have been reporting using ICG, some studies also reported the usefulness of the other dye fluorescein. In one study to evaluate the feasibility of confocal laser endomicroscopy (CLE) during RARP, 21 patients with biopsy-proven prostate cancer scheduled for RARP were included. After intravenous administration of fluorescein, 15 patients underwent *in vivo* intraoperative CLE of prostatic and periprostatic structures using probes. Standard robotic instruments were used to grasp and maneuver the CLE probes for image acquisition. Intraoperative CLE imaging of NVB prior to and following NS dissection revealed characteristic features including dynamic vascular flow and intact axon fibers. *Ex vivo* confocal imaging of the prostatic parenchyma demonstrated the normal prostatic glands, stroma, and prostate carcinoma [51].

3.3.2. Adding fluorescein in additional to ICG

A couple studies also demonstrated the feasibility and potential of using two fluorescent dyes, ICG and fluorescein, or three fluorescent dyes, ICG, fluorescein, and Cy5 to provide multispectral fluorescence guidance during prostate cancer surgery [52,53]. In one study with 10 patients who underwent RARP, following ICG-^{99m}Tc-nanocolloid administration, lymphangiographic tracer fluorescein was injected into prostate immediately after the patient was anesthetized. In eight out of ten patients, fluorescein imaging allowed bright and accurate identification of lymphatic ducts, although higher background staining and tracer washout were observed [52]. In another report from the same group, laparoscopic three-color multispectral

imaging in combination with white light imaging was demonstrated in a phantom set-up. Nerve fibers, SLNs, and tumor lesions were able to be differentiated [53].

3.4. Safety profile

No adverse effects have been reported from published studies. In the study with a large cohort of 495 patients, the use of the hybrid tracer or the fluorescence-guidance procedure was found to be safe [45].

4. Conclusion

In summary, it seems that there is a value of fluorescent SLN detection with ICG for the detection of LN metastases in intermediate- and high-risk patients undergoing robot-assisted prostatectomy and ePLND. It also implicates a value in better preserving NVB and defining surgical margin during radical prostatectomy. However, more and larger randomized prospective studies are warranted.

Author contributions

Study concept and design: Donghua Xie, Yongda Liu.
Data acquisition: Donghua Xie.
Data analysis: Donghua Xie.
Drafting of manuscript: Donghua Xie.
Critical revision of the manuscript: Yongda Liu, Guohua Zeng, Donghua Xie, Di Gu, Ming Lei, Cong Cai, Wen Zhong, Defeng Qi, Wenqi Wu.

Conflicts of interest

The authors declare no conflict of interest.

References

[1] Leung K. Quenched indocyanine green-anti-prostate-specific membrane antigen antibody J591 [Internet]. In: Molecular imaging and contrast agent database (MICAD). Bethesda (MD): National Center for Biotechnology Information (US); 2011 Dec 08 [updated Mar 01, 2012]. https://pubmed.ncbi.nlm.nih.gov/22400137/.

[2] Achilefu S, Dorshow RB, Bugaj JE, Rajagopalan R. Novel receptor-targeted fluorescent contrast agents for *in vivo* tumor imaging. Invest Radiol 2000;35:479—85.

[3] Kneipp J, Kneipp H, Rice WL, Kneipp K. Optical probes for biological applications based on surface-enhanced Raman scattering from indocyanine green on gold nanoparticles. Anal Chem 2005;77:2381—5.

[4] Bates AS, Patel VR. Applications of indocyanine green in robotic urology. J Robot Surg 2016;10:357—9.

[5] Nakajima T, Mitsunaga M, Bander NH, Heston WD, Choyke PL, Kobayashi H. Targeted, activatable, *in vivo* fluorescence imaging of prostate-specific membrane antigen (PSMA) positive tumors using the quenched humanized J591 antibody-indocyanine green (ICG) conjugate. Bioconjugate Chem 2011;22:1700—5.

[6] Kothapalli SR, Sonn GA, Choe JW, Nikoozadeh A, Bhuyan A, Park KK, et al. Simultaneous transrectal ultrasound and photoacoustic human prostate imaging. Sci Transl Med 2019;11:eaav2169. https://doi.org/10.1126/scitranslmed.aav2169.

[7] Xia L, Zeh R, Mizelle J, Newton A, Predina J, Nie S, et al. Near-infrared intraoperative molecular imaging can identify metastatic lymph nodes in prostate cancer. Urology 2017;106:133—8.

