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REVIEW

Nanomaterial-Enhanced Immunotherapy: Advancing T-Cell-Based Treatments for Bladder Cancer

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Abstract: Bladder cancer (BC) is a prevalent urinary malignancy characterized by high recurrence rates and suboptimal long-term outcomes from traditional treatments such as surgery, chemotherapy, and radiotherapy. T-cell-based immunotherapy has emerged as a promising approach, harnessing T cells' capacity to target and destroy tumor cells, yet it faces challenges from the immunosuppressive tumor microenvironment (TME), immune evasion, and T-cell exhaustion. Nanomaterials offer innovative solutions by enabling targeted delivery of antigens, checkpoint inhibitors, and immunomodulators; remodeling the TME through metabolic interventions (eg, hypoxia alleviation and adenosine reduction); and enhancing T-cell infiltration and persistence with stimulus-responsive systems like pH-sensitive nanoparticles and biomimetic vesicles. This review systematically examines nanomaterial integration to amplify T-cell-mediated immunity in BC, covering T-cell origins, differentiation (eg, CD8+ cytotoxic and CD4+ helper subsets), roles in the TME, and exhaustion mechanisms driven by factors like PD-1 and TOX. We discuss key strategies including direct immune enhancement via immunogenic cell death induction, metabolic reprogramming to optimize T-cell function, and sustained activation for improved persistence. In conclusion, these nanomaterial-enhanced therapies address critical barriers, promoting precise and synergistic immune responses. Future prospects highlight AI-driven designs, personalized medicine, and clinical translation to tackle heterogeneity, biosafety, and resistance for durable BC remission.

Keywords: bladder cancer, T cell, immunotherapy, nanotechnology, nanomaterials, precision medicine

Introduction

BC is a common malignancy of the urinary system that primarily affects the epithelial lining of the bladder. The most prevalent subtype is bladder urothelial carcinoma (BUC), accounting for over 90% of all BC cases.[1,2] BC typically manifests as a mass within the bladder, disrupting its normal function. Due to the subtlety of early symptoms, it is often diagnosed at an advanced stage.[3] Non-muscle-invasive bladder cancer (NMIBC) exhibits a particularly high recurrence rate, with approximately 70–80% of patients experiencing tumor recurrence after initial treatment. In a subset of patients, NMIBC may progress over time, infiltrating the bladder muscle layer and developing into muscle-invasive bladder cancer (MIBC). MIBC is associated with increased invasiveness, a higher risk of metastasis, and significantly worsened prognosis.[4] If left untreated, MIBC can spread to distant organs such as lymph nodes, lungs, and bones.[5] Standard treatment options for BC include surgery, chemotherapy, and radiotherapy, all of which can exert adverse effects on patients. Although cystectomy can eradicate the tumor, it profoundly impacts the patient's quality of life due to the need

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for urinary diversion or neobladder reconstruction. Chemotherapy and radiotherapy are also associated with side effects such as immunosuppression, alopecia, and nausea, which further affect overall health and life quality.[6]

In recent years, immunotherapy has emerged as a promising strategy in cancer treatment due to its unique mechanism of action and reduced systemic toxicity. BC cells often exhibit robust immune evasion capabilities, making immunotherapy an attractive treatment modality. Bacillus Calmette-Guérin (BCG) immunotherapy remains the gold standard for high-risk NMIBC, especially in patients with high-grade or recurrent tumors. BCG induces a strong localized immune response and effectively prevents tumor recurrence and progression. Approximately 70% of high-risk NMIBC patients benefit from BCG therapy.[7,8] The advent of immune checkpoint inhibitors has further expanded the role of immunotherapy in BC. Clinical trials have demonstrated that PD-1/PD-L1 inhibitors significantly improve overall survival in patients with metastatic BC.[9] For example, pembrolizumab has been approved for patients ineligible for surgery or chemotherapy, particularly those with high PD-L1 expression.[10] In patients with advanced or metastatic disease, PD-1/PD-L1 blockade has been approved as a treatment option, especially when BCG therapy fails or resistance develops. From the perspective of T cells, BC—being a prevalent malignancy of the urinary system—exhibits a high degree of immune evasion, making T cell function and activation central to effective immunotherapeutic strategies. BC cells evade immune surveillance through multiple mechanisms, among which suppression of T cell activity is one of the most critical.[11] Tumor cells frequently suppress T cell function by expressing immune checkpoint molecules, such as PD-L1, thereby facilitating immune evasion. The central objective of immunotherapy is to activate or restore T cell function in order to potentiate the immune system's capacity to eliminate BC cells. Current immunotherapeutic modalities include immune checkpoint inhibitors, BCG immunotherapy, and chimeric antigen receptor T (CAR-T) cell therapy, among others. T cells play a central role in the antitumor immune response against BC, primarily through the recognition and elimination of tumor cells, regulation of immune signaling pathways, and maintenance of immunological memory. Immunotherapy enhances the antitumor activity of T cells by promoting their activation, alleviating immunosuppressive mechanisms, and modulating the tumor microenvironment. With continuous advancements in immunotherapeutic strategies, T cells are anticipated to play an increasingly critical role in the treatment of BC, particularly in patients with BCG-resistant, advanced-stage, or metastatic disease.

With the rapid development of nanotechnology, nanomaterials have demonstrated tremendous potential in the field of cancer therapy. Beyond serving as carriers for targeted drug delivery to improve intratumoral accumulation of therapeutic agents, nanomaterials can also synergize with immunotherapy to enhance antitumor immune responses. For instance, rationally designed nanocarriers can precisely deliver tumor antigens, immune adjuvants, or gene-editing tools, thereby promoting robust and sustained activation of T cells. Furthermore, nanomaterials have the capacity to reshape the tumor immune microenvironment, facilitating T cell infiltration and enhancing cytotoxic activity. In the context of BC, the integration of nanotechnology with T cell-based immunotherapy offers a promising strategy to overcome the limitations of conventional treatments. This combinatorial approach holds the potential to provide more efficient and safer therapeutic outcomes, paving the way for personalized and precision immunotherapy.[12] In this review, we focus on recent advances in the application of nanomaterials for enhancing T cell-mediated immunotherapy in BC. We further discuss current research gaps and future directions in this emerging field, with the aim of accelerating the development of effective T cell-based immunotherapeutic strategies against BC.

T Cells as the Biological Foundation of Immunotherapy in BC

Origin and Functional Differentiation of T Cells

T lymphocytes (T cells) are a vital subset of leukocytes within the human immune system, primarily responsible for recognizing and eliminating pathogens, tumor cells, and other abnormal cells. As integral components of the adaptive immune system, T cells play a pivotal role in mounting antigen-specific immune responses against infections and malignancies. T cells originate from hematopoietic stem cells in the bone marrow; however, their maturation and functional differentiation predominantly occur in the thymus. Upon completion of thymic development, mature T cells enter the peripheral circulation and migrate to various tissues, where they participate in immune surveillance and responses. Based on their functional roles and surface receptor expression, T cells can be classified into several subtypes. Among these, the two most common and well-characterized subsets are cytotoxic

T cells (CD8+ T cells) and helper T cells (CD4+ T cells).[13,14] Among T cell subsets, cytotoxic T lymphocytes (CD8+ T cells) are the primary effector cells responsible for antiviral and antitumor immunity. These cells eliminate infected or malignant cells by recognizing and attacking those that present specific antigens on their surface. Recognition is mediated through T cell receptors (TCRs), which bind to antigenic peptides displayed by infected or tumor cells, initiating a cytolytic immune response. Activated cytotoxic T cells release effector molecules such as perforin and granzymes, leading to apoptosis of the target cells.[15] TCRs recognize foreign antigens presented by major histocompatibility complex (MHC) molecules. Specifically, CD8+ T cells interact with antigens presented by MHC class I, while CD4+ T cells recognize antigens presented by MHC class II molecules.[16] Helper T cells (CD4+ T cells) play a crucial immunoregulatory role by assisting other immune cells. They promote B cell differentiation and antibody production, activate CD8+ T cells, and enhance macrophage-mediated phagocytosis. These functions are mediated via the secretion of cytokines such as interleukin-2 (IL-2) and interferon-gamma (IFN-γ). Based on their cytokine expression profiles, CD4+ T cells are further categorized into subsets including Th1, Th2, Th17, and regulatory T cells (Tregs).[17] Tregs are a specialized CD4+ T cell subset that function to suppress immune responses and prevent autoimmunity. They maintain immune tolerance by inhibiting overactive immune cells through the secretion of immunosuppressive cytokines such as transforming growth factor-beta (TGF-β) and interleukin-10 (IL-10)[18] (Figure 1).

T cells originate from the bone marrow, which serves as the primary site for hematopoiesis and the production of all immune cells, including stem cells and hematopoietic progenitors. Hematopoietic stem cells (HSCs) within the bone marrow give rise to various blood cell lineages, including red blood cells, platelets, and leukocytes such as T cells and B cells.[20] T cell precursor cells, referred to as thymic progenitor T cells or pro-T cells, originate in the bone marrow and, following partial differentiation, migrate via the bloodstream to the thymus, where they undergo further maturation and selection. Upon antigen recognition, the T cell receptor (TCR) engages with antigen–MHC complexes presented on the surface of antigen-presenting cells. This interaction triggers the phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) located within the CD3 complex, which is physically associated with the TCR. This phosphorylation event is mediated by Src family kinases, primarily Lck, and leads to the activation of several downstream signaling cascades, including the phosphoinositide 3-kinase (PI3K)/Akt, mitogen-activated protein kinase (MAPK), and nuclear factor-kappa B (NF-κB) pathways. These signaling networks coordinate the transcriptional and functional reprogramming of T cells, ultimately resulting in their proliferation, differentiation, and cytotoxic activity against target cells.[21,22] In the thymus, T cells undergo a process known as TCR rearrangement, during which the genes encoding the T cell receptor are somatically recombined to generate a diverse repertoire of antigen specificities. As a result of this process, each mature T cell expresses a unique TCR, enabling it to recognize a specific antigen. The TCR thus serves as the physiological antigen recognition structure common to all T cells. In contrast, chimeric antigen receptor (CAR) T cells are genetically engineered T cells that express synthetic transmembrane receptors. A CAR typically consists of an extracellular antigen-binding domain derived from a monoclonal antibody (usually in the form of a single-chain variable fragment, scFv), a hinge region, and one or more intracellular signaling domains derived from native T cell receptor components. Similar to monoclonal antibody-based therapies, CAR-expressing T cells exhibit high antigen specificity. However, unlike natural TCRs—which require peptide antigen presentation by MHC molecules—CARs directly bind to tumor-associated antigens (TAAs) on the cell surface independently of MHC presentation. This MHC-unrestricted recognition enables CAR-T cells to target antigens that may evade traditional TCR-mediated detection, thus providing a complementary and potent approach to conventional T cell-based immunotherapy.[23,24] Through this mechanism, CAR-T cells bypass the restriction imposed by MHC molecules, enabling them to recognize antigens that conventional TCRs are unable to detect. The single-chain variable fragment (scFv) region of the CAR mediates direct recognition and binding to tumor-associated antigens expressed on the surface of cancer cells, regardless of whether these antigens are presented by MHC molecules. This MHC-independent recognition endows CAR-T cells with the capacity to target tumor-specific or tumor-associated markers that may otherwise evade immune surveillance. Upon antigen engagement, the intracellular signaling domain—typically composed of the CD3ζ chain—triggers downstream activation cascades. These include the MAPK, PI3K/Akt, and NF-κB pathways, which collectively enhance T cell proliferation, cytokine production, and cytotoxic activity.[25] Through CAR-mediated recognition, T cells can rapidly identify and eliminate tumor cells. In addition to direct cytotoxicity, CAR-T cells also secrete pro-inflammatory cytokines, such as interferon-gamma (IFN-γ), which contribute to remodeling the TME and amplifying local immune responses, thereby potentiating antitumor immunity.

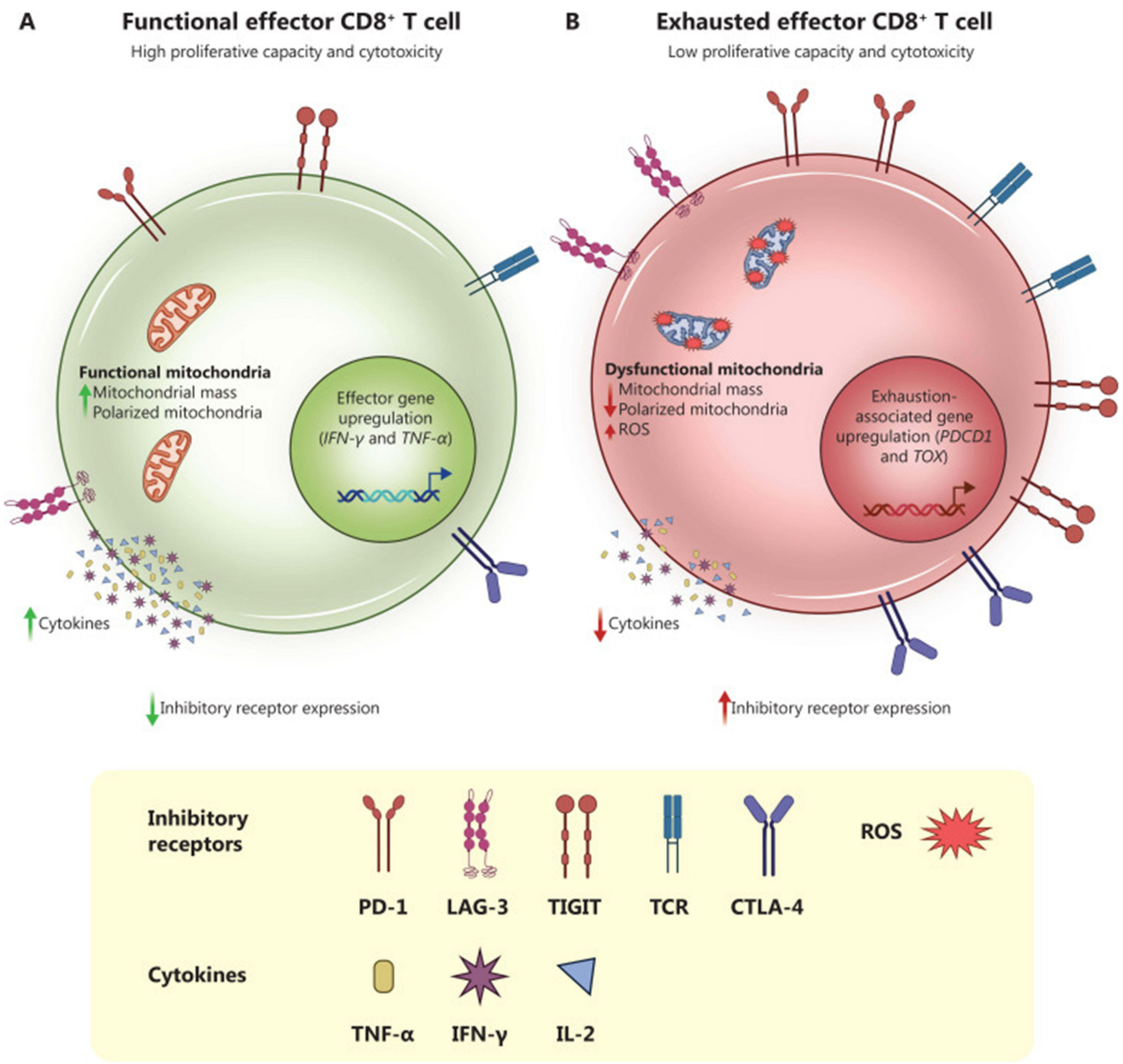

Figure 1 Phenotypic Comparison Between Functional and Exhausted Effector CD8+ T Cells. (**A**) Functional effector CD8+ T cells exhibit high proliferative capacity and cytotoxicity. They maintain functional mitochondria with increased mitochondrial mass and proper polarization, leading to upregulation of effector genes (eg, IFN-γ and TNF-α) and robust cytokine production. Expression of inhibitory receptors is low. (**B**) Exhausted effector CD8+ T cells display impaired proliferation and cytotoxicity. They have dysfunctional mitochondria with depolarization and increased ROS (reactive oxygen species) accumulation. These cells upregulate exhaustion-associated genes such as PDCD1 and TOX, produce fewer cytokines, and express high levels of inhibitory receptors (PD-1, LAG-3, TIGIT, CTLA-4). Adapted from Zhong, Tao et al. The mechanisms and clinical significance of $CD8^+$ T cell exhaustion in anti-tumor immunity. Cancer biology and medicine vol. 22,5 (2025), under the Creative Commons Attribution-NonCommercial 4.0 International License.[19]

Mechanisms of T Cells and Immunotherapy in BC

CD8+ T

CD8+ T cells are key effector cells in antitumor immune responses and are referred to as cytotoxic T lymphocytes (CTLs) due to their ability to directly kill tumor cells. As part of the αβ T cell lineage, they express T cell receptors (TCRs) that recognize tumor-associated antigens presented by MHC class I molecules on the surface of tumor cells, mediating antigen-specific cytotoxicity.[26] Studies have shown that the spatial distribution of CD8+ T cells in the TME is closely associated with clinical outcomes in BC. High intratumoral CD8+ T cell density correlates with prolonged overall survival, whereas high CD8+ T cell density in the tumor stroma is often associated with poorly differentiated tumors and

worse prognosis.[27] In BC, CD8+ T cells can differentiate into various functional subsets, including effector CD8+ T cells and exhausted CD8+ T cells (Table 1).

Effector CD8+ T

Effector CD8+ T cells are the principal cytotoxic effectors in adaptive immunity. Upon antigen recognition and activation, naive CD8+ T cells differentiate into this subset, which is characterized by potent cytolytic activity and robust secretion of inflammatory cytokines. Structurally, effector CD8+ T cells express both TCRs and the CD8 co-receptor. The TCR binds to antigen–MHC I complexes, while CD8 facilitates this interaction and recruits Lck kinase to initiate intracellular signaling. Functionally, they highly express cytotoxic mediators such as perforin, granzyme B, IFN-γ, and TNF-α, enabling them to directly kill target cells.[26] Phenotypically, these cells are typically CD44+, CD69+, CD25+, and $CCR7^{-}$, indicating their activation and migration from lymphoid organs. Effector CD8+ T cells kill tumor cells by releasing perforin, which forms pores in the target cell membrane, allowing granzyme B to enter and activate caspase-dependent apoptosis. Simultaneously, secretion of IFN-γ and TNF-α enhances antigen presentation, inhibits tumor angiogenesis, and strengthens local immune responses.[28] This mechanism enables effector CD8+ T cells not only to directly kill tumor cells but also to serve as immunological coordinators, amplifying the antitumor immune response. In BC, the presence and activation status of effector CD8+ T cells are critical determinants of effective tumor clearance. Studies have demonstrated that a higher intratumoral density of granzyme B+ and IFN-γ+ CD8+ T cells correlates with improved patient outcomes, indicating that these cells have entered an active effector state with cytotoxic capability. However, T cell infiltration alone is insufficient; what matters is whether these T cells express perforin and granzyme B and are functionally active. Several studies have revealed that the bladder TME often suppresses perforin expression in CD8+ T cells via the TGF-β2 and ICAM-1 signaling pathways, leading to a state of phenotypic activation but functional silencing, rendering the T cells ineffective. For instance, immunosuppressive mediators such as TGF-β and indoleamine 2,3-dioxygenase (IDO) have been shown to markedly reduce perforin expression and impair cytotoxic activity.[29] Moreover, chronic antigen exposure and inhibitory signaling—notably through the PD-L1 /PD-1 axis—can drive effector CD8+ T cells into functional exhaustion, transitioning them into a non-cytolytic state. Modern immunotherapies, particularly immune checkpoint inhibitors (ICIs), are designed to restore the function of effector CD8+ T cells by relieving such suppressive signals. As such, both the presence and functional activation of effector CD8+ T cells have become key predictive markers of response to immunotherapy.

