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Advances in preclinical approaches for intravesical therapy of bladder cancer

Introduction

Bladder cancer (BC) represents a prevalent form of urinary malignancy, predominantly manifesting as non-muscle-invasive bladder cancer (NMIBC)(1-3). The established treatment protocol for NMIBC entails transurethral resection of bladder tumors followed by intravesical therapy(4-6). However, approximately 70% of patients exhibit resistance to initial intravesical instillation therapy, subsequently developing recurrent or progressive tumors within a 5-year timeframe, thereby correlating with a notably unfavorable prognosis(7). Notwithstanding treatment involving transurethral resection of bladder tumor (TURBT) in conjunction with intravesical Bacillus Calmette-Guerin (BCG) or chemotherapy, between 55% to 85% of NMIBC patients encounter recurrence within 5 years(8, 9). Consequently, there exists an urgent imperative for supplementary therapeutic modalities to ameliorate the prevailing predicament in NMIBC therapy.

The unique structure of the bladder makes it difficult to deliver drugs systemically to the bladder site, so intravesical therapy is commonly used for treating BC(10). However, the protective barrier within the bladder, which consists of a glycosaminoglycan (GAG) layer and densely packed BC tissue, hinders drug penetration during intravesical therapy(11-13). Researchers have tried using mucus-penetrating nanoparticles to transport drugs to tumor regions(14-17), but the outcomes in practical use have been disappointing in orthotopic BC models(18). Moreover, off-targeting of normal urothelial cells increases the risk of side effects(4). There is an urgent need for new therapeutic strategies to improve the current challenges of NMIBC therapy.

Novel Therapeutic Approaches for NMIBC

Although several carriers for transmucosal delivery have shown promise in improving the movement of intravesical drugs across the epithelial layer, they have struggled to differentiate between cancerous and nearby healthy bladder tissues. To address this challenge, a research study developed a novel material called PGFCS, which is synthesized using PEGylated fluorinated chitosan (PFCS) and then modified with glutaraldehyde to obtain PGFCS(1). This material aims to enhance transepithelial penetration by targeting collagen within tumors. PGFCS promotes the opening of barriers in the epithelial layer at tumor sites by modulating tight junctions through fluorinated chitosan, which forms an adhesive interface specific to tumors by reacting with collagen amines found in tumor tissue(1). The study reported that intravesical administration of PGFCS significantly improved the localized efficacy of intravesical pirabucin (THP) and interleukin-12 (IL-12) in mouse models with orthotopic bladder tumors and patient-derived xenograft (PDX) bladder tumors. This enhancement was achieved through selective adherence to tumor surfaces and regulation of tight junctions at the tumor site, thus facilitating chemoimmunotherapy. Antitumor efficacy tests demonstrated that the administration of intravesical PGFCS/THP + IL-12 treatment resulted in an 80% cure rate in mice with aggressive orthotopic bladder tumors, surpassing the outcomes observed in control groups, including those that received combined THP and IL-12 treatment without prior administration of PGFCS(1). It is worth mentioning that the mice healed with intravesical PGFCS/THP + IL-12 treatment demonstrated significant immunological memory, as indicated by the complete rejection of re-challenged tumors. This study presents a new tumor-specific enhancer for transepithelial

penetration that has great potential for safe and effective intravesical administration in the treatment of NMIBC(1). A recent investigation delved into the efficacy and safety of RC48-ADC, a HER2-targeted antibody-drug conjugate, when administered intravesically for NMIBC(8). The study revealed that RC48-ADC operates by inducing G2/M arrest and caspase-dependent apoptosis, effectively curbing tumor growth in orthotopic bladder cancer models compared to disitamab, monomethyl auristatin E (MMAE), epirubicin, or control groups(8). Moreover, the study observed a positive correlation between higher HER2 expression in bladder cancer cell lines and increased sensitivity to RC48-ADC, a relationship validated by NMIBC organoid models, which demonstrated superior inhibition of proliferation and apoptosis induction in high-HER2 expression organoids treated with RC48-ADC(8). In vivo results demonstrated the effectiveness of RC48-ADC in inhibiting tumor growth, especially in cases with a high tumor burden, while disitamab, MMAE, and epirubicin exhibited limited inhibitory effects. Additionally, the study assessed the toxicity of intravesical RC48-ADC through dose escalation in nude mice and confirmed its safety within effective therapeutic doses. The findings of this study indicate that RC48-ADC holds promise as a safe and effective intravesical treatment option for NMIBC(8).