[8] van Leeuwen AC, Buckle T, Bendle G, Vermeeren L, Valdés Olmos R, van de Poel HG, et al. Tracer-cocktail injections for combined pre- and intraoperative multimodal imaging of lymph nodes in a spontaneous mouse prostate tumor model. J Biomed Opt 2011;16:016004. https://doi.org/10.1117/1.3528027.

[9] Kim G, Huang SW, Day KC, O'Donnell M, Agayan RR, Day MA, et al. Indocyanine-green-embedded PEBBLEs as a contrast agent for photoacoustic imaging. J Biomed Opt 2007;12:044020. https://doi.org/10.1117/1.2771530.

[10] Ranjan AP, Zeglam K, Mukerjee A, Thamake S, Vishwanatha JK. A sustained release formulation of chitosan modified PLCL: poloxamer blend nanoparticles loaded with optical agent for animal imaging. Nanotechnology 2011;22:295104. https://doi.org/10.1088/0957-4484/22/29/295104.

[11] Souchek JJ, Wojtynek NE, Payne WM, Holmes MB, Dutta S, Qi B, et al. Hyaluronic acid formulation of near infrared fluorophores optimizes surgical imaging in a prostate tumor xenograft. Acta Biomater 2018;75:323—33.

[12] Yuen D, Gonder J, Proulx A, Liu H, Hutnik C. Comparison of the *in vitro* safety of intraocular dyes using two retinal cell lines: a focus on brilliant blue G and indocyanine green. Am J Ophthalmol 2009;147:251—9.e2. https://doi.org/10.1016/j.ajo.2008.08.031.

[13] Patel RH, Wadajkar AS, Patel NL, Kavuri VC, Nguyen KT, Liu H. Multifunctionality of indocyanine green-loaded biodegradable nanoparticles for enhanced optical imaging and hyperthermia intervention of cancer. J Biomed Opt 2012;17:046003. https://doi.org/10.1117/1.JBO.17.4.046003.

[14] Sano K, Nakajima T, Ali T, Bartlett DW, Wu AM, Kim I, et al. Activatable fluorescent cys-diabody conjugated with indocyanine green derivative: consideration of fluorescent catabolite kinetics on molecular imaging. J Biomed Opt 2013;18:101304. https://doi.org/10.1117/1.JBO.18.10.101304.

[15] Reichel D, Tripathi M, Butte P, Saouaf R, Perez JM. Tumor-activatable clinical nanoprobe for cancer imaging. Nanotheranostics 2019;3:196—211.

[16] Ji C, Yuan A, Xu L, Zhang F, Zhang S, Zhao X, et al. Activatable photodynamic therapy for prostate cancer by NIR dye/photosensitizer loaded albumin nanoparticles. J Biomed Nanotechnol 2019;15:311—8.

[17] Matsuoka D, Watanabe H, Shimizu Y, Kimura H, Yagi Y, Kawai R, et al. Structure-activity relationships of succinimidyl-Cys-C(O)-Glu derivatives with different near-infrared fluorophores as optical imaging probes for prostate-specific membrane antigen. Bioorg Med Chem 2018;26:2291—301.

[18] Mahounga DM, Shan L, Jie C, Du C, Wan S, Gu Y. Synthesis of a novel L-methyl-methionine—ICG-Der-02 fluorescent probe for *in vivo* near infrared imaging of tumors. Mol Imag Biol 2012;14:699—707.

[19] Watanabe R, Sato K, Hanaoka H, Harada T, Nakajima T, Kim I, et al. Minibody-indocyanine green based activatable optical imaging probes: the role of short polyethylene glycol linkers. ACS Med Chem Lett 2014;5:411—5.

[20] Bai J, Wang JT, Rubio N, Protti A, Heidari H, Elgogary R, et al. Triple-modal imaging of magnetically-targeted nanocapsules in solid tumours *in vivo*. Theranostics 2016;6:342—56.

[21] Meershoek P, KleinJan GH, van Oosterom MN, Wit EM, van Willigen DM, Bauwens KP, et al. Multispectral fluorescence imaging as a tool to separate healthy and disease related lymphatic anatomies during robot-assisted laparoscopic procedures. J Nucl Med 2018;59:1757—60.