While earlier studies did not yet distinguish functional effector CD8+ T cells from other subsets, they laid the foundation by establishing a relationship between CD8+ T cell infiltration and tumor prognosis. Ferris et al[30] identified immunogenic peptides derived from p53 (8–11 amino acids in length) and tested their binding affinity and stability with common HLA class I molecules (eg, HLA-A2, A24, B44, B51). CD8+ T cells from 16 BC patients were stimulated with these peptides, and IFN-γ secretion was measured using ELISPOT assays. Their findings indicated that tumor-infiltrating CD8+ T cells could recognize and respond to p53-derived tumor-associated antigens, demonstrating antigen-specific reactivity and establishing a basis for subsequent functional analyses. Later research shifted focus toward the cytotoxic function of these cells. Tajima et al[31] reported that upregulation of granzyme B and IFN-γ was a defining feature of functional effector CD8+ T cells. In murine models, initially non-cytotoxic Tc17 cells (IL-17+CD8+ T cells) could be reprogrammed by IL-12 to acquire an effector phenotype characterized by expression of IFN-γ and granzyme B (Tc17/ IFN-γ), gaining cytotoxic potential comparable to conventional Tc1 cells. This study highlighted that the expression of cytotoxic molecules, rather than the cell's original phenotype, is the true determinant of effector function. This functional reprogramming was confirmed to be IL-12–induced and associated with the upregulation of granzyme B and perforin, rather than being a superficial phenotypic change. Nonetheless, subsequent findings revealed that even CD8+ T cells with appropriate phenotypic markers often lose cytotoxic function due to complex immunosuppressive signals within the TME. Subsequent research efforts have increasingly focused on elucidating how tumors selectively silence cytotoxic T cells that are otherwise equipped with effector potential. In the context of BC, immunological investigations have begun to uncover that tumor cells exploit immunosuppressive signaling pathways—most notably those involving transforming growth factor-beta (TGF-β) and intercellular adhesion molecule-1 (ICAM-1)—to suppress the expression of perforin and granzyme B in CD8+ T cells, thereby establishing a mechanism of immune evasion. Critically, studies conducted during this period not only confirmed the existence of such effector function suppression, but also

Table 1 The Role of Different T Cell Subsets in the Immunity of BC and Its Immunotherapeutic Mechanism

T Cell Type	Mechanism/Function	Role in BC	Immunotherapy Mechanism	Ref.
CD8+ T cells (Cytotoxic T cells)	Recognize tumor antigens presented by MHC I via TCR, release perforin and granzyme B to directly kill tumor cells; secrete IFN-γ and TNF-α to enhance the local immune environment.	High-density infiltration in tumor tissue is associated with better prognosis, but often falls into an exhausted state (PD-1+TOX+), leading to functional limitations; effector subtypes determine immune clearance efficiency.	Use immune checkpoint inhibitors (eg, PD-1/PD-L1 antibodies) to relieve inhibition and restore killing function; CAR-T therapy bypasses MHC restrictions to directly target tumor surface antigens.	[2,3]
Effector CD8+ T cells	Differentiate from naive T cells upon activation, highly express Perforin, Granzyme B, and IFN-γ; phenotype CD44+CD69+CCR7^{-}, with strong killing and inflammatory cytokine secretion capabilities.	Directly kill tumor cells in BC and amplify immune responses; high density correlates with good prognosis, but susceptible to TME suppression (eg, TGF-β reduces Perforin expression), leading to functional silencing.	ICIs (eg, PD-1 blockade) restore effector function; nanoparticle vaccines (eg, PLGA loaded with antigens) enhance activation and proliferation, synergizing with BCG therapy to improve efficacy.	[1,4,5]
Exhausted T cells (Mainly CD8+ subtype)	Progressive functional exhaustion under chronic antigen stimulation, high expression of PD-1, TIM-3, TOX; reduced killing factors (eg, Granzyme B), metabolic disorders (eg, mitochondrial damage).	Widely present in BC TME, leading to immunotherapy resistance; progenitor exhausted type (TCF1+) can respond to ICIs for recovery, while terminal type indicates poor prognosis.	Dual checkpoint blockade (eg, PD-1+TIGIT) reactivates exhausted T cells; nanoparticle carriers deliver metabolic regulators (eg, rapamycin) to optimize mitochondrial function and reverse exhaustion.	[6,7]
CD4+ Th1 cells	Secrete IFN-γ and IL-2 to activate CD8+ T cells and macrophages; induced by IL-12 differentiation, regulated by T-bet transcription factor.	Promote antitumor cell-mediated immunity; Th1 dominance is associated with BCG treatment response and better prognosis, while deficiency leads to treatment failure.	BCG immunotherapy relies on Th1 responses to induce local IFN-γ expression; combined with ICIs to enhance Th1 bias and improve overall immune response rates.	[8]
CD4+ Th2 cells	Secrete IL-4, IL-10, and IL-13 to promote humoral immunity and immune suppression; regulated by GATA3 transcription factor.	Th2 shift leads to immune escape and tumor progression; high Th2-score may indicate immune plasticity, associated with BCG response (dual role).	Use nanoparticle carriers to regulate Th1/Th2 balance, inhibit Th2 to enhance cytotoxic immunity; ICIs can reverse Th2-dominated suppressive microenvironment.	[9]
CD4+ Treg cells (Regulatory T cells)	Regulated by Foxp3, secrete TGF-β and IL-10 to suppress effector T cells; maintain immune homeostasis but promote tumor escape.	Enrichment in tumors is associated with poor prognosis; secrete inhibitory factors to build an "immune cold" microenvironment, inducing T cell exhaustion.	Target Tregs (eg, CCR8 antibodies or nanoparticle depletion strategies) combined with ICIs to reduce inhibition and restore T cell activity; predict immunotherapy response.	[10]
γδ T cells (Non-classical T cells)	Recognize phosphorylated antigens independent of MHC; rapidly respond to mucosal threats, secrete IFN-γ and granzyme.	Reside in bladder mucosa, providing early immune surveillance; low abundance but can mediate rapid tumor killing, enhancing BCG efficacy.	Oncolytic viruses or nanoparticle vaccines activate γδ T cells, synergizing with CD8+ T cells to enhance antitumor responses; potential CAR-γδ T therapy targeting tumor markers.	[8,9]

demonstrated that this dysfunction is potentially reversible. These findings point to actionable molecular targets and underscore the therapeutic potential of interventions aimed at reinvigorating T cell cytotoxicity within the TME. Hartana et al[29] described an immune evasion mechanism in BC mediated by ICAM-1 and TGF-β2, which suppresses perforin expression in CD8+ T cells. In their study, CD8+ T cells were isolated from the peripheral blood, sentinel lymph nodes, and tumor tissues of patients with urothelial bladder cancer (UBC). Notably, perforin expression was significantly reduced in CD8+ T cells derived from the sentinel lymph nodes. Mass spectrometry analysis revealed that UBC cells secrete ICAM-1 and TGF-β2. CD8+ T cells were treated with tumor cell-conditioned medium or ICAM-1/TGF-β2, and it was confirmed that these treatments suppressed perforin expression. Under Tc1-promoting conditions (such as with IL-12), perforin expression could be partially restored. The tumor induces CD8+ T cell exhaustion and functional inactivation through ICAM-1 and TGF-β2 signaling, which represents a mechanism of immune escape, suggesting that dual targeting of these two molecules may improve the efficacy of immunotherapy. This key study found that ICAM-1 and TGF-β2 secreted by BC cells can suppress perforin expression in CD8+ T cells within tumor-draining sentinel lymph nodes (SNs), while granzyme B levels remain unchanged. Most of these perforin-deficient CD8+ T cells exhibited an exhausted effector memory phenotype (TEM/PD-1+/GATA3+), indicating that the tumor had induced a population of "functionally hollow" T cells. This reveals an important immune escape pathway: even when T cells appear phenotypically activated, they have in fact lost their cytotoxic function.

T Cell Exhaustion

Under conditions of chronic antigen stimulation, such as persistent viral infections or tumors, CD8+ T cells remain continuously activated but eventually enter a functionally impaired state known as T cell exhaustion.[32] This phenomenon was first described in chronic viral infections and has since been recognized as a major mechanism underlying the failure of antitumor immunity in solid tumors, including BC. Exhausted CD8+ T cells arise under prolonged antigen exposure in the tumor or chronic infection environment. Although they are not completely inactive, their cytotoxic function is significantly diminished. Specifically, they exhibit reduced expression of key cytolytic molecules such as granzyme B, perforin, IFN-γ, and TNF-α, leading to impaired antitumor activity. Additionally, they show weakened proliferative capacity, limiting their clonal expansion. These cells are also characterized by persistent high expression of multiple co-inhibitory receptors, including PD-1, TIM-3, LAG-3, CTLA-4, and TIGIT, which restrain their activation. At the transcriptional level, they undergo reprogramming, with upregulation of transcription factors such as TOX, NFAT, and Eomes, and downregulation of T-bet. Moreover, exhausted T cells display metabolic dysregulation, including mitochondrial dysfunction and reduced glucose metabolism, further contributing to their functional impairment.[33] Together, these alterations constitute the molecular basis of CD8+ T cell exhaustion. T cell exhaustion is not a binary state but a progressive continuum, generally categorized into progenitor exhausted and terminally exhausted stages. Early exhausted cells are characterized by a PD-1+TCF1+SLAMF6+TOX^{-} phenotype, retain self-renewal capacity, and are responsive to PD-1 blockade therapy, making them the primary targets of immune checkpoint inhibitors (ICIs). In contrast, terminally exhausted cells exhibit a PD-1+TOX+$TCF1^{-}$TIM-3+ profile, are functionally impaired, possess weak cytotoxicity, and respond poorly to ICIs. In BC, sustained tumor antigen exposure and an immunosuppressive microenvironment (eg, TGF-β, PD-L1) promote the progression of CD8+ T cells toward exhaustion. Studies have shown a high abundance of PD-1+TOX+CD8+ T cells within bladder tumors. Although these cells can infiltrate tumors, their expression of multiple co-inhibitory receptors—such as PD-1 and TIGIT—severely limits their effector function.[34] Further single-cell transcriptomic analyses have revealed that exhausted CD8+ T cells in tumors commonly express high levels of TOX, LAG-3, and CTLA-4, which are closely associated with immunotherapy resistance and poor prognosis.[35] Nevertheless, these cells are still considered potentially reactivatable therapeutic targets. For example, Han et al[34] discovered that PD-1+TOX+CD8+ T cells harbor tumor antigen–specific TCR clones, and following combined PD-1 and TIGIT blockade, these cells could re-express effector cytokines such as IFN-γ and TNF-α, suggesting that a subset belongs to the progenitor exhausted population and may respond to checkpoint-based immunotherapy.

CD4+ T

CD4+ T cells are key regulators within the adaptive immune system, orchestrating and guiding immune responses through diverse mechanisms. They play central roles in antiviral and antitumor immunity, maintenance of immune homeostasis, and tissue repair. These cells become activated upon recognizing peptide antigens presented by major histocompatibility complex class II (MHC II) molecules on antigen-presenting cells (APCs) such as dendritic cells, in the presence of costimulatory signals. Following activation, CD4+ T cells differentiate into distinct functional subsets depending on the local cytokine milieu, including Th1, Th2, and regulatory T cells (Tregs).[36] In terms of cellular immunity, Th1 cells promote antitumor and antiviral defense primarily by secreting interferon-gamma (IFN-γ) and interleukin-2 (IL-2), which activate macrophages and CD8+ cytotoxic T lymphocytes, enhancing the clearance of intracellular pathogens. In contrast, Th2 cells are involved in humoral immunity by producing IL-4, IL-5, and IL-13, which facilitate B cell activation, class-switch recombination, and responses against extracellular parasites.[36] BC tissues are often enriched with regulatory T cells (Tregs), a subset of CD4+ T cells characterized by FOXP3 expression. Tregs exert immunosuppressive functions by producing cytokines such as IL-10 and TGF-β, which inhibit the activity of effector T cells and dendritic cells, thereby dampening antitumor immune responses. High levels of Treg infiltration are generally associated with poor prognosis, suggesting that tumors may exploit Tregs to sustain an immunosuppressive microenvironment conducive to tumor progression. In addition, BC cells can directly impair CD4+ T cell function to promote immune evasion. Studies have shown that BC cell lines such as T24 can significantly suppress T cell activity and even induce apoptosis in vitro, indicating that tumor cells themselves possess the capacity to actively inhibit immune cell function.[37]

Th1

The differentiation of Th1 cells is regulated by specific cytokine signals. Naïve CD4+ T cells, upon encountering antigens presented by APCs such as dendritic cells and being stimulated by IL-12 and IFN-γ, initiate Th1 lineage commitment through activation of the key transcription factor T-bet.[38] This process is also regulated by the STAT4 signaling pathway. In the context of tumor immune surveillance, Th1 cells are considered a central force in initiating and maintaining antitumor immune responses. Enhanced Th1 responses have been associated with improved treatment efficacy and prognosis in cancer patients. Conversely, a shift in the Th1/Th2 balance toward Th2 dominance often correlates with immune suppression, tumor immune escape, and disease progression. In BC, the correlation between Th1 immune activity and therapeutic response has also been demonstrated.[39] For instance, the efficacy of BCG immunotherapy depends on a strong Th1 response that induces local expression of IFN-γ and IL-12, which in turn activates tumor-associated immune responses. The absence of an effective Th1 response is often associated with BCG treatment failure or higher recurrence risk.[40]

Research on Th1 cells in BC was initially driven by efforts to understand the mechanism of BCG immunotherapy. Since the clinical application of BCG for NMIBC in the 1980s, it has become evident that its efficacy is closely related to host immune system activation.[41] In the early 2000s, studies showed that BCG treatment induces a Th1-type immune response, characterized by upregulation of inflammatory cytokines such as IFN-γ and IL-2, establishing the central role of Th1 cells in antitumor immunity.[42] Subsequent work by Luo et al[42] confirmed that BCG can significantly elevate Th1-type cytokines, including IFN-γ and TNF-α, which are critical for promoting local immune activation within the TME. These findings laid the foundation for the positive correlation between Th1 responses and BCG efficacy. Attention then shifted to the regulatory role of Th1/Th2 balance in BC immunity. For example, Satyam et al[43] observed a Th2-skewed immune profile in BC patients, marked by elevated Th2 cytokines (eg, IL-4, IL-10) and reduced Th1 cytokines (eg, IFN-γ), a shift associated with immune evasion and therapeutic resistance. Concurrently, researchers began exploring ways to enhance Th1 responses via recombinant genetic engineering. Luo and colleagues[44] developed recombinant BCG strains that secrete Th1-promoting cytokines, which led to enhanced antitumor immunity and improved therapeutic outcomes. With the clinical introduction of immune checkpoint inhibitors (ICIs) such as PD-1/PD-L1 antibodies, interest has grown in Th1 cells as predictive biomarkers of immunotherapy response. In 2014, Ingels et al[45] reported that bladder tumors with high levels of Th1-associated markers (eg, IFN-γ expression, CD8+ T cell infiltration) were associated with better prognosis and stronger therapeutic responses. Subsequent studies linked the Th1 gene expression signature within tumors to both treatment

efficacy and overall survival. Several research groups have analyzed BC samples from the TCGA database and found that patients with upregulated expression of Th1-related genes, particularly IFNG, tend to exhibit higher immune activity, better response rates to immunotherapy, and improved overall survival.[46] This finding has promoted interest in developing "Th1 gene signatures" as potential tools for personalized treatment stratification in BC. In recent studies, Th1-based vaccines have emerged as promising strategies to actively induce antitumor immune responses in BC therapy. For example, Dang et al.[47] A multi-epitope Th1-selective vaccine platform was developed specifically targeting TAAs that are highly expressed in BC patients, including CDC20, TOPO2A, PBK, and MELK. The researchers screened TCGA BC datasets to identify a panel of TAAs that are highly expressed in tumors, strongly immunogenic, and minimally expressed in normal tissues. They then applied MHC class I and class II binding prediction tools to precisely select Th1-biased epitope peptides capable of inducing both CD8+ cytotoxic T cell and CD4+ helper T cell responses. Their findings suggested that such tumor epitope–based Th1-inducing vaccines can effectively activate antigen-specific immune responses, bypass tumor immune escape mechanisms, and hold potential as an alternative to BCG therapy or as a component of combination immunotherapy strategies for BC.

Th2

T helper type 2 (Th2) cells, a major subset of CD4+ T cells, play a key role in regulating humoral immunity, promoting B cell differentiation, antibody production, and immune tolerance. Th2 cells differentiate under the influence of interleukin-4 (IL-4) and characteristically secrete cytokines such as IL-4, IL-5, IL-6, IL-10, and IL-13.[48,49] Under physiological conditions, Th2 cells are involved in anti-parasitic responses, allergic reactions, and tissue repair. However, in the TME, overactivation of Th2 cells may contribute to the formation of an immunosuppressive niche by suppressing Th1-type responses and promoting the recruitment of tumor-associated macrophages (TAMs) and regulatory T cells (Tregs), thereby facilitating immune evasion. In BC, increased expression of Th2-associated cytokines, especially IL-4 and IL-10, has been observed. This Th2-skewed immune imbalance is strongly associated with tumor progression, immune escape, and failure of BCG immunotherapy.[50] Furthermore, some studies suggest that a tumor-driven Th2-dominant environment can suppress effective T cell responses and limit the efficacy of immune checkpoint inhibitors (ICIs).[51] In recent years, restoring Th1/Th2 balance and reversing Th2 polarization has emerged as a therapeutic strategy in cancer immunotherapy. Inhibiting Th2 cytokines or enhancing Th1 responses may improve clinical responses to immunotherapy in BC patients.

