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ADVANCES IN IMAGING TECHNOLOGIES IN THE EVALUATION OF HIGH-GRADE BLADDER CANCER

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Abstract

Bladder cancer is a heterogeneous disease that ranges from low-grade variant with an indolent course, to high-grade subtype with a recurrent, progressive, and potentially lethal outcome. Accurate assessment for individualized treatment depends critically on the diagnostic accuracy of white light cystoscopy. Despite its central role, white light cystoscopy has several well-documented shortcomings including difficult flat lesion detection, imprecise tumor delineation that limits complete resection, differentiation between inflammation and malignancy, and grade and stage determination. As the limitations of white light cystoscopy contribute to the risk of cancer persistence, recurrence, and progression, there is a need for improved visualization of flat, multifocal, high-grade, and muscle-invasive lesions. Optical imaging technologies have emerged as an adjunct to white light cystoscopy with the goal to guide more effective treatment by improving cancer detection and patient stratification on the basis of grade and stage. Photodynamic diagnosis and narrow band imaging are macroscopic imaging modalities similar to white light cystoscopy, but provide additional contrast enhancement of bladder tumors and have been shown to improve detection rates. Confocal laser endomicroscopy and optical coherence tomography are microscopic imaging technologies that enable real-time high resolution, subsurface tissue characterization with spatial resolutions similar to histology. Molecular imaging offers the potential for the combination of optical imaging technologies with cancer-specific molecular agents to improve the specificity of disease detection.

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CONFLICT OF INTEREST

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Keywords

Bladder cancer; confocal laser endomicroscopy; fluorescence cystoscopy; molecular imaging; narrow band imaging; optical coherence tomography; photodynamic diagnosis

INTRODUCTION

Bladder cancer is the sixth most common cancer in the United States with 74,690 new cases and 15,580 deaths expected in 2014.[1] The natural history of bladder cancer is heterogeneous, ranging from low-grade variant that does not recur after local resection to high-grade that recurs and progresses to metastatic, lethal disease.[2] Although 80% of patients present at a non-muscle-invasive stage (Ta, T1, TIS) that may be managed endoscopically, recurrence rate reaches 61% at one year and 78% at five years.[3, 4] As a result of its high recurrence rate and associated need for lifelong surveillance and repeat resections, the healthcare cost for bladder cancer is among the highest of all malignancy.[3, 5]

White light cystoscopy (WLC) is the standard for evaluation of bladder urothelium. In the office setting, flexible cystoscopes are used for initial identification of suspected lesions and subsequent surveillance for recurrence. In the operating room, complete transurethral resection (TUR) with larger rigid cystoscopes is performed for tissue diagnosis and local staging. Despite its central role, WLC has well-recognized limitations.[6, 7] While sufficient for the identification of papillary lesions, visual appearance under white light is unreliable for the determination of low- and high-grade cancer and cannot assess level of invasion.[8] Additionally, non-papillary and flat malignant lesions such as carcinoma-in-situ (CIS) can be difficult to differentiate from inflammation,[6] with detection rates of CIS only 58–68% by WLC.[9–11] Smaller or satellite tumors can be missed, which contributes to the up to 40% rate of residual bladder cancer found at the time of ‘second-look’ TUR.[12, 13] Finally, indistinct borders and difficult visualization of submucosal tumor margins during TUR can lead to incomplete tumor resection and understaging of bladder cancer.[14, 15] These limitations of WLC contribute to the increased risk of cancer persistence, recurrence, and in the case of high-grade bladder cancer, progression to metastatic lethal disease.[2, 16, 17]

To address the shortcomings of WLC, several adjunctive optical imaging technologies have emerged with the goal to improve bladder cancer detection and resection (Table 1). The imaging technologies can be broadly categorized based on their field of view. Photodynamic diagnosis (PDD) and narrow band imaging (NBI) are examples of macroscopic imaging modalities that survey a large area of mucosa similar to WLC, but provide additional contrast enhancement to distinguish suspicious lesions from non-cancerous mucosa. Microscopic modalities including optical coherence tomography (OCT) and confocal laser endomicroscopy (CLE) provide high-resolution, subsurface tissue characterization similar to histology and thus offer the potential for ‘optical biopsy’ of bladder cancer. Molecular imaging, through coupling of optical imaging technologies with fluorescently labeled binding agents (e.g. antibodies), may enable real time cancer imaging with molecular specificity. These technological advances have the potential to improve optical diagnosis and endoscopic management of bladder cancer. The most recent literature on adjunctive

optical imaging technologies is reviewed, with consideration for the evaluation of high-grade bladder cancer highlighted.

