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Nanotechnology in bladder cancer: current state of development and clinical practice

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Abstract

Nanotechnology is being developed for the diagnosis and treatment of both nonmyoinvasive bladder cancer (NMIBC) and invasive bladder cancer. The diagnostic applications of nanotechnology in NMIBC mainly focus on tumor identification during endoscopy to increase complete resection of bladder cancer while nanotechnology to capture malignant cells or their components continues to be developed. The therapeutic applications of nanotechnology in NMIBC are to reformulate biological and cytotoxic agents for intravesical instillation, combine both diagnostic and therapeutic application in one nanoformulation. In invasive and advanced bladder cancer, magnetic resonance imaging with supraparamagnetic iron oxide nanoparticles can improve the sensitivity and specificity in detecting small metastasis to lymph nodes. Nanoformulation of cytotoxic agents can potentially decrease the toxicity while increasing efficacy.

Keywords

bladder cancer; nanoparticle; photodynamic diagnosis; photodynamic therapy

Bladder cancer is the fourth most common cancer in men and tenth in women [1]. In the USA, approximately 72,570 new cases were diagnosed in 2013 with 15,000 deaths [2]. At diagnosis, approximately 80% of the patients present with nonmyoinvasive bladder cancer (NMIBC) [3,4]. The current standard therapy for NMIBC is complete transurethral resection followed by intravesical therapy to prevent recurrence. Bacillus Calmette–Guérin (BCG) is the only first-line intravesical therapeutic agent approved by the US FDA. This combination

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treatment is associated with a recurrence rate of 60% at 2 years and 25% disease progression into advanced stages [5–7]. Valrubicin is the only FDA-approved drug after failure of BCG with a response rate only around 20% [8]. Because of its high recurrence rate, all patients will need frequent intrusive, costly and uncomfortable cystos-copy examinations. Secondary to the long-term survival rate and costs associated with intensive disease surveillance, bladder cancer has the highest lifetime cost per patient of all cancer types, ranging from US $96,000 to US$187,000 (2001 values) per case [9–11]. For those patients whose disease has progressed to a locally advanced stage, radical cystectomy with urinary diversion is usually performed. This treatment modality is associated with the worst health-related quality of life among all cancer survivors [12,13]. For those patients with metastatic bladder cancer, combination systemic chemotherapy is the treatment of choice with a response rate around 50% [14]. There is an unmet need in diagnosis of bladder cancer, tumor localization during intravesical resection, decrease of disease recurrence, prevention of disease progression to advanced stages, monitor and early detection of disease recurrence. In addition, there are critical needs for better detection and more effective treatment of advanced disease.

Nanotechnology has been widely developed for the management of cancer, from diagnosis, detection, to treatment of cancer. Research into nanoparticles for cancer care has yielded a tremendous amount of possible applications, though most remain in the preclinical state. Many particles, such as gold nanoparticles, can be useful for *in vitro* assays for cancer diagnostics, enhancement of *in vivo* imaging, and as potential drug loading techniques. Moreover, with different manufacturing techniques, particles have demonstrable flexibly in size, shape and structure that changes their potential utility [15]. Some nanoparticles may serve as carriers for potential photosensitizers for therapeutics [16]. There is tremendous potential for nanoparticles as drug delivery tools. Liposomes, polymeric micelles, self-assembling drug-polymer particles have all been formulated as potential drug delivery mechanisms [17]. There is an increasing interest in theranostics, multipurpose nanoparticles that both enhance imaging and deliver therapeutic agents in the same particle [18–20]. In this review, we examine the current state of nanotechnology in the diagnosis and treatment of both NMIBC and advanced bladder cancer (Table 1).

Nonmyoinvasive bladder cancer

Diagnosis: nanotechnology in photodynamic diagnosis & screening

Fluorescent or photodynamic diagnosis has been one of the more promising techniques to improve on contemporary white light microscopy for detection of NMIBC. Standard white light cystoscopy often has difficulty distinguishing benign from malignant lesions and has particular challenges detecting flat lesions such as carcinoma *in situ*. Hence, transurethral resection with white light cystoscopy is associated with residue tumor in at least a third of cases regardless of the experience of surgeon [35]. Fluorescent cystoscopy utilizes a photosensitizer such as 5-aminolevulinic acid (5-ALA) or hexaminolevulinate which is administered intravesically prior to blue light cystoscopy (380–440 wavelength) [36]. These agents are preferentially absorbed by cancer cells and will appear red compared with surrounding tissue. Although these agents demonstrate high sensitivity, specificity may be poor, particularly after BCG therapy [37]. Nanotechnology may improve specificity of

photosensitizers to tumor tissue despite prior intravesical therapy and provide improved identification of carcinoma *in situ* [38]. Some groups have shown increased uptake of nanoparticles within cancer cells. But although many authors highlight the promise of nanotechnology-enhanced diagnosis, few articles actually describe attempts to use this technology specifically in bladder cancer [39,40]. We previously reported the development of a bladder cancer-specific ligand named PLZ4 (amino acid sequence: cQDGRMGFc) and a porphyrin-based nanometer-scale micelles (Figure 1) [21,32,41]. PLZ4 can specifically bind to both human and dog bladder cancer cells, but not to normal urothelial cells or cells from the urine after intravesical BCG treatment. PLZ4 decorated on the micelles could dramatically enhance PLZ4 mediated bladder cancer cell uptake into the cytoplasm (Figure 1C). Micelles decorated with PLZ4 on their surface could specifically target bladder cancer cells, but not normal cells, suggesting it can be potentially used for photodynamic diagnosis. Its clinical application is being explored [22,33]. In an orthotopic bladder cancer model, nanoporphyrin decorated with PLZ4 on the surface could specifically bind to bladder cancer, but not to adjacent normal urothelial cells [41].