Early studies revealed a Th2-biased immune phenotype in the BC immune microenvironment. This skewing not only contributes to immune escape but may also impact immunotherapeutic efficacy. For instance, Abhigyan et al[43] elevated expression of Th2 cytokines such as IL-4 and IL-10, along with a marked decrease in Th1 cytokines (eg, IFN-γ and IL-2), has been observed in BC patients. This immunological skewing is thought to contribute to tumor immune evasion, as Th2 cytokines often suppress Th1 responses, thereby weakening cell-mediated antitumor immunity. Researchers have found that such an imbalance may foster immune tolerance within the TME, suppressing effective antitumor immune responses. With the advent of BC immunotherapies such as BCG treatment, increasing attention has been paid to the role of the Th2 response in modulating therapeutic efficacy. BCG acts primarily by promoting a Th1-dominant immune profile to exert antitumor effects. However, in some patients, an enhanced Th2 response may counteract BCG's efficacy. Pichler et al[52] observed that BCG responders exhibited a higher GATA-3/T-bet ratio, suggesting that interactions between Th2 cells (marked by GATA-3) and Th1 cells (marked by T-bet) within the TME may enhance overall immune activation and improve clinical outcomes. Therefore, Th2 cells may exert dual roles in immunotherapy response: both supporting Th1 responses and modulating the immune milieu to enhance efficacy. Immune evasion in BC is not solely due to suppressed Th1 activity. Th2 cells may directly regulate immune escape via the secretion of immunosuppressive cytokines. It has been shown that Th2-polarized immune responses are frequently hyperactivated in bladder tumors and closely linked to mechanisms of tumor immune escape. For instance, Th2 cytokines like IL-4 and IL-10 inhibit effective Th1 responses, thereby attenuating antitumor immunity and promoting tumor progression. Activation of eosinophils, a hallmark of Th2 responses, may further support immune escape by secreting cytokines and participating in immunomodulation. In 2023, Villoldo et al[53] introduced the concept of a "Th2-score", a quantitative metric based on the expression ratio of GATA3 to T-bet, to classify the immune status of BC patients. Given that GATA3 is a canonical

Th2 transcription factor and T-bet a key Th1 lineage marker, this ratio reflects the Th2/Th1 immune inclination. Analyzing samples from NMIBC patients undergoing BCG therapy, they found that patients with a high Th2-score had greater response rates to BCG and longer recurrence-free survival, suggesting that a Th2-dominant state may not simply indicate immune suppression, but rather a plastic and responsive immune system, offering a new prognostic biomarker for therapeutic efficacy. Meanwhile, the application of single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics has significantly advanced the in-depth identification of intratumoral Th2 cells. Traditional immunohistochemistry can only measure bulk expression levels, whereas these emerging technologies allow researchers to precisely localize T cells within tumor tissues, as well as to determine their quantity and functional status. Specifically, studies utilizing spatial transcriptomic platforms have observed that Th2 cells may cooperate with regulatory T cells (Tregs) and M2-type tumor-associated macrophages (TAMs) to form a coordinated suppressive network, collectively shaping an immunosuppressive microenvironmentTME.[54–57] This "spatial clustering effect" suggests that Th2 cells are not merely bystanders but rather critical nodes within the immune evasion axis. Through the IL-4/IL-13 signaling axis, Th2 cells can induce macrophage polarization, promote Treg stabilization, and suppress CD8+ T cell activity. In addition, some studies have identified a population of Th2-like CD4+ T cells within tumor tissues that exhibit a functionally exhausted phenotype, characterized by high expression of inhibitory receptors such as PD-1 and CD200. This indicates that prolonged antigen stimulation may drive these Th2 cells into a loss-of-function state.[58] This finding links Th2 cells to resistance against immune checkpoint blockade, highlighting the possibility that successful combination immunotherapy may require simultaneously targeting Th2-mediated immune escape mechanisms to restore global T cell functionality. In summary, the development of Th2 scoring systems and advances in spatial transcriptomics mark a shift from merely qualitative descriptions of Th2 cells to a new era of quantitative, spatially resolved, and functionally defined immunological profiling. These innovations lay a robust theoretical and technical foundation for future personalized immunotherapeutic strategies.

Regulatory T Cells

Regulatory T cells (Tregs) are a critical immunosuppressive subset responsible for maintaining immune homeostasis, preventing excessive immune activation, and limiting autoimmune responses. Most Tregs belong to the CD4+CD25+Foxp3+ lineage, with the transcription factor Foxp3 (forkhead box P3) serving as the master regulator of Treg development and function.[59] Tregs can be categorized into two groups based on their origin: naturally occurring thymic Tregs (nTregs) and peripherally induced Tregs (iTregs). The former develop tolerance to self-antigens within the thymus, while the latter arise in peripheral tissues under the influence of TGF-β and IL-2, particularly in inflammatory or tumor environments. In BC, elevated proportions of Tregs have been observed in both the peripheral blood and tumor tissues of patients, especially those with high-grade or MIBC. Tregs exert immunosuppressive effects through multiple mechanisms: high CD25 expression allows them to consume IL-2 competitively, limiting effector T cell expansion; CTLA-4 on Tregs binds to CD80/CD86 on APCs, inhibiting costimulatory signaling; and they can induce indoleamine 2,3-dioxygenase (IDO) expression in dendritic cells, further suppressing immune activity via metabolic reprogramming.[60,61] Together, these mechanisms establish a prototypical "immune-suppressive niche" within the bladder TME. Although Tregs are predominantly viewed as promoters of tumor immune evasion, their role is not exclusively detrimental. Some studies have shown that Tregs may limit tumor invasiveness by suppressing matrix metalloproteinase-2 (MMP2) expression, thereby exerting protective, anti-invasive effects under specific contexts.[62] Recent studies have begun to explore the potential of regulatory T cells (Tregs) as predictive biomarkers for immunotherapy responsiveness. By establishing an immune scoring model based on the infiltration levels of Tregs and natural killer (NK) cells, researchers have demonstrated its effectiveness in predicting the sensitivity of BC patients to immune checkpoint inhibitors (ICIs), thereby offering promise for personalized treatment strategies.[63] In BC, Tregs contribute to tumor progression and therapeutic resistance through multiple immunosuppressive mechanisms. However, their functions appear to exhibit a degree of context dependency, varying with the TME. As such, Treg-targeted immunotherapeutic approaches—including combinatorial checkpoint blockade, chemokine axis inhibition, and selective depletion techniques—are emerging as important avenues to enhance treatment response rates in BC.

The immunological importance of regulatory T cells (Tregs) in tumors was initially recognized through the discovery of CD4+Foxp3+ cells exerting immunosuppressive functions across multiple cancer types. In a foundational study in 2007, Brandau et al[64] first reported a marked accumulation of Tregs in both tumor tissues and peripheral blood of BC patients. These Tregs expressed Foxp3, and their presence was associated with high levels of IL-10 and TGF-β, suggesting that Tregs play a central role in both local and systemic immunosuppression, contributing to the formation of a so-called "immune-cold" TME in BC. Subsequent research shifted from simply quantifying Tregs to dissecting their suppressive mechanisms and regulatory pathways. It became widely accepted that Tregs not only increase in number but also reshape the TME via multiple immunosuppressive mechanisms. Early studies demonstrated that BC-associated Tregs suppress the cytotoxic activity of CD8+ T cells and NK cells by secreting IL-10 and TGF-β. Additionally, they inhibit antigen presentation by dendritic cells through CTLA-4–mediated competition for CD80/CD86, collectively forming the core of the tumor immunosuppressive network.[65] Meanwhile, researchers have begun to recognize that the function of Tregs is not entirely detrimental. Winerdal[66] reported in a 2015 study that FOXP3+ cells in BC tissues exhibit considerable heterogeneity. Notably, a subset of these cells appeared to suppress tumor invasiveness by downregulating pro-invasive factors such as MMP2, suggesting that Tregs may exert a "restrictive regulatory" role in specific TME. This bidirectional modulatory capacity has led to a paradigm shift in the understanding of Tregs, transforming them from being regarded solely as "immunosuppressive agents" to "complex regulators." With the widespread application of immune checkpoint inhibitors (ICIs) and BCG immunotherapy in BC, Tregs have increasingly been identified as a critical mechanism contributing to immunotherapy resistance. While BCG can robustly activate T cell-mediated immunity, it also induces the expansion of PD-L1+Foxp3+ Treg populations, potentially undermining therapeutic efficacy. Fenner et al[67] demonstrated that suppressive PD-L1-expressing Treg subsets were significantly elevated in tumor tissues following BCG treatment, indicating that these cells may counteract BCG-induced antitumor responses. The authors proposed that combining BCG with immune checkpoint blockade could enhance therapeutic outcomes. Simultaneously, Treg trafficking mechanisms have emerged as a focus of research. Maeda et al[68] found in a canine BC model that Tregs are highly dependent on the CCR4 chemotactic axis. Treatment with a CCR4-blocking antibody, such as mogamulizumab, significantly depleted intratumoral Tregs and prolonged the survival of the animals. This study proposed a Treg migration blockade strategy, providing a basis for clinical translational application. With the advancement of technologies such as single-cell sequencing and spatial transcriptomics, researchers are now able to precisely localize Treg cells within the tissue microenvironment and identify their subpopulation states. For example, studies have demonstrated the presence of functionally heterogeneous Treg subsets in BC tissues, including populations expressing PD-1+, ICOS+, and LAG-3+, some of which exhibit metabolically active or exhausted phenotypes. Yang et al[63] developed an immune risk prediction model by integrating the infiltration scores of Tregs and NK cells, which can effectively predict the responsiveness of BC patients to immunotherapy and provide a foundation for individualized treatment stratification.

T Cells in the Tumor Microenvironment of BC

The tumor microenvironment (TME) plays a pivotal role in tumor initiation, progression, metastasis, and invasion. T cells not only act as direct effectors of anti-tumor immunity, but also serve as key indicators of therapeutic efficacy and prognosis due to their abundance, spatial distribution, and functional state within the TME (Figure 2). Investigating the interplay between T cells and the BC microenvironment has thus become a crucial avenue for developing targeted therapies. TheTME of BC comprises tumor cells, cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), vascular and lymphatic endothelial cells, as well as a variety of immune cell subsets. These components interact through chemokines, metabolic intermediates, and extracellular matrix (ECM) components to determine whether T cells can effectively infiltrate the tumor and maintain their effector functions. Recent studies have highlighted that immunosuppressive cytokines (such as TGF-β), metabolic competition (eg, lactic acid accumulation), and inhibitory surface molecules (eg, PD-L1) collectively impair the function of CD8+ T cells and T helper 1 (Th1) responses, thereby facilitating immune evasion by the tumor.[69,70] Within this context, T cells play multifaceted and dual roles in the BC microenvironment—not only as central mediators of anti-tumor immunity but also as key contributors to immune escape and therapeutic failure. Emerging evidence suggests that in many bladder tumors, T cells fail to effectively eliminate malignant cells, and may even exacerbate local inflammation or

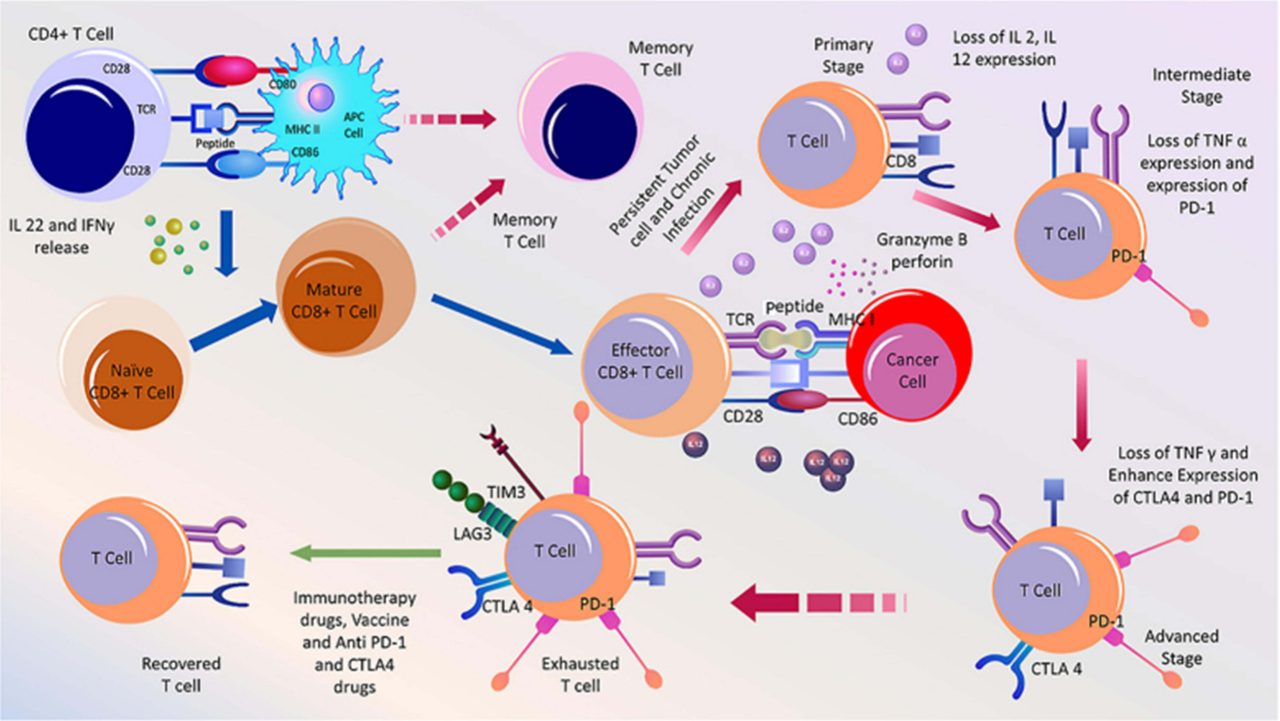

Figure 2 T cell subsets in the BC tumor microenvironment. Naïve CD8+ T cells are activated by antigen-presenting cells (APCs) through MHC–TCR and CD28–CD80/86 interactions, with cytokine support from CD4+ T cells. Activated effector CD8+ T cells release granzyme B and perforin to kill cancer cells. During chronic tumor antigen exposure, CD8+ T cells progressively lose effector functions and express inhibitory receptors (PD-1, CTLA-4, TIM3, LAG3), leading to T cell exhaustion. Immunotherapeutic agents such as anti–PD-1 and anti–CTLA-4 antibodies can restore T cell function and promote tumor clearance. Adapted from Ahmed, Hossain et al. Role of T cells in cancer immunotherapy: Opportunities and challenges. Cancer pathogenesis and therapy vol. 1,2 116–126. 20.[72]

autoimmune-like tissue injury. The functional activity of T cells within the bladder is subject to modulation by local mucosal immune factors, commensal microbiota, and tumor-derived signals. In in vitro studies, BC cell lines such as EJ and T24 have been shown to directly suppress T cell activity and induce apoptosis.[71] This indicates that BC cells possess intrinsic immunosuppressive properties, enabling them to evade recognition and destruction by the immune system.

In addition, Lv et al[73] utilized human BC specimens to demonstrate that downregulation of SIRT4 enhances glycolytic activity and promotes an immunosuppressive phenotype in tumor cells, leading to a significant reduction in CD8+ T cell cytotoxicity. Another study involving deep sequencing of T cell receptors (TCRs) from 119 patients with MIBC revealed that low TCR diversity and a low frequency of circulating T cells were closely associated with poor overall survival.[74] Furthermore, TCR sequencing of tumor-infiltrating T cells in BC tissues showed that a high clonal expansion of TCRs correlated with favorable responses to immunotherapy. These findings suggest that in BC, T cell-mediated immunity is not solely dependent on cell quantity, but is also critically influenced by antigen specificity and clonal expansion dynamics.[75,76] To date, single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics have demonstrated that T cells within the bladder TME exhibit high heterogeneity among BC patients (Table 1). Adoptive T-cell therapy, particularly tumor-infiltrating lymphocyte (TIL) therapy, has been extensively studied in the context of BC. This therapy involves extracting T-cells from the patient's tumor, expanding them ex vivo, and reintroducing them back into the patient. When combined with interleukin-2 (IL-2), a cytokine that promotes T-cell proliferation, TIL therapy has shown promise in improving anti-tumor responses and prolonging survival in patients with advanced BC. Although clinical studies involving TIL and IL-2 combination therapies for metastatic BC are being pursued, similar approaches using TIL therapy have been tested in other cancers, and promising results are still emerging.[77,78] Immune checkpoint inhibitors, such as PD-1/PD-L1 inhibitors like atezolizumab, nivolumab, and pembrolizumab, have also become critical components of T-cell-based treatments for BC. These inhibitors work by blocking the immune checkpoints that suppress T-cell activation, thereby enabling T-cells to better recognize and attack BC cells. Clinical trials have demonstrated that these drugs significantly improve progression-free survival and overall response

rates, especially in patients with locally advanced or metastatic BC. Notable trials include the IMvigor210 study, which found that atezolizumab provided durable responses in patients with advanced BC who were unresponsive to standard therapies.[79] A variety of combination strategies are currently being tested in clinical trials. One promising direction is combining T-cell therapies, such as CAR-T cell therapy, with immune checkpoint inhibitors. Early-phase studies have shown that this combination may help overcome the tumor's resistance mechanisms and improve the efficacy of T-cell therapies.[80] Additionally, combination treatments with chemotherapy or anti-angiogenic therapies are being explored to enhance T-cell infiltration into the tumor and to counteract tumor-induced immune suppression.[81] These trials collectively highlight the growing potential of T-cell-based immunotherapies for BC. While more research is needed to confirm long-term efficacy, these therapies offer new hope for more targeted and effective treatment options, particularly for patients with advanced or metastatic BC.

T cell exhaustion (TEX) within the TME of BC has become a focal point of current research, offering new insights for clinical immunotherapy strategies. For instance, Xue et al[82] employed single-cell RNA sequencing (scRNA-seq) and bulk transcriptomic analysis to investigate TEX-associated immunosuppressive signatures in the BC TME. In their study, a 28-gene TEX signature score was constructed to stratify patients into TEX^high and TEX^low subgroups. It was found that patients in the TEX^high group had worse prognosis and exhibited reduced responsiveness to immune checkpoint inhibitors (ICIs). This study provided a meaningful link between molecular profiling and clinical decision-making, suggesting potential applications in personalized immunotherapy. Moreover, Liu et al[83] used single-cell transcriptomic mapping to reveal that cancer-associated fibroblast (CAF)-derived CXCL12/CXCL14 interact with CXCR4 on exhausted T cells, forming a CAF–CXCL axis that spatially sequesters exhausted T cells within the TME, restricting their access to tumor target cells. These findings underscore the dual role of T cells in BC—as both guardians of immune surveillance and facilitators of immune evasion when functionally impaired. Although T cells have the capacity to recognize and eliminate tumor cells, the cancer microenvironment may induce exhaustion, suppress immune function, and remodel cellular context, allowing tumors to escape immune control. Therefore, restoring T cell quantity, diversity, and functionality remains a key therapeutic objective in enhancing immunotherapy efficacy for BC (Table 2).

Nanomaterials Directly Enhance T Cell–Mediated Immunotherapy in BC

Immunotherapy for BC, particularly T cell–based strategies, has made remarkable progress in recent years. However, multiple challenges remain that hinder its overall efficacy and clinical applicability.[84–86] Against this backdrop, the

Table 2 High Heterogeneity of T Cells in BC Tumor Microenvironment of BC

T Cell Subtype	Function/Feature	Relatively Enriched Sites	Clinical Association	Ref
CD8+GZMK+ pre-effect /memory sample	Both killing and storage potential	MIBC > NMIBC > Normal	Potential increases with rising grading, prompt early reply	[14]
CD8+Tex (PD-1+TOX+)	Functional failure	Hypoxic and high-lactic acid zone	TEX^high = poor prognosis + ICI low sensitivity	[12]
Resident memory (TRM, CD103+CD69+)	Residence mucosa, rapid immune surveillance	Epithelial-basal layer	The most high recurrence rate is low	[15]
Treg, FOXP3+	Inhibit effector T cells	Caf-rich area	IFI27 mediates tumor-Treg interaction, with high IFI27 expression → elevated Treg → ICI resistance	[16]
Helper T cells (Th1/Th2/Th17)	Cytokine network	Dispersed distribution	Th1↑ → Good prognosis, Th2/Th17↑ → inflammatory tumor-promoting	[17,18]
γδ T & MAIT	Non-classical antigen recognition	Mucosa-microbiota associated zone	γδ T cells can mediate rapid tumor killing, but their quantity is low	[20]

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integration of nanomaterials into immunotherapeutic regimens has emerged as a novel approach to overcoming these immunological barriers.[87,88] Nanomaterials offer a versatile platform for precise delivery of immunomodulators, tumor antigens, or siRNA, and have been shown to promote immunogenic cell death (ICD) within the TME. Additionally, they can help alleviate metabolic stress, remodel the physical barriers of the tumor stroma, and enhance T cell infiltration and cytotoxic function[12,89] (Figure 3). In the following sections, we highlight the recent advances and underlying mechanisms by which nanomaterials are being applied to potentiate T cell–mediated immunotherapy in BC, with a particular focus on distinct T cell subsets.