Photodynamic Diagnosis (PDD)

PDD, also known as fluorescence cystoscopy or blue light cystoscopy, provides wide-field fluorescence imaging of the bladder with a field of view comparable to WLC. PDD requires pre-operative intravesical administration of a photosensitive protoporphyrin IX precursor as the contrast agent, a blue light source that illuminates at 375 – 440 nm, and a specialized lens and camera head. Once taken up by the bladder urothelium, the protoporphyrin accumulates preferentially in neoplastic cells and emits a fluorescence in the red part of the spectrum under blue light excitation, allowing visualization of the tumor (Figure 1).[18] Two protoporphyrin analogues, 5-aminolevulinic acid (5-ALA) and its ester derivative hexaminolevulinate (HAL), have been investigated clinically. HAL is the more potent analogue, with greater local bioavailability and superior fluorescence intensity, and is approved for single clinical use in Europe and the United States for patients with suspected or known bladder cancer. Due to a false-positive fluorescence from inflammatory lesions, previous biopsy sites, or in patients previously treated with bacillus Calmette-Guérin (BCG),[7, 19] HAL is not approved currently for patients who received intravesical immunotherapy or chemotherapy within 90 days.

Multi-institutional randomized clinical studies have demonstrated that PDD improves the detection of papillary bladder tumors.[19, 20] Although PDD does not distinguish high-grade from low-grade bladder cancer, several studies have shown that PDD has an increased rate of detection of flat-appearing CIS.[19, 20] In a meta-analysis of three phase III studies, the detection of CIS was significantly higher by PDD + WLC compared to WLC alone (87% vs. 75% pooled sensitivity, $p = 0.006$).[21] Additionally, several meta-analyses have found significantly reduced residual tumor rates in patients treated with PDD, with relative risk of residual tumor 2.77-fold higher for WLC compared to PDD.[22, 23]

The recurrence rate of PDD-guided TUR of bladder tumor remains to be determined. In a meta-analysis that reviewed raw data from prospective studies on 1345 patients with known or suspected non-muscle-invasive bladder cancer (NMIBC), overall recurrence rates up to 12 months were significantly lower with PDD compared to WLC (34.5% vs. 45.4% pooled sensitivity, $p = 0.006$) and independent of the level of risk.[24] However, a prospective randomized multi-institutional trial found no significant difference in tumor recurrence and progression between PDD and WLC in patients with NMIBC.[25] Additional studies with longer follow-up time periods are needed to better define the optimal indications for PDD use and evaluate the long-term efficacy of PDD in regards to recurrence-free and progression-free survival.

Narrow Band Imaging (NBI)

Narrow band imaging (NBI) is a high-resolution wide-field endoscopic technique that improves detection of bladder neoplasia through enhanced visualization of mucosal and submucosal vasculature without the use of exogenous dye. NBI devices (Olympus Corp, Tokyo, Japan) filter out the red spectrum from white light, with the resultant blue (415 nm)

and green (540 nm) spectra absorbed by hemoglobin, thus highlighting the contrast between capillaries and mucosa. Under NBI, the more vascularized CIS or tumor areas are accentuated in appearance as green or brown (Figure 2). NBI is approved for clinical use in the United States and is available either in an integrated videocystoscope or through a camera head that can be attached to standard cystoscopes. These devices include a toggling functionality between WLC and NBI, thereby facilitating rapid real-time evaluation of suspected lesions. Although NBI provides a subjective impression of abnormal areas of bladder mucosa, there does not appear to be a significant difference in detection rate of bladder tumor between new and experienced users.[26, 27]

In a recent meta-analysis of eight studies including 1022 patients, the detection of bladder cancer was higher by NBI compared to WLC on both per-person (94% vs. 85% pooled sensitivity) and per-lesion basis (95% vs. 75% pooled sensitivity), however the pooled specificity on a per-lesion basis was lower by NBI compared to WLC (55% vs. 72%).[28]

Similarly to PDD, NBI does not distinguish bladder cancer grade but does improve CIS detection. In a study of 427 patients that compared NBI + WLC with WLC alone for recurrent bladder cancer, the detection of CIS was significantly improved by NBI over WLC (100% vs 83% sensitivity).[29] A single-center prospective randomized controlled trial involving 220 patients with bulky non-muscle invasive bladder tumor demonstrated an improved detection rate of CIS for NBI compared with WLC (95% vs. 68%).[30] A multicenter prospective study reported a significantly increased sensitivity for the detection of CIS from 50% for WLC to 90% for NBI in 104 patients.[31]

