TGF-β signaling-mediated immune microenvironment remodeling in urological malignancies: A contributor to immunotherapy resistance and a candidate therapeutic target (Review)

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Abstract. Immune checkpoint inhibitors (ICIs) have transformed the treatment of bladder cancer (BC) and renal cell carcinoma (RCC), whereas prostate cancer (PC) remains intrinsically insensitive to ICIs. In patients with BC and RCC, primary and acquired resistance remain formidable clinical challenges. Transforming growth factor-β (TGF-β) signaling has emerged as a contributor to immunotherapy refractoriness through its multifaceted remodeling of the tumor immune microenvironment. This pathway orchestrates $CD8^+$ T cell exhaustion, regulatory T cell expansion, macrophage polarization toward immunosuppressive phenotypes, cancer-associated fibroblast activation with consequent extracellular matrix remodeling, and metabolic reprogramming that sustains a tolerogenic niche. In patients with BC, PC and RCC, TGF-β pathway activation is correlated with immune exclusion and poor responses to immune checkpoint blockade. Although TGF-β-targeted agents, including ligand-neutralizing antibodies, receptor kinase inhibitors, and TGF-β-resistant cellular immunotherapies, have shown encouraging preclinical and early clinical activity, challenges persist regarding on-target toxicity, the context-dependent dual functionality of TGF-β, and the lack of validated predictive biomarkers for patient selection. The present review comprehensively summarizes the mechanisms underlying of TGF-β-mediated immune evasion in urological malignancies, critically evaluates current therapeutic strategies and their limitations, and discusses emerging approaches to overcome resistance, thereby informing the rational design of TGF-β-targeted immunotherapies.

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Abbreviations: TGF-β, transforming growth factor-beta; ICIs, immune checkpoint inhibitors; BCG, Bacillus Calmette-Guérin; PC, prostate cancer; CRPC, castration-resistant PC; RCC, renal cell carcinoma; ccRCC, clear cell RCC; TβRII, type II TGF-β receptor; TβRI/ALK5, type I TGF-β receptor/activin receptor-like kinase 5; R-Smads, receptor-regulated Smads; EMT, epithelial-mesenchymal transition; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; Tregs, regulatory T cells; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TAMs, tumor-associated macrophages; NK, natural killer; CAFs, cancer-associated fibroblasts; ECM, extracellular matrix; MHC-I, major histocompatibility complex class I; HIF, hypoxia-inducible factor; BC, bladder cancer; NMIBC, non-muscle-invasive BC; MIBC, muscle-invasive BC; NED, neuroendocrine differentiation; NEPC, neuroendocrine PC; AR, androgen receptor; UTUC, upper tract urothelial carcinoma; ORR, objective response rate; MNTR, mature neutrophil-to-T-cell ratio; iCAF, inflammatory CAFs

Key words: TGF-β signaling, urological malignancies, immune microenvironment remodeling, immunotherapy resistance, tumor immunosuppression, BC, PC, RCC, targeted therapy

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1. Introduction

Urological malignancies, encompassing bladder cancer (BC), prostate cancer (PC) and renal cell carcinoma (RCC), constitute a substantial global health burden. Globally, an estimated 573,278 new cases of BC and 212,536 related deaths occurred in 2020, with incidence and mortality rates exhibiting marked geographic variation (1). PC remains the second most commonly diagnosed cancer among men worldwide, accounting for ~1.4 million new cases and 375,000 deaths annually. RCC similarly poses a significant clinical challenge; in the United States alone, ~81,000 new kidney cancer cases

were estimated for 2025, with incidence rates continuing to increase. Despite therapeutic advances across all three tumor types, patients with advanced or metastatic disease continue to face poor prognoses. In muscle-invasive BC (MIBC), for example, the 5-year survival rate for patients with nodal involvement remains below 20%, highlighting persistent unmet clinical needs (1,2).