CD8+ T

In recent years, CD8+ T cells have emerged as central effectors of antitumor immunity, particularly in the context of cancer immunotherapy. However, within the immunosuppressive TME of BC, CD8+ T cells often exhibit functional exhaustion or suppression, leading to diminished cytotoxic activity. To overcome these limitations, nanomaterial-based strategies have been explored to reactivate and sustain CD8+ T cell functions. Due to their high surface-area-to-volume ratio, programmability, and excellent biocompatibility, nanomaterials can serve as precision delivery systems for tumor antigens, adjuvants, immune checkpoint inhibitors, and regulatory agents. These platforms enhance cross-presentation by dendritic cells (DCs), improve T cell priming, and promote robust antigen-specific CD8+ T cell activation. For example, nanovaccines co-delivering TLR7/8 agonists and peptide antigens have demonstrated superior immunostimulatory capacity compared to conventional adjuvants, significantly increasing the population of antigen-specific CD8+ T cells.[90] Moreover, smart nanocarriers have been employed to transport small-molecule drugs, checkpoint inhibitors, or redox modulators directly into tumor sites, facilitating local immune activation, CD8+ T cell infiltration, and sustained cytotoxic

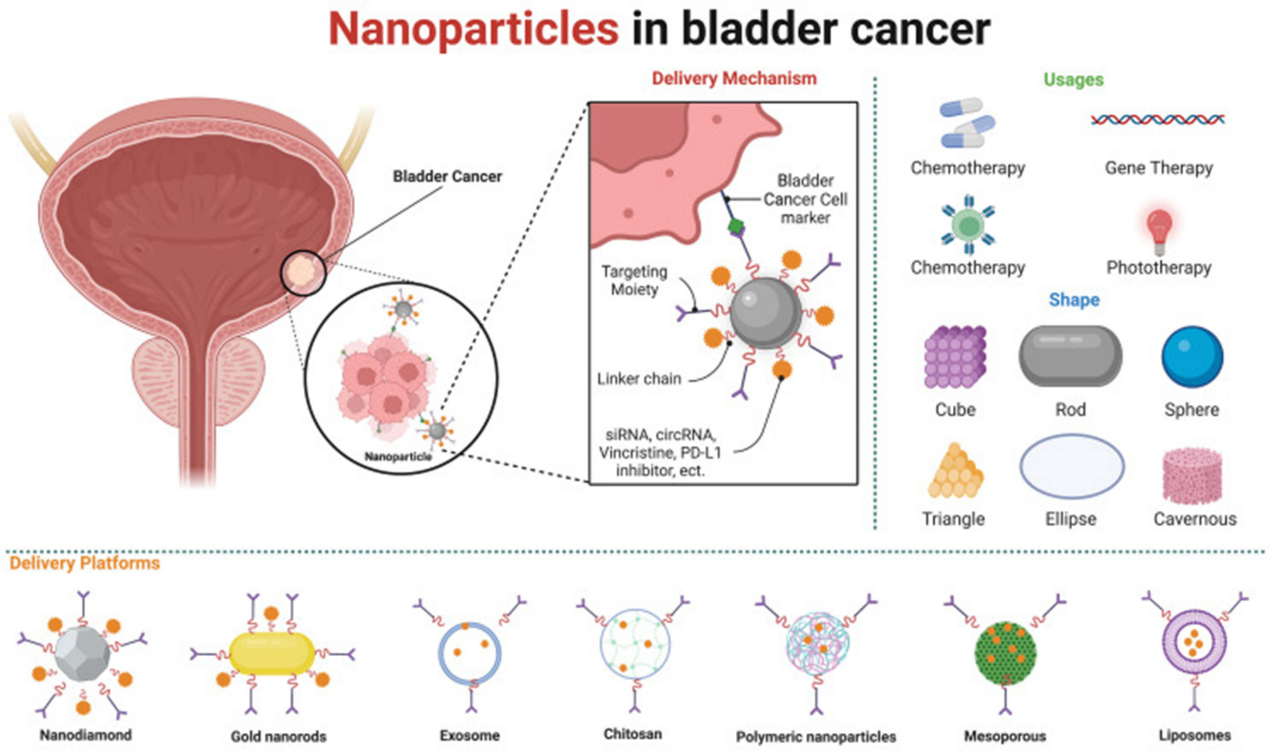

Figure 3 Application of Nanomaterials in BC Therapy. Nanoparticles have emerged as a promising strategy for targeted BC treatment. By incorporating surface ligands that recognize specific biomarkers on BC cells, these nanocarriers can precisely deliver therapeutic agents—such as small interfering RNA (siRNA), circular RNA (circRNA), chemotherapeutic drugs (eg, vincristine), and PD-L1 inhibitors—into tumor cells via linker-mediated conjugation. The morphology of nanoparticles significantly influences their biodistribution and cellular uptake efficiency. A variety of nanodelivery platforms have been explored, including nanodiamonds, gold nanorods, exosomes, chitosan, polymeric nanoparticles, porous materials, and liposomes. Each of these systems offers distinct advantages in enhancing drug stability, targeting specificity, and bioavailability, thereby contributing to improved therapeutic efficacy in BC treatment. Adapted from Zhao, Xinming et al. A Novel Approach for BC Treatment: Nanoparticles as a Drug Delivery System. International journal of nanomedicine, Copyright © 2025 by the authors.[86]

responses.[91] Some multifunctional platforms integrate photothermal therapy (PTT), chemotherapy, and immune stimulation. These systems induce immunogenic cell death (ICD) and release tumor-associated antigens, further boosting CD8+ T cell priming and memory formation, resulting in durable antitumor immunity.[92]

In the BC context, conventional therapies such as BCG or PD-1/PD-L1 blockade may partially activate T cell responses, yet often fall short due to T cell exhaustion or suboptimal antigen delivery. Nanomaterial-enabled immunomodulation has thus emerged as a promising strategy to overcome these limitations. Early designs focused on PLGA-based nanovaccines, co-loading tumor antigens with TLR agonists for efficient delivery to lymphoid tissues. Kim et al[90] developed a PLGA nanoparticle containing a TLR7/8 agonist and tumor peptide, which significantly increased CD44 +CD8+ T cells in murine BC and outperformed BCG in durability of immune response. With the continuous advancement of nanoparticle delivery strategies, researchers have developed multifunctional biomimetic nanomaterials that enable synergistic targeting of therapeutic agents and antibodies. For instance, Zhou et al[93] engineered exosome-like nanovesicles cloaked with macrophage membranes, co-loaded with PD-L1 monoclonal antibodies and CD73 inhibitors. These vesicles mimic innate immune cells' tumor-infiltrating behavior, facilitating deep penetration across the tumor barrier and significantly enhancing CD8+ T cell recruitment and cytotoxic activation within the TME, ultimately suppressing tumor progression. Recent breakthroughs have also incorporated nanomotor propulsion systems and tissue-penetrating mechanisms to further improve T cell access. Choi et al[94] designed a urease-driven nanomotor, whose surface was functionalized with a STING pathway agonist. This self-propelling system enabled non-catheter-based mucosal delivery, penetrating the bladder epithelium autonomously and delivering immune-stimulatory signals directly to the tumor site, thereby robustly enhancing CD8+ T cell activation and accumulation—an approach significantly superior to conventional instillation methods (Figure 4). Moreover, researchers have explored the integration of magneto-responsive nanomaterials with localized hyperthermia, inducing immunogenic tumor cell death (ICD), releasing tumor-associated antigens, and promoting the activation of local dendritic cells (DCs). This cascade enhances subsequent CD8+ T cell proliferation and memory formation, presenting a unified "therapy-plus-vaccine" immunoengineering strategy.[92] Collectively, these studies underscore the evolution of the "nanomaterials + CD8+ T cell modulation" paradigm in BC immunotherapy—from simple adjuvant delivery to targeted, penetrating, and multi-modal smart systems. This transition marks a pivotal shift in nanomedicine's role, from an auxiliary component to a central element in immune-centric therapeutic designs, unlocking new avenues for precision and efficacy in BC treatment.

CD4+ T

Th1

Recent studies have increasingly explored how nanomaterials can potentiate Th1-mediated immune responses to enhance the efficacy of BC immunotherapy, particularly in the context of BCG-based immunotherapy and nanovaccine strategies. One of the key mechanisms involves the use of nanomaterials as adjuvant-antigen delivery systems, which activate dendritic cells (DCs) and promote the secretion of Th1-polarizing cytokines such as IL-12 and IFN-γ, thereby directing naive T cell differentiation toward a Th1 phenotype. For example, MgAl-layered double hydroxide (LDH) nanoparticles conjugated with CpG oligodeoxynucleotides significantly increased the IgG2a/IgG1 ratio in murine models, indicating a shift from Th2 to Th1 immunity. This strategy not only enhanced antigen-specific humoral responses but also improved antitumor efficacy in melanoma models through reinforced Th1 polarization, a mechanism with potential translatability to BC therapy.[95] In the field of BC, Nano-BCG represents one of the most promising advancements to date. Conventional Bacillus Calmette–Guérin (BCG) therapy exerts its anti-tumor effect primarily through the induction of a Th1-type immune response. However, the application of nanodelivery systems significantly enhances the local stability and cellular uptake of BCG, thereby boosting the expression of Th1-associated cytokines such as interferon-gamma (IFN-γ) and improving the overall efficacy of immunotherapy.[12] In addition to delivering conventional agents, certain functional nanomaterials themselves exhibit intrinsic immunomodulatory properties. For instance, Gd@C82(OH)22, a fullerene-based nanoparticle, can promote Th1 immune polarization and induce the release of cytokines such as tumor necrosis factor-alpha (TNF-α) and IFN-γ. This enhanced Th1 bias may help activate cytotoxic CD8+ T cells and macrophages, leading to more effective clearance of BC cells.[96] Recent studies have begun to explore how to reconstruct a Th1-dominant TME through genetic engineering, gut microbiota modulation, or localized immune delivery systems. For

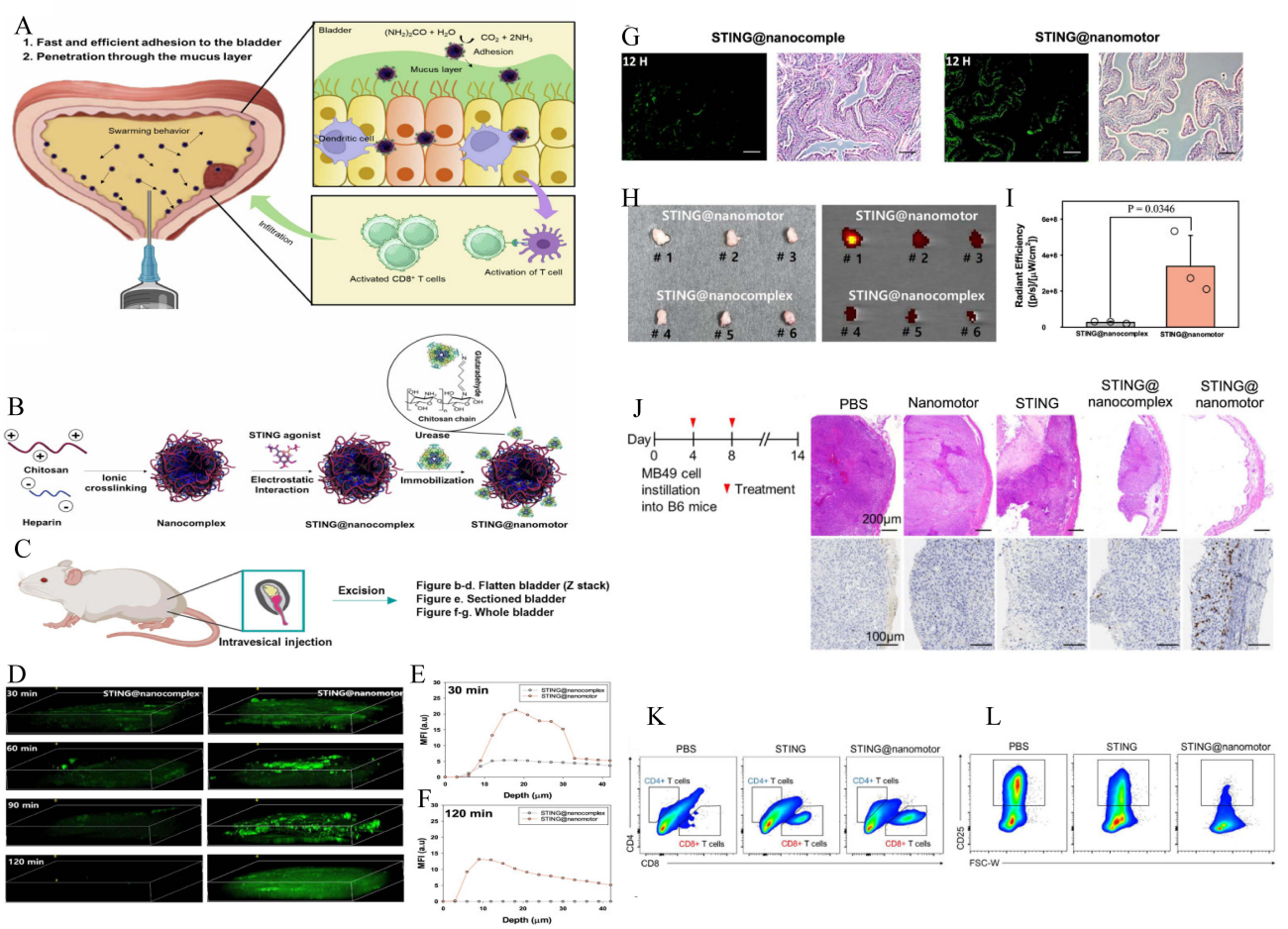

Figure 4 Intravesical Delivery and Immune Activation by STING@Nanomotors. (**A** and **B**) Schematic illustration of urease-powered nanomotor. (**C–I**) In vivo penetration and retention of STING@nanomotor after intravesical instillation. (**C**) Illustration of three administration methods for assessing nanomotor penetration and retention post-intravesical instillation. (**D**) 3D fluorescence imaging and corresponding mean fluorescence intensity (MFI) at (**E**) 30 min and (**F**) 120 min show enhanced bladder accumulation of STING@nanomotors compared to STING@nanocomplex. (**G**) Bladder sections at 12 h post-instillation reveal deeper tissue penetration by STING@nanomotors (scale bar = 100 μm). (**H**) Ex vivo bladder images and IVIS imaging, and (**I**) quantification of total radiant efficiency confirm superior retention. Data represent mean ± S.D. (n = 3); statistical significance determined by two-sided *t*-test. (**J**): STING@nanomotor to inhibit BC growth by inducing antitumor immunity. (**K** and **L**): Immune response and pro-tumor response of STING@nanomotor. The representative flow cytometry plots of (**K**) $CD4^+$ and $CD8^+$ T cells, and (**L**) regulatory T cells. Adapted from Choi, Hyunsik et al. Urease-powered nanomotor containing STING agonist for BC immunotherapy. Nature communications vol. 15,1 9934. 15 Nov, under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License. Copyright © 2024 by the authors.[94]

example, some research teams are using targeted nanoparticles to deliver IL-12 or IFN-γ in order to enhance local Th1 responses; others are attempting to systemically improve anti-tumor Th1 immunity by regulating the gut microbiota (such as increasing Th1-inducing bacteria). Recent studies have begun to explore how to reconstruct a Th1-dominant tTME through genetic engineering, gut microbiota modulation, or localized immune delivery systems. Some teams are using targeted nanoparticles to deliver IL-12 or IFN-γ to enhance local Th1 responses; others are attempting to systemically boost anti-tumor Th1 immunity by modulating the gut microbiota (such as enhancing Th1-inducing bacteria). Although current studies on Th1-oriented nanomaterials in BC are mostly confined to animal models, accumulating evidence supports their potential in immune polarization and tumor suppression. Future investigations may focus on integrating Th1-associated transcription factors (eg, T-bet) or chemokines (eg, CXCL10) into nano-platforms to enable more precise activation of Th1 immunity and improved therapeutic outcomes in BC.

Th2

Although direct studies specifically targeting "nanomaterial-mediated regulation of Th2 immunity in BC immunotherapy" are currently lacking, existing research offers relevant clues and mechanistic insights suggesting that nanomaterials may influence Th2-type responses and thereby affect therapeutic efficacy. On one hand, nanomaterials have been

extensively utilized to improve the delivery efficiency, antigen presentation, and local immune activation of BCG immunotherapy. In particular, while enhancing Th1 responses, they may concurrently suppress Th2 polarization, thereby disrupting the immunosuppressive TME. For example, Zeng et al[12] reviewed that Nano-BCG, via stabilized delivery, can promote dendritic cell maturation and cross-presentation of antigens, inducing stronger IL-12 and IFN-γ expression, which in turn may suppress Th2-biased responses. On the other hand, certain intelligent nano-platforms (such as metallic nanoparticles and polymer-based nanocarriers) have been developed to construct "nanovaccines" or to deliver Toll-like receptor (TLR) agonists and immunomodulatory cytokines. Although most of these studies focus on Th1 activation or CD8+ T cell stimulation, they may also indirectly modulate the function or frequency of Th2 cells by remodeling the local immune microenvironment. For instance, Tian et al reported that some nano-systems can induce tumor necrosis and release pro-inflammatory factors, potentially transforming "cold tumors" into "hot tumors"—a process that might involve Th1/Th2 immune reprogramming.[97] Taken together, although current research on direct targeting or modulation of Th2 cells by nanomaterials in BC remains in the exploratory stage, emerging mechanisms suggest that nanotechnology could alleviate Th2-dominant immunosuppression by promoting Th1-skewed polarization, thus improving immunotherapeutic outcomes. Future studies are warranted to identify specific Th2-related targets and regulatory strategies for nanoplatform design.