In a recent single center randomized controlled trial to assess whether NBI improved TUR of bladder tumors in 254 patients with 2-year follow-up, a reduced recurrence rate (22% vs. 33%, $p = 0.05$) and improved recurrence-free survival (22 months vs. 19 months, $p = 0.02$) were reported by NBI compared to WLC.[32] A multicenter randomized controlled trial to compare the recurrence rate at 1 year between NBI- and WLC-assisted TUR of bladder tumor in patients with NMIBC is ongoing.[33]

Confocal Laser Endomicroscopy (CLE)

CLE is an optical biopsy technology that provides dynamic, high resolution microscopy of mucosal lesions.[34] Recently approved for clinical use in the urinary tract, image acquisition is performed through fiber-optic probes ranging from 0.85-mm to 2.6-mm diameter compatible with working channels of standard cystoscopes.[35] Fluorescein, an FDA-approved drug, is used as the contrast agent and can be administered intravesically or intravenously with minimal toxicity.[36] In the current CLE clinical system (Mauna Kea Technologies, Paris, France), illuminating light from a 488-nm laser fiber source is focused by an objective lens, with scattered light from the in-focus tissue plane converged back into the fiber and subsequent signal processing into an image. CLE provides the highest spatial resolution (1–5 μm) of clinically available technologies, with images comparable to conventional histopathology. Video sequences are acquiring at 12 frames/second, allowing for real-time dynamic imaging of physiologic processes such as vascular flow.

Given spatial resolution sufficient to resolve microarchitectural and cellular features, CLE is capable of differentiating high-grade and low-grade bladder cancer. Following the initial pilot study that demonstrated the feasibility of using CLE in *ex vivo* bladders after cystectomy[37], CLE was conducted in 27 patients undergoing cystoscopy and TUR and differences between normal urothelium, low-grade tumors and high-grade tumors were visualized.[34] In normal urothelium, larger umbrella cells were seen most superficially followed by organized smaller intermediate cells with distinct cell borders. Low-grade papillary tumors demonstrated densely arranged but normal-shaped small cells extending outward from fibrovascular cores, whereas high-grade tumors and CIS showed markedly irregular architecture and cellular pleomorphism (Figure 3).

An imaging atlas based on 66 patients has been created to establish criteria for CLE diagnosis and grading of bladder cancer.[38] A recent study that analyzed 31 bladder regions with CLE and WLC demonstrated moderate interobserver agreement in image interpretation between novice and experienced CLE urologists with respect to cancer diagnosis; interestingly, experienced CLE urologists were found to have higher agreement for image interpretation with CLE compared to WLC alone (90% vs. 74%).[39] Multi-center studies examining the diagnostic accuracy of CLE for real-time cancer diagnosis and grading remain to be completed.

Optical Coherence Tomography (OCT)

OCT is another optical biopsy technology that provides high-resolution, real-time, subsurface imaging of tissues. Analogous to B mode ultrasound, OCT relies on information gathered by reflected energy. Unlike ultrasound, however, OCT utilizes near-infrared light (890–1300 nm) and measures the backscatter properties of different tissue layers to provide a cross-sectional image with 2 mm depth of penetration and 10–20 μm spatial resolution.[40] Under OCT, normal urothelium is seen as a weakly scattering darker layer, the lamina propria is a bright layer with the highest scattering intensity, and the muscularis propria is a less scattering layer beneath the lamina propria. In cancerous tissue, anatomical layers of the urothelium are lost.[41, 42] Limitations of OCT include false-positive results possibly due to disruptions of the bladder wall from erosion, scarring or granuloma formation.[40, 43]

Multiple studies have evaluated the real-time classification by OCT-assisted cystoscopy of bladder lesions as benign or malignant, with overall sensitivity 84% – 100% and overall specificity 65% – 89%.[40, 43–47] Although OCT does not distinguish neoplastic bladder lesions by tumor grade, it is the only optical imaging technology with sufficient subsurface tissue penetration for real-time assessment of invasiveness of bladder lesions (i.e. staging).[43–45] In a single center study involving 24 patients at high risk for bladder cancer, the positive predictive value for tumor invasion into the lamina propria diagnosed by OCT was 90%.[40] Other recent studies have reported an automated image-processing algorithm to detect bladder cancer from OCT images[48] and application in the upper tract.[49] Larger multi-center studies are needed to evaluate the diagnostic value of tumor invasion by OCT as an adjunct to cytology, WLC, and histopathology.