A recent study investigated the potential of using immunotherapy with Protein Aggregate magnesium-ammonium phospholinoleate-palmitoleate anhydride (P-MAPA) for NMIBC in an animal model(19). The study induced NMIBC in female Fischer 344 rats by administering N-methyl-N-nitrosourea (MNU). After the MNU treatment, the rats were divided into four experimental groups: control (not administered MNU), MNU (cancer), MNU-BCG, and MNU-P-MAPA(19). The results showed that intravesical P-MAPA treatment led to better histological recovery from the cancerous state compared to BCG treatment. The Western blot analysis revealed that rats treated with P-MAPA showed higher levels of cellular myelocytomatosis oncogene (c-Myc), chicken ovalbumin upstream promoter transcription factor II (COUP-TFII), and wild-type p53 proteins compared to rats treated with BCG(19). This study found that rats given P-MAPA intravenous immunotherapy had higher levels of Bcl-2-like protein 4 (BAX) and a lower proliferation/apoptotic ratio than rats given BCG, indicating a prevalence of apoptosis. The study's findings demonstrated that P-MAPA therapy enhanced the expression of wild-type p53 and promoted c-Myc/COUP-TFII-induced apoptosis, crucial processes for the histopathological recovery of cancer and the suppression of abnormal cell proliferation. These findings highlight the potential of P-MAPA as an innovative approach for NMIBC treatment, particularly in terms of its impact on apoptotic pathways(19).

Advancements in Immunotherapy for NMIBC

The study of the activation of immune receptor stimulator of interferon genes (STING) by natural or synthetic agonists has gained attention due to its potential to trigger immune responses against tumors. This is achieved through the production of type I interferons (IFNs) and inflammatory cytokines(20). BCG is the established treatment for high-risk NMIBC, but there are limited options for patients who do not respond to BCG(20). Huang and colleagues investigated the effects of E7766, a macrocyclic-bridged STING agonist, administered directly into the bladder, in two mouse models of NMIBC that were resistant to BCG and anti-PD-1 treatments(20). The study discovered that E7766 effectively stimulated the STING protein, triggering IFNβ expression in human immune cells and demonstrating efficacy across various STING genotypes. The results revealed that intravesical E7766 showed effectiveness in both NMIBC models, inducing immunological memory. Furthermore, the study revealed that the STING pathway in the bladder

activates the interferon (IFN) pathway. This activation leads to the infiltration of T cells and natural killer cells, the activation of dendritic cells, and the presentation of antigens in the bladder epithelium. This results in anti-tumor effects and immunity(20). The study also demonstrated that E7766 showed significant activity when administered intravesically in NMIBC models that were resistant to BCG or mouse surrogate anti-PD-1 agents, even at doses equivalent to or higher than their respective human therapeutic doses. The results of this study emphasize that E7766 may be valuable as an immune modulator for treating BCG-unresponsive NMIBC(20).