Regulatory T Cells (Treg)

Nanomaterials have shown great promise in remodeling the TME in BC immunotherapy, with regulatory T cells (Tregs) emerging as a critical target. Tregs facilitate immune evasion in BC and are often associated with therapeutic resistance. Therefore, nanomaterial-mediated targeting of Tregs to attenuate their immunosuppressive activity has become an emerging strategy. A cutting-edge study introduced a "Reconstructed Synthetic Nanopathogen" (RSnP) system, which combines an inactivated Mycobacterium cell wall skeleton with a TLR7/8 agonist to elicit potent anti-tumor immune responses without pathogen-associated toxicity. This system significantly enhances the activation of CD8+ T cells, natural killer (NK) cells, and Th17 cells while reducing the proportion of immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs) and M2-type macrophages. However, the robust immune activation also leads to a compensatory expansion of CCR8+FoxP3+ Tregs within the TME. Co-administration of anti-CCR8 monoclonal antibodies can effectively deplete tumor-specific Tregs, further improving therapeutic efficacy and reducing the risk of autoimmune side effects.[98] Moreover, Zeng et al highlighted that the Nano-BCG platform enhances T cell activation, improves antigen presentation, and boosts local immune responses, potentially reprogramming immune lineages within the TME and indirectly suppressing Treg expansion to enhance treatment response.[12] In terms of delivery strategies, intelligent nanocarriers have been engineered to deliver TLR agonists, antibodies, or siRNAs that target Treg differentiation or recruitment. For instance, Li et al developed multifunctional PICO nanoparticles (PICO NPs) that co-deliver a TLR7 agonist and OKT3 antibody. This platform not only inhibits Treg function but also boosts the activity of APCs, leading to robust antigen-specific immune responses.[99] In summary, nanomaterials have become a pivotal strategy in modulating Treg cell function and abundance, beyond merely improving drug delivery. Treg-targeted nanoplatforms hold potential to overcome resistance barriers in current immunotherapy and offer more precise and effective clinical interventions.

Nanomaterial-Mediated T Cell Delivery and Activation

In recent years, the development of nanomaterials has introduced new strategies for BC immunotherapy. Through rational design, nanocarriers can efficiently deliver drugs or biological agents to tumors and immune organs, enhancing anti-tumor immune responses while minimizing systemic side effects.[100,101] Although numerous preclinical studies have demonstrated the promise of nanomaterials in enhancing T cell–mediated immunotherapy for BC, the translation into clinical trials remains limited and primarily focused on drug delivery or tumor ablation, rather than immune modulation. For instance, a Phase I trial investigated nab-paclitaxel, a nanoparticle-bound formulation of paclitaxel, for non-muscle-invasive BC. This approach improved local drug retention and reduced systemic toxicity.[102] Another example is VAX014, a genetically engineered oncolytic bacterial nanoparticle designed for integrin-targeted delivery. It has completed early-phase trials showing preliminary safety and potential to stimulate anti-tumor immune responses.[103]

Recent studies also highlight developments in Nano-BCG immunotherapy, where nanotechnology enhances the delivery and immune activation of Bacillus Calmette-Guérin. These platforms aim to improve treatment durability while reducing toxicity.[12] Despite these advances, most clinical applications are still in early stages and have not yet addressed the more complex goals of T cell activation, metabolic reprogramming, or precise immunosuppression reversal that are being demonstrated in preclinical nanomedicine platforms.[91,104] Various types of nanomaterials—with distinct structures and functionalities—have been developed to improve the therapeutic efficacy of BC immunotherapy. These materials can be broadly categorized into organic nanomaterials, inorganic nanomaterials, biomimetic membrane-coated systems, and stimuli-responsive nanocarriers. In the following section, we provide an in-depth review of the design principles, targeting performance, and research progress of nanomaterials for enhancing T cell–mediated immunotherapy against BC.

Organic Nanomaterials

Organic nanomaterials are typically composed of biocompatible constituents such as polymers, lipids, or proteins—examples include poly(lactic-co-glycolic acid) (PLGA) nanoparticles, chitosan nanoparticles, liposomes, and dendrimers. These carriers are commonly prepared using methods like self-assembly or emulsion–solvent evaporation and can be surface-modified to introduce functional moieties.[105] Their advantages include excellent biocompatibility, biodegradability, high drug loading capacity, and chemical modifiability, making them suitable for co-delivery of multiple drugs or nucleic acid molecules.[106–108] For example, PLGA-based nanoparticles have been used to encapsulate gene therapeutics or hydrophobic drugs, enabling controlled release.[109] The typical size range of organic nanoparticles (tens to hundreds of nanometers) allows for passive accumulation in tumor tissues via the enhanced permeability and retention (EPR) effect.[110] For example, we have observed that such nanomaterials are commonly employed to deliver tumor neoantigens or adjuvants in a targeted manner to dendritic cells (DCs) and lymph nodes, thereby activating APCs and inducing CD8+ T cell responses.[86,111] For instance, PLGA nanovaccines carrying TLR7/8 agonists (eg, IMDQ, R848) have been shown to upregulate co-stimulatory molecules on DCs, promote CD8+ T cell expansion, and inhibit tumor growth in BC models.[112] Additionally, surface functionalization enables the conjugation of peptides, antibodies, or ligands for active targeting. In the context of BC, considering the unique characteristics of intravesical drug administration, organic nanocarriers can be engineered to enhance mucosal adhesion and tissue penetration. For instance, cationic chitosan nanomicelles can electrostatically adsorb to urothelial mucins, thereby improving intravesical retention and tumor tissue penetration.[113] Moreover, chitosan can transiently disrupt tight junctions, improving trans-epithelial drug permeability, making it an ideal high-molecular-weight carrier for intravesical applications.[114] These properties allow organic nanomaterials to deliver immunotherapeutics more effectively to the bladder tumor site, improving treatment specificity.

In one study, attenuated BCG was formulated into chitosan nanoparticles for intravesical immunotherapy. The resulting nano-BCG formulation demonstrated good biocompatibility and reduced toxicity; bladder instillation of nano-BCG significantly enhanced anti-tumor immune responses and prolonged survival in mice, while minimizing systemic side effects.[115,116] In another study, researchers constructed a liposome-encapsulated formulation of BCG cell wall skeleton (CWS) as an alternative to live BCG. This nano-CWS system overcame the poor solubility and cellular uptake limitations of free CWS; approximately 95% of BC (MBT-2) cells internalized the CWS nanoparticles, resulting in marked tumor growth inhibition.[117] In animal studies, intravesical instillation of nano-CWS led to a dose-dependent reduction in tumor volume and induced a Th1-type immune response, as evidenced by increased IFN-γ–producing cells and decreased IL-4 expression. These findings indicate that nano-CWS elicits superior immune activation compared to conventional BCG. Collectively, the results demonstrate that organic nanomaterials possess favorable in vivo adaptability and targeted drug delivery capabilities for BC immunotherapy and gene therapy applications.

Inorganic Nanomaterials

Inorganic nanomaterials include metallic or non-metallic nanoparticles such as gold nanoparticles (AuNPs), magnetic iron oxide nanoparticles (Fe_3O_4), silica nanoparticles (SiNPs), and manganese dioxide nanozymes (MnO_2). These materials typically possess a stable inorganic framework, offer precise control over size and morphology, and feature a large surface area that facilitates conjugation with a variety of functional groups or biomolecules.[118,119]

Moreover, inorganic nanomaterials often exhibit unique physicochemical properties: for instance, gold nanorods (AuNRs) efficiently convert near-infrared (NIR) light into thermal energy (photothermal effect); magnetic iron oxide nanoparticles respond to external magnetic fields, enabling tumor targeting or magnetic hyperthermia; and manganese dioxide nanoparticles can generate oxygen to relieve tumor hypoxia.[120–122] These characteristics position inorganic nanomaterials as promising platforms for theranostics in cancer immunotherapy. For example, gold nanoparticle–Listeriolysin O (LLO) peptide-based nanovaccines can mimic pathogenic patterns to enhance immunogenicity, leading to significantly increased intratumoral infiltration of CD8+ T cells and dendritic cells (DCs), while concurrently suppressing immunosuppressive Tregs and myeloid-derived suppressor cells (MDSCs).[123] Additionally, Paolo Armanetti et al demonstrated that AuNR-assisted photoacoustic imaging combined with photothermal therapy enabled visualization and ablation of residual tumor foci smaller than 1 mm in a mouse BC model. This approach offers a potential strategy for monitoring and treating residual disease post-surgery, providing valuable prognostic insights for BC patients.[124] Manganese dioxide or iron oxide nanoparticles can be employed for magnetic hyperthermia and co-delivery of STING agonists such as cyclic GMP–AMP (cGAMP), thereby activating the stimulator of interferon genes (STING) pathway and promoting dendritic cell (DC) maturation and T cell activation.[125]

Despite their tunable size and surface characteristics, inorganic nanoparticles are typically rigid and poorly degradable, leading to partial clearance by the mononuclear phagocyte system during circulation.[126] To enhance tumor targeting, surface modification with polymers or targeting ligands can prolong circulation time and improve active accumulation. For instance, mesoporous silica nanoparticles functionalized with RGD peptides targeting tumor vasculature showed preferential accumulation in bladder tumors and significant tumor growth inhibition in nude mice.[104] Moreover, inorganic nanoparticles can deposit within the bladder cavity. For example, glucose aldehyde-functionalized AuNR/Fe_3O_4 hydrogels can selectively adhere to collagen in bladder tumor tissue, enabling localized intravesical drug delivery.[126]

Héctor Terán-Navarro et al[127] designed a gold nanoparticle–bacterial toxin peptide composite nanovaccine (GNP–LLO_91–99) for BC immunotherapy. In this system, the immunostimulatory peptide (LLO_91–99) from Listeriolysin O was conjugated to the surface of AuNPs to enhance anti-tumor immunity. In a subcutaneous BC mouse model, a single injection of the nanovaccine reduced tumor burden by approximately 4.7-fold and elicited a potent Th1-biased immune response.[127] Mechanistically, GNP–LLO was internalized by DCs, which subsequently secreted pro-inflammatory cytokines and induced immunogenic tumor cell apoptosis. This led to increased intratumoral infiltration of cytotoxic T cells and DCs, along with a reduction in immunosuppressive cell populations. In animal models, the nanovaccine alone significantly inhibited tumor growth and exhibited synergistic efficacy when combined with PD-1/CTLA-4 immune checkpoint blockade. Another study utilized a composite hydrogel containing AuNRs and iron oxide nanoparticles (IONs) to construct a "tri-modal" therapy platform that achieved near-complete tumor eradication in a mouse BC (MB49) model.[126] Under NIR irradiation, AuNRs generated localized heat for photothermal ablation, while high concentrations of iron ions induced ferroptosis and reprogrammed tumor-associated macrophages (TAMs) from an M2 to an anti-tumor M1 phenotype. Immunofluorescence analysis revealed a marked reduction in CD11b+ M2 macrophages and an increase in CD80+ M1 macrophages. Following AuNR&ION hydrogel treatment and NIR exposure, tumors became nearly undetectable within 20 days, and the survival rate significantly improved. Manganese dioxide (MnO_2) nanozymes have also demonstrated promising applications in BC photodynamic therapy (PDT). Lin et al developed a HSA–MnO_2–Ce6 nanoplatform capable of generating oxygen from endogenous H_2O_2 within tumors to relieve hypoxia, thereby enhancing the production of reactive oxygen species (ROS) by the photosensitizer Ce6.[128] In an orthotopic mouse BC PDT model, this system increased intratumoral oxygenation and significantly prolonged survival, improving therapeutic outcomes. Collectively, these examples highlight the diverse applications of inorganic nanomaterials in multi-modal BC immunotherapy. Through rational design, these platforms not only mediate direct tumor destruction but also remodel the local immune microenvironment to enhance T cell–mediated anti-tumor responses (Figure 5).

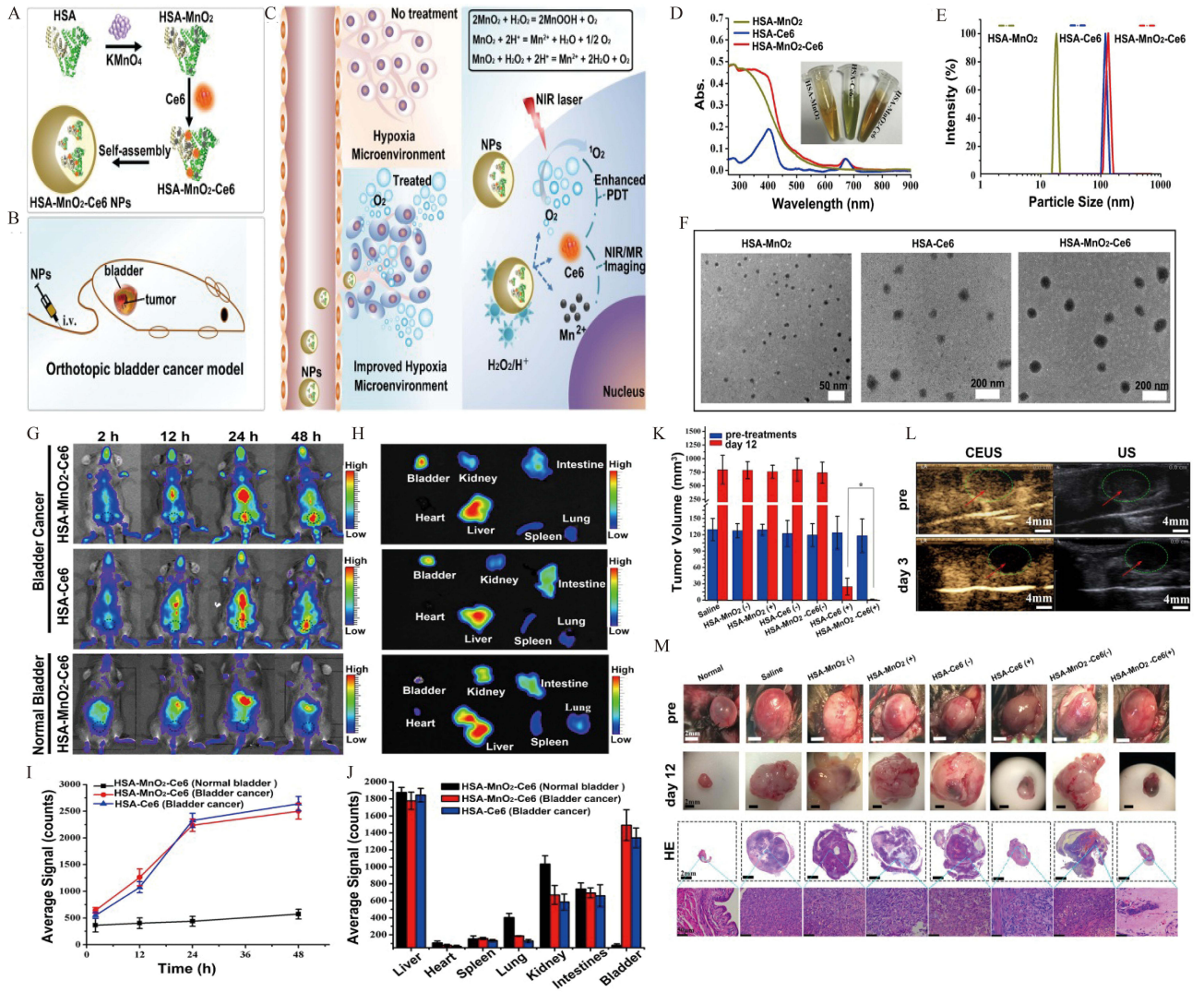

Figure 5 O_2-generating MnO_2 nanoparticles for enhanced photodynamic therapy of BC by ameliorating hypoxia. (**A**) Schematic representation of the synthesis of HSA-MnO_2-Ce6 NPs. (**B** and **C**) HSA-MnO 2-Ce6 NPs' application in enhanced PDT therapy for orthotopic BC by ameliorating hypoxia. (**D**–**F**) The physicochemical properties of HSA-MnO_2, HSA-Ce6, and HSA-MnO_2-Ce6 nanoparticles were characterized by UV–vis spectroscopy, DLS, and TEM, confirming their distinct optical features, size distributions, and morphologies. (**G**–**J**) The biodistribution and bladder-targeting ability of HSA-MnO_2-Ce6 nanoparticles were validated by in vivo and ex vivo fluorescence imaging, showing enhanced bladder accumulation and reduced off-target distribution compared to HSA-Ce6. (**K**–**M**) HSA-MnO_2-Ce6 nanoparticles combined with photodynamic therapy significantly reduced bladder tumor volume, as evidenced by imaging, CEUS/US monitoring, and histological analysis, demonstrating effective therapeutic response within 12 days. Adapted from Lin, Tingsheng et al. O_2-generating MnO_2 nanoparticles for enhanced photodynamic therapy of BC by ameliorating hypoxia. *Theranostics* vol. 8,4 990–1004. (https://creativecommons.org/licenses/by-nc/4.0/). Copyright © 2020 by the authors.[128]

Membrane Biomimetic Nanomaterials

Cell membrane– or biomimetic membrane–coated nanoplatforms achieve immune-targeted delivery by cloaking nanoparticles with T cell membranes, tumor cell membranes, or other biological membranes.[100] For instance, nanoparticles coated with T cell membranes can utilize native surface receptors to target tumor cells while evading immune clearance.[129] In addition, stimuli-responsive and composite nanoplatforms can release payloads under specific conditions of the TME—such as pH, enzymatic activity, or redox potential—thus enabling precise drug delivery. Peng et al[125] developed a "nanomotor" system that forms in situ within the bladder via self-polymerization, generating nanoparticles (DMCU) loaded with a STING agonist. The system is propelled by urease-driven motion, which enhances mucosal retention and intravesical delivery efficiency, resulting in robust immune activation (Figure 6). Similarly, Choi et al[94] designed a urease-powered nanomotor functionalized with STING agonists. These autonomous particles penetrate the urothelium and deliver immunostimulatory signals without requiring catheterization, thereby inducing potent activation