Multimodal Imaging

The combination of imaging modalities harbors the potential to increase diagnostic accuracy. For example, macroscopic imaging (PDD, NBI) could be utilized to identify suspicious lesions, whereas microscopic imaging (CLE, OCT) could provide grading or staging information via high-resolution tissue characterization.[7, 50] A recent study reported the feasibility of simultaneous PDD and CLE, however intraoperative tumor grading was not done as TUR was performed using PDD followed by *ex vivo* histologic analysis with CLE.[51] Another study evaluated 232 lesions from 66 patients with suspected bladder cancer using WLC, PDD alone, and PDD + OCT.[52] The combination of PDD and OCT compared to PDD alone significantly increased per-patient specificity from 62% to 87%. In another study, PDD in combination with standard OCT did not significantly improve diagnostic accuracy in detecting non-invasive bladder cancer, but the combined use of cross-polarization OCT and PDD improved the positive predictive and negative predictive values in detecting bladder cancer in flat suspicious areas.[53]

Other Emerging Technologies

In addition to the above modalities, a number of emerging technologies are at advanced prototype phase or early clinical feasibility stage. *Raman Spectroscopy (RS)* is based on the principle of scattering of photons following interaction with molecular bonds. As near infrared light (785–845 nm) illuminates the tissue, the donation of energy to molecular bonds results in a different wavelength of the photons exiting the sample (Raman shift).[54] Detection of these scattered photons is then plotted to create a spectrum of peaks, producing a molecular “fingerprint” of the examined sample without the requirement of an exogenous contrast agent.[55] In the *ex vivo* setting, RS has been shown to differentiate the normal bladder wall layers, assess invasiveness, and identify low- and high-grade bladder cancer.[56, 57] The first *in vivo* study utilized a Raman probe compatible with endoscopic working channels and reported sensitivity of 85% and specificity of 79% for the detection of bladder cancer in 62 suspected lesions.[55] Surface-enhanced Raman scattering (SERS) nanoparticles have been shown to augment weak signals and allow for conjugation to cancer-specific antibodies to enable Raman-based molecular imaging.[58]

Ultraviolet (UV) Autofluorescence is based on the ultraviolet laser excitation of the fluorescence of molecules naturally present in tissue. A recent pilot *in vivo* study compared spectroscopic results with histological findings in 14 patients who underwent cystoscopy.[59] Normal urothelium, papillary tumors and suspicious flat lesions were interrogated with a UV probe via the working channel of a standard cystoscope. The diagnostic signal was then converted into an intensity ratio of the emitted light at approximately 360 nm and 450 nm and color-coded to facilitate real-time interpretation.[59] Differentiation of bladder cancer from normal urothelium was demonstrated. Additional studies are required to investigate signal intensity across low- and high-grade bladder cancer, reproducibility, and potential for UV-induced toxicity.

Multiphoton Microscopy (MPM) is a laser-scanning microscopy technique based on the simultaneous absorption of two (or three) near-infrared photons (700–800 nm) to cause a localized nonlinear excitation that reduces the potential for cellular damage. MPM enables

imaging of unstained tissue at sub-micron resolution in three dimensions to a depth of up to 0.5 mm by utilizing intrinsic tissue emissions signals from autofluorescence (NADH and in cells, elastin in connective tissue, lipofuscin in fat) and second harmonic generation (collagen).[60] The interpretation of acquired images is simplified by color-coding the detected signals. A recent *in vitro* study reported MPM imaging on 77 fresh bladder biopsies, with 88% accuracy in differentiation between benign and neoplastic lesions and 68% accuracy in the assignment of cytologic grading.[61] Similarly to CLE, limitations of MPM include limited depth of penetration that is not sufficient for cancer staging and a lack of nuclear detail. Current research is focused on the minituarization of the MPM system for *in vivo* application.[62, 63]

Scanning Fiber Endoscopy (SFE) is an ultrathin flexible endoscope containing an optical fiber to provide wide-angle, full-color high-resolution images. [64, 65] The 1.2-mm diameter of the probe decreases invasiveness and allows versatility of use as either a standalone miniaturized endoscope or as a probe in conjunction with other imaging modalities.[66] SFE has been demonstrated in *ex vivo* bladder models.[67] Additional studies are required to investigate the feasibility of *in vivo* SFE. A potential application of SFE involves automated integration with an “image stitching” algorithm to generate a panoramic view of the urothelium that could be used for tumor mapping and surveying the bladder longitudinally.[67]