In addition to intravesical applications, nanoparticles may provide more effective options for improved diagnostics or potentially even screening high risk populations for bladder cancer. Gold nanoparticles, for example, have a unique property called surface plas- mon resonance that results in a very different visible color to the naked eye depending on the distance of the nanoparticles from each other. This property might be exploited to develop rapid laboratory techniques where potentially malignant urine specimens could be quickly screened. An example of this technique involves detection of hyaluronidase with nanoparticles. Cationic gold nanoparticles aggregate with polyanionic hyaluronic acid and lead to a blue color change. Bladder cancer often produces high levels of hyaluronidase, and so urine from bladders with tumors will degrade hyaluronic acid and result in dispersion of the nanoparticles and color change easily visible to the naked eye [42]. Nanoparticles have also been developed to target-specific RNA sequences that could be easily detected if overexpressed in clinical cancer specimens. Eissa *et al.* describe a gold nanoparticle assay in which target RNA is purified using magnetic nanoparticles (Fe_2O_3) and then rapidly detected with standard molecular techniques. This study analyzed the hepatoma upregulated protein RNA that is upregulated in bladder cancer tumor cells in urine samples of 50 bladder carcinoma patients, 25 benign bladder lesions and 25 controls. A sensitivity of 88.5% and specificity of 94% was obtained [23]. Considering the low incidence of bladder cancer, the value of these technologies in cancer screen is questioned. Their clinical value lies more in follow-up and disease monitoring after initial treatment considering the high disease recurrence rate (around 60% in 2 years), and needs to be defined in large clinical studies in comparison to the gold standard of cystoscopy examinations with biopsy.

Therapeutic: nanoformulations for intravesical delivery of biologic & chemotherapeutic therapy

Intravesical instillation of live BCG bacteria remains a standard of care for high grade NMIBC, but signifi-cant side effects can occur, including BCG infections, sepsis and death. Repackaging components of the BCG bacteria has been investigated to substitute for live bacteria with the hopes of achieving similar efficacy without the risks. For example, the cell

wall skeleton (CWS) of BCG was evaluated for clinical use, but was found to be very difficult to dissolve in water and deliver to the site of interest. By encapsulating BCGCWS within 166 nm liposomes, Nakamura *et al.* were able to demonstrate efficacy in rat models against the development of bladder cancer [24].

Other nanoformulations of biological agents have also been assessed in treating bladder cancer. Martin *et al.* packaged poly-lactic-co-glycolic acid nanoparticles with small interfering RNA targeting survivin, an antiapoptosis protein often overexpressed in bladder cancer, and decorated the nanoparticle surface with chitosan, a polysaccharide that increases the nanoparticle adhesion to cells. More than ten-times increased small interfering RNA delivery were observed in mouse bladder and human ureter, as well as a 65% reduction in tumor volume and a 75% decrease in survivin expression relative to tumors treated with blank chitosan nanoparticles in xenograft tumors [43]. Poly-lactic-co-glycolic acid nanoparticles linked with poly(guanidinium oxanorbornene) of 140–160 nm in size and loaded with the histone deacetylase inhibitor beninostat have already entered human clinical trials. These nanoparticles were readily taken into bladder cancer cells at high enough concentrations to be effective, and had demonstrable effects in xenograft mouse models [44]. RNA delivery can also be achieved with lipid based nanoparticles. 1,2-dilinoleyloxy-3-dimethylaminopropane is a cationic lipid that can be used to form nanoparticles around RNA. Kang *et al.* demonstrated that this could enhance delivery of small activating RNA by increasing the expression of the tumor suppressor gene p21 in *in vitro* models. This group further tested these RNA-containing nanoparticles in a xenograft mouse model of NMIBC. Intravesical delivery of these lipid nanoparticles resulted in both reduced growth and improved survival in the mouse model using this system [45].

In addition to BCG, chemotherapeutic agents, such as mitomycin C, are also commonly used for intravesical administration in patients with bladder cancer. Polysaccharide-based nanoparticles made with chitosan, chitosan combined with polylactic acid or chitosan combined with poly(ε-caprolactone) can be loaded with mitomycin C and these nanoparticle drug-delivery systems have showed at least a similar efficacy in delivery of pure drug. Drug release kinetics with these nanoparticles revealed that the drug was slowly leached out of the particles and as the polysaccharide structure might act as a bioadhesive, exposure of the drug to the bladder surface can be increased, even after voiding [25].

Cationic particles loaded with mitomycin C, for example, demonstrate longer bladder retention in rat models than the pure drug [46]. Epirubicin-loaded poly(ethyl-2-cyanoacrylate) nanoparticles have been studied *in vitro* and demonstrate increased cytotoxicity, but also increased drug concentrations within superficial tissue of treated porcine bladders [27]. In addition to nanoparticles, single-walled carbon nanotubes (each ~150 nm in length) decorated with PEG and loaded them with pirarubicin demonstrated much higher drug retention within the bladder than free pirarubicin in a mouse model, and histologic examination of treated bladder tumors showed higher apoptosis rates of apoptosis than free drug [47].

