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The Central Role of Immune Checkpoint Receptors in Genitourinary Tumor Immunotherapy: Mechanisms, Biomarkers, and Therapeutic Landscape

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Abstract: Immune checkpoint receptors (ICRs) play a pivotal role in modulating antitumor immunity and have become central targets in the immunotherapy of genitourinary (GU) malignancies. This review provides a comprehensive overview of the fundamental mechanisms of ICR signaling, the expression and pathophysiological roles of these receptors in GU cancers (kidney, bladder, prostate, testicular, and penile), and the evolving therapeutic landscape. Key ICRs, including PD-1, CTLA-4, LAG-3, TIM-3, and TIGIT, orchestrate complex signaling cascades that can lead to T-cell exhaustion and tumor immune evasion. Their expression varies significantly across GU cancer types, histological subtypes, and tumor stages, influencing prognosis and therapeutic response. Immune checkpoint inhibitors (ICIs) reinvigorate antitumor immunity by disrupting these inhibitory pathways and remodeling the tumor microenvironment (TME); however, resistance mechanisms (primary, adaptive, and acquired) and immune-related adverse events (irAEs) pose significant clinical challenges. Established biomarkers such as PD-L1 expression, tumor mutational burden (TMB), and microsatellite instability (MSI)/deficient mismatch repair (dMMR) status guide ICI use, but their predictive power has limitations. Consequently, emerging tissue-based (e.g., immune cell signatures, multiplex IHC/IF, spatial transcriptomics), liquid biopsy-based (e.g., ctDNA, CTCs, exosomes), and imaging-based (radiomics, AI-driven analysis) biomarkers are under active investigation to refine patient selection and monitor treatment efficacy. The therapeutic armamentarium is rapidly expanding with novel ICIs targeting new receptors, bispecific antibodies, and innovative combination strategies involving ICIs with chemotherapy, targeted therapies, radiotherapy, and other immunotherapies. Furthermore, ICIs are increasingly explored in neoadjuvant, adjuvant, and maintenance settings. This review highlights the dynamic progress in understanding ICR biology and its clinical translation, emphasizing the ongoing efforts to develop more personalized and effective immunotherapeutic strategies for patients with genitourinary tumors.

Keywords: immune checkpoint inhibitors; immune checkpoint receptors; genitourinary malignancies; tumor microenvironment; biomarkers; immunotherapy; combination therapy

1. Introduction

The intricate interplay between the immune system and cancer cells is substantially modulated by immune checkpoint receptors (ICRs), which are critical regulators of immune homeostasis. In the context of malignancy, these receptors and their signaling pathways are frequently exploited by tumors to evade immune surveillance and destruction, primarily by inducing a state of T-cell dysfunction, such as exhaustion or anergy [1]. A comprehensive understanding of the ligands, downstream signaling cascades, structural interactions, and regulatory mechanisms governing ICR expression is fundamental to advancing cancer immunotherapy.

1.1. Key Immune Checkpoint Receptors and T-Cell Regulation

Several key ICRs, including programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), lymphocyte activation gene 3 (LAG-3), T-cell immunoglobulin and mucin

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domain 3 (TIM-3), and T-cell immunoreceptor with Ig and ITIM domains (TIGIT), play pivotal roles in dampening antitumor immune responses.

PD-1, prominently expressed on activated T cells, interacts with its ligands PD-L1 and PD-L2. This engagement recruits the phosphatase SHP-2 to cytoplasmic immunoreceptor tyrosine-based switch motifs (ITSMs) within PD-1, leading to the dephosphorylation of crucial signaling molecules like ZAP-70 and the attenuation of PI3K/AKT pathways, thereby suppressing T-cell activation [2–5]. Notably, PD-L1 can also directly promote tumor invasiveness by activating signaling pathways such as β-catenin and WIP [5,6].

CTLA-4 primarily functions during the initial T-cell activation phase by competing with the co-stimulatory receptor CD28 for binding to CD80/CD86 on antigen-presenting cells. This interaction diminishes T-cell survival and proliferation [1,7]. Inhibition of CTLA-4 can enhance T-cell activity, evidenced by increased secretion of cytokines like IL-2 and IFN-γ [1].

LAG-3 negatively regulates T-cell proliferation and function by binding to MHC class II molecules, inhibiting T-cell receptor (TCR) signaling and downregulating CD40L expression [8]. LAG-3 often acts synergistically with PD-1, amplifying immunosuppression within the tumor microenvironment (TME), suggesting that co-blockade strategies may be particularly effective [1,9].

TIM-3 contributes to T-cell exhaustion through interaction with ligands such as galectin-9, which can induce T-cell apoptosis or skew T-cells towards a regulatory phenotype [10]. TIM-3 expression is frequently upregulated in chronic infections and tumors, indicative of its role in maintaining immune tolerance and T-cell anergy [8,11].

TIGIT suppresses the activation of both CD8+ T cells and Natural Killer (NK) cells. Its ligand, PVR (Poliovirus receptor), is frequently expressed on tumor cells, providing a direct mechanism for immune inhibition [1,12]. Blockade of TIGIT has shown promise in rejuvenating exhausted T cells [13,14].

The concerted action of these ICRs, often involving overlapping downstream pathways like PI3K/AKT [15], and the chronic upregulation of immunosuppressive cytokines such as IL-10 and TGF-β, solidify T-cell exhaustion within the TME [16,17].

1.2. Immune Checkpoint Receptor Signaling Beyond T-Cells

While T-cells are primary targets, ICR signaling also profoundly impacts other immune cell populations, contributing to tumor immune evasion. NK cells, crucial for innate antitumor defense, can be inhibited by receptors like NKG2A, which binds to HLA-E on tumor cells [18,19]. PD-1 is also expressed on NK cells, and its engagement with PD-L1 diminishes NK cell cytotoxicity and cytokine secretion [20,21]. Similarly, CTLA-4 blockade can augment NK cell effector functions [22].