Molecular Imaging

Molecular imaging is the visualization and characterization of biologic processes at the molecular and cellular levels.[68] Molecular specificity may be conferred through coupling of optical imaging technologies with fluorescently labeled binding agents such as antibodies, peptides, or small molecules. Bladder, given the ease of access and an established track record of intravesical therapy, is a promising target organ for endoscopic molecular imaging. Fluorescently labeled cancer-specific molecular imaging agents may provide enhanced differentiation between tumor and adjacent normal or benign tissues. Thus, the combination of optical imaging technologies with cancer-specific molecular imaging agents holds the potential for real-time endoscopic cancer detection.[69, 70]

A recent study used fluorescently labeled CD47 antibody (anti-CD47) to successfully demonstrate *ex vivo* endoscopic molecular imaging of bladder cancer using CLE and PDD in 25 intact bladders derived from radical cystectomy.[71] All of the bladders were derived from patients with invasive high-grade bladder cancer. CD47 is a surface marker of human solid tumors and is expressed on more than 80% of bladder cancer cells.[72, 73] A monoclonal CD47 antibody under development as a targeted therapy agent was labeled with a fluorescent tag, either fluorescein isothiocyanate (FITC) or quantum dot, a semi-conductor nanocrystal, was instilled intravesically as a topical molecular imaging agent (Figure 4A). After allowing sufficient time for antibody binding, excess antibody was removed by bladder irrigation. Bladders incubated with anti-CD47–FITC were imaged with CLE (Figure 4B) and those incubated with anti-CD47-$Qdot_{625}$ were imaged by PDD (Figure 5). In all bladders imaged with CLE, anti-CD47–FITC binding to cancer lesions was between 95- and 1100-fold greater than binding to normal urothelium in the same bladder. In the bladders

imaged with PDD, the sensitivity and specificity for CD47-targeted imaging were 82.9 and 90.5%, respectively, indicating the potential of CD47 to serve as a bladder cancer imaging agent with improved disease specificity. Further studies to assess the *in vivo* binding and toxicity of labeled anti-CD47 will be required prior to clinical translation. The study offers the promising possibility that targeted therapy may be combined with targeted imaging.

CONCLUSIONS

New optical imaging technologies have emerged and hold the potential to revolutionize the detection and management of bladder cancer beyond white light cystoscopy. Macroscopic technologies such as PDD and NBI improve the detection of bladder cancer and are already implemented in the clinical setting. Microscopic technologies such as OCT and CLE provide optical biopsy techniques that enable subsurface imaging comparable to standard histopathology. CLE is the only clinically available imaging technique capable of differentiating between low-grade and high-grade bladder cancer. Molecular imaging represents an exciting innovation that combines optical imaging technologies with cancer-specific molecular agents to improve the specificity of disease detection and potentially grading differentiation. Additional studies are needed to further define the role of imaging technologies in the evaluation and management of high-grade bladder cancer.

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KEY POINTS

- Improved optical imaging of the bladder can lead to more effective use of bladder sparing management for low-grade cancer and more aggressive treatment for high-grade cancer.
- Photodynamic diagnosis and narrow band imaging are examples of macroscopic imaging modalities that survey bladder areas similar in size to white light cystoscopy, however provide additional contrast enhancement of suspicious lesions that improve detection rates.
- Confocal laser endomicroscopy is an example of a microscopic optical biopsy technology that provides high-resolution and subsurface tissue characterization similar to histology, and is the only clinical technology capable of differentiating high-grade and low-grade bladder cancer.
- The highest image resolution is inferred by molecular specificity and the development of molecular markers and binding agents for molecular imaging can potentially serve as a means to differentiate low-grade and high-grade disease.

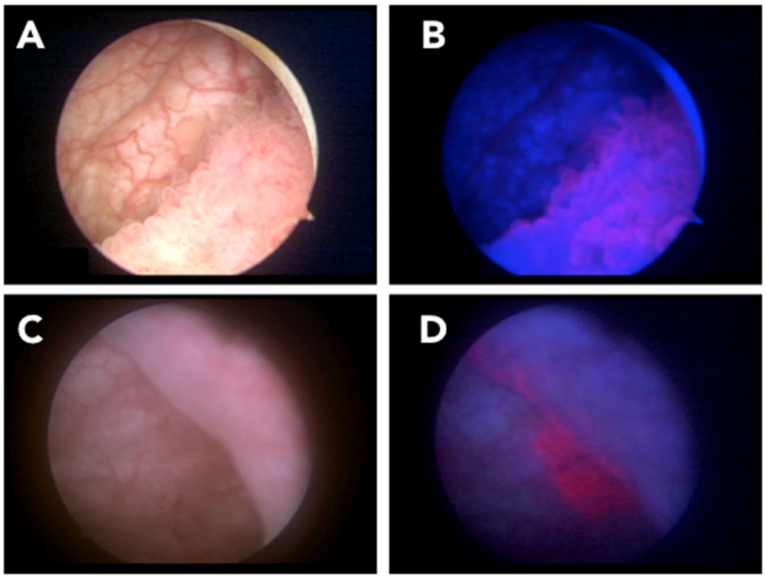

Figure 1.