Taxane agents (paclitaxel [PTX] and docetaxel) are active in treating advanced bladder cancer. Therefore, multiple studies focused on nanoformulation of taxane agents to improve

intravesicular delivery of this class of chemotherapeutic agents. Current intravenous formulation of PTX forms micelles around the drug when exposed to urine which may prevent tumor exposure to the drug [26]. Nanotechnology may be leveraged to overcome this issue by creating nanoparticles with gelatin. PTX-loaded nanoparticles can be created from basic polysorbate gelatin as polysorbate is commonly used in many human medicines given to humans, such as vaccines. Resulting particles are 600–1000 nm (large by most nanotechnology standards), but researchers were able to demonstrate rapid release of PTX within the bladders of dogs using these nanoparticles [48]. Sub-sequent pharmacokinetic studies in dogs with NMIBC showed that PTX bound to gelatin nanoparticles when instilled into the bladder, resulted in concentrations of drug within the urothelium and lamina propria 4.4-times greater than that observed with standard PTX formulations [49]. Tsallas *et al.* performed *ex vivo* pharmacokinetic studies of PTX- and docetaxel-loaded micelles of PEG-poly lactic acid using porcine bladder tissue. Tissue penetration of these drugs were compared with control chemotherapy solvents in the bladder and found to be significantly higher for the drug combination [50,51].

Mugabe *et al.* propose that hyperbranched polyglycerols (HPG) and polyethyleneimine linked with PEG polymers could be mucin adhesive, potentially allowing for longer exposure to drug in the NMIBC setting. These polymers have been shown to bind PTX as well and permit removal of organic solvents, making them safe for bladder instillation. Bound with PTX, these polymers form particles <10 nm in diameter on average and in murine models with NMIBC xenografts, the PTX-loaded particles were statistically more effective at slowing tumor growth compared with free PTX [52]. The same group tested similar HPG–PEG polymers loaded with docetaxel in a murine bladder cancer model and, using both fluorescent and histopatho-logic techniques, was able to demonstrate increased efficacy after bladder instillation. The treatment was most effective with the addition of amine groups to the external ends of the nanoparticle, which they also demonstrated was associated with increased mucoadhesive properties [31,53].

Some of the taxane nanoformulations have already reached clinical trials. N-albumin bound PTX (nab-PTX) is a commercially available nanoparticle formulation with proven successful in treatment of other tumor types [54,55]. It is also an attractive agent for intravesicular administration as it is more soluble in water than standard PTX. McKiernan reported a Phase I trial of intravesical instillation of nab-PTX at 5 mg/ml in patients with recurrent NMIBC after a minimum of one complete course of intravesical therapy [56]. No systemic toxicity was observed, and five out of 18 patients responded. Bassi *et al.* reported another Phase I clinical trial in which PTX-hyaluronic acid was used for the treatment of BCG-refractory cancer *in situ* [57]. The PTX concentration ranged from 0.6 to 3.0 mg/ml. Nine out of 15 patients responded with minimal systemic toxicity. More clinical trials are needed to determine the efficacy of these two formulations in NMIBC.

Drug loaded nanoscale moieties can be combined with other approaches to further increase the therapeutic efficacy. Huang *et al.* combined two different forms of nanotechnology in an attempt to create novel delivery devices for chemotherapy. Poly(ε-caprolactone)-bpoly-(propargyl methacrylate-click-mercaptosuccinic acid-co-poly-(ethylene glycol) methyl ether methacry-late) particles were developed and loaded with super-paramagnetic iron oxide

nanoparticles in combination with cisplatin [58]. This group was able to demonstrate improved slow release of cisplatin over a longer period of time, permitting for longer drug exposure and that they could modify the release effect by carefully controlling the treatment temperature. Incorporation of iron oxide nanoparticles might allow for *in vivo* controlled hyper-thermia by utilizing an external alternating magnetic field. Men *et al.* described creating 35-nm nanoparticles composed of a combination of 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), a cationic lipid, and monomethoxypoly(ethylene glycol)–poly(3-caprolactone) incorporated in a hydrogel matrix for bladder instillation [59]. The hydrogel served to slow elimination from the bladder and permit prolonged exposure, while the nanoparticles permitted delivery of otherwise hydrophobic compounds. Using a fluores-cent surrogate for drug compounds, they demonstrated increased delivery to the bladder wall. Additionally, high intravesical doses in mice were well tolerated, whereas systemic doses resulted in significant toxicity.

Therapeutic: nanoformulation for photodynamic & photothermal therapy

Nanotechnology may eventually permit the wide application of photodynamic therapy for treatment of bladder cancer. The prospect of photodynamic therapy for superficial bladder cancer has been discussed since the 1950s and 1960s. The basic concept involves use of a photosensitizer, typically large cyclic compounds such as porphyrins, chlorins and phthalocyanines. In addition to the potential for photodynamic diagnosis as described before, these compounds produce free radicals when exposed to visible or near infrared light. Current products on the market such as porfimer sodium have major limitations – in particular poor specificity for targeting the tumor alone [28]. It is approved for the treatment of bladder cancer in Canada, but only for esophageal and non-small cell lung cancer in the USA. Several authors have reported developing nanoparticles to increase the stability and enhance delivery of these large macrocyclic molecules to tumor cells specifically for bladder cancer applications. Ghosh *et al.* demonstrated that meso-tetra-4-pyridyl porphyrin molecules could form nanoparticles when complexed with DNA. These porphyrin:DNA nanocomplexes release DNA in hydrophobic environments including cell membrane, produce reactive oxygen species and cytotoxicity in a light-dependent manner, and *in vivo* antitumor activity in bladder cancer xenografts [16].