[22] Jacquart A, Kéramidas M, Vollaire J, Boisgard R, Pottier G, Rustique E, et al. LipImage™ 815: novel dye-loaded lipid

nanoparticles for long-term and sensitive *in vivo* near-infrared fluorescence imaging. J Biomed Opt 2013;18:101311. https://doi.org/10.1117/1.JBO.18.10.101311.
[23] Chen Y, Pullambhatla M, Banerjee SR, Byun Y, Stathis M, Rojas C, et al. Synthesis and biological evaluation of low molecular weight fluorescent imaging agents for the prostate-specific membrane antigen. Bioconjugate Chem 2012;23:2377–85.
[24] Ruhi MK, Ak A, Gülsoy M. Dose-dependent photochemical/photothermal toxicity of indocyanine green-based therapy on three different cancer cell lines. Photodiagnosis Photodyn Ther 2018;21:334–43.
[25] Colasanti A, Kisslinger A, Quarto M, Riccio P. Combined effects of radiotherapy and photodynamic therapy on an *in vitro* human prostate model. Acta Biochim Pol 2004;51:1039–46.
[26] Chen WR, Liu H, Ritchey JW, Bartels KE, Lucroy MD, Nordquist RE. Effect of different components of laser immunotherapy in treatment of metastatic tumors in rats. Cancer Res 2002;62:4295–9.
[27] Joniau S, Van den Bergh L, Lerut E, Deroose CM, Haustermans K, Oyen R, et al. Mapping of pelvic lymph node metastases in prostate cancer. Eur Urol 2013;63:450–8.
[28] Heck MM, Retz M, Bandur M, Souchay M, Vitzthum E, Weirich G, et al. Topography of lymph node metastases in prostate cancer patients undergoing radical prostatectomy and extended lymphadenectomy: results of a combined molecular and histopathologic mapping study. Eur Urol 2014;66:222–9.
[29] Chennamsetty A, Zhumkhawala A, Tobis SB, Ruel N, Lau CS, Yamzon J, et al. Lymph node fluorescence during robot-assisted radical prostatectomy with indocyanine green: prospective dosing analysis. Clin Genitourin Cancer 2017;15:e529–34. https://doi.org/10.1016/j.clgc.2016.10.014.
[30] Inoue S, Shiina H, Arichi N, Mitsui Y, Hiraoka T, Wake K, et al. Identification of lymphatic pathway involved in the spreading of prostate cancer by fluorescence navigation approach with intraoperatively injected indocyanine green. Can Urol Assoc J 2011;5:254–9.
[31] Nguyen DP, Huber PM, Metzger TA, Genitsch V, Schudel HH, Thalmann GN. A specific mapping study using fluorescence sentinel lymph node detection in patients with intermediate- and high-risk prostate cancer undergoing extended pelvic lymph node dissection. Eur Urol 2016;70:734–7.
[32] Hruby S, Englberger C, Lusuardi L, Schätz T, Kunit T, Abdel-Aal AM, et al. Fluorescence guided targeted pelvic lymph node dissection for intermediate and high risk prostate cancer. J Urol 2015;194:357–63.
[33] Miki J, Yanagisawa T, Tsuzuki S, Mori K, Urabe F, Kayano S, et al. Anatomical localization and clinical impact of sentinel lymph nodes based on patterns of pelvic lymphatic drainage in clinically localized prostate cancer. Prostate 2018;78:419–25.
[34] Shimbo M, Endo F, Matsushita K, Hattori K. Impact of indocyanine green-guided extended pelvic lymph node dissection during robot-assisted radical prostatectomy. Int J Urol 2020;27:845–50.
[35] Yuen K, Miura T, Sakai I, Kiyosue A, Yamashita M. Intraoperative fluorescence imaging for detection of sentinel lymph nodes and lymphatic vessels during open prostatectomy using indocyanine green. J Urol 2015;194:371–7.
[36] Ramírez-Backhaus M, Mira Moreno A, Gómez Ferrer A, Calatrava Fons A, Casanova J, Solsona Narbón E, et al. Indocyanine green guided pelvic lymph node dissection: an efficient technique to classify the lymph node status of patients with prostate cancer who underwent radical prostatectomy. J Urol 2016;196:1429–35.
[37] Manny TB, Patel M, Hemal AK. Fluorescence-enhanced robotic radical prostatectomy using real-time lymphangiography and tissue marking with percutaneous injection of unconjugated indocyanine green: the initial clinical experience in 50 patients. Eur Urol 2014;65:1162–8.