A new urinary drug-disposing (UDD) strategy was developed by Bellat et al.(21) to improve the treatment for NMIBC. Their aim was to reduce the accumulation of drugs in unintended organs and provide more comprehensive therapy for the urinary system compared to traditional intravesical chemotherapy (ITC). They achieved this by using a 12-amino acid peptide (Bdd) that can be filtered exclusively by the kidneys, along with a microtubule inhibitor called DM1. When administered intravenously to mice with human bladder tumors, this approach led to longer survival times(21). The study showed that administering the DM1-peptide combination (DM1-Bdd) intravenously significantly increased overall survival in mice with bladder cancer compared to intravesical mitomycin (MIT), with fewer side effects because the drugs were cleared more efficiently by the kidneys. In contrast, mice treated with DM1 or cisplatin (CIS) experienced hepatic and renal damage, highlighting the importance of enhancing renal clearance to reduce drug-related toxicity(21). Furthermore, they demonstrated the flexibility of the UDD approach by using a different drug called aldox-Bdd (aldox-DOX with a hydrazone linker) for bladder cancer treatment. Although this improved survival rates without causing death, it was not as effective at eliminating tumors as DM1-Bdd, likely because DM1 is more potent than doxorubicin (DOX). These findings show that the UDD approach has great potential for enhancing NMIBC therapy while reducing drug-induced toxicity, providing a promising alternative to traditional intravesical chemotherapy(21).

Intravesical chemotherapy is a widely used treatment for BC, but its effectiveness is limited by off-target effects on normal urothelial cells and the permeability barrier. A recent study explores the use of BC cell-derived membrane nanovesicles as carriers for drugs, taking advantage of their ability to target similar tumors(4). The study found that by functionalizing a hendeca-arginine peptide that targets BC, the nanovesicles gained the ability to penetrate mucus, allowing them to overcome the permeation barrier(4). These nanovesicles, created using dual-targeting and mucus-penetrating techniques, were shown to be stable in urine, have a high ability to penetrate the glycosaminoglycan layer, precisely target BC, and be successfully internalized through caveolin-mediated endocytosis. *In vivo* results from the study demonstrated that using these nanovesicles for intravesical chemotherapy led to chemo-resection in murine orthotopic BC models. This innovative drug delivery system has the potential to improve the effectiveness and safety of intravesical chemotherapy for BC(4).

Novel Drug Delivery Systems

The use of intravesical therapy is often faced with challenges such as short retention periods and insufficient drug penetration into the bladder. As a result, patients may require frequent. high-dose treatments, which can lead to significant adverse effects and financial burdens. To address these issues, Ma et al. developed a chitosan (CS) carrier system named LRO-BCG/CS(22). This system combines oxaliplatin (OXA) prodrug liposomes (LRO) and Bacillus Calmette-Guerin (BCG) to

treat orthotopic BC. The study has revealed that CS prolonged the presence of LRO and BCG in the bladder beyond 24 hours and improved LRO's ability to penetrate the bladder(22). In an orthotopic bladder tumor model using MB49 cells, the study has demonstrated that LRO released OXA in response to tumor cell reductants, resulting in immunogenic cell death (ICD), while BCG further enhanced the systemic anti-tumor immune response(22). The results of the study demonstrated that LRO-BCG/CS exhibited superior anti-tumor activity and significantly prolonged the survival of tumor-bearing mice, even at relatively low doses of oxaliplatin and BCG. This combined chemoimmunotherapy approach has demonstrated minimal side effects, highlighting its potential as a promising and well-tolerated treatment strategy for individuals with BC(22).

Bladder cancer treatment often relies on surgery, but it's not always effective due to high recurrence rates and poor outcomes. To address this challenge, Liu et al.(23) developed an approach involving the use of a thermo-sensitive triblock polymer called poly(D, L-lactide)-poly(ethylene glycol)-poly(D, L-lactide) (PLEL) hydrogel drug delivery system. PLEL hydrogel loaded with gemcitabine (GEM) was injected into the body to specifically target tumor cells. PLEL hydrogel and cytosine-phosphate-guanine (CpG) were then injected under the skin in both groins to boost immune responses, along with GEM. The results of this study demonstrate that the PLEL hydrogel, which contains the drug, undergoes a phase transition from a sol to a gel state at normal body temperature, thus providing continuous and controlled drug release. The *in vivo* experiments of this study showed that this combined therapy effectively suppressed tumors and boosted the immune system. This method holds promise for bladder cancer treatment by offering longer-lasting drug delivery, improved effectiveness against cancer, enhanced immune response, and reduced side effects.