Photodynamic diagnosis (PDD) of high-grade papillary and flat bladder cancer. (A) White light cystoscopy (WLC) showed a large broad-based papillary tumor. (B) PDD showed diffuse pink fluorescence over the tumor. Subsequent pathology for the lesion imaged in (A) and (B) confirmed non-invasive high-grade urothelial carcinoma. In a different patient, (C) WLC showed minimal erythema on the bladder mucosa along the left lateral wall, however (B) PDD showed a pink fluorescence over the region that was confirmed to be non-invasive high-grade urothelial carcinoma and CIS on histopathology.

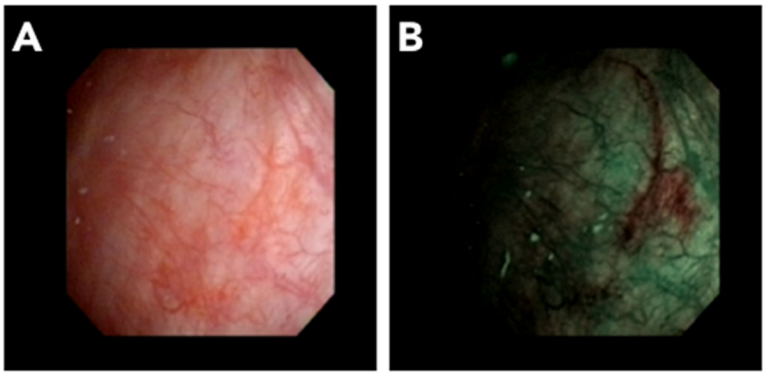

Figure 2.

Narrow band imaging (NBI) of the bladder. (A) WLC of the left lateral wall regions showed only mild erythema. (B) Under NBI, brown fluorescence delineated the extent of more vascularized neoplastic areas, subsequently confirmed on pathology to be high-grade non-invasive urothelial carcinoma.

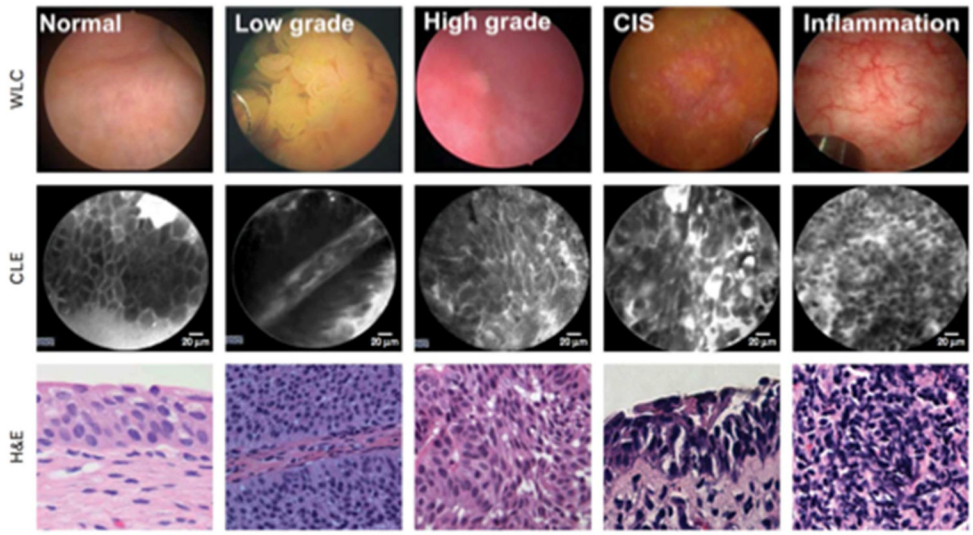

Figure 3.