B-cells, increasingly recognized for their role in tumor immunity, express ICRs like PD-1 and CTLA-4. PD-1 expression on activated B-cells limits their proliferation and antibody production in the TME [23,24]. Soluble forms of ICRs, such as soluble PD-1, can also inhibit B-cell activities [25], highlighting the complexity of checkpoint-mediated B-cell regulation [26,27].

Myeloid-Derived Suppressor Cells (MDSCs) promote tumor growth by suppressing antitumor immunity, and their expansion and function are enhanced by ICR expression, including PD-1 and CTLA-4 [28,29]. PD-1 blockade can decrease MDSC accumulation, thereby improving T-cell responses [28].

Dendritic Cells (DCs), essential for antigen presentation, can also be functionally impaired by ICR signaling. PD-1 and CTLA-4 engagement can hinder DC maturation and their T-cell activating capacity [21,24,29]. Modulating ICR signals in DCs, potentially in combination with DC vaccines, is an emerging therapeutic strategy [30,31].

1.3. Structural Insights into Immune Checkpoint Receptor-Ligand Interactions

Advanced structural biology techniques, particularly X-ray crystallography and cryoelectronic microscopy (cryo-EM), have provided high-resolution insights into ICR-ligand interactions, informing the development of novel therapeutics. Crystallographic studies of the PD-1/PD-L1 complex have detailed the binding interface, revealing key residues and conformational changes crucial for inhibitory signaling [32,33]. This structural knowledge underpins the design of small molecule inhibitors targeting this interaction [33].

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Cryo-EM offers complementary insights, particularly regarding the multimeric states and dynamic interactions of ICRs like PD-1/PD-L1 within their physiological membrane environment [34,35]. Similar structural characterizations of CTLA-4, LAG-3, and TIM-3 have elucidated their binding mechanisms with respective ligands (e.g., CD80/CD86 for CTLA-4, MHC class II for LAG-3, galectin-9 for TIM-3), thereby guiding the development of specific blocking antibodies and small molecule inhibitors [32,36].

The integration of these structural data with computational methods, such as molecular docking and time-resolved crystallography, accelerates the discovery and optimization of therapeutic compounds [37–39]. Future advancements, including serial femtosecond crystallography (SFX) and high-throughput crystallography, promise to further refine our understanding of these dynamic molecular interactions and uncover new therapeutic targets [40–42].

1.4. Impact of Antigen Exposure Chronicity on Immune Checkpoint Receptor Function

The expression and function of ICRs are dynamically regulated by the nature of antigen exposure. During acute antigen exposure, T-cells mount robust responses with typically low ICR expression. However, chronic antigen exposure, characteristic of the TME or chronic infections, leads to sustained ICR upregulation (e.g., PD-1, CTLA-4, TIM-3) and progressive T-cell exhaustion, characterized by diminished effector functions and proliferative capacity [43–46]. This physiological response, aimed at preventing immunopathology, is co-opted by tumors for immune evasion [47–49].

The state of T-cell exhaustion driven by chronic antigen stimulation, often involving underlying epigenetic modifications, poses a significant challenge for immunotherapy [50–52]. While checkpoint blockade can be effective, particularly in inflamed TMEs, deeply exhausted T-cells may require combination therapies that address multiple inhibitory pathways or T-cell metabolic reprogramming to restore function [53–56].

1.5. Epigenetic and Transcriptional Regulation of Immune Checkpoint Receptor Expression

The expression of ICRs on both tumor and immune cells is tightly controlled by a complex network of epigenetic and transcriptional mechanisms. DNA methylation plays a crucial role; for instance, hypermethylation of the PDCD1 (PD-1) gene promoter can correlate with reduced PD-1 expression and potentially improved antitumor immunity, while hypomethylation of CD274 (PD-L1) may predict better responses to PD-1/PD-L1 blockade [57–59].

Transcriptional regulation is heavily influenced by the TME. Hypoxia, a common feature, can drive PD-L1 expression via hypoxia-inducible factors (HIFs) like HIF1α [60]. Pro-inflammatory cytokines, such as IL-6, can also enhance PD-1 and PD-L1 expression through pathways like JAK/STAT, contributing to a chronic inflammatory state conducive to T-cell exhaustion [61]. Non-coding RNAs, including long noncoding RNAs (lncRNAs) and microRNAs (miRNAs), are emerging as significant regulators. Specific lncRNAs can modulate ICR gene expression, while certain miRNAs can target ICR mRNAs (e.g., PD-L1 mRNA) for degradation, thereby influencing protein expression and immune cell activity [62]. These regulatory layers offer potential therapeutic targets, with epigenetic modifying drugs being explored in combination with immunotherapy to resensitize tumors [63].

2. Expression and Pathophysiological Roles

The expression patterns and pathophysiological significance of ICRs exhibit considerable heterogeneity across different genitourinary (GU) malignancies, including kidney, bladder, prostate, testicular, and penile cancers. These variations, influenced by histological subtype, tumor stage, and the unique TME, have profound implications for disease progression, prognosis, and the efficacy of immunotherapeutic interventions.

2.1. Comparative Expression Patterns of Immune Checkpoint Receptors Across Genitourinary Cancers

Recent analyses reveal distinct ICR expression profiles in GU cancers. In renal cell carcinoma (RCC), particularly clear cell RCC (ccRCC), PD-L1 expression is often elevated and correlates with higher tumor burden and advanced stage [64,65]. Conversely, CTLA-4 expression in papillary RCC (pRCC) does not

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appear to vary significantly with tumor subtype or stage [66]. Non-clear cell RCC types may show common elevation of PD-1 and PD-L1, while other checkpoints like LAG-3 are variably expressed [64].