Another study explored implantable systems to tackle the challenges of drug retention in bladder cancer chemotherapy. A novel polysaccharide supramolecular injectable hydrogel, abbreviated as CCA hydrogels, was synthesized by blending cationic chitosan, anionic sulfobutyl ether β-cyclodextrin, and silver ions(24). The study reported that the hydrogel quickly regained its form upon injection and maintained suitable elasticity for biological applications. By directly encapsulating DOX, the gel achieved a high drug concentration, demonstrating the potential for localized drug delivery. *In vivo* experiments using bladder tumor models such as MB49-luc cells revealed that CCA-DOX gel had a pronounced inhibitory effect on tumor growth, surpassing the efficacy of free DOX. Thus, this self-healing injectable hydrogel presents a promising approach for bladder cancer treatment (24).

Jing et al.(25) have introduced a new intravesical instillation nanoparticle hydrogel hybrid system aimed at controlling the metabolic process of intracellular ammonia to fight BC. This system includes a biomimetic fusogenic liposomalized nanoporter (FLNP) with urea transporter-B (UT-B) and protonated chitosan oligosaccharide, which delivers urease and small interfering RNA (siRNA) to bladder tumors, targeting CPS1 specifically. Their research has shown that FLNP-mediated immobilization of UT-B significantly improves urea transport into tumor cells. Furthermore, the simultaneous administration of urease and siRNA targeting CPS1 (siCPS1) leads to a notable increase in ammonia accumulation within tumors, ultimately causing cell apoptosis. Importantly, using this hybrid approach has demonstrated exceptional anti-cancer effects in both orthotopic bladder tumor animal models and patient-derived xenograft models. Additionally, a

high-protein diet increases urea synthesis in the urine, enhancing ammonia accumulation within BC cells and improving the prevention of tumor growth. This study emphasizes the potential of manipulating urinary urea tumor-tropistic transport and its ammonia transformation as a practical approach to eliminate TP53-mutated bladder cancer.

The development of cancer treatment through stimulating the immune system faces challenges due to the limited effectiveness of anticancer vaccines. Recent progress in cancer immunotherapy suggests that biodegradable polymers such as chitosan could enhance the effectiveness of weak antigens, providing a potential solution(26). This study aims to assess the effectiveness of a polymeric gel matrix (TPG) consisting of poloxamer 407 and chitosan, along with MB49 cells, as an intravesical anticancer vaccination using a C57BL/6 mouse model of bladder urothelial carcinoma(26). This study examined the efficacy of vaccination by dividing participants into three groups: control, TPG, and TPG+MB49. The study revealed that animals immunized with the TPG+MB49 vaccine showed higher cumulative survival rates, significantly reduced bladder weight and size compared to the other groups, and more antitumor cytotoxicity in their splenocytes (**Figure 1**)(26). The findings of this study shows that incorporation of MB49 cells into a thermoreversible polymeric gel matrix that contains chitosan and using it as an intravesical vaccine can effectively trigger an immune response and slow the growth of bladder tumors in a C57BL/6 mouse model(26).