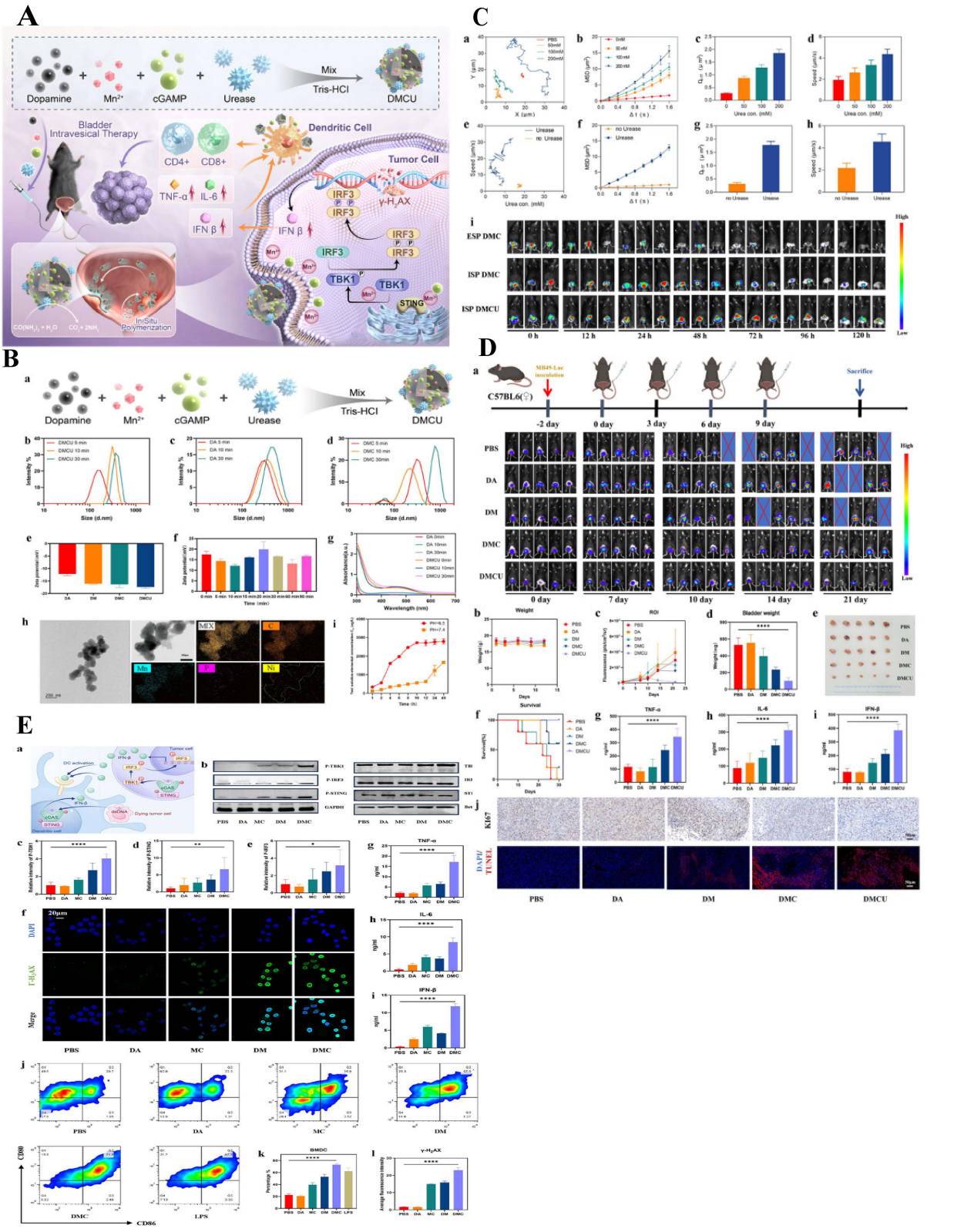

Figure 6 Design, characteristics and effects of Mn-cGAMP@PDA-urease (DMCU) nanoparticles. (**A**) Schematic diagram of the preparation of in-situ polymerized nanomedicine and the mechanism of nanoparticle formation. (**B**) The construction and characterization of self-propelled in situ polymerized nanodrugs were systematically illustrated, including a schematic of the polymerization process, variations in particle size and zeta potential, UV–vis absorption changes, TEM imaging with elemental mapping, and drug release profiles under different conditions. (**C**) The bladder retention time of nanomedicine formulations in vivo was evaluated to assess the exercise performance of DMCU. (**D**) DMCU inhibits the growth of BC in vivo (**E**) In vitro investigation of DNA damage mechanisms and STING pathway activation with DMC. *p < 0.05, **p < 0.01, ***p < 0.001. Adapted from Peng, Lei et al. Self-Propelled In Situ Polymerized Nanoparticles Activating the STING Pathway for Enhanced BC Immunotherapy. Advanced science (Weinheim, Baden-Wurttemberg, Germany) vol. 12,25 (2025): e2502750. Copyright © 2025 by the authors.[125]

https://doi.org/10.2147/IJN.S557690

and recruitment of CD8+ T cells—markedly outperforming traditional infusion methods. Due to the presence of source cell-derived surface receptors and ligands, biomimetic membrane-coated nanoparticles can target specific microenvironments. For example, tumor cell membrane–camouflaged nanovaccines can carry tumor-specific antigens and home to their parental tumor site, enabling personalized immune activation. Exosome-like vesicles (EMVs) derived from macrophage membranes possess intrinsic inflammation-homing properties and can transport payloads to inflamed tumor regions.[130,131] In BC, recent reports have described the use of macrophage-derived EMVs to deliver agents that modulate the immunosuppressive microenvironment.[93] These vesicles inherit membrane proteins from macrophages, allowing them to evade immune clearance and selectively target immunosuppressive components in tumor tissue, thereby improving drug delivery efficiency[132] For instance, Zhou et al[93] constructed macrophage membrane–derived biomimetic nanovesicles (EMVs) capable of co-delivering the CD73 inhibitor AB680 and anti–PD-L1 monoclonal antibodies (αPD-L1) for BC treatment. This EMV-based nanosystem exhibited excellent biocompatibility and stability both in vitro and in vivo, along with strong bladder tumor–targeting capability. Mechanistically, AB680 carried by the macrophage membrane vesicles inhibits CD73 within the tumor, reducing the production of immunosuppressive adenosine. Meanwhile, αPD-L1 antibodies released from the vesicles bind to tumor cells, reversing PD-L1–mediated immune escape. In tumor-bearing mice, this dual-delivery biomimetic nano-therapy significantly enhanced the activation and proliferation of tumor-infiltrating T lymphocytes. A robust increase in cytotoxic T cell infiltration was observed within the tumor, leading to inhibited tumor growth and prolonged survival.[133] In another study, Hu et al designed a platelet membrane–camouflaged hydrogel delivery system incorporating CSPG4-targeted CAR-T cells, IL-15–loaded PLGA nanoparticles, and platelets conjugated with anti–PD-L1 antibodies.[134] Upon postoperative implantation into the tumor bed, local inflammation triggered the platelets to release PD-L1 antibodies, neutralizing tumor-derived PD-L1, while the hydrogel slowly released IL-15 to sustain CAR-T cell activity. In a melanoma model, this biomimetic platform exhibited superior tumor suppression compared to conventional CAR-T cell infusion. Notably, due to prolonged CAR-T cell retention within the hydrogel, the system also suppressed contralateral untreated tumors in a bilateral tumor model—suggesting a durable systemic immune response.[134] These findings imply that locally implanted biomimetic carriers may prolong the in vivo persistence and function of T cells, thereby sustaining anti-tumor immune pressure. In summary, membrane-biomimetic nanomaterials, by mimicking the natural attributes of immune or tumor cells, enable precise targeting of the TME and co-delivery of multiple immunotherapeutic agents. They represent a promising emerging strategy to enhance the efficacy of innate and adaptive cancer immunotherapy.

Stimuli-Responsive Nanomaterials

Stimuli-responsive nanomaterials are engineered to incorporate functional units that undergo physicochemical transformations upon exposure to specific endogenous or exogenous stimuli, thereby enabling on-demand drug release or therapeutic activation. Stimuli can be broadly categorized into tumor-internal microenvironmental triggers (eg, acidic pH, elevated levels of reducing glutathione [GSH], overexpressed enzymes, or hypoxia and high hydrogen peroxide) and exogenous physical triggers (eg, light, ultrasound, magnetic fields, or temperature changes).[135,136] Common design strategies include: acid-labile linkers that release drugs under acidic conditions, redox-sensitive polymers that disassemble in response to high GSH levels, enzyme-cleavable peptides sensitive to tumor-associated proteases (eg, MMPs), and photothermal/magnetothermal conversion nanoparticles.[136,137] These designs allow nanomaterials to remain inert and stable under physiological conditions but become activated upon reaching the tumor site, significantly increasing local drug concentration while minimizing toxicity to normal tissues.

Moreover, stimuli-responsive behavior itself can serve as a targeting mechanism by exploiting the aberrant features of the TME. Most solid tumors exhibit mildly acidic pH (~6.5–6.8), elevated reductive potential (high GSH), increased H_2O_2 levels, and hypoxia.[138] Accordingly, various smart nanocarriers have been developed, such as pH-sensitive release systems, GSH-triggered drug-releasing micelles, and H_2O_2-driven oxygen-generating nanozymes. Once these carriers reach the TME, they are "unlocked" by specific stimuli to release payloads or exert therapeutic effects precisely at the tumor site.[139,140] For instance, Hunter et al[141] utilized an HSA–MnO_2 nanozyme that responded to high intratumoral H_2O_2 levels to release oxygen, enhancing photodynamic therapy (PDT) in bladder tumors. As the bladder is a surface-accessible organ, it is also suitable for exogenous physical triggers such as light. NIR light can penetrate the bladder wall

to activate photosensitizers or photothermal agents, enabling localized energy release and tumor ablation.[142] This facilitates novel opportunities for multimodal BC therapy.

Chen et al[143] developed a multifunctional nanoparticle system (GOx@MBSA–PPy–MnO_2) responsive to both glucose and GSH for combination BC therapy. In this system, polypyrrole (PPy) components generate heat under NIR light for photothermal therapy (PTT); meanwhile, the encapsulated glucose oxidase (GOx) catalyzes glucose into H_2O_2 and gluconic acid, lowering pH and triggering MnO_2 decomposition to release Mn^2+. Mn^2+ contributes in two ways: (1) it facilitates Fenton-like reactions under high H_2O_2 levels, generating cytotoxic hydroxyl radicals (·OH) for chemodynamic therapy (CDT); and (2) it enhances oxygen generation by decomposing H_2O_2, thereby alleviating hypoxia.[144] Similarly, Hao et al designed an RGD-modified platinum nanozyme system (PtNPs) co-loaded with a GSH-responsive prodrug (PTX–SS–HPPH/Pt@RGD–NP) for targeted BC therapy. Their in vitro and in vivo studies demonstrated that the synergistic effects of PTT, CDT, and metabolic modulation achieved significantly better tumor ablation than monotherapies.[145] Guo et al's AuNR&ION hydrogel system is another classic example of stimuli-responsive design: the gold nanorod (AuNR) component requires NIR irradiation to exert photothermal effects, while the ferrite nanoparticles (IONs) are activated by H_2O_2 and iron ions in the TME to trigger lipid peroxidation chain reactions, leading to ferroptosis and reactive oxygen species (ROS) production.[126] In another study, urease-driven nanomotors were developed for BC therapy. These nanocarriers had surface-anchored urease that catalyzed urea in bladder urine to generate gas bubbles, propelling the particles deep into tumor tissues.[146] When labeled with radioactive iodine-131 (^131I), these nanorobots enabled intravesical radionuclide therapy, reducing tumor volume by ~90% and increasing nanoparticle accumulation within the tumor eightfold compared to passive diffusion.[146] In summary, stimuli-responsive nanomaterials provide a powerful strategy for spatiotemporal control of therapeutic activation. By sensitively responding to endogenous or exogenous cues, they enable precise delivery of potent therapies to tumor sites, enhancing efficacy and minimizing adverse effects in BC immunotherapy (Figure 7).

Functional Nanomaterials for Enhancing T Cell Immunity in BC

BC is characterized by a high recurrence rate and notable resistance to therapy, largely attributable to its highly immunosuppressive TME and the unique anatomical features of the urinary tract, which together impede T cell infiltration and effector function.[147] Although current T cell–based therapies—such as PD-1/PD-L1 immune checkpoint inhibitors and adoptive cell transfer approaches (eg, CAR-T, TCR-T, TIL)—have demonstrated durable responses in certain advanced cases, their overall response rates remain limited.[113] To overcome these challenges, nanotechnology has emerged as a novel platform for drug delivery and immune modulation owing to its unique biological advantages. It is rapidly becoming a central strategy in optimizing T cell–based immunotherapy.[106,135,148] The use of engineered nanomaterials is now reshaping the therapeutic landscape for BC T cell immunotherapy.[149] In general, both organic (eg, liposomes, polymeric micelles) and inorganic (eg, mesoporous silica, gold nanoparticles) nanomaterials ranging from 1 to 100 nm in size offer innovative solutions to address the limitations of conventional approaches. Their high surface area, programmable surface chemistry, and intelligent, stimuli-responsive drug release capabilities are particularly advantageous.[150] Its core advantages include the following: First, through surface functionalization with targeting ligands—such as tumor-homing peptides or anti-CD3 antibodies—nanocarriers can achieve precise accumulation of therapeutic molecules (eg, antigens, adjuvants, cytokines, or immune checkpoint inhibitors) at tumor sites or in the vicinity of T cells, thereby significantly reducing systemic toxicity.[151] Second, their capacity for multi-component co-delivery enables synergistic T cell activation and reversal of microenvironmental immunosuppression. For example, co-delivery of tumor antigens and PD-L1 inhibitors, in combination with pH-, enzyme-, or ROS-responsive release mechanisms, enhances localized immune stimulation within tumors.[152] Furthermore, the nanoscale characteristics of these materials facilitate penetration through the dense stromal barriers of bladder tumors, thereby addressing the issue of insufficient T cell infiltration. Meanwhile, biomimetic modifications—such as T cell membrane coating—can enhance the ability of the nanocarriers to evade immune clearance. What is particularly important is that the anatomical accessibility of the urinary system makes nanomedicine especially suitable for intravesical administration, greatly improving bioavailability. For instance, liposome-encapsulated BCG antigens can enhance dendritic cell cross-presentation, while pH-responsive hydrogel-based sustained release of IL-15 helps maintain the activity of tissue-resident memory T cells in the

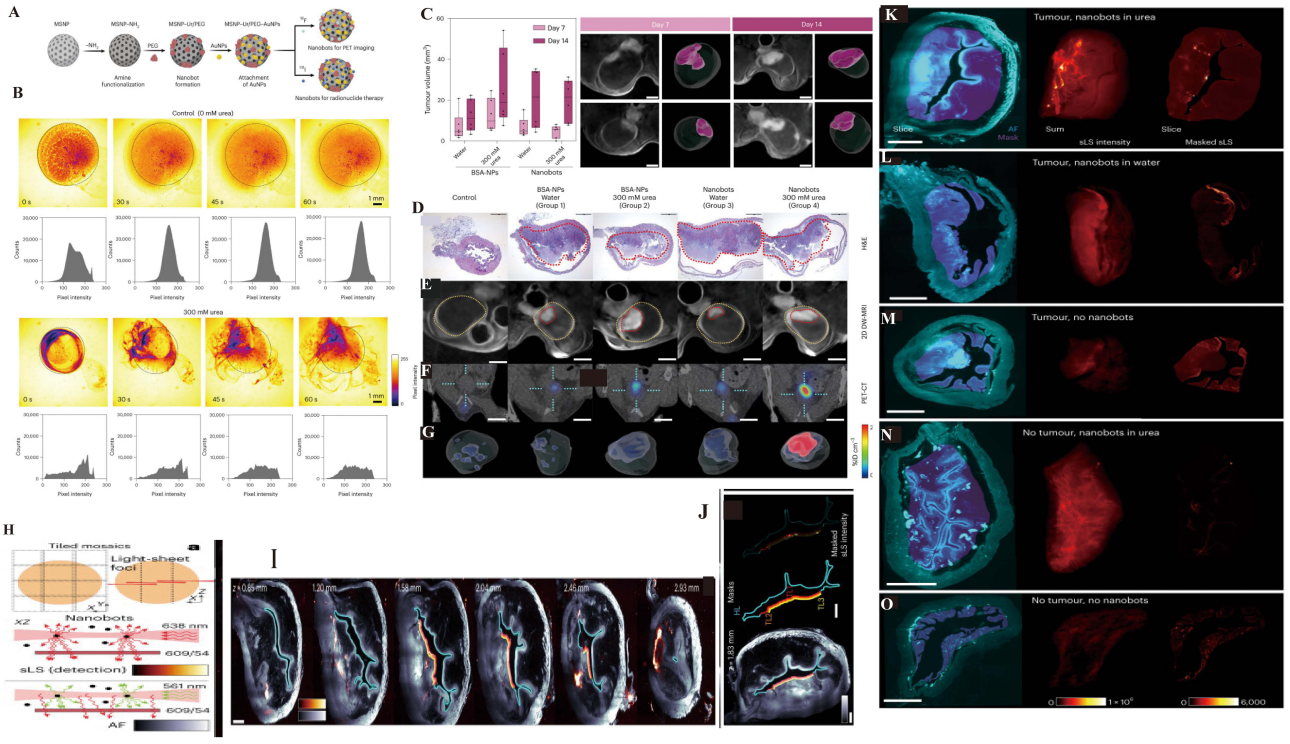

Figure 7 Urea-Driven Nanobot Design, Motion, and Multimodal Imaging Reveal Enhanced Bladder Tumor Targeting and Retention. (**A**) Schematic representation of the nanobot fabrication process and radiolabelling. (**B**) Snapshots depicting the nanobot motion dynamics in the absence and presence (300 mM) of urea as fuel, and corresponding pixel intensity histograms for the ROI marked by a circle. (**C**) Tumor volumes on days 7 and 14 after cell implantation for the different study groups. (**D**–**G**) Multimodal imaging including H&E staining, DW-MRI, PET/CT, and 3D reconstruction demonstrated tumor presence, bladder localization, and enhanced radioactive accumulation in representative animals across treatment groups. (**H**) The principle of laser being scattered by particles (such as nanorobots) and detected through a filter. (**I**) Optical sections of bladder tissue at different depths with tissue layer annotations for quantification. (**J**) tumour and healthy layers of tissues along bladder cavity, detected and annotated (masks) in three dimensions. (**K**–**O**) Volumetric light-sheet imaging and voxel intensity analysis revealed that nanobots administered with urea achieved markedly higher retention and signal intensity in both tumor-bearing and healthy bladders, whereas nanobots without urea or control groups showed minimal signal, confirming urea-driven active accumulation. Adapted from Simó, Cristina et al. Urease-powered nanobots for radionuclide BC therapy. Nature nanotechnology vol. 19,4 (2024): 554–564. https://creativecommons.org/licenses/by-nc/4.0/. Copyright © 2024 by the authors.[146]

bladder mucosa.[153] Recent progress has further confirmed these advantages. Self-propelling, in situ–polymerized nanoparticles significantly boosted the immunotherapeutic efficacy against BC by activating the STING pathway.[125]

Nanomaterials for the Delivery of siRNA and Antibodies

In BC immunotherapy, a critical strategy involves regulating the expression of immune-related molecules in tumor cells and the TME, such as silencing immunosuppressive genes (eg, PD-L1, IDO) or delivering exogenous antibodies/receptors (eg, anti–PD-1 antibodies or immune-stimulatory cytokines).[154,155] However, small interfering RNA (siRNA) and plasmid DNA are highly susceptible to degradation in vivo and have poor membrane permeability, while systemic delivery of macromolecular antibodies is often limited by poor tumor targeting and adverse side effects.[97,156] Nanocarriers serve as ideal platforms for delivering siRNA and antibodies, as they protect biomacromolecules, enhance targeting, and facilitate cellular uptake.