In bladder cancer, specifically urothelial carcinoma, PD-L1 expression is significantly higher in muscle-invasive bladder cancer (MIBC) compared to non-muscle invasive bladder cancer (NMIBC), especially in basal subtypes [67]. Higher PD-L1 expression generally correlates with advanced stages and an immune-infiltrated TME [67,68].

Prostate cancer typically exhibits lower PD-L1 expression compared to other GU malignancies. However, metastatic castration-resistant prostate cancer (mCRPC) with high microsatellite instability or mismatch repair deficiencies can respond to immunotherapy [69]. While CTLA-4 and PD-1 expression may vary with tumor aggressiveness, their overall levels remain relatively low [70].

Testicular cancer generally shows lower ICR expression, though some non-seminomatous germ cell tumors may exhibit increased levels [64]. Penile cancer research is less extensive, but initial studies suggest high PD-L1 expression may correlate with advanced disease [70].

These expression patterns are further nuanced by histological subtypes, such as the basal versus luminal subtypes in bladder cancer showing varied PD-L1 expression and distinct TMEs [65,67]. Tumor stage also plays a critical role, with progressive ICR expression often observed with advancing disease across various GU cancers [64,68].

2.2. Immune Checkpoint Receptor Expression, Localization, and Clinical Outcomes

The expression levels and localization of ICRs within the TME of GU cancers are increasingly linked to patient prognosis and immunotherapy response. In MIBC, high PD-L1 expression is associated with better outcomes following treatment with PD-1/PD-L1 inhibitors like pembrolizumab and atezolizumab [71]. Higher densities of PD-L1-expressing cells often correlate with increased CD8+ T-cell infiltration, a hallmark of an active antitumor immune response [71].

In RCC, high PD-L1 expression can be a negative prognostic marker, correlating with advanced tumor stage [72]. However, it can also predict response to combination immunotherapies [73]. For prostate cancer, CTLA-4 expression on infiltrating T-cells is associated with T-cell dysfunction and poor prognosis, suggesting CTLA-4 as a therapeutic target in mCRPC [73,74]. In urothelial carcinoma, elevated LAG-3 expression, often co-expressed with PD-L1, suggests an additional layer of immune suppression and may predict benefit from dual checkpoint blockade [75]. Similarly, high T-cell immunoglobulin and mucin domain 3 (TIM-3) expression in bladder cancer correlates with a suppressive TME and poor prognosis, marking TIM-3 as a prognostic factor and therapeutic target [76]. These findings underscore the potential of ICR expression and localization as biomarkers to guide personalized therapy, potentially complemented by radiographic assessments of the TME [77].

2.3. Interplay of the Tumor Microenvironment with Immune Checkpoint Receptor Function

The unique cellular and cytokine milieu of the TME in GU cancers critically influences ICR engagement and the functional consequences of their blockade. In MIBC, a high density of tumor-infiltrating lymphocytes (TILs) expressing PD-1 and T-cell immunoreceptor with Ig and ITIM domains (TIGIT) on CD8+ T-cells correlates with immune exhaustion and poor prognosis [78]. Inflammatory cytokines like IL-6 and TGF-β within the bladder cancer TME further promote immune suppression by enhancing checkpoint expression [78].

In RCC, factors like vascular endothelial growth factor (VEGF) within the TME can increase PD-L1 expression on tumor cells, impairing T-cell function [79]. This provides a rationale for combining immunotherapy with anti-angiogenic agents [80]. The prostate cancer TME is often characterized by elevated IL-10 and TGF-β, which upregulate PD-1 and CTLA-4 on T-cells, fostering a suppressive environment [81,82]. Blockade of PD-1 combined with therapies targeting these cytokines shows promise in treatment-resistant models [83]. Even in testicular cancer, which generally has a favorable immune landscape, alterations in local cytokines can lead to adaptive resistance by modulating PD-L1 and TIM-3 expression [79,84].

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Engagement of ICRs like PD-1 and LAG-3 on TILs leads to an exhausted phenotype with diminished effector functions [85,86]. Conversely, checkpoint blockade, particularly dual blockade (e.g., PD-1/CTLA-4), can revert these impairments and enhance antitumor immunity [87,88].

2.4. Novel and Less-Studied Immune Checkpoint Receptors in Genitourinary Malignancies

Beyond the well-established ICRs, research is uncovering the roles of novel or less-studied checkpoints in GU cancer pathogenesis. Molecules like CD24 and CD200 are overexpressed in various GU tumors, contributing to immune evasion by inhibiting T-cell activation and promoting a suppressive TME [26]. Telomerase, specifically human telomerase reverse transcriptase (hTERT), has been identified as a potential checkpoint, as its activity can modulate immune responses by inhibiting cytotoxic T-lymphocyte activation in RCC and bladder cancer [89].

Checkpoints on NK cells, such as KIR, NKG2A, and TIM-3, also regulate antitumor immunity [22]. For instance, elevated NKG2A expression in prostate cancer is linked to reduced NK cell activity. Glyco-immune checkpoints, involving cell-surface glycan interactions with lectin-like receptors, represent another emerging evasion mechanism, with antibody-lectin chimeras (AbLecs) being explored as therapeutics [90]. Phagocytosis checkpoints, notably CD47 (the "don't eat me" signal), are expressed in GU cancers and inhibit macrophage-mediated tumor cell clearance; targeting CD47 is a promising strategy [91]. These novel targets offer potential for combinatorial approaches, possibly with epigenetic modulators, to overcome complex immune evasion mechanisms [90,91].

2.5. Differential Immune Checkpoint Receptor Expression in Primary Versus Metastatic Genitourinary Cancers

Significant differences in ICR expression exist between primary GU tumors and their metastatic sites, impacting treatment strategies. In RCC, the checkpoint CD73, which suppresses T-cell responses, shows notably higher expression in metastatic lesions compared to primary tumors, suggesting a mechanism for enhanced immune evasion at distant sites [92]. This highlights CD73 as a potential target in metastatic RCC [93].