Precision Medicine Approaches

The global prevalence and recurrence rates of bladder cancer have spurred significant efforts to develop effective treatments. Intravesical chemotherapy following tumor resection faces challenges due to the rapid degradation and elimination of chemotherapy drugs. A recent study created externally thiolated hollow mesoporous silica nanoparticles (MSN-SH(E)) as a viable platform for improving intravesical therapy(27). The thiolated nanovector improved mucoadhesive properties on porcine bladders and increased permeability, as shown by the fragmented distribution of the tight junction protein claudin-4. Furthermore, the *in vitro* experiments conducted in this study demonstrated that MSN-SH(E) facilitated the transformation of M2 macrophages into M1-like cells, and that the mitomycin C (MMC)-loaded nanovector (MMC@MSN-SH(E)) exhibited superior anticancer effects compared to MMC alone. Immunohistochemical investigation of this study further emphasized the significance of MMC@MSN-SH(E) in enhancing anticancer activity(27). They reported that many benefits of MMC@MSN-SH(E), such as its ability to adhere to mucosa, improve permeation, modulate the immune system, and provide prolonged drug exposure, make it a promising candidate for intravesical therapy in NMIBC. The emergence of MMC@MSN-SH(E) introduces an innovative approach to tackle the requirement for improved effectiveness in the treatment of NMIBC, hence providing prospects for further advancements in this field(27). The ability of nano- and micropollen-type materials to mimic the adhesive properties of natural pollen, allowing them to adhere to cell or tissue surfaces(28), has led to their utilization in biomedical applications. Wang et al.(29) conducted a study in which they created hollow silica (HS) and hollow pollen silica (HPS) nanoparticles (NPs) and then modified the HPS NPs with CPBA to produce CHPS NPs, chosen for their strong affinity towards sialic acid residues overexpressed on bladder cancer (BC) cells (**Figure 2**). Pirarubicin (THP) was used as a model agent and loaded into the hollow interiors of CHPS, HPS, and HS, resulting in the formation of THP@CHPS, THP@HPS, and THP@HS NPs, respectively(29). The study revealed that both THP@CHPS and THP@HPS nanoparticles

displayed comparable tissue adhesion capabilities, surpassing THP@HS nanoparticles. Moreover, the authors found that THP@CHPS NPs exhibited the highest uptake by MB49 cells, leading to increased cell mortality. The study's in vivo results showed that intravesical administration of THP@CHPS NPs in BC mice resulted in their highest accumulation in the bladder, causing significant damage and reductions in bladder volume and weight(29). The authors noted that THP@CHPS NPs demonstrated excellent biocompatibility, inducing only minor alterations in blood parameters and negligible effects on body weight. These findings underscore the potential of THP@CHPS NPs for intravesical therapy in BC(29).

In a recent study, Qi et al.(30) examined the potential of cellular microvesicles (MVs) produced from bladder cancer cells as a therapeutic strategy for bladder cancer due to their adhesion abilities, homologous targeting, and parental cell-specific properties. The authors reported that pirarubicin (THP) encapsulated MVs (THP-MVs) demonstrated improved adhesion and targeting capabilities towards bladder cancer cells, thus enabling intravesical therapy administration(30). Results of the study showed that MB49 bladder cancer cells absorbed THP-MVs more efficiently than free THP, which led to a stronger induction of apoptosis. In vivo studies using a bladder cancer mouse model showed that THP-MVs accumulated significantly after being injected intravenously, leading to significant reductions in bladder weights and volumes, highlighting promising therapeutic potential(30). This study proposes THP-MVs as an innovative approach for formulating intravesical agents for the treatment of bladder cancer(30). Another study has proposed a novel approach to enhance intravesical chemotherapy by utilizing a drug-loaded TA@Fe thin film, formed through the self-assembly of tannic acid (TA) and ferric ions (Fe^{3+}), and was in situ fabricated on the bladder wall in vivo(31). The results of this investigation demonstrated that the TA@Fe film, adjustable in thickness, effectively prolonged the presence of anticancer drugs in the bladder, facilitating sustained release and significantly enhancing antitumor efficacy(31). The study also showed that the TA@Fe film is biocompatible, indicating no adverse effects on bladder function, and has strong antibacterial properties that could help prevent infections after surgery(31). Furthermore, the results demonstrated that the T_2 contrast effect of Fe^{3+} was employed to continuously monitor the TA@Fe film's breakdown and subsequent drug release processes through magnetic resonance imaging. This study underscores the importance of the TA@Fe-based drug delivery platform, which exhibits enhanced bladder retention, in the treatment of various bladder diseases(31).