Optical biopsy of the bladder using confocal laser endomicroscopy (CLE). Normal, low-grade, high-grade papillary bladder cancer, CIS and inflammation CLE images are shown with corresponding WLC images and hematoxylin and eosin (H&E) staining from subsequent biopsy. Low-grade cancer shows characteristically organized papillary structures, in contrast to high-grade cancer and CIS that display pleomorphic cells and distorted microarchitecture. (From Hsu, M., Gupta, M., Su, L. M. et al.: Intraoperative optical imaging and tissue interrogation during urologic surgery. Curr Opin Urol, 24: 66, 2014; with permission.)

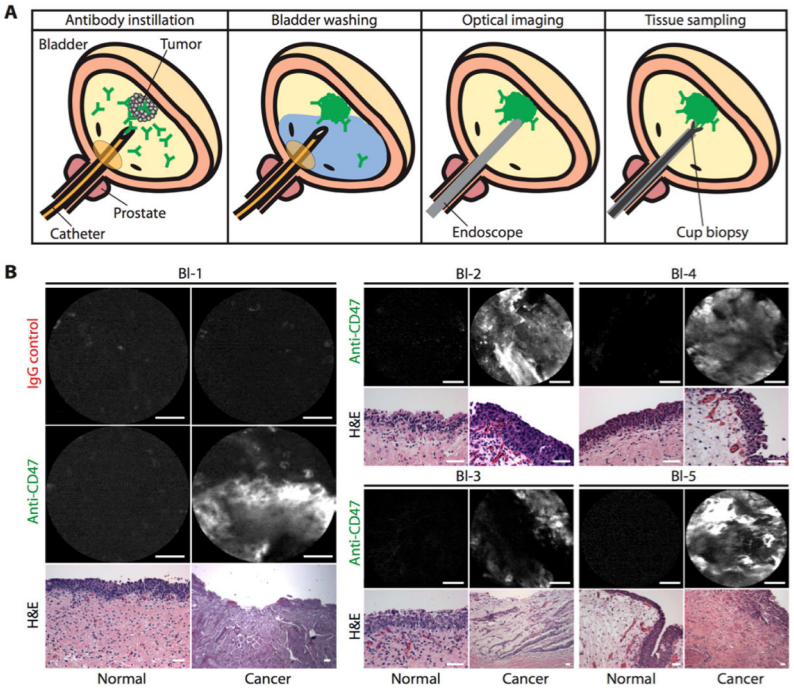

Figure 4.

Endoscopic molecular imaging of human bladder cancer using fluorescein labeled anti-CD47 as the imaging agent and CLE as the imaging modality. (A) Immediately after radical cystectomy, the ex vivo intact bladder was instilled with the molecular imaging agent via a urinary catheter and incubated for 30 minutes to allow antibody binding. After irrigation with saline, bound anti-CD47 was detected by endoscopic imaging of the bladder mucosa and normal and suspicious regions were biopsied for histopathological analysis. (B) Representative frames of CLE videos acquired from normal and cancer lesions in 5 bladders (Bl) with corresponding H&E images. Scale bars, 50 mm. (From Pan, Y., Volkmer, J. P., Mach, K. E. et al.: Endoscopic molecular imaging of human bladder cancer using a CD47 antibody. Sci Transl Med, 6: 260ra148, 2014; with permission.)