In bladder cancer, primary tumors often exhibit higher TIL infiltration and ICR expression (e.g., PD-L1) than their metastases. Metastatic lesions may feature altered cytokine profiles, such as increased IL-8, associated with immunosuppression and reduced response to PD-1/PD-L1 blockade [94]. Similarly, metastatic castration-resistant prostate cancer frequently displays elevated PD-L1 levels in metastases compared to primary tumors, contributing to a more immunosuppressive microenvironment [69,95].

These disparities necessitate tailored therapeutic approaches. For instance, the upregulation of specific checkpoints like CD73 in metastases could guide the use of targeted therapies with conventional ICIs. The heterogeneous immune infiltration at different metastatic sites, such as brain metastases regularly having fewer TILs, also influences ICI efficacy [96]. Furthermore, immune evasion mechanisms involving factors like DUX4, an embryonic transcription factor suppressing IFN-γ signaling, are prevalent in metastatic GU cancers and correlate with poor immunotherapy responses, marking DUX4 as another potential therapeutic target [97].

3. Mechanistic Insights into Immune Checkpoint Blockade in Genitourinary Tumor Immunotherapy

ICIs have revolutionized the treatment landscape for various malignancies, including GU cancers. Their efficacy hinges on reinvigorating the host's antitumor immune responses by disrupting inhibitory signaling pathways. However, the mechanisms underlying their action, the dynamic remodeling of the TME, the emergence of resistance, the influence of systemic factors like the gut microbiome, and the occurrence of immune-related adverse events (irAEs) are complex and multifaceted. Understanding these mechanistic insights is crucial for optimizing ICI therapy in GU cancers.

3.1. Molecular and Cellular Mechanisms of Immune Reinvigoration by ICIs

Recent research has identified several key mechanisms by which ICIs specifically reinvigorate antitumor immunity in GU cancers, considering their distinct immune landscapes. Beyond direct T-cell

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derepression, ICIs induce broader changes within the TME. Reprogramming of myeloid cells is one such mechanism; for instance, JAK inhibition with ruxolitinib can modify the myeloid cell compartment, enhancing T-cell responses and improving therapeutic outcomes when combined with PD-1/CTLA-4 blockade [98]. This suggests that targeting myeloid subsets can create a more permissive environment for T-cell engagement.

The regulation of immune checkpoint gene expression itself is also critical. The innate immune-modulating gene OAS1 has been shown to influence the expression of various checkpoint genes, thereby directly affecting antitumor immune responses and potentially serving as a prognostic biomarker [99]. Furthermore, emerging checkpoint molecules like CD24 and CD200, which can regulate immune cell interactions and promote immune evasion, represent novel targets whose inhibition may restore T-cell functionality and augment the efficacy of existing ICIs [26].

Activation of innate immune pathways can synergize with ICIs. Toll-like receptor (TLR) agonists, such as TLR5 agonists, can shift macrophage polarization from a pro-tumor (M2) to an antitumor (M1) state and promote CD8+ T-cell priming, thereby enhancing the efficacy of anti-PD-1 therapy [100]. Moreover, inducing specific forms of immunogenic cell death, like pyroptosis with agents such as nigericin, can bolster antitumor immunity and increase the effectiveness of PD-1 inhibitors [101]. These findings highlight the importance of modulating both innate and adaptive immune responses, as well as the TME itself (e.g., counteracting immunosuppressive cytokines like TGF-β and IL-10), to maximize ICI efficacy [100,102].

3.2. Dynamic Remodeling of the Tumor Microenvironment by ICI Therapy

ICI therapy induces significant dynamic remodeling of the TME, which differs between responsive and non-responsive GU cancer patients. These changes involve alterations in immune cell infiltrates, cytokine profiles, and tumor cell characteristics.

In responsive bladder cancer patients, ICIs enhance the infiltration of TILs, particularly CD8+ cytotoxic T-cells, and increase pro-inflammatory cytokines like IFN-γ [103]. Conversely, non-responders often show reduced TIL functionality and elevated levels of immunosuppressive cells like Tregs [104]. Similar patterns are seen in renal cell carcinoma (RCC), where increased densities of effector T-cells and NK cells in the TME correlate with improved progression-free survival following PD-1 blockade [105,106].

Cytokine networks within the TME are also profoundly affected. In prostate cancer, elevated IL-6 and IL-2 levels are linked to T-cell activation post-PD-1/PD-L1 blockade [107]. Responders generally exhibit increased anti-inflammatory cytokines, promoting T-cell effector functions [108], while non-responders typically maintain high levels of immunosuppressive cytokines like TGF-β and IL-10 [109].

Tumor cells themselves adapt to ICI therapy. In bladder cancer, resistant tumor cells frequently upregulate PD-L1 expression, an adaptive resistance mechanism [103]. ICIs can also modify the expression of chemokines and their receptors; for example, upregulation of CXCL8 post-ICI treatment can recruit additional immune cells to the TME, potentially enhancing therapeutic responses [110]. These dynamic changes underscore the need for personalized approaches, potentially combining ICIs with agents targeting immunosuppressive cytokines or informed by tumor mutational burden (TMB) and cytokine profiles [111,112].

3.3. Mechanisms of Resistance to Immune Checkpoint Blockade

Resistance to ICIs, whether primary, adaptive, or acquired, remains a significant clinical challenge in GU cancers. Primary resistance can be driven by intrinsic tumor characteristics. Genetic alterations, such as mutations in the B2M gene (essential for MHC class I antigen presentation), impair tumor antigen display and T-cell recognition in bladder and renal cancer [113]. Downregulation of MHC molecules further contributes to this antigen presentation deficiency [114].