The clinical status of immunotherapy differs substantially among urological malignancies. In patients with BC, immune checkpoint inhibitors (ICIs) have shown clinical activity across various disease settings, including non-muscle-invasive disease, where a recent meta-analysis revealed that ICIs, particularly pembrolizumab and atezolizumab, may offer favorable efficacy and safety outcomes compared than Bacillus Calmette-Guérin (BCG) in BCG-refractory patients (3). In that analysis, compared with BCG, pembrolizumab was associated with significantly improved tumor control [odds ratio (OR), 4.67; 95% confidence interval (CI), 1.43-15.25; P=0.01], progression-free survival (PFS; OR, 4.85; 95% CI, 1.5-14.85; P=0.006), and overall survival (OS; OR, 3.61; 95% CI, 1.28-10.19; P=0.02) relative to BCG, and atezolizumab showed superior efficacy in patients with metastatic disease (OR, 0.19; 95% CI, 0.06-0.59; P=0.004) and lymph node involvement (OR, 0.43; 95% CI, 0.20-0.93; P=0.03). However, these findings should be interpreted cautiously given that most included studies were single-arm or nonrandomized, the comparison involved ICI monotherapy vs. BCG monotherapy, the patient populations and prior treatment histories were heterogeneous, and confirmatory randomized controlled trials are still lacking (3). In MIBC, the evolving perioperative landscape continues to refine treatment approaches, with ICIs increasingly integrated into neoadjuvant and adjuvant regimens (3,4). Similarly, in RCC, combination strategies integrating ICIs with tyrosine kinase inhibitors (TKIs) or other immunomodulatory agents are under active investigation (5).

Unlike BC and RCC, in which primary or acquired ICI resistance can emerge, PC exhibits intrinsic low sensitivity to ICIs, with the immune desert phenotype of castration-resistant PC (CRPC) presenting a major barrier. Single-cell sequencing has revealed that TGF-β signaling and extracellular matrix (ECM) remodeling pathways are remarkably upregulated in CRPC-associated fibroblasts, and elevated CAF abundance correlates with diminished recurrence-free survival (RFS) (6). These features, including few intratumor effector T cells, low tumor mutation burden (TMB), high density of immunosuppressive myeloid cells, and suppressive cytokines such as TGF-β, collectively render CRPC largely refractory to current immunotherapies (7). In total, ~30-40% of patients with BC exhibit primary resistance to BCG immunotherapy, and relapse rates can reach up to 50%, underscoring the urgent need to understand and overcome resistance mechanisms (8). A substantial proportion of patients across urological malignancies fail to derive durable benefit from ICI-based regimens, particularly in PC where response rates remain disappointingly low (7).

Among the various immunosuppressive pathways implicated in ICI resistance, TGF-β signaling has emerged as a contributor to immune evasion (9). While myeloid-derived suppressor cells (MDSCs), LAG-3 and VEGF represent important downstream effectors of immunosuppression, TGF-β distinguishes itself as a master upstream regulator that may coordinate their activation and coordinates multi-compartmental immune evasion, simultaneously suppressing $CD8^+$ T cells, NK cells and dendritic cells (DCs), while activating cancer-associated fibroblasts (CAFs) and remodeling the ECM, a breadth of action not shared by these other pathways (9-11). TGF-β is a pleiotropic cytokine that exerts context-dependent and often paradoxical functions in cancer. While TGF-β signaling can suppress tumor growth by inducing apoptosis in premalignant cells, it also promotes tumor progression by inducing epithelial-mesenchymal transition (EMT), stimulating neo-angiogenesis, and suppressing antitumor immune responses (12). In the tumor microenvironment (TME), TGF-β drives immunosuppression through multiple convergent mechanisms. It promotes T cell exclusion and exhaustion, induces the polarization of tumor-associated macrophages (TAMs) toward immunosuppressive phenotypes, and activates CAFs that remodel the ECM and form physical barriers to immune infiltration (10). In BC, TGF-β-rich tumors inhibit natural killer (NK) cell-mediated antibody-dependent cellular cytotoxicity by downregulating CD16 expression, while M2 TAM-derived TGF-β promotes programmed death-ligand 1 (PD-L1)-mediated immune evasion through PKM2-dependent metabolic reprogramming (13,14). In RCC, TOPK mediates immune evasion by upregulating PD-L1 expression through activation of the extracellular signal-regulated kinase 2 (ERK2) and TGF-β/Smad pathways, thereby significantly suppressing $CD8^+$ T cell infiltration and function (15). Furthermore, TGF-β-mediated T cell exclusion, regulatory T cell expansion, and adaptive checkpoint upregulation collectively contribute to immune evasion across urologic malignancies (11). Notably, TGF-β contributes to an immunosuppressive network by modulating functions of both adaptive and innate immune cells, inhibiting effector T cell activation and impairing NK-cells proliferation, migration and effector functions (16). Emerging evidence indicates that tumor cells frequently secrete elevated levels of TGF-β, which restricts the activation and growth of T cells, establishing a host-tolerant environment that facilitates immune evasion (10).