Conjugation of specific ligands to the surface of nanoparticles encapsulating photosensitizer is another strategy to improve the efficacy and tumor specificity of this treatment modality. Derycke *et al.* generated PEG-based liposomes loaded with the photosensitizer aluminum phthalocyanine tetrasulfonate ($AlPcS_4$).

Transferrin was conjugated to the surface of these liposomes, with the hope of increasing cellular uptake, taking advantage of transferrin receptor overexpression in certain cancer cells. *In vitro*, bladder cancer cells demonstrated high uptake of the photosensitizer when utilizing transferrin-conjugated liposomes. This high uptake then correlated with increased photocytotoxicity in the treated cells. In an orthotropic rat model, tumor cells demonstrated increased uptake in tumor cells over surrounding tissue after treatment with transferrin-conjugated liposomes whereas untargeted liposomes and free AlPcS4 were uniformly absorbed. A particular challenge to this approach was that lipsome-loaded $AlPcS_4$ did not

demonstrate significant uptake without pretreatment of bladder with chondroitinase, highlighting that liposome penetration of the bladder mucosa may be difficult. Additionally, *in vivo*, the free $AlPcS_4$ was absorbed systemically at higher concentrations which they speculated was due to its small particle size [60].

One important limitation of photodynamic therapy is the poor penetration of light through tissue. Most photosensitizers require blue or green light in order to release oxygen species. Ideally, near-infrared wavelengths can be used to penetrate deeper into tissues. Nanotechnology can also be used to create nanoparticle carriers of photosensitizers that can upconvert near infrared to the visible spectrum – and then activate the sensitizers within them. One such nanoparticle with these properties is sodium yttrium fluoride with a mesoporous silica coat. These particles are uniformly about 90 nm in size and can be loaded with zinc phthalocyanine (ZnPc) [29]. *In vitro* studies with human bladder cancer cells demonstrated cellular uptake of nanoparticle-loaded ZnPc. Exposure to infrared light then resulted in increased caspase-dependent apoptosis of bladder cancer cells [61]. In addition, another group attempted to enhance the photodynamic effect using porphyrin and iron oxide magnetic particles and demonstrated that external magnetic fields could enhance *in vitro* apoptosis [62]. Further studies are needed to determine these approaches can be translated into *in vivo* applications.

Photothermal therapy takes advantage of some nanoparticles' ability to absorb infrared light leading to localized increases in temperature. In these cases, the nanoparticle itself becomes the therapeutic agent when combined with light. Gold-based nanoparticles show promise in this area of photothermal therapy. In a proof of principle experiment, Cheng *et al.* demonstrated that silica-Au nanoshell, Au/Ag nanospheres and Au nanorods modified with tumor targeting antibodies, such as anti-EGF receptor and anti-HER2 antibodies, were internalized by cells, and resulted in photothermal-induced death of urothelial cancer cells *in vitro* [63]. FGF-1 has also been conjugated to the surface of gold nanoparticles, leading to increased uptake by tumor cells. This model also demonstrated hyperthermia with exposure to near infrared light [64].

Locally advanced & metastatic bladder cancer

The standard of care for myoinvasive bladder cancer is neoadjuvant chemotherapy followed by radical cystectomy or definitive chemotherapy in the setting of a metastatic disease [14,65,66]. There are two major regimens commonly used in treating bladder cancer, both developed decades ago: gemcitabine and cisplatin/carboplatin (GC regimen) and regular or dose-dense methotrexate, vinblastine, doxorubicin and cisplatin [14]. The prognosis of bladder cancer has not changed significantly over the last three decades. After the initial treatment, computed tomography, MRI and sometimes, PET are commonly used in evaluating disease response and monitoring recurrence. However, these imaging modalities are far from optimal. For example, the pooled sensitivity and specificity of PET is only around 80% [30].

Diagnosis: iron nanoparticles for MRI contrast to improvement detection of lymph node metastasis

Current imaging technologies, such as computed tomography and MRI, heavily rely on the size change of lymph nodes and/or appearance of anatomic abnormalities which usually is the primary criteria used to establish the diagnosis. Nanoformulation of contrast agents showed some promising results. One of these agents is ultra-small superparamagnetic particles of iron oxide (USPIO). These particles are 30–50 nm in diameter that are passively collected within normal lymph node architecture when injected into the blood stream. In the lymph node, macrophages in the reticuloendothelial system take up these particles, which lead to decreased intensity with some MRI sequences. Lymph nodes infiltrated with tumor will fail to uptake these particles, potentially improving their ability to detect metastases. In the largest study of USPIO in patients with bladder cancer [67], 58 patients were recruited with known myoinvasive bladder cancer who were planned to have cystectomy. MRI enhanced with USPIO ferumoxtran-10 was performed to evaluate pelvic lymph nodes before surgery. A total of 172 nodes were found and correlated with the node dissection in these 58 patients. Compared with the precontrast imaging which used the size as the only criteria, post-USPIO contrast MRI increased the sensitivity from 76 to 96% ($p < 0.001$) with pathology as the gold standard, and the overall negative predictive value was increased 91 to 98% ($p < 0.01$). Post-contrast MRI detected small metastases (4–9 mm) in ten of 12 normal-sized nodes (10 mm or less) that were otherwise not detected on precontrast images, and lymph nodes that were normal in size, and outside of the normal surgical field in two patients. In another study that included a broader subset of genitourinary malignancies, 75 patients, including 19 with bladder cancer, had USPIO enhanced MRI for detection of lymph nodes prior to extended template pelvic lymph node dissection, with results correlated with histopathology [68]. Patients were clinical stage N0 at the time of surgery. Out of 2993 lymph nodes removed, 54 metastatic lymph nodes were identified with pathologic cancer metastases. Compared with the histopathology, the enhanced MRI had a sensitivity of 55.0% and a specificity of 85.5%. Most of the missed lymph nodes had the short axis <3 mm.