Adaptive resistance involves changes within the TME that allow tumors to evade ICI effects. The TIGIT/CD155 axis, for example, mediates immune suppression; elevated CD155 levels can inhibit T-cell activity via TIGIT engagement [115]. Immunosuppressive cytokines like IL-10 and TGF-β also promote Treg activity and inhibit effector T-cells, facilitating escape from ICI efficacy [114].

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Acquired resistance develops as tumors evolve under therapeutic pressure. ICIs can lead to the upregulation of alternative immune checkpoints (e.g., CTLA-4, LAG-3) on TILs, diminishing therapeutic effects over time [116]. Activation of oncogenic signaling pathways, such as Wnt/β-catenin, can decrease T-cell infiltration [117]. Furthermore, increased recruitment of immunosuppressive myeloid cells and enhanced PD-L1 expression on resistant tumor cells contribute to acquired resistance [116,118]. Addressing these multifaceted resistance mechanisms necessitates combination therapies targeting multiple pathways, personalized approaches based on tumor and TME profiling, and strategies to remodel the TME, such as using nanoparticles or oncolytic viruses with ICIs [119–122].

3.4. Influence of the Gut Microbiome on ICI Efficacy

The gut microbiome has emerged as a critical modulator of ICI efficacy in GU cancers. Microbial diversity is positively associated with favorable responses to PD-1 blockade, potentially by enhancing T-cell priming [123]. Specific bacterial species, such as certain strains of Bifidobacterium and Lactobacillus, correlate with increased CD8+ T-cell infiltration and activation, leading to better treatment outcomes [124].

Mechanistically, the gut microbiome influences systemic immunity by modulating cytokine production. Responders to ICIs often exhibit upregulated pro-inflammatory cytokines like IL-12 and TNF-α, while dysbiosis can promote immunosuppressive cytokines like IL-10 [124]. Microbial metabolites, such as short-chain fatty acids (SCFAs), can enhance T-cell activation and cytokine production [125]. The microbiome can also shape antigen presentation machinery and even upregulate PD-L1 expression on tumor cells [126,127].

Conversely, certain microbial species can contribute to ICI resistance. Bacteroides fragilis has been associated with increased Treg levels in the TME [128]. Imbalanced microbiomes may also lead to nutrient competition, impairing T-cell function [129]. These insights are paving the way for personalized microbiome-based therapies, such as fecal microbiota transplantation (FMT) or specific pre/probiotics, often in combination with ICIs, to improve clinical outcomes [124,130].

3.5. Off-Target Effects and Mechanisms of Immune-Related Adverse Events

While ICIs unleash antitumor immunity, they can also trigger irAEs by disrupting self-tolerance. Blockade of PD-1, PD-L1, and CTLA-4 can lead to immune activation against normal tissues, causing conditions like dermatitis, colitis, hepatotoxicity, and endocrinopathies [131–133]. For example, renal tubular cells in RCC patients can express PD-L1, potentially leading to immune-mediated nephritis upon PD-1 blockade [131].

The concept of shared antigens between tumors and normal tissues is a leading hypothesis for irAEs; T-cells activated against tumor antigens may cross-react with similar antigens on healthy tissues [134,135]. Pre-existing autoantibodies and specific immune profiles (e.g., Treg frequency, cytokine ratios) may predict irAE risk [136,137].

The TME's cytokine milieu also influences irAE severity. Elevated pro-inflammatory cytokines like IL-6 and TNF-α are associated with increased irAE incidence [138]. Furthermore, the phenotype of TILs post-ICI therapy can impact irAE risk; dysfunctional T-cell phenotypes in RCC have been linked to a higher likelihood of irAEs [139]. Managing irAEs involves identifying predictive biomarkers, developing preventive strategies, and exploring combination therapies that mitigate toxicity while preserving antitumor efficacy [136,140,141].

4. Biomarkers to Guide Immune Checkpoint Inhibitor Therapy

The advent of ICIs has significantly advanced the treatment of GU malignancies. However, patient responses to ICIs are variable, underscoring the critical need for robust biomarkers to predict efficacy, monitor response, and guide personalized therapeutic strategies. This section reviews the current clinical utility and limitations of established biomarkers, as well as promising emerging tissue-based, liquid biopsy-based, and imaging-based biomarkers in GU oncology.

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4.1. Established Biomarkers: Clinical Utility and Limitations

Several biomarkers are currently utilized or investigated for their predictive value in ICI therapy for GU cancers, including PD-L1 expression by immunohistochemistry (IHC), TMB, and MSI or deficient mismatch repair (dMMR) status.

PD-L1 expression by IHC is a widely adopted biomarker, particularly in bladder cancer and RCC. Higher PD-L1 expression levels generally correlate with improved responses and survival outcomes in patients treated with PD-1/PD-L1 inhibitors [142]. However, its utility is hampered by variability in assay platforms (e.g., 22C3 vs. SP142), scoring algorithms, and cutoff values, necessitating standardization [143]. Furthermore, the spatiotemporal heterogeneity of PD-L1 expression within tumors and between primary and metastatic sites can limit the predictive accuracy of a single biopsy [144,145].

TMB, reflecting the number of somatic mutations per megabase of DNA, has emerged as a predictor of ICI response. A higher TMB is hypothesized to generate more neoantigens, thereby enhancing immunogenicity. In RCC, high TMB has been associated with greater benefit from ICI therapy [142]. Nevertheless, the lack of a standardized definition for "high TMB" across studies poses a challenge [146]. Moreover, TMB does not always correlate with immune infiltration or response, as other factors within the TME or defects in antigen presentation machinery can override the potential benefit of a high neoantigen load [145].