A recent study has developed an intravesical drug delivery system aimed at improving the effectiveness of drugs in treating bladder cancer by directly targeting the bladder and bypassing systemic distribution limitations(32). The study focused on formulating an intravesical delivery system made of mucoadhesive in situ gel loaded with quercetin-solid lipid nanoparticles (SLNs), either uncoated or coated with chitosan. These SLNs, which are spherical and approximately 250 nm in size, demonstrated high entrapment efficiency (>97%) and sustained drug release for up to 142 hours(32). The cytotoxicity assessment of this study revealed dose-dependent toxicity, with an IC_{50} range of 1.6–8.9 µg/mL of quercetin. The authors reported that the developed gels exhibited appropriate gelation temperatures (around 25°C) and prolonged erosion durations (24–27 hours), with SLNs-loaded gels showing enhanced retention on bladder tissues compared to SLNs alone. Furthermore, they observed that the coated SLNs displayed improved tissue penetration, especially when dispersed in gel, reaching depths of up to 350 µm. Thus, coated SLNs present a promising approach for direct quercetin delivery to the bladder. The study also demonstrated sustained release

patterns and significant toxicity against T-24 human bladder cancer cells, along with effective penetration into the bladder wall, strong adhesion to the bladder mucosa, and the short-term safety of bladder tissue following artificial urine irrigation(32). This research explores the potential of utilizing SLNs-loaded in situ gel as a strategy for managing bladder cancer intravesically.

The utilization of intravenous Mycobacterium Bovis BCG therapy in NMIBC has shown efficacy in preventing cancer spread and slowing its progression. However, reports have indicated instances of treatment failure, recurrence, and adverse effects(33). To tackle these challenges, a study investigated the potential of synthetic selenium nanoparticles (SSeNPs) and biogenic selenium nanoparticles (BSeNPs) as supplements to enhance intravesical BCG therapy in mice with bladder tumors(33). The findings of the study revealed that both types of SeNPs reduced the activity of certain genes related to cell function, namely HMGB1 and iNOS, with no apparent distinction between them. Additionally, rats with bladder cancer treated with either SSeNPs or BSeNPs exhibited similar anti-tumor effects. The sequential intravesical administration of SSeNPs, BCG, BCG/SSeNPs, and BCG/BSeNPs triggered a robust immune response characterized by increased expression of IFN-γ, IL-12, and IL-6, coupled with decreased expression of IL-10 and TGF-β cytokines(33). Furthermore, they observed that treatment with these nanoparticles alongside BCG therapy led to increased activity of certain proteins associated with cell death, reduced activity of genes related to cell function, and decreased activity of a protein involved in suppressing the immune response, indicating improved treatment effectiveness. The findings of this study suggest that both synthetic and biogenic selenium nanoparticles hold promise as additional treatments to enhance the effectiveness of intravesical BCG therapy for BC(33).

Advancements in Noninvasive Diagnosis and Imaging

Bladder cancer diagnosis traditionally relies on macroscopic methods like white-light cystoscopy (WLC) and fluorescence blue-light cystoscopy (BLC), yet the demand persists for more accurate noninvasive optical imaging techniques(34). Although BLC can detect early-stage bladder cancer, including carcinoma in situ (CIS), it falls short in evaluating cancer infiltration due to visible light's limited permeability through bladder tissue(34). Additionally, WLC's limitations include its inability to classify cancer stages and detect early-stage cancers like CIS(35, 36). Addressing these challenges, recent research has delved into real-time bladder cancer imaging using a near-infrared fluorescence (NIRF) probe, ASP5354, in an MB49 mouse bladder cancer orthotopic model(34). The study revealed ASP5354's specific absorption by cancerous tissue following intravesical administration, with NIRF imaging distinctly delineating boundaries between cancerous and normal tissues(34). These innovative techniques, coupling ASP5354 with NIRF imaging devices, show potential for real-time and sensitive bladder cancer detection in clinical diagnosis and surgery, thereby offering promising avenues for advancing diagnostic accuracy and treatment outcomes(34). Another notable advancement in bladder cancer treatment is the development of an innovative adhesive multifunctional nano-transformer termed CTMF NPs by Liu et al.(37). This nano-transformer is designed specifically for targeted therapy in bladder cancer. Their work showcases a series of structural modifications facilitating tumor microenvironment (TME)-responsive zero-background 19F-magnetic resonance imaging (MRI) and ratiometric photoacoustic imaging-guided synergistic tumor therapy. Through a process of encapsulation and dynamic coordination, they synthesized CTMF NPs by incorporating copper sulfide nanoparticles (CuS NPs) and perfluoro-15-crown-5-ether (PFCE) into a dopamine-grafted polymer, enveloped with a metal-polyphenol shell(37). This design allows for activatable 19F-MRI and photoacoustic