In general, nanomaterials designed for gene delivery are expected to possess high loading capacity, efficient cellular uptake, and produce no toxic byproducts upon degradation. Commonly used carriers include cationic polymeric nanoparticles, which form complexes with nucleic acids to facilitate cellular internalization, and mesoporous silica nanoparticles (MSNs), which load nucleic acids within their porous structures and protect them via pore sealing.[157,158] These platforms improve the targeting specificity and therapeutic efficacy of gene therapies and are regarded as promising strategies for nucleic acid delivery in BC. For protein-based therapeutics such as antibodies, nanomaterials can be employed via surface conjugation or encapsulation to enhance their accumulation in tumor tissues.[159] A representative example is the delivery of PD-L1 antibodies using macrophage-derived biomimetic extracellular vesicles (EMVs). Researchers successfully utilized these vesicles to enrich antibodies at bladder tumor sites while

facilitating rapid clearance from the circulatory system, thereby reducing systemic exposure. In vivo studies demonstrated that compared with free antibodies, EMV-delivered PD-L1 antibodies elicited comparable immune activation—such as enhanced T cell activity following PD-L1 blockade in the TME—while significantly reducing systemic toxicity.[97] In addition, several stimuli-responsive nanocarriers have been developed to further improve the performance of antibody-based therapies. For example, near-infrared photoimmunotherapy (NIR-PIT) uses antibodies conjugated with photosensitive moieties that, upon binding to tumor cells and subsequent light exposure, release localized cytotoxic effects.[160] Applications of NIR-PIT in BC have been reported, targeting molecules such as EGFR and PD-L1, resulting in enhanced local immune-mediated tumor killing with minimal side effects.[161,162]

Broadly speaking, the delivery of siRNA/genes and antibodies via nanocarriers can relieve immunosuppression at the molecular level, thereby indirectly enhancing T cell–mediated anti-tumor responses.[163] For instance, one study demonstrated that PD-L1 downregulation via RGD-functionalized mesoporous silica nanoparticles (RGD-MSNs) increased tumor susceptibility to cytotoxic T lymphocyte (CTL)–mediated killing. At the same time, delivery of miR-34a was shown to reduce cancer stem cell–like properties, helping to eliminate antigen-negative, drug-resistant clones. Furthermore, a combination therapy involving EMV-based co-delivery of PD-L1 antibodies and a CD73 inhibitor successfully reversed two key immunosuppressive mechanisms—PD-L1 expression and adenosine accumulation—in the TME. This led to a substantial increase in the number of intratumoral CD8+ cytotoxic T cells and significantly inhibited tumor growth.[164] Currently, multiple nanomedicine-based strategies for the delivery of immune-related genes and antibodies have entered preclinical or clinical stages. Lipid nanoparticle (LNP)–based mRNA technology, for example, has already been utilized in personalized cancer vaccines for other malignancies and holds promise for translation to BC.[165] In parallel, an innovative approach has been proposed using amphiphilic polymeric micelle carriers to deliver plasmids encoding chimeric antigen receptors (CARs) directly into T cells.[113,166] This strategy aims to "nano-transfect" patient-derived T cells in vivo, converting them into CAR-T cells capable of targeting BC and other solid tumors—representing a frontier in the convergence of gene-engineered immunotherapy and nanotechnology. Looking ahead, continued translation of these nanocarrier platforms from bench to bedside is anticipated to broaden therapeutic options and improve the efficacy of T cell–based immunotherapy for BC.

Nanomaterials for Inducing Immunogenic Cell Death (ICD)

Immunogenic cell death (ICD) refers to a form of tumor cell death triggered by specific therapeutic interventions, during which highly immunostimulatory damage-associated molecular patterns (DAMPs)—such as ATP, HMGB1, and heat shock proteins—are released along with tumor-associated antigens. This process initiates and enhances anti-tumor immune responses by the host.[167] ICD plays a pivotal role in the concept of "in situ tumor vaccination," as it promotes antigen uptake, maturation of dendritic cells (DCs), and subsequent activation of T cell–mediated immunity[168] However, the efficacy of conventional radiotherapy or chemotherapy in inducing ICD is often suboptimal, and tumor cells may escape immune surveillance by upregulating tolerance mechanisms.[140] To overcome these limitations, nanomaterials have been employed to amplify the immunogenicity of ICD through multimodal interventions—including physical, chemical, and biological strategies. These materials not only directly kill tumor cells but also deliver abundant danger signals and antigenic stimuli to support robust T cell activation, thereby significantly enhancing the effectiveness of T cell–based immunotherapy in BC.[169,170]

Physically, nanomaterial-induced ICD primarily relies on photothermal therapy (PTT) and photodynamic therapy (PDT), both of which exploit the photoresponsive properties of nanoparticles to generate localized heat or reactive oxygen species (ROS), inducing immunogenic damage to BC cells.[171] For example, gold nanorods (AuNRs) under near-infrared (NIR) irradiation can efficiently convert light into heat (photothermal conversion efficiency >90%), causing disruption of the tumor cell membrane and subsequent release of DAMPs[172] Moreover, when AuNRs are combined with iron oxide nanoparticles (IONPs), they can further induce ferroptosis—an iron-dependent, lipid peroxidation–driven form of non-apoptotic cell death. This enhances the release of ROS and oxidized phospholipids, amplifying DAMP exposure and polarizing tumor-associated macrophages (TAMs) toward a pro-inflammatory M1 phenotype. This immune remodeling creates a more favorable environment for T cell infiltration and activation.[173] In BC models, such composite systems have not only achieved direct tumor ablation but also enhanced cytotoxic T lymphocyte (CTL) infiltration and immune

memory formation, providing resistance against tumor rechallenge.[124] In addition, Deng et al recently reported a T cell membrane–camouflaged nanoparticle system (Tim3@PHSM@IC), which combines PTT-induced ICD with blockade of the Tim-3 checkpoint pathway. This dual-functional nanoplatform overcomes the immunosuppressive TME and significantly improves the response rate to T cell–based therapies (Figure 8).[129] These physical mechanisms offer the advantage of precise spatial and temporal control. However, they often require further surface modification (eg, PEGylation) to enhance biocompatibility and tumor targeting efficiency.

Chemically, ICD can be enhanced through the delivery of pro-oxidative or pro-apoptotic agents via nanocarriers, which directly modulate cell death pathways to increase the immunogenicity of dying tumor cells.[174] For example, polymeric nanoparticles such as PLGA loaded with anthracycline-based chemotherapeutics (eg, doxorubicin) or oxidized phosphonates can be used to locally release these agents within the tumor, inducing surface exposure of calreticulin (CRT) and ATP efflux. These DAMP signals promote dendritic cell (DC) maturation and activate downstream T cell responses[175] Additionally, Nectin-4–targeted PLGA nanoparticles delivering gene therapies such as MT50 have demonstrated dual functionality—suppressing tumor proliferation (with >70% inhibition) and chemically inducing ICD to release tumor-associated antigens, thereby stimulating antigen-specific T cell responses[176] Another innovative platform is the hybrid membrane–coated nanoparticle (HM-MPS NP), which co-delivers oxidative agents alongside anti–PD-1 antibodies. This system amplifies ICD, reverses immune suppression in the TME, and enhances T cell–mediated tumor clearance.[177] These chemically functionalized nanomaterials are typically synthesized via emulsion–solvent

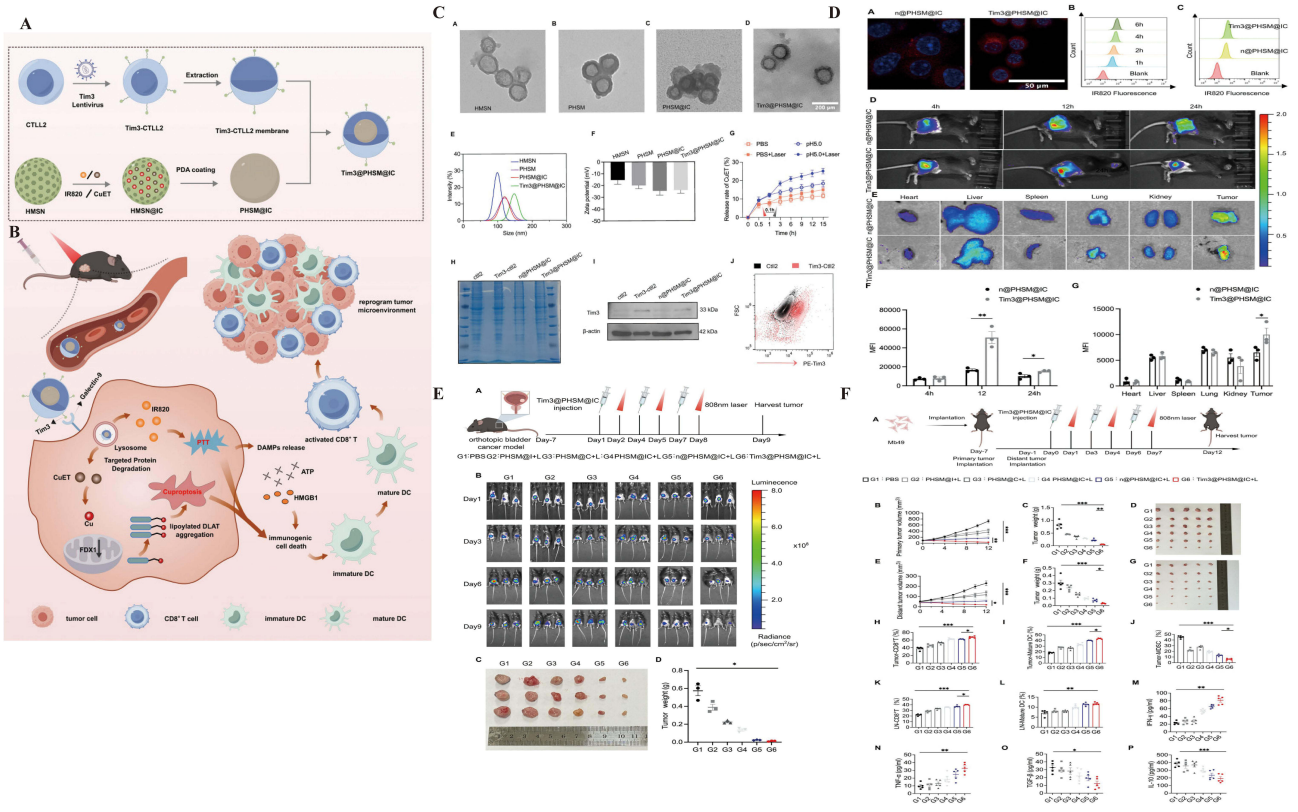

Figure 8 Therapeutic Design, Characterization, and Antitumor Efficacy of Tim3\@PHSM\@IC Nanoparticles for BC Treatment Schematic illustration showing the preparation (**A**) of Tim3@PHSM@IC and the proposed mechanism (**B**) of Tim3@PHSM@IC to treat BCa. (**C**) Tim3@PHSM@IC nanoparticles were successfully constructed and characterized by TEM, size and zeta-potential measurements, drug release profiling, protein staining, Western blot, and flow cytometry, confirming their structural integrity, protein incorporation, and Tim3 expression. (**D**) Tim3@PHSM@IC nanoparticles showed enhanced cellular uptake and tumor targeting compared to controls, as demonstrated by fluorescence imaging, flow cytometry, and biodistribution analyses in cells and mice, with significantly higher accumulation in tumors. (**E**) Tim3@PHSM@IC nanoparticles significantly inhibited orthotopic bladder tumor growth in mice, as evidenced by reduced tumor fluorescence, smaller tumor size and weight, and statistical analysis confirming superior therapeutic efficacy over control treatments. (**F**) In a subcutaneous BC model, Tim3@PHSM@IC nanoparticles markedly suppressed tumor growth, reduced tumor weight, improved survival, and showed minimal toxicity, with histological analysis confirming decreased proliferation and effective therapeutic impact. *p < 0.05, **p < 0.01, ***p < 0.001. Adapted from Deng, Wen et al. Genetically engineered T cell membrane-camouflaged nanoparticles triggered cuproptosis for synergistic BC photothermal-immunotherapy. Journal of nanobiotechnology vol. 23,1 425. 7. https://creativecommons.org/licenses/by-nc/4.0/. Copyright © 2025 by the authors.[129]

evaporation techniques, with particle sizes controlled between 50–200 nm to optimize the enhanced permeability and retention (EPR) effect. However, parameters such as drug loading efficiency (>80%) and potential toxicity must be carefully evaluated to ensure safe delivery.[178]

From a biological perspective, nanomaterials can mimic infection signals or deliver microbial components to further strengthen ICD-associated immune stimulation. For instance, gold nanoparticles loaded with Listeriolysin O (LLO) peptides simulate bacterial membrane perforation, triggering stress-induced necrosis and DAMP release. This facilitates DC recruitment and debris clearance, followed by activation of secondary immunogenic apoptosis. Experiments have shown that 35–55% of tumor cells undergo DC-mediated ICD, while direct cytotoxic effects remain minimal.[125] Moreover, self-propelling, in situ–polymerized nanoparticles (eg, DMCU) can activate the STING pathway by delivering microbial mimics, promoting both DC maturation and T cell recruitment, thereby enhancing BC immunotherapy outcomes.[179] These biologically inspired nanomaterials are often biomimetically modified (eg, with membrane coatings) to improve targeting accuracy and evade immune clearance, enabling efficient ICD induction within the bladder's mucosal environment. Through a combination of physical, chemical, and biological strategies, nanomaterials can synergistically induce ICD, effectively transforming tumor cells into "vaccine donors" that recruit and activate T cells. Such strategies have been validated in BC models for their capacity to enhance anti-tumor T cell responses.[126] For instance, the AuNR&ION nanoplatform has been shown to induce systemic immune memory capable of suppressing distant tumors.[127] GNP–LLO nanovaccines increase infiltration of CTLs and DCs while reducing immunosuppressive cells,[180] and HM-MPS NPs in combination with anti–PD-1 therapy further amplify therapeutic outcomes.[177] Therefore, nanomaterial-induced ICD represents a promising approach to substantially improve the responsiveness of BC to T cell–based immunotherapy, and to facilitate the transition from an immunologically "cold" to a "hot" tumor phenotype.

Nanomaterials for Dendritic Cell Activation

Dendritic cells (DCs) are the key antigen-presenting cells responsible for initiating tumor antigen–specific T cell responses. Effective BC immunotherapy requires the uptake of tumor antigens by a large number of mature DCs, followed by their migration to lymph nodes to activate T cells.[181] However, DCs in the bladder TME are often poorly activated and sparsely infiltrated. Moreover, conventional vaccines—such as tumor lysates and peptide vaccines—typically lack sufficient adjuvant stimulation, resulting in suboptimal DC maturation.[182,183] Nanotechnology offers powerful tools to construct novel vaccine and adjuvant delivery systems that markedly enhance antigen uptake and maturation of DCs in BC.

One widely studied strategy involves nanoparticle-based cancer vaccines that co-deliver tumor antigens and immune adjuvants directly to DCs. For example, Nie et al designed a hybrid membrane nanoparticle vaccine (HM@NP) composed of fused tumor cell membrane and bacterial (E. coli) membrane components, thereby combining autologous tumor antigens with microbial adjuvants on a single nanoplatform.[182] This vaccine elicited robust anti-tumor immunity and long-term immune memory in murine models of colon and breast cancers.[182] For BC, patient-derived tumor cell membranes could be similarly fused with bacterial components (eg, *Acinetobacter, Mycobacterium*) to formulate autologous nanovaccines aimed at DC activation.[184] Another strategy is the use of nanoparticle-based adjuvant delivery, in which potent immunostimulatory molecules are encapsulated in nanocarriers for targeted delivery to DCs.[181] For instance, CpG oligodeoxynucleotides (TLR9 agonists) or STING agonists encapsulated in nanoparticles can be injected intratumorally or intravesically to locally trigger DC maturation and type I interferon production, converting "cold tumors" into "hot tumors" that are more readily recognized by T cells.[185] A representative example is hafnium-based metal–organic frameworks (Hf-MOFs) loaded with CpG; combined with radiotherapy, this nano-adjuvant eliminated primary tumors, inhibited metastasis, and established strong anti-tumor immune memory in murine models.[186]

BCG, a classical BC immunotherapy, functions partly by stimulating DC uptake of BCG and tumor antigens in the bladder epithelium, thereby initiating both innate and adaptive immunity.[113] Based on this mechanism, nanotechnology has been applied to optimize BCG delivery and safety. As reviewed by Yu et al, chitosan-based nano-BCG significantly increased recruitment and activation of immune cells such as DCs. In mice, intravesical instillation of nano-BCG induced stronger local inflammatory responses and lower systemic toxicity, suggesting more efficient DC activation within the bladder mucosa and reduced risk of systemic BCG infection.[187] In another study, Ren et al constructed chitosan/graphene

oxide nanoparticles (CS/GO-NPs) loaded with GM-CSF for combined photothermal therapy (PTT) and immunotherapy in an orthotopic mouse model of BC. These nanoparticles were anchored to the tumor surface via biotinylation, enabling sustained local release of GM-CSF, effective recruitment and activation of DCs, and enhanced anti-tumor immunity. The thermal effects of PTT further increased DC and T cell infiltration, reinforcing the immune response.[188] Additionally, using nanoparticles as artificial antigen-presenting cells (aAPCs) is an emerging concept. For instance, PLGA nanoparticles functionalized with MHC-I/peptide complexes, co-stimulatory molecules, and cytokines have been developed to directly interact with and activate T cells in vitro, mimicking the role of natural APCs.[189] Although not yet applied to BC specifically, this proof-of-concept highlights the potential for nano-platforms to perform antigen presentation without relying on host cells, paving the way for future antigen-specific vaccine design.

The ultimate outcome of DC activation via nanotechnology is the efficient cross-priming of naïve T cells and enhancement of both effector and memory T cell quantity and quality.[190] In the case of nano-delivered BCG, improved DC activation correlated with stronger anti-tumor responses and superior long-term tumor-free survival compared to conventional BCG.[191] Similarly, Th1 polarization induced by nano-formulated CWS indicates that the resulting CD8+ cytotoxic and Th1 helper T cells exhibit heightened effector functions. For example, in the study by Li et al, the hybrid membrane nanoparticle vaccine HM@NP induced long-lasting immune memory in various tumor models, significantly extending postoperative tumor-free survival. Upon tumor rechallenge, these mice rapidly cleared cancer cells, demonstrating durable immune protection.[192] In conclusion, effective DC activation through nanotechnology can be seen as the establishment of an "immune arsenal" within the host, continuously generating high-quality tumor-specific T cells and significantly amplifying the depth and breadth of BC immunotherapy.[189]

Nanomaterials for Enhancing the Formation of Memory T Cells

The generation and maintenance of memory T cells are critical for the long-term success of cancer immunotherapy, as these cells provide sustained tumor surveillance and rapid immune responses that help prevent tumor recurrence.[193] However, inducing durable memory T cells in solid tumors remains challenging. Persistent tumor antigen exposure can lead to T cell exhaustion,[194] while the absence of post-treatment immune stimulation may cause gradual apoptosis or functional decline of memory T cells.[195] Thus, therapeutic strategies aimed at promoting memory T cell differentiation and survival have become a key focus of current research. Nanomaterials contribute to this objective primarily through two mechanisms: (1) serving as sustained-release depots to provide prolonged antigen/adjuvant exposure, mimicking chronic infection to drive memory differentiation;[196] and (2) delivering survival factors such as cytokines (eg, IL-7, IL-15) or co-stimulatory signals to T cells, preventing apoptosis and reinforcing the memory phenotype.[197]

Currently, hydrogel-based nanocomposites are frequently utilized as post-surgical implantable vaccine depots. One notable example is the CAR-T hydrogel system developed by Gu et al, which incorporates sustained release of IL-15 to support the long-term proliferation of CAR-T cells, effectively mimicking the function of a memory T cell–nourishing cytokine.[134] Results demonstrated that CAR-T cells retained within the hydrogel remained viable and functional over extended periods in vivo. Remarkably, tumor suppression was also observed in contralateral, untreated tumor sites, indicating that the persistent effector T cells exerted systemic protective effects.[134] Similarly, controlled release of nanovaccines can avoid transient immune stimulation caused by single-dose immunization, instead enabling continuous antigen exposure that promotes memory T cell differentiation.[198] In one study, tumor-derived neoantigen peptides were co-encapsulated with CpG adjuvant in PLGA nanoparticles and injected intramuscularly. Compared with free vaccines, the slow-release formulation significantly increased the proportion of CD8+ memory T cells in mice and improved tumor-free survival following rechallenge.[199] Moreover, certain immunoregulatory cytokines—such as IL-2, IL-7, and IL-15—are known to favor the development of memory T cell responses.[200] Nanocarriers offer a means to deliver these potent cytokines locally, thereby minimizing systemic toxicity. For example, in a murine BC study, IL-12 and the chemotherapeutic agent pirarubicin (THP) were co-encapsulated in fluorinated chitosan nanoparticles for intravesical instillation. The chitosan derivative enhanced drug penetration into tumor tissues, while IL-12 activated local lymphocytes. The combination effectively eradicated primary tumors and significantly reduced recurrence rates.[93] These findings suggest that IL-12 promoted T cell differentiation and IFN-γ secretion within the bladder TME, making residual cancer cells less likely to survive, and thus exerting a "relapse-preventing" effect. Similarly, local delivery of cytokines such as

IL-15 and IL-21 using nanoparticle platforms may further enhance the memory phenotype of effector T cells. Notably, nanoparticle-mediated delivery of the IL-15 superagonist ALT-803 has been shown to dramatically expand the pool of tumor-specific memory T cells, which persisted in vivo and exhibited rapid recall responses upon re-exposure to tumor antigens.[201]

To evaluate the impact of nanomaterial-based strategies on memory T cell formation, tumor rechallenge experiments or long-term survival assessments are typically conducted.[202] To date, multiple murine studies have demonstrated that following nanoparticle-based immunotherapeutic interventions, animals often exhibit delayed or completely inhibited tumor growth upon rechallenge with tumor cells.[100] For example, in a 4T1 breast cancer model, mice treated with a nanocomposite therapy co-delivering PD-L1–targeted siRNA and oncolytic viral proteins showed complete clearance of the primary tumor on the left flank. When rechallenged with 4T1 cells on the contralateral side, 25% of the mice developed no secondary tumors, while the remainder showed significantly delayed tumor growth—providing strong evidence of robust immune memory.[199] Similarly, in the aforementioned hybrid vaccine model composed of *E. coli* membrane and tumor membrane components, mice that received postoperative vaccination remained tumor-free for up to 90 days, whereas most control animals experienced recurrence within several weeks.[182] Collectively, these findings suggest that nanomaterials, by providing sustained antigenic stimulation and targeted immune modulation, can effectively promote the formation and maintenance of memory T cells. This offers a powerful immunological foundation for achieving long-term immune surveillance and relapse prevention in BC therapy.