MSI and dMMR status are established pan-cancer biomarkers for ICI response. Tumors with MSI-high (MSI-H) or dMMR status often exhibit a hypermutated phenotype and respond favorably to PD-1 blockade [147]. However, the prevalence of MSI-H/dMMR is relatively low in most GU cancers compared to malignancies like colorectal cancer, limiting its broad applicability [142]. The limitations of these individual biomarkers highlight the need for integrated, multi-biomarker approaches to improve predictive accuracy [145,147].

4.2. Emerging Tissue-Based Biomarkers

Beyond established markers, several promising tissue-based biomarkers are under investigation. Specific immune cell signatures, derived from detailed immunoprofiling of TILs, are gaining traction. Higher densities of CD8+ cytotoxic T-lymphocytes (CTLs) within the TME, particularly those expressing PD-1, correlate with better ICI responsiveness in bladder cancer and RCC [148,149]. Multiomics approaches integrating genomic, transcriptomic, and immunological data are being used to define comprehensive immune signatures predictive of ICI benefit [150].

Multiplex IHC and immunofluorescence (IF) techniques allow for the simultaneous visualization and spatial characterization of multiple immune checkpoint proteins and immune cell types within the tumor tissue. This provides crucial insights into the immune contexture and cellular interactions within the TME [151]. In bladder cancer, for instance, specific immune cell compositions (e.g., high CD8, CD4, and PD-L1 expressing cells) identified by multiplex IHC have shown a significant correlation with improved responses to PD-1 inhibitors [152].

Spatial transcriptomics represents a cutting-edge approach that maps gene expression patterns within the spatial context of the tissue. This technology can reveal distinct immune microenvironments and cellular interactions associated with treatment response [153]. In bladder tumors, spatial transcriptomics has identified regions with active T-cell responses that correlate with higher ICR expression and improved ICI efficacy [154].

The validation of these emerging tissue-based biomarkers is ongoing through clinical trials and the development of multi-biomarker platforms, often integrated with genomic profiling to enhance predictive power [155–157].

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4.3. Liquid Biopsy-Based Biomarkers

Liquid biopsies, analyzing analytes such as circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and exosomes from bodily fluids, offer a non-invasive means to predict and monitor ICI efficacy.

ctDNA analysis can reveal tumor-specific genetic alterations and TMB. High TMB detected in ctDNA has been validated as a favorable biomarker for ICI response in RCC and bladder cancer [158,159]. Dynamic changes in ctDNA levels during therapy can also serve as a real-time indicator of treatment response, with decreasing levels associated with better clinical outcomes [160,161]. Furthermore, ctDNA methylation patterns are emerging as epigenetic biomarkers, with specific methylation signatures correlating with ICI response [160,161].

CTCs, though less established than ctDNA, hold potential for monitoring disease progression and treatment response. Changes in CTC counts and characteristics during ICI therapy may provide prognostic insights [162,163].

Exosomes, nano-sized vesicles released by cells, carry molecular cargo (proteins, RNAs, metabolites) that can reflect tumor biology and immune status. Profiling exosomal contents, such as immune-related microRNAs or proteins like TGF-β, may predict ICI efficacy and reveal resistance mechanisms [164–166].

Analysis of circulating immune cells using advanced techniques like flow cytometry and single-cell sequencing can reveal dynamic changes in immune cell populations (e.g., activated T-cells, Tregs) that correlate with ICI response [167,168]. While promising, the clinical implementation of liquid biopsy biomarkers faces challenges in standardization, reproducibility, and the integration of multiomic data [166,168–172].

4.4. Radiomics and AI-Driven Analysis of Medical Imaging

Radiomics, the high-throughput extraction of quantitative features from medical images (CT, MRI, PET), coupled with artificial intelligence (AI)-driven analysis, is emerging as a powerful non-invasive tool for predicting ICI response. Machine learning models can correlate radiomic features reflecting tumor phenotype, burden, and heterogeneity with treatment outcomes [173–175].

Deep learning algorithms enhance the ability to identify unique imaging biomarkers and monitor temporal changes in tumor characteristics, facilitating early assessment of ICI efficacy [176–179]. The integration of radiomics with liquid biopsy data, such as ctDNA profiles, offers a synergistic approach to comprehensively assess tumor burden and response [180].

Challenges in radiomics include the need for standardization of imaging protocols and feature extraction methods, as well as validation in large, multi-institutional cohorts to ensure generalizability [181–183]. Future directions involve advanced computational techniques, multi-modal data integration (e.g., imaging with spatial transcriptomics), and the application of these tools in personalized medicine [184–186].

5. Therapeutic Landscape and Evolving Strategies

The therapeutic landscape for GU cancers has been substantially reshaped by the advent of ICIs targeting receptors such as PD-1, PD-L1, and CTLA-4. More recently, novel ICIs targeting emerging receptors like LAG-3, TIM-3, and TIGIT, as well as innovative bispecific antibodies and combination strategies, are further expanding the therapeutic armamentarium. This section reviews pivotal clinical trial results, evolving combination strategies, long-term outcomes, and the impact of ICIs in neoadjuvant, adjuvant, and maintenance settings for GU malignancies.

5.1. Novel Immune Checkpoint Inhibitors and Bispecific Antibodies

Recent clinical development has focused on ICIs targeting novel co-inhibitory receptors to overcome resistance to established PD-1/PD-L1 or CTLA-4 blockade and to enhance antitumor immunity.

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LAG-3 Inhibition: The combination of anti-LAG-3 antibodies, such as relatlimab, with PD-1 inhibitors like nivolumab has shown promising efficacy. A Phase II trial in advanced urothelial carcinoma reported an objective response rate (ORR) of 30% with this combination, notably higher than historical controls for anti-PD-1 monotherapy [187]. Mechanistically, LAG-3 blockade is thought to reinvigorate exhausted T-cells and enhance immune activation, particularly in tumors with high PD-L1 expression and dense TIL infiltration [188].