One of the challenges of the aforementioned studies with USPIO contrast agents is the need for a high level of technical expertise of the physicians reading the MRI. Some studies have sought to improve the sensitivity, specificity and interoperator variability. In a pilot study, Thoeny *et al.* evaluated the effectiveness of combining USPIO with diffusion weighted MRI in an initial pilot study, and demonstrated a potential improvement in interobserver variability and detection of 24 out of 26 lymph node metastases as small as 2.0 × 3.0 mm [69].

While promising in the ability to give better preoperative staging for bladder cancer, these iron nanoparticles currently lack the evidence that surgical or chemotherapeutic strategies could be changed on their results [70]. More clinical trials will be needed to define the role of nanoparticle contrast enhanced imaging in bladder cancer management.

Therapeutics: nanoformulations of standard therapeutic agents

Nanoparticles can enhance drug delivery to cancer sites because tumor vasculature is often porous enough to allow particles in the 10–100-nm range to penetrate preferentially into tumors. This characteristic is called the enhanced permeability and retention effect for tumor-targeting drug delivery [71,72]. Luo *et al.* showed that small-size nanoparticles (17–60 nm) had better drug delivery to tumor sites than large (150 nm) ones [73]. Albumin nanoformulation of PTX (nab-PTX) has the size around 130–150 nm, larger than the cut-off size of 100 nm by the definition of nanoparticle [74,75]. In this formulation, PTX binds noncovalently to albumin which serves a carrier in the blood stream and eliminates the use of special solvents for intravenous administration. The major advantage of nab-PTX over PTX is its elimination of the organic solvents polyoxyethylated castor oil and alcohol. These solvents work as carriers for the hydrophobic PTX and can cause severe allergic reaction even with corticosteroid and antihistamine premedication. It has been approved by the FDA for metastatic breast cancer, advanced nonsmall cell lung cancer and pancreatic cancer [54,55]. In a Phase II study with bladder cancer, 48 patients who had progressed after prior platinum-based therapy received 260 mg/m^2 of nab-PTX every three weeks. This study demonstrated an overall response rate of 28% that is within the range of other second-line therapy. Even though the dose is slightly higher than PTX, its toxicity was similar to previous studies, with neuropathy and fatigue being common during treatment [76]. Currently, nab-PTX has not been approved by the FDA for the treatment of bladder cancer.

Other nanoparticles in development to improve systemic deliver include micelle-based structures. Dr Lam's group developed a novel micelle drug-delivery system [30,73,77–82]. The building blocks of micelles are called telodendrimers, which are composed of linear PEG and dendritic oligomers of cholic acid. The biphasic structure of cholic acid allows the loading of hydrophobic drugs in the core while exposing the hydrophilic side and PEG on the surface. These micelles possess the characteristics that optimal nanocarriers should have: well-defined structure and functionality, good physical and chemical stability, biocompatibility, biodegradability, high drug loading capacity, easy manufacturing and narrow polydispersity. To develop bladder cancer-targeting micelles, Lin *et al.* decorated the micelle surface with a bladder cancer-targeting ligand named PLZ4 that resulted in bladder cancer-targeting micelles with the size around 23 ± 8 nm. Micelles were loaded with fluorescent dye as well as PTX or daunorubicin [33]. *In vitro* studies showed that these bladder cancer-targeting micelles were more effective than daunorubicin in a dog bladder cancer cell line. *In vivo* imaging study in the subcutaneous patient-derived xenografts (PDXs) bladder cancer models showed high accumulation of targeting micelles at the tumor site with a long retention time, but not other major organs (Figure 2) or other lung cancer xenografts in the same mice, highlighting the potential for a high degree of specificity [22,33]. Figure 3 showed a therapeutic example of bladder cancer PDX bearing mice treated with free PTX and different doses of PTX formulated in the targeting micelles for a total of six-times of intravenous injection. Targeting PTX showed a dose-dependent antibladder cancer efficacy and cell cycle arrest. Formulation of PTX in these targeting micelles could significantly decrease the toxicity that allowed the administration of PTX at three-times the therapeutic dose without increasing the toxicity, and prolong the overall survival from 27 days with free PTX at the therapeutic dose of 10 mg/kg to 76 days with bladder cancer-

targeting micelles ($p < 0.0001$, Figure 3) [22]. In those studies, instead of using cell lines that have been cultured and maintained in laboratory for a long time, PDXs were used that were developed directly from unselected and unmanipulated clinical patient bladder cancer specimens. Therefore, human cancer cells and PDXs share the same genetic background and the efficacy study in PDXs more reflects what happens in clinic.