[38] Aoun F, Albisinni S, Zanaty M, Hassan T, Janetschek G, van Velthoven R. Indocyanine green fluorescence-guided sentinel lymph node identification in urologic cancers: a systematic review and meta-analysis. Minerva Urol Nefrol 2018;70:361–9.
[39] KleinJan GH, Bunschoten A, van den Berg NS, Olmos RA, Klop WM, Horenblas S, et al. Fluorescence guided surgery and tracer-dose, fact or fiction? Eur J Nucl Med Mol Imag 2016;43:1857–67.
[40] van Oosterom MN, van der Poel HG, van Leeuwen FWB, Meershoek P, Welling MM, Pinto F, et al. Extending the hybrid surgical guidance concept with freehand fluorescence tomography. IEEE Trans Med Imag 2020;39:226–35.
[41] van der Poel HG, Buckle T, Brouwer OR, Valdés Olmos RA, van Leeuwen FW. Intraoperative laparoscopic fluorescence guidance to the sentinel lymph node in prostate cancer patients: clinical proof of concept of an integrated functional imaging approach using a multimodal tracer. Eur Urol 2011;60:826–33.
[42] KleinJan GH, van den Berg NS, Brouwer OR, de Jong J, Acar C, Wit EM, et al. Optimisation of fluorescence guidance during robot-assisted laparoscopic sentinel node biopsy for prostate cancer. Eur Urol 2014;66:991–8.
[43] Jeschke S, Lusuardi L, Myatt A, Hruby S, Pirich C, Janetschek G. Visualisation of the lymph node pathway in real time by laparoscopic radioisotope- and fluorescence-guided sentinel lymph node dissection in prostate cancer staging. Urology 2012;80:1080–6.
[44] KleinJan GH, van den Berg NS, de Jong J, Wit EM, Thygessen H, Vegt E, et al. Multimodal hybrid imaging agents for sentinel node mapping as a means to (re)connect nuclear medicine to advances made in robot-assisted surgery. Eur J Nucl Med Mol Imag 2016;43:1278–87.
[45] KleinJan GH, van Werkhoven E, van den Berg NS, Karakullukcu MB, Zijlmans HJMAA, van der Hage JA, et al. The best of both worlds: a hybrid approach for optimal pre- and intraoperative identification of sentinel lymph nodes. Eur J Nucl Med Mol Imag 2018;45:1915–25.
[46] Hinsenveld FJ, Wit EMK, van Leeuwen PJ, Brouwer OR, Donswijk ML, Tillier CN, et al. Prostate-specific membrane antigen positron emission tomography/computed tomography combined with sentinel node biopsy for primary lymph node staging in prostate cancer. J Nucl Med 2020;61:540–5.
[47] Buckle T, Brouwer OR, Valdés Olmos RA, van der Poel HG, van Leeuwen FW. Relationship between intraprostatic tracer deposits and sentinel lymph node mapping in prostate cancer patients. J Nucl Med 2012;53:1026–33.
[48] Harke NN, Godes M, Wagner C, Addali M, Fangmeyer B, Urbanova K, et al. Fluorescence-supported lymphography and extended pelvic lymph node dissection in robot-assisted radical prostatectomy: a prospective, randomized trial. World J Urol 2018;36:1817–23.
[49] Kumar A, Samavedi S, Bates A, Coelho R, Rocco B, Marquinez J, et al. V36 Use of intra-operative indocyanine green and Firefly® technology to visualize the "landmark artery" for nerve sparing robot assisted radical prostatectomy. Eur Urol Suppl 2015;14:eV36. https://doi.org/10.1016/S1569-9056(15)61120-4.
[50] Mangano MS, De Gobbi A, Beniamin F, Lamon C, Ciaccia M, Maccatrozzo L. Robot-assisted nerve-sparing radical prostatectomy using near-infrared fluorescence technology and indocyanine green: initial experience. Urologia 2018;85:29–31.

[51] Lopez A, Zlatev DV, Mach KE, Bui D, Liu JJ, Rouse RV, et al. Intraoperative optical biopsy during robotic assisted radical prostatectomy using confocal endomicroscopy. J Urol 2016; 195:1110—7.

[52] van den Berg NS, Buckle T, KleinJan GH, van der Poel HG, van Leeuwen FWB. Multispectral fluorescence imaging during robot-assisted laparoscopic sentinel node biopsy: a first step towards a fluorescence-based anatomic roadmap. Eur Urol 2017;72:110—7.

[53] van Willigen DM, van den Berg NS, Buckle T, KleinJan GH, Hardwick JC, van der Poel HG, et al. Multispectral fluorescence guided surgery; a feasibility study in a phantom using a clinical-grade laparoscopic camera system. Am J Nucl Med Mol Imaging 2017;7:138—47.