TIM-3 Inhibition: Targeting TIM-3 is another strategy under investigation. Early trial data in metastatic RCC suggest that TIM-3 monoclonal antibodies, especially in combination with PD-1 or CTLA-4 inhibitors, can enhance antitumor activity compared to monotherapies [189]. TIM-3 blockade appears to foster a more inflammatory T-cell environment within tumors, restoring T-cell functionality [190].

TIGIT Inhibition: TIGIT has emerged as a significant target, particularly for patients who have developed resistance to prior ICIs. Preliminary Phase II trial results of a TIGIT-targeting agent, alone or with PD-1 blockade, demonstrated an ORR of approximately 40% in previously treated metastatic bladder cancer patients [191]. TIGIT blockade can remodel the TME by increasing CD8+ T-cell infiltration and pro-inflammatory cytokine levels [192].

Bispecific Antibodies: Bispecific antibodies, engineered to engage multiple immune targets simultaneously (e.g., PD-L1 and TIGIT), represent an innovative therapeutic class. Preclinical models and early clinical trials in GU malignancies have shown that these agents can improve T-cell activation and reduce tumor growth, offering potential for enhanced efficacy, especially in resistant settings [193,194].

5.2. Combination Strategies Involving ICIs

Combining ICIs with other therapeutic modalities is a key strategy to improve efficacy and overcome resistance in GU cancers.

ICIs with Chemotherapy: The combination of ICIs with chemotherapy has demonstrated synergistic effects. For instance, pembrolizumab plus chemotherapy improved overall survival (OS) and progression-free survival (PFS) in metastatic urothelial carcinoma compared to chemotherapy alone [195]. Chemotherapy can increase tumor antigenicity and enhance TIL infiltration, thereby priming the TME for ICI activity [196–198].

ICIs with Targeted Therapies: Combining ICIs with targeted agents, such as multi-kinase inhibitors, is showing considerable promise. The combination of pembrolizumab with lenvatinib has improved efficacy in advanced RCC and is under evaluation in bladder cancer [199,200]. Similarly, combining ICIs with drugs targeting the VEGF pathway aims to create a less immunosuppressive TME [201].

ICIs with Radiotherapy: Radiotherapy can induce immunogenic cell death and release tumor antigens, potentially synergizing with ICIs to elicit systemic antitumor immunity (the abscopal effect). Combining pembrolizumab with localized radiotherapy has shown improved response rates in some GU cancer settings [202–204].

Novel Immunotherapy Combinations: The integration of ICIs with other immunotherapies, such as Chimeric Antigen Receptor (CAR) T-cell therapy or cancer vaccines, is being explored. CAR-T cells engineered to express PD-1 inhibitors or personalized cancer vaccines combined with PD-1 blockade aim to boost antitumor T-cell responses and overcome resistance [199,205].

Microbiome Modulation: Given the influence of the gut microbiome on ICI efficacy, strategies like fecal microbiota transplantation (FMT) from responders to non-responders are being investigated to enhance treatment outcomes [206].

5.3. Durability of Response and Long-Term Survival Outcomes

Long-term follow-up from pivotal ICI trials provides crucial insights into the durability of responses and survival benefits. In advanced RCC, nivolumab demonstrated sustained OS benefit over everolimus in previously treated patients [207]. Pooled analyses of ipilimumab trials in melanoma showed a plateau in OS rates around three years, suggesting long-term survival for a subset of patients [208], a trend also

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observed in GU cancers treated with ICIs [209]. The KEYNOTE-001 trial of pembrolizumab in advanced melanoma reported durable response rates, with similar trends emerging in GU cancer cohorts [210].

Combination therapies, such as avelumab plus axitinib in treatment-naïve advanced RCC, have also shown significant improvements in long-term PFS and OS compared to sunitinib monotherapy [211]. Durable responses are often associated with robust immune activation, increased TILs, and favorable cytokine profiles (e.g., high IFN-γ, low IL-10/TGF-β) [212].

5.4. ICIs in Neoadjuvant, Adjuvant, and Maintenance Settings

The role of ICIs is expanding beyond metastatic disease into earlier stages of GU cancer treatment.

Neoadjuvant ICI Therapy: Trials like SWOG S1314 (atezolizumab in MIBC) and CheckMate 016 (nivolumab plus ipilimumab in high-risk localized RCC) are evaluating the potential of neoadjuvant ICIs to improve pathological complete response (pCR) rates and surgical outcomes [3,5].

Adjuvant ICI Therapy: In the adjuvant setting, ICIs aim to reduce recurrence rates and improve survival post-definitive local treatment. Trials such as NCT03187305 (pembrolizumab in locally advanced penile cancer) and NCT03249700 (pembrolizumab plus chemotherapy post-cystectomy in MIBC) are exploring this approach [2,4].

Maintenance ICI Therapy: Following first-line therapy for advanced disease, maintenance ICI treatment is being investigated to prolong disease-free survival and maintain quality of life, as seen in trials like KEYNOTE-045 for advanced urothelial carcinoma [6]. Sequential therapies, using ICIs after targeted agents in RCC, are also being explored [7].

The integration of ICIs into these earlier treatment settings, often guided by biomarkers, has the potential to significantly alter future treatment paradigms for GU cancers [213–218].

6. Conclusions

The advent of ICR-targeted immunotherapy has undeniably transformed the therapeutic paradigm for a spectrum of GU malignancies. This review has underscored the central and multifaceted roles of ICRs—including established players like PD-1, CTLA-4, LAG-3, TIM-3, and TIGIT, as well as emerging novel checkpoints—in dictating the intricate balance between antitumor immunity and immune evasion. A fundamental understanding of their signaling pathways, which extend beyond T-cells to modulate the functions of NK cells, B-cells, myeloid cells, and dendritic cells, is paramount. Structural biology insights continue to refine our comprehension of receptor-ligand interactions, informing the development of next-generation therapeutics, while epigenetic and transcriptional mechanisms reveal complex layers of ICR regulation influenced by factors such as chronic antigen exposure.