Therapeutic: nanoparticle delivery of biological agents

Delivery of biological agents is another potential area of promise where nanotechnology may help improve the transfection rate. Uptake of target genes can be improved by including targeting receptors for ligands that are highly expressed on the cell surface. Plasmids with target genes can be complexed with cationic liposomes of DOTAP combined with 1,2-dioleoyl-*sn*glycero-phosphoethanolamine or with cholesterol. Xu *et al.* described the addition of transferrin to the surface of these liposomes to enhance uptake within tumors [83]. The surface ligand transferrin was further modified to be an antibody fragment against a cell surface receptor. Pirollo *et al.* demonstrated that the tumor suppressor gene *RB94* could be transfected into human bladder cancer cell lines, but not at high rates in cell lines from normal tissues using this novel cell ligand. *In vitro* tumor cells after transfection with the tumor suppressor gene were 31-fold more sensitive to gemcitabine. Bladder cancer murine xenograft models were treated systemically with the transferrin-liposome or transferrin receptor short chain antibody fragment-liposomes complexed with plasmids of the *RB94* gene. Histologic examination of tumors suggested strong expression of the tumor suppressor gene – which was absent if the surface ligands were not included on the liposome. Tumors in these models were demonstrated to be more sensitive to gemcitabine for a longer period of time if transfected with *RB94*. This gene and vector is now being evaluated in Phase I clinical trials [34].

Conclusion & future perspective

There are two distinct subtypes of bladder cancer: nonmyoinvasive bladder cancer and locally advanced or metastatic. The nanotechnology clinical application for nonmyoinvasive cancer is mainly for intravesical instillation. Nanotechnology-based diagnostic approach using urine specimens can potentially have clinical impacts in disease monitoring after treatment. This approach may prove less useful during the staging workup as muscle-deep biopsies are needed to dictate the clinical management. The major diagnostic application of nanotechnology for NIMBC is tumor localization during cystoscopy. Current white light cystoscopy sometimes cannot distinguish flat lesions from nonmalignant cells. This may explain why approximately a third of patients have residual cancer after tumor resection utilizing white light cystoscopy alone. Nanotechnology combined with cancer-specific targeting ligands can potentially deliver cancer-detecting agents specifically to cancer cells, improve cancer detection and resection, potentially leading to decreased tumor recurrence.

There is much room for improvement with intravesical therapy for NMIBC utilizing nanotechnology. Currently, BCG and valrubicin are the only two drugs approved by the FDA for first- and second-line intravesical therapy, respectively. The recurrence rate after resection and BCG instillation is around 60% at 2 years and the response rate for valrubicin is only 20%. Nano-formulations of taxane have already demonstrated some promising

efficacy after failure with first-line intravesical therapy. These nanoformulations and others, especially those coated with cancer-targeting ligand on the surface, are expected to enter into clinical trials and can possibly increase response rate, decrease recurrence and mitigate the need for cystectomy.

The management of advanced bladder cancer has not changed over the last three decades. Even though MRI with USPIO improves the sensitivity and specificity of detecting small metastasis to lymph nodes, its clinical impact in guiding chemotherapy and surgery is unknown. There have been no new intravenous drugs for advanced bladder cancer for decades. Micelles loaded with PTX and coated with the PLZ4 bladder cancer-specific ligand can improve the overall survival of mice carrying PDXs, and are expected to enter a Phase I trial within the next year.

The future of nanoparticles in NMIBC and advanced bladder cancer will likely be determined by properties of new particles in development. Metal-organic frameworks and hydroxyapatite-based particles may offer improved loading with large hollow spaces and the ability to increase the amount drug release in the intracellular space, with potential applications for both systemic administration and intravesicular therapy [84–88]. Continued development of polymer-based nanoparticles may improve *in vivo* stability of drug carriers and ability to both therapeutic and diagnostic agents to improve on current theranostic approaches, such as core-shell-corona-type polymeric micelles, or mesoporous silica nanoparticles [19,89]. Tribock terpolymer films suggest at efficient and cost effective methods of production [20]. All of these advances will have to be tested in the clinic and continue to add to hopeful new strategies for the diagnosis and treatment of bladder cancer.

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Executive summary

Nonmyoinvasive bladder cancer: diagnostic applications

- Cancer-targeting nanoparticles to deliver imaging agents can potentially facilitate identification of bladder cancer during cystoscopy, and guide tumor resection.
- Nanoparticle-based capturing of malignant cells and/or their subcellular components is promising and might have a role during follow-up.

Nonmyoinvasive bladder cancer: therapeutic applications

- Nanoformulation of biological agents can potentially be used to treat nonmyoinvasive bladder cancer or as adjuvant therapy.
- Nanoformulation of cytotoxic agents has already showed promising results in Phase I trials and can possibly be used to treat nonmyoinvasive bladder cancer after bacillus Calmette–Guérin failure.

Nonmyoinvasive bladder cancer: theranostic applications

- Nanoformulation of large cyclic photosensitizing compounds can be used for both photodynamic diagnosis and therapy as these compounds, upon light activation, can emit light with different wave length for cancer detection and produce reactive oxygen species for cell killing.
- Photothermal therapy can be integrated as these large cyclic compounds can absorb light and convert into heat locally.

Invasive & advanced bladder cancer: diagnostic applications

- MRI with ultra-small superparamagnetic particles of iron oxide can significantly improve the detection sensitivity and specificity of small metastasis to lymph nodes.

Invasive & advanced bladder cancer: therapeutic applications

- Formulation of therapeutic agents in nanoparticles takes advantage of the enhanced permeability and retention effect, and preferentially delivers these agents to cancer sites.
- Formulation of paclitaxel in bladder cancer-targeting micelles significantly decreases the toxicity that allows the administration of paclitaxel at three-times the therapeutic dose without increasing the toxicity, and prolongs the overall survival by almost three-times in mice carrying patient-derived xenografts. A Phase I clinical trial is being planned.