imaging, thereby improving precision in tumor localization. Notably, the polyphenol component enhances cell adherence and, upon decomposition, generates ROS-induced reactive quinones that enhance the nanoprobes' adhesion to the bladder urothelium. Additionally, the depletion of glutathione (GSH) and ROS generated from the Fenton reaction heightens oxidative stress in cancer cells, leading to mitochondrial damage and dysfunction(37). The study further reveals that laser-induced conversion of encapsulated copper sulfides into copper ions triggers cuproptosis, disrupting the lipoylated tricarboxylic acid cycle and inducing significant immunogenic cell death, thus offering a synergistic therapeutic approach through multiple anticancer mechanisms within a single therapeutic agent (**Figure 3**). The observed cascade of morphological changes, including the collapse of metal-polyphenol shells and the decomposition of the nano-transformer, overcomes drug permeation barriers specific to bladder cancer. Overall, these findings underscore the promising potential of CTMF NPs with their multifaceted capabilities for the precise treatment of BC(37).

Concluding Remarks

Bladder cancer poses significant challenges for treatment and patient outcomes, especially in NMIBC where recurrence rates remain high despite standard treatments. To address these challenges, various novel approaches have been explored, including improving drug delivery systems, using immunotherapy, and developing targeted medicines. Promising results in preclinical studies have been seen with materials such as polyethylene glycol (PEG) and glutaraldehyde co-modified fluorinated chitosan (PGFCS), antibody-drug conjugates targeting HER2, and immunotherapeutic agents like protein aggregate magnesium-ammonium phospholinoleate-palmitoleate anhydride (P-MAPA). Advancements in drug delivery technologies, such as nanoparticles and hydrogels, offer opportunities for better drug retention and precise distribution to bladder tissues, potentially reducing off-target effects and improving treatment outcomes. Additionally, exploring imaging techniques like near-infrared fluorescence (NIRF) probes and multifunctional nano-transformers could provide new ways for accurate diagnosis and precise treatment delivery. These collaborative efforts represent a rapidly evolving area of research focused on transforming bladder cancer treatment and enhancing patient outcomes. The ongoing exploration and application of these innovative methods show significant potential for addressing the unmet clinical needs in bladder cancer treatment.

Despite significant progress in drug delivery methods and treatment modalities, bladder cancer therapy still faces challenges. The effectiveness of intravesical therapy is limited by issues such as poor drug penetration and off-target effects on healthy urothelial cells. Current intravesical therapy also struggles with obstacles like limited drug penetration through the bladder's protective barriers and inadequate retention times, requiring frequent and high-dose treatments. Additionally, the diversity of bladder tumors makes targeted therapy difficult, often resulting in treatment resistance and tumor recurrence. To overcome these challenges, advanced drug delivery systems need to be developed to penetrate bladder barriers, selectively target tumors, and enhance therapeutic efficacy while minimizing adverse effects. Furthermore, optimizing immunotherapeutic approaches to harness the immune system's potential in combating bladder cancer remains crucial. Innovative strategies such as nanotechnology-based drug delivery systems, immunomodulators, and combination therapies offer promising ways to address these challenges and improve outcomes in bladder cancer treatment. As research progresses, additional efforts are essential to translate

promising findings into clinically viable treatments that can substantially improve outcomes for patients with bladder cancer.

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