The expression and pathophysiological significance of ICRs are markedly heterogeneous across different GU cancers (kidney, bladder, prostate, testicular, and penile), varying significantly with histological subtype, tumor stage, and the specific TME. This heterogeneity, observed even between primary tumors and metastatic sites, directly impacts prognosis and the predictive value of ICR expression for therapeutic response. The unique cellular and cytokine milieu within each GU cancer TME further dictates the functional consequences of ICR engagement and blockade, highlighting the need for tumor-specific considerations in immunotherapy.

Mechanistically, ICIs reinvigorate antitumor immune responses not only by direct T-cell derepression but also through broader TME remodeling, including myeloid cell reprogramming, modulation of cytokine profiles, and even influencing tumor cell characteristics. However, the clinical success of ICIs is often challenged by primary, adaptive, and acquired resistance mechanisms. These involve genetic alterations in tumor cells, defects in antigen presentation machinery, dynamic changes in the TME (such as the upregulation of alternative checkpoints or the influx of immunosuppressive cells), and the influence of systemic factors like the gut microbiome. Furthermore, the very mechanism of immune reactivation can lead to irAEs, driven by off-target effects and shared antigen recognition, necessitating careful management and predictive strategies.

The quest for robust biomarkers to guide ICI therapy is critical. While established biomarkers such as PD-L1 IHC, TMB, and MSI/dMMR status offer clinical utility, their limitations—including assay

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variability, tumor heterogeneity, and low prevalence in some GU cancers—are apparent. Consequently, the field is rapidly advancing towards emerging tissue-based biomarkers (e.g., specific immune cell signatures, multiplex IHC/IF, spatial transcriptomics), liquid biopsy-based analytes (ctDNA mutations and methylation patterns, circulating immune cells, exosomes), and non-invasive imaging biomarkers (radiomics and AI-driven image analysis). The integration of these multiomic data streams holds immense promise for developing more accurate predictive and monitoring models.

The therapeutic landscape for GU tumors continues to evolve at a rapid pace. Pivotal clinical trials are evaluating novel ICIs targeting LAG-3, TIM-3, and TIGIT, as well as innovative bispecific antibodies. Combination strategies—pairing ICIs with chemotherapy, targeted therapies, radiotherapy, and other immunotherapies like CAR-T cells or cancer vaccines—are demonstrating enhanced efficacy and are actively being investigated to overcome resistance. Importantly, the role of ICIs is expanding beyond advanced disease into neoadjuvant, adjuvant, and maintenance settings, with the potential to significantly improve long-term survival outcomes and alter standard treatment paradigms.

In conclusion, ICR-targeted immunotherapy represents a cornerstone in the management of GU cancers. Future progress will undoubtedly rely on a deeper mechanistic understanding of ICR biology, the complex tumor-immune interplay within the diverse GU cancer TMEs, and the sophisticated integration of multi-modal biomarkers. These advancements will be instrumental in refining patient selection, overcoming resistance, minimizing toxicities, and ultimately realizing the full potential of personalized and more effective immunotherapeutic strategies for patients with genitourinary malignancies.

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Abbreviations

The following abbreviations are used in this manuscript:

AI	Artificial Intelligence
CAR	Chimeric Antigen Receptor
ccRCC	Clear Cell Renal Cell Carcinoma
CTCs	Circulating Tumor Cells
CTLA-4	Cytotoxic T-Lymphocyte-Associated Protein 4
ctDNA	Circulating Tumor DNA
CTLs	Cytotoxic T-Lymphocytes
DCs	Dendritic Cells
dMMR	Deficient Mismatch Repair
FMT	Fecal Microbiota Transplantation
GU	Genitourinary
HIFs	Hypoxia-Inducible Factors
hTERT	Human Telomerase Reverse Transcriptase
ICIs	Immune Checkpoint Inhibitors
ICRs	Immune Checkpoint Receptors
IF	Immunofluorescence
IFN-γ	Interferon Gamma
IHC	Immunohistochemistry
IL	Interleukin

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irAEs	Immune-Related Adverse Events
ITSMs	Immunoreceptor Tyrosine-Based Switch Motifs
LAG-3	Lymphocyte Activation Gene 3
lncRNAs	Long Noncoding RNAs
MDSCs	Myeloid-Derived Suppressor Cells
MIBC	Muscle-Invasive Bladder Cancer
miRNAs	MicroRNAs
mCRPC	Metastatic Castration-Resistant Prostate Cancer
MSI	Microsatellite Instability
MSI-H	Microsatellite Instability-High
NK	Natural Killer
NMIBC	Non-Muscle Invasive Bladder Cancer
ORR	Objective Response Rate
OS	Overall Survival
PD-1	Programmed Cell Death Protein 1
PD-L1	Programmed Death-Ligand 1
PFS	Progression-Free Survival
pCR	Pathological Complete Response
pRCC	Papillary Renal Cell Carcinoma
PVR	Poliovirus Receptor
RCC	Renal Cell Carcinoma
SCFAs	Short-Chain Fatty Acids
SFX	Serial Femtosecond Crystallography
TCR	T-Cell Receptor
TGF-β	Transforming Growth Factor Beta
TIGIT	T-Cell Immunoreceptor with Ig and ITIM Domains
TILs	Tumor-Infiltrating Lymphocytes
TIM-3	T-Cell Immunoglobulin and Mucin Domain 3
TLR	Toll-Like Receptor
TMB	Tumor Mutational Burden
TME	Tumor Microenvironment
TNF-α	Tumor Necrosis Factor Alpha
VEGF	Vascular Endothelial Growth Factor
WIP	WASP-Interacting Protein

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