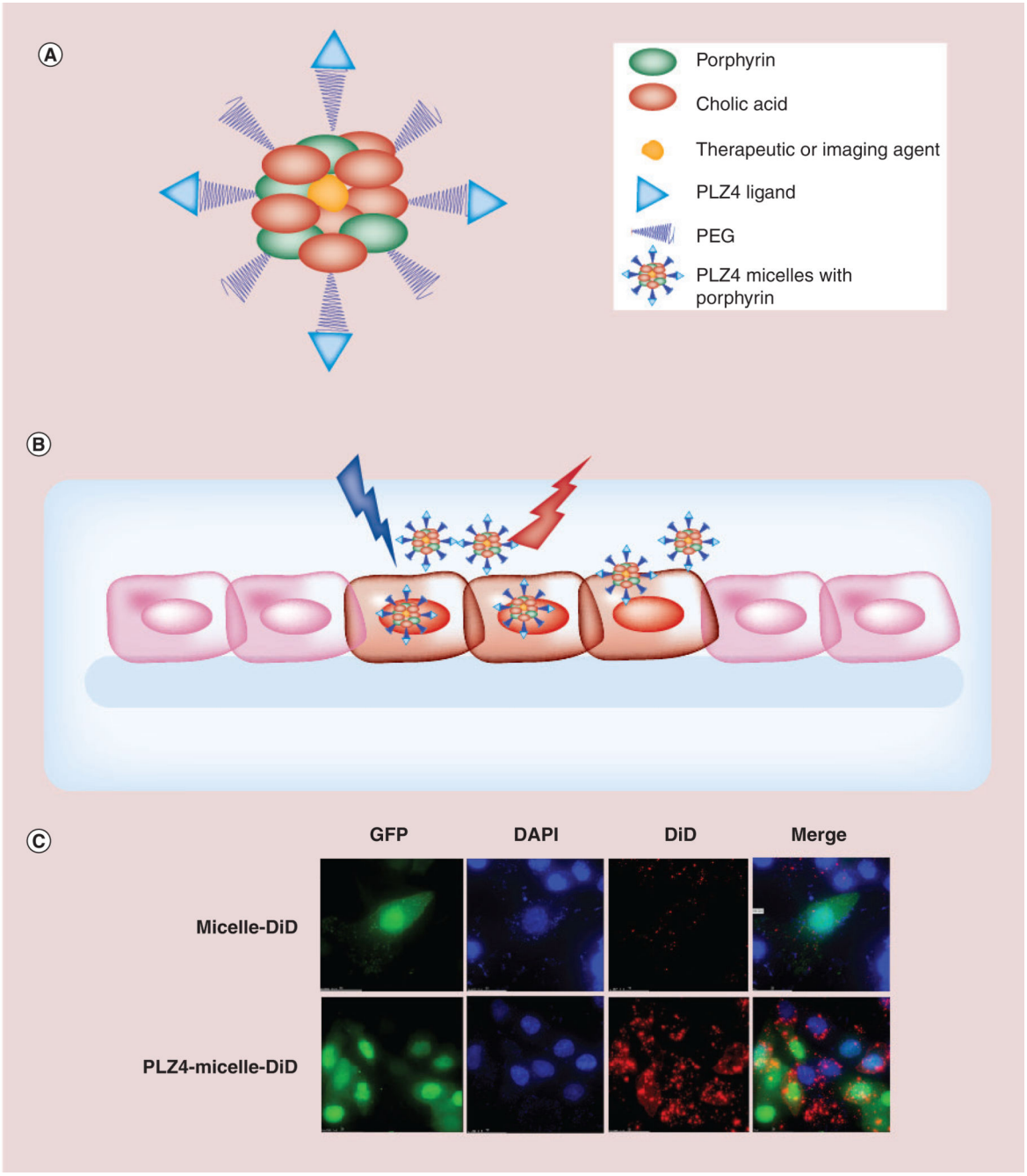

Figure 1. Photodynamic diagnosis and therapy of PLZ4-coated micelles

(A) Structure of PLZ4-micelles and their building polymer telodendrimer. A telodendrimer is synthesized through conjugation of cholic acid at one terminus of PEG and PLZ4 at the other terminus. To synthesize nanoporphyrin, porphyrin analogs can either be conjugated to PEG or loaded in micelles. To synthesize micelles, hydrophobic agents (therapeutic or diagnostic) and telodendrimers are mixed and dissolved in aqueous solution. The hydrophobic agents and the biphasic cholic acid units will form the core while exposing more hydrophilic PEG and PLZ4 on the surface for cancer-specific targeting. **(B)** Illustration of photodynamic diagnosis and therapy. PLZ4 can not only attach the nanoporphyrin on the

surface of bladder cancer cells, but more important, induce uptake of the whole micelles into cells. Upon activation with blue light, porphyrin can yield red fluorescence for photodynamic diagnosis and produce reactive oxygen species to damage macromolecules and kill cells for photodynamic therapy. **(C)** PLZ4-guided specific drug delivery. A bladder cancer cell line 5637 cells transfected with GFP were incubated with PLZ4-coated micelles or nontargeting micelles, both loaded with fluorescent DiD dye. After incubation for 15 min, cells were fixed and incubated with 4′,6-diamidino-2-phenylindole for nuclear staining. Scarce scattered red fluorescence from DiD was observed when cells were incubated with nontargeting micelles. By contrast, cells incubated with PLZ4 targeting micelles showed strong multifocal aggregated fluorescence not only on cell membrane, but also in the cytoplasm with a peri-nucleus pattern.

DiD: 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicar bocyanine.