Nanomaterials for Reshaping the Tumor Metabolic Environment

In the TME of BC, metabolic dysregulation is often intricately linked with immune suppression. Tumor cells and immunosuppressive myeloid-derived cells competitively consume essential nutrients such as glucose, tryptophan, and arginine, while producing high levels of lactic acid and adenosine. This leads to a nutrient-deprived, acidic, and hypoxic environment that impairs T cell survival and function, thereby posing a significant challenge to effective immunotherapy.[203,204] In addition, several metabolic enzymes—such as indoleamine 2,3-dioxygenase (IDO), arginase, and CD73—are overexpressed by tumor cells or suppressive immune cells. These enzymes deplete amino acid pools critical for T cell proliferation or generate immunosuppressive metabolites, rendering T cells dysfunctional.[205] Encouragingly, recent research has shown that remodeling the metabolic landscape of the TME—essentially reshaping its "soil"—can significantly enhance the efficacy of immunotherapy.[84,206] Based on this, nanomaterials—with their inherent targeting capabilities—are increasingly being applied to precisely modulate intratumoral metabolic parameters and alleviate T cell–suppressive conditions.

One approach involves the delivery of small-molecule inhibitors or genetic regulators via nanocarriers to directly block immunometabolic suppressive pathways within the (TME.[207] For instance, Zhou et al utilized extracellular vesicle–mimetic (EMV) nanovesicles to deliver the CD73 inhibitor AB680. CD73 catalyzes the degradation of ATP into adenosine, a potent suppressor of T cell activity. Nano-enabled delivery of AB680 effectively reduced adenosine accumulation in the TME and restored T cell functionality.[93] Similarly, loading IDO inhibitors (eg, NLG-919) or siRNA targeting IDO into nanocarriers can suppress local tryptophan catabolism, thereby relieving restrictions on T cell proliferation.[168] Although not yet demonstrated in BC, such approaches have been validated in melanoma and other tumor models, highlighting their translational potential.[168,208] A second strategy focuses on the clearance or reprogramming of metabolic byproducts. For example, MnO_2 nanoparticles act as nanozymes that decompose H_2O_2 to produce oxygen, thereby relieving tumor hypoxia and acidity and enhancing oxygen availability for T cells and NK cells.[128] Glucose oxidase (GOx)-loaded nanoparticles simultaneously deplete glucose—limiting energy supplies to tumor and regulatory cells—and generate H_2O_2, which can synergistically induce tumor cell death and immune activation.[209] In one system, GOx was co-encapsulated with catalase (CAT) to convert H_2O_2 into O_2 immediately upon generation, minimizing oxidative damage to T cells while maintaining local oxygen concentration to support T cell effector function.[143] Compared to the aforementioned strategies, a third approach focuses on reprogramming metabolism-associated immune cells to counteract the immunosuppressive conditions of the TME.[210] Tumor-associated macrophages (TAMs), which are abundant in the TME, play a pivotal role in immunometabolic regulation by secreting arginase—which depletes arginine—and vascular endothelial growth factor (VEGF)—which promotes abnormal angiogenesis and hypoxia.[211] To target

TAMs, Feng et al developed upconversion nanoparticles camouflaged with TAM membranes (NPR@TAMM) to deliver a colony-stimulating factor 1 receptor (CSF1R) inhibitor into tumors. This strategy exerts a dual function: on one hand, it depletes the TAM survival factor CSF-1, disrupting TAM–tumor cell interactions; on the other, it induces immunogenic cell death (ICD) via photodynamic therapy, thereby directly eliminating a subset of TAMs.[212] As a result, TAMs were repolarized from a tumor-promoting M2 phenotype to an antitumor M1 phenotype, with concurrent blockade of the CSF-1/CSF1R axis, leading to significant attenuation of immunosuppression within the TME.[212] This dual modulation of metabolism and immunity markedly enhanced T cell infiltration and activation. Similarly, neutrophil membrane–mimetic nanoparticles have been designed to adsorb and deplete myeloid-derived suppressor cells (MDSCs), another key suppressive population in the TME. Wang et al reported that granulocyte membrane–coated nanoparticles (pCS) significantly reduced MDSC accumulation in both tumor tissues and lymphoid organs in murine tumor models. This intervention restored T lymphocyte function and, when combined with anti–PD-1 therapy, led to a substantial extension in overall survival.[213] Collectively, these findings demonstrate that targeting metabolism-regulatory immune cells via nanomaterials provides an effective means to reshape the immunometabolic landscape of tumors, thereby indirectly optimizing the metabolic fitness of T cells and enhancing their antitumor activity.

Remodeling the tumor metabolic environment confers multifaceted benefits to T cells. For instance, in a murine model treated with nano-delivered AB680 (a CD73 inhibitor) in combination with αPD-L1 antibody, the reduction of adenosine and PD-L1 blockade synergistically led to a marked increase in tumor-infiltrating cytotoxic T lymphocytes (CTLs), eliciting potent antitumor activity.[93] Similarly, oxygen-releasing MnO_2 nanozymes alleviated tumor hypoxia, thereby enhancing the efficacy of photodynamic therapy (PDT) in bladder tumors. This not only resulted in direct tumor ablation but also downregulated hypoxia-inducible immunosuppressive factors such as HIF-1α and VEGF, thereby improving the responsiveness of subsequent immunotherapeutic interventions.[128] In the context of TAM/MDSC depletion or repolarization, murine models frequently exhibit simultaneous inhibition of both primary and metastatic tumor growth. These outcomes are largely attributed to global immunological reprogramming, characterized by reduced regulatory T cell (Treg) populations and elevated levels of Th1-polarizing cytokines such as IL-12, ultimately enhancing T cell–mediated immune surveillance.[214] In conclusion, precise metabolic intervention using nanomaterials offers a robust strategy to eliminate physiological barriers impeding T cell functionality. By targeting both tumor metabolism and immunosuppressive cellular components, these platforms provide the necessary conditions for unleashing effective antitumor immunity—thus representing a powerful paradigm in the integration of immunotherapy and metabolic modulation.

Improve T-Cell Tumor Infiltration and Persistence

Effective tumor infiltration and sustained functionality of T cells are pivotal for the long-term success of immunotherapy in BC. However, the tumor immune microenvironment is typically enriched with immunosuppressive cells, inhibitory cytokines, and physical barriers such as the extracellular matrix (ECM), which collectively restrict T cell infiltration and induce functional exhaustion.[202] To address these challenges, nanomaterials have emerged as multifunctional regulatory platforms that enhance T cell infiltration and persistence by precisely delivering immunomodulators and remodeling the TME.[215] For instance, as reviewed by Suphiya Parveen et al, organic nanomaterials such as poly(lactic-co-glycolic acid) (PLGA) nanoparticles can be used to encapsulate interleukin-12 (IL-12), promoting T cell proliferation and interferon-γ (IFN-γ) secretion, thereby reversing the immunosuppressive TME and significantly increasing CD8+ T cell infiltration into tumors. The biodegradability and controlled release properties of PLGA (particle size ~100–200 nm; drug loading efficiency >80%) ensure sustained IL-12 exposure at the tumor site while minimizing systemic toxicity and enhancing tumor targeting via the enhanced permeability and retention (EPR) effect.[216] However, organic nanomaterials alone may not suffice to overcome physical barriers within tumors. Inorganic nanomaterials such as mesoporous silica nanoparticles (MSNs) are commonly employed to deliver ECM-degrading enzymes (eg, hyaluronidase) to break down stromal components and facilitate T cell penetration.[135] MSNs, with a high surface area (>800 m^2/g) and pH-responsive release profiles, allow for precise enzyme delivery under acidic TME conditions, markedly improving T cell infiltration into tumor cores and enhancing antitumor efficacy in BC models.[217] Moreover, membrane-biomimetic nanomaterials enhance tumor targeting via cell membrane

camouflage (eg, macrophage membrane coating). These materials retain critical surface markers such as CD47, which transmits a "don't phagocytose me" signal to immune cells, thereby prolonging systemic circulation (>48 h) and enabling the targeted depletion of myeloid-derived suppressor cells (MDSCs), ultimately promoting sustained T cell infiltration and functional persistence.[218]

In terms of persistence, nanomaterials can mitigate T cell exhaustion by modulating cellular metabolism. For instance, inorganic gold nanoparticles (AuNPs) loaded with rapamycin have been shown to inhibit the mTOR pathway, thereby promoting the differentiation of T cells into memory phenotypes and providing antioxidant protection to preserve mitochondrial function—ultimately extending T cell survival within the TME. In addition, stimulus-responsive nanomaterials—such as pH- or ROS-sensitive systems—can locally release metabolic supplements (eg, glucose) within tumors, enhancing glycolytic metabolism in T cells and downregulating the expression of exhaustion markers such as PD-1 and LAG-3.[219] Oxidative stress is another major contributor to T cell dysfunction. Nanomaterials with intrinsic antioxidant properties, such as cerium oxide nanoparticles (CeNPs), are capable of scavenging reactive oxygen species (ROS), thereby preserving mitochondrial integrity and sustaining T cell effector functions.[220] One study demonstrated that CeNPs significantly reduced intracellular ROS levels in T cells and maintained their cytotoxic capacity within tumors.[221] This approach is particularly beneficial in oxidative-stress-prone tumors such as BC.[86] Furthermore, nanomaterials can be engineered into scaffolds or hydrogels to mimic lymphoid niches, supporting T cell metabolism and functional maintenance.[222] For example, nanofiber-based hydrogel scaffolds have been shown to enhance the metabolic activity of T cells in vitro while downregulating exhaustion markers like PD-1 and LAG-3.[223] In the context of BC therapy, such technologies may be integrated with adoptive T cell transfer strategies to further improve clinical efficacy.[91] Collectively, these multifunctional nanoplatforms not only alleviate physical and biological barriers to T cell infiltration but also enhance metabolic adaptability and long-term functionality, offering promising avenues for improving both the response rate and durability of immunotherapy in BC models.

The Risks and Limitations of Nanomaterials

While nanomaterials hold great potential in enhancing the efficacy of cancer therapies, it is essential to consider their potential risks and limitations. The size, shape, and surface characteristics of nanomaterials play a crucial role in their biocompatibility and toxicity.[224] Long-term exposure to nanomaterials may lead to their accumulation in the body, causing immune responses, cytotoxicity, or tissue damage.[225] For instance, certain nanoparticles, such as metallic oxide and metal nanoparticles, have been shown to induce oxidative stress or damage cellular structures, resulting in inflammation or tissue injury. In addition to their direct impact on tissues, the long-term accumulation of nanomaterials may pose chronic toxicity risks, especially with repeated usage in cancer therapies. Incomplete clearance or retention of nanomaterials in the body can lead to adverse effects on various organs. Therefore, the safety profile of nanomaterials must be thoroughly evaluated in preclinical and clinical trials to ensure that their benefits outweigh any potential harm. Moreover, the environmental impact of nanomaterials must not be overlooked. The production, use, and disposal of nanomaterials can lead to their release into the environment, where they may affect ecosystems, particularly aquatic organisms. This environmental concern necessitates careful monitoring and regulation of nanomaterial use to prevent ecological disruptions. Efforts to mitigate these risks include designing nanomaterials with improved biodegradability, lower toxicity, and enhanced biocompatibility.[226] Personalized treatment approaches, such as precision medicine, may also help reduce adverse effects by tailoring therapies to individual patients, thus optimizing safety. Additionally, more research is needed to develop methods for the safe disposal or recycling of nanomaterials, ensuring their environmental sustainability. These considerations highlight the need for continued research on the safety and environmental impact of nanomaterials in cancer treatment. By improving their design and developing better safety protocols, nanomaterials can be utilized more effectively while minimizing risks.

Conclusions and Future Research Directions

Nanotechnology is rapidly reshaping the paradigm of T cell–based immunotherapy for BC. Innovations span from urease-powered nanomotors capable of self-propelling deep into bladder mucosa to deliver STING agonists—achieving over a 10-

fold increase in CD8+ T cell infiltration and a tumor inhibition rate of 94.2% in murine models[94] —to macrophage-mimicking membrane vesicles that co-deliver CD73 inhibitors and αPD-L1 antibodies, effectively disrupting the adenosine–PD-L1 dual immunosuppressive axis.[93] Other advances include immune–photothermal hybrid nanoplatforms that encapsulate cuproptosis inducers and photothermal agents within Tim-3+ T cell membranes, simultaneously overcoming thermotolerance and T cell exhaustion.[129] These frontier approaches highlight the potential of multifunctional, programmable, and biomimetic nanocarriers to penetrate bladder barriers, modulate metabolic and checkpoint axes, and catalyze a shift toward the "fourth-generation" of logic-gated, multimodal immunotherapeutics.[86,227,228]

Despite these promising advancements, several critical challenges continue to hinder the clinical translation of nanomaterial-assisted T cell immunotherapy for BC.[86] One of the foremost obstacles lies in the intratumoral heterogeneity across BC subtypes, particularly between NMIBC and MIBC. These subtypes exhibit distinct TME compositions and immune evasion mechanisms, which may lead to inconsistent therapeutic responses.[135,229,230] From the perspective of nanomaterials, biosafety concerns remain paramount, especially regarding their long-term biocompatibility and immunogenicity.[231,232] For instance, although metallic nanoparticles such as gold nanorods (AuNRs) have demonstrated robust photothermal activation of immune responses, they may also induce off-target inflammatory reactions or accumulate in non-tumor tissues, thereby exacerbating adverse effects. Moreover, the enhanced permeability and retention (EPR) effect, a foundational principle for passive tumor targeting by nanoparticles, has shown limited reliability in human tumors compared to xenograft models. This underscores an urgent need for more physiologically relevant, patient-derived tumor models to more accurately predict clinical outcomes and nanoparticle performance.[219] The promising potential of nanomaterial-based therapies for BC, but several barriers to their clinical application remain. The scalability of nanoparticle production remains an obstacle. Achieving consistent quality and reproducibility at a large scale is a significant hurdle, as even small variations in particle size, surface properties, or formulation can affect their therapeutic efficacy and biodistribution. Additionally, tumor targeting and penetration are still major challenges. The dense extracellular matrix and high interstitial pressure of the TME hinder effective delivery and penetration of nanoparticles. Lastly, regulatory considerations pose a significant challenge, as nanomedicines do not fully fit within existing drug approval frameworks.[104,233] Regulatory agencies, such as the FDA and EMA, are still developing appropriate guidelines for nanomedicines, requiring tailored testing protocols for long-term toxicity, immune responses, biodegradability, and manufacturing standards. In conclusion, while nanomaterial-based therapies for BC show great promise, addressing these barriers—ranging from safety and production to regulatory frameworks—will be essential for their clinical translation. Overcoming these challenges will enable the development of safer and more effective therapeutic options for BC patients.

We propose that future research in nanomaterial-enhanced T cell immunotherapy for BC should focus on four key directions: First, integrating multi-dimensional single-cell and spatial transcriptomics to decode the complex crosstalk among nanomaterials, T cells, metabolism, and the microbiome. This will guide personalized nanotherapeutic design, offering tailored treatment and prognosis strategies for BC patients and advancing precision medicine;[234,235] Second, the development of cascade-responsive or logic-gated nanoplatforms capable of on-demand release of metabolic modulators, immune checkpoint inhibitors, and memory-supporting cytokines, thereby enabling multifunctional theranostics and achieving a one-stop transition from "cold" to "hot" to "memory" tumors;[146,236] Third, employing 3D organoid–microfluidic systems combined with real-time imaging to dynamically assess pharmacokinetics, pharmacodynamics, and immune responses, facilitating a seamless translation from bench to bedside.[237,238] In parallel, clinical challenges in BC treatment should actively inform basic science, uncovering new questions and inspiring novel mechanistic insights.[166] In recent years, artificial intelligence (AI) and machine learning (ML) have rapidly emerged as a "second engine" in nanomedicine research. Their potential is especially prominent in BC T cell immunotherapy, where multi-parametric optimization—encompassing nanoparticle size, surface ligands, delivery kinetics, and immunomodulatory profiles—is essential. Emerging studies suggest that AI-driven nanomaterial design is shifting from "predict-and-optimize" to "generate-and-self-evolve", with the prospect of creating ultra-personalized delivery systems adapted to the high-salinity, flushing-prone bladder environment and patient-specific antigenic landscapes.[239–242] However, current models remain constrained by limited datasets, measurement noise, and batch-to-batch variability across laboratories. Most datasets lack negative results and long-term toxicity annotations, limiting model generalizability and

interpretability, and failing to meet GMP-grade safety and quality standards for regulatory approval.[149,243] Looking forward, we believe that only under a triadic framework combining precision delivery, systemic immune modulation, and industrial-grade quality control, can nanotechnology truly break through the ceiling of recurrence and therapeutic resistance. This would ultimately lead to a new era of reproducible, affordable, and sustainable precision immunotherapy for BC.[219,244] We look forward to future breakthroughs and the emergence of diverse, effective strategies to combat BC.

Data Sharing Statement

No datasets were generated or analysed during the current study.

Consent for Publication

All the authors have read and approved the manuscript.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests.

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