Given the increasingly recognized involvement of TGF-β signaling in immune evasion and ICI resistance across BC, PC and RCC, therapeutic targeting of this pathway has emerged as a promising strategy to enhance immunotherapy efficacy (12). The present review provides a comprehensive overview of the molecular basis of TGF-β signaling, its core mechanisms of immune microenvironment remodeling, and its tumor type-specific roles in urological malignancies. Furthermore, the current TGF-β-targeted therapeutic strategies, predictive biomarkers for patient selection, and the challenges and future perspectives in translating TGF-β inhibition into clinical practice for urological cancers are critically evaluated.

A literature search was performed in PubMed (including MEDLINE) from database inception to March 31, 2026. The search terms were as follows: ('TGF-β' OR 'transforming growth factor beta' OR 'TGF-beta') AND ('urological malignancies' OR 'BC' OR 'PC' OR 'RCC' OR 'urothelial carcinoma') AND ('immune microenvironment' OR 'immunotherapy resistance' OR 'ICI' OR 'tumor immune evasion' OR 'cancer-associated fibroblast' OR 'regulatory T cell' OR 'TAM' OR 'biomarker'). Peer-reviewed articles published in

English were included, comprising clinical trials, meta-analyses, cohort studies, single-cell or spatial omics studies, animal models, and *in vitro* mechanistic studies relevant to TGF-β signaling, immune remodeling, immunotherapy resistance, or therapeutic targeting in urological malignancies. Conference abstracts, non-English publications, studies without retrievable full text, and reports unrelated to TGF-β signaling or urological malignancies were excluded. Titles and abstracts were screened, full texts were assessed, and additional references were identified by manual screening of the reference lists of key reviews and included articles.

2. Molecular basis of TGF-β signaling pathways

TGF-β signaling constitutes a complex and finely regulated signaling network that governs diverse cellular processes, including proliferation, differentiation, apoptosis, migration and immune regulation. The pathway transduces extracellular signals through a core receptor-Smad axis yet also engages multiple non-canonical cascades that collectively determine the contextual outcomes of TGF-β stimulation. In urological malignancies, the molecular architecture of TGF-β signaling has been extensively characterized, revealing how its canonical and non-canonical branches converge to remodel the tumor immune microenvironment (TIME). These interconnected signaling modules are integrated in Fig. 1, illustrating the core receptor-Smad axis, parallel non-canonical pathways, and the regulatory feedback loops that govern the contextual output of TGF-β stimulation.

Canonical Smad-dependent signaling. The canonical TGF-β signaling cascade is initiated upon ligand binding to the type II TGF-β receptor (TβRII), a constitutively active serine/threonine kinase that subsequently recruits and phosphorylates the type I receptor (TβRI, also known as ALK5) within a tetrameric receptor complex (17). Activated TβRI propagates the signal by phosphorylating receptor-regulated SMADs (R-SMADs), specifically SMAD2 and SMAD3, at their C-terminal SSXS motif. Following phosphorylation, SMAD2/3 forms a heterotrimeric complex with the common mediator Smad4, which translocates into the nucleus to regulate the transcription of target genes involved in cell cycle control, EMT and ECM