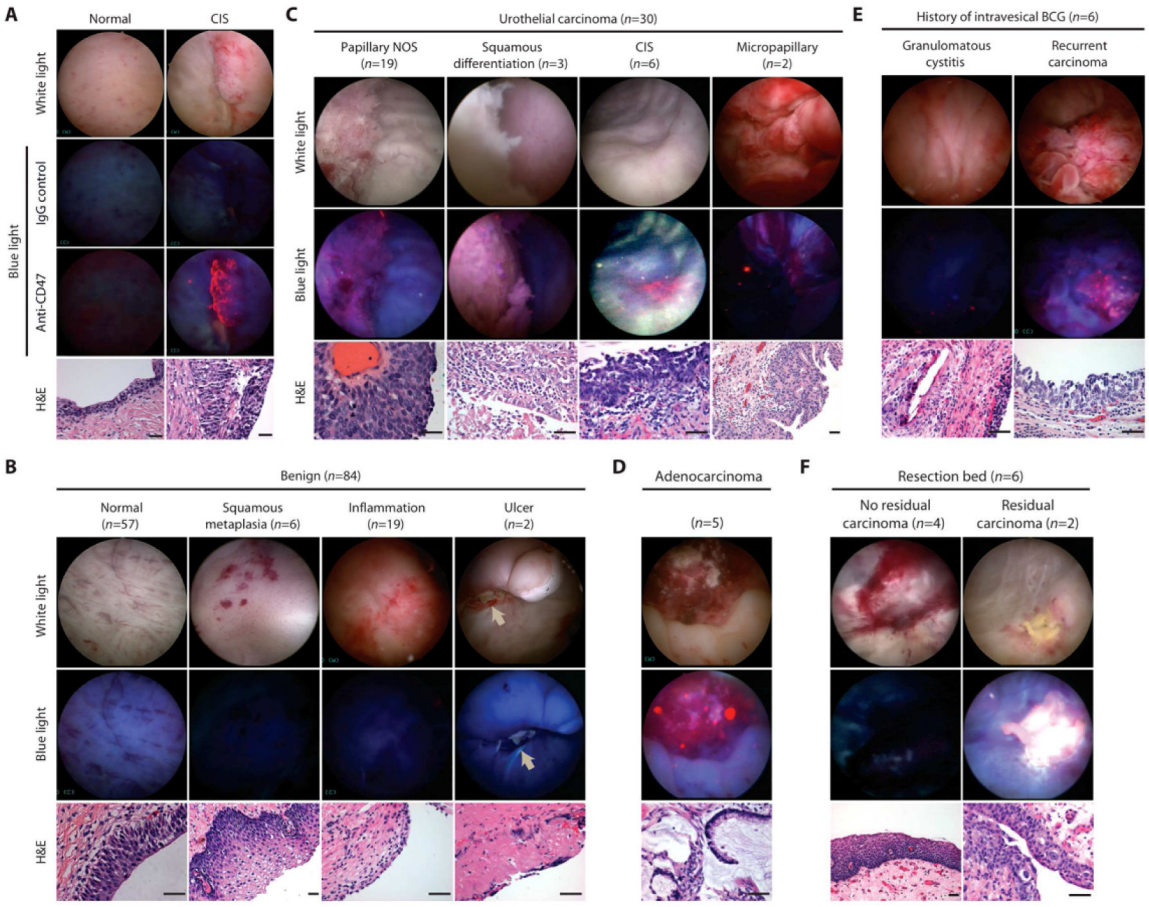

Figure 5.

Endoscopic molecular imaging of human bladder cancer using anti-CD47 labeled with a quantum dot nanocrystal ($Qdot_{625}$) and imaged with blue light cystoscope from a clinical PDD system. Representative WLC and PDD images with corresponding H&E staining for co-localization of anti-CD47-$Qdot_{625}$ binding and histopathology. (A) Cancer-specific binding of anti-CD47-$Qdot_{625}$ in a bladder with normal mucosa and a CIS lesion. (B) Benign regions of normal urothelium, squamous metaplasia, inflammation, and ulcer with no detectable anti-CD47-$Qdot_{625}$ binding. (C) Anti-CD47-$Qdot_{625}$ binding detected under PDD on urothelial carcinomas. (D) Anti-CD47-$Qdot_{625}$ bound to adenocarcinoma of the bladder. (E) In a bladder with a history of BCG treatment, anti-CD47-$Qdot_{625}$ bound to a region with recurrent carcinoma but did not bind to a region of cystitis. (F) Anti-CD47-$Qdot_{625}$ bound to residual tumor in a prior resection bed but not to benign scar tissue. Scale bars, 50 mm. (Adapted from Pan, Y., Volkmer, J. P., Mach, K. E. et al.: Endoscopic molecular imaging of human bladder cancer using a CD47 antibody. Sci Transl Med, 6: 260ra148, 2014; with permission.)

Table 1

Characteristics and properties of adjunct optical imaging technologies for bladder cancer

Name	Mechanism	Contrast agent	Resolution	Depth	Scope or probe (diameter)	Status
PDD	Fluorescence	HAL	mm – cm	Surface	Standard rigid cystoscope	Clinical
NBI	Absorption	None	mm – cm	Surface	Flexible cystoscope (5.5 mm) or standard rigid cystoscope	Clinical
CLE	Fluorescence	Fluorescein	1 – 3.5 μm	120 μm	Probe (0.85 – 2.6 mm)	Clinical/Investigational (*in vivo*)
OCT	Scattering	None	10–20 μm	~2.5 mm	Probe (2.7 mm)	Clinical/Investigational (*in vivo*)
Raman	Scattering	Optional (SERS)	-	2 mm	Probe (2.1 mm)	Investigational (*in vivo*)
UV	Fluorescence	None	mm – cm	Surface	Probe (3 mm)	Investigational (*in vivo*)
SFE	Reflectance + Fluorescence	None	mm – cm	Surface	Scope/Probe (1.2 mm)	Investigational (*ex vivo*)