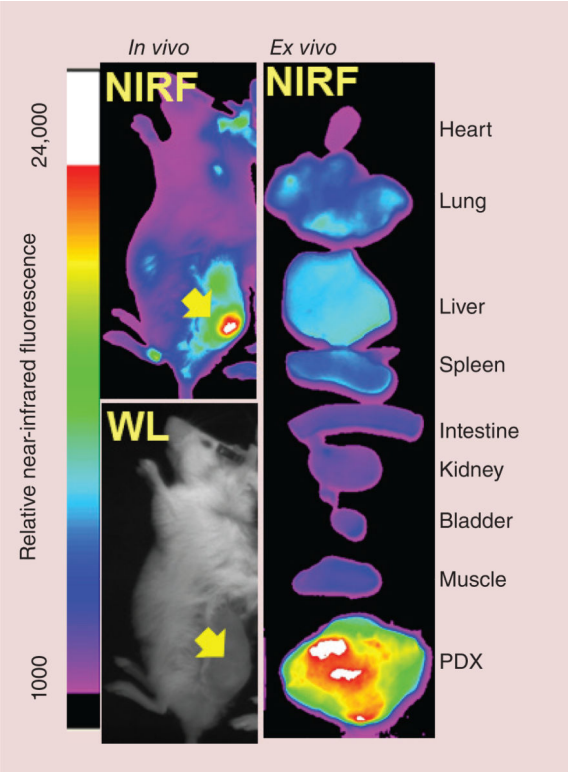

Figure 2. *In vivo*bioimaging of targeting micelles on patient-derived xenograft bearing mice

Mice carrying patient-derived xenografts were intravenously injected DiD loaded PLZ4 micelles (50 mg/kg of DiD) for 3 days. *In vivo* whole mouse and *ex vivo* imaging showed high retention of PLZ4-micelles-DiD at the PDX (yellow arrow) but only minimal signals were noted in other major organs.

NIRF: Near infrared fluorescence; PDX:Patient-derived xenograft; WL: White light.

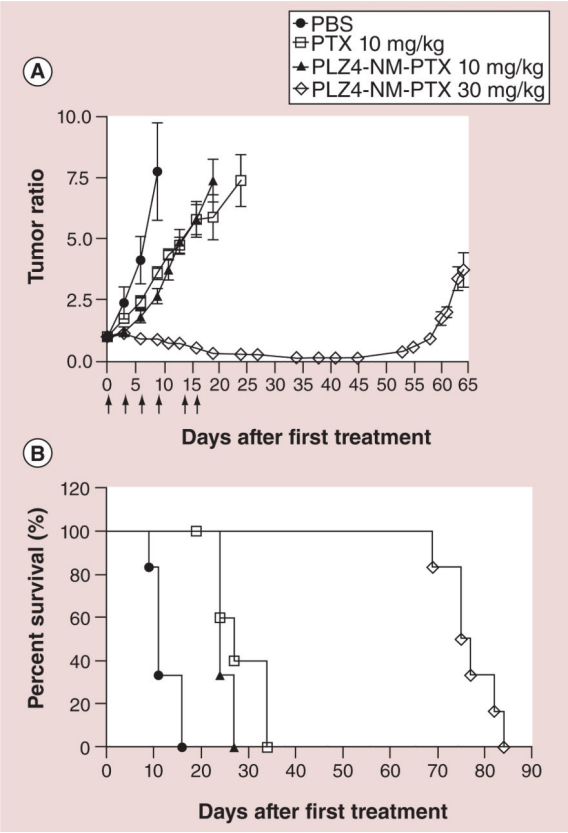

Figure 3. Comparison of antitumor activity in mice carrying patient-derived xenografts

Mice carrying patient-derived xenografts were treated with phosphate-buffered solution, free PTX at 10 mg/kg (therapeutic dose), PTX formulated in PLZ4 micelles (PLZ4-NM-PTX) at 10 or 30 mg/kg for six times with a 3-day interval. PTX formulated in the PLZ4 micelles showed a dose-dependent anticancer efficacy and G2/M phase cell cycle arrest rate on histopathologic examination. At the same dose of 10 mg/kg, PTX in PLZ4 micelles was associated with significantly reduced toxicity than free PTX evaluated at the 3 days after six doses [22]. PTX in PLZ4 micelles at 30 mg/kg had comparable toxicity as free PTX at 10 mg/kg, but significantly prolonged the progression-free survival ($p < 0.0001$) **(A)**, and overall survival from 27 to 76 days ($p < 0.0001$) **(B)**.

PBS: Phosphate-buffered saline; PTX: Paclitaxel.

Table 1

Applications of nanotechnology in bladder cancer.

Stage	Categories	Examples
Nonmyoinvasive bladder cancer	Diagnosis Therapy	PLZ4-coated nanoporphyrin [21] Urine diagnostics: gold nanoparticles to detect urine hyaluronidase [22] Nanoformutation of biological agents: bacillus Calmette-Guerin cell wall skeleton [23], siRNA [24] Nanoformulation of cytotoxic agents: mitomycin C [25], taxanes [26], anthracyclines [27] Nanoparticle carriers of photosensitizers: cancer targeting with transferrin [28], upconversion of fluorescent light [29], PLZ4-coated nanoporphyrin [21]
Advanced bladder cancer	Diagnosis Therapy	MRI with ultra-small superparamagnetic particles of iron oxide [30] Nab-paclitaxel [31] PLZ4-micelles loaded with paclitaxel [32,33] Nanoparticles for targeted gene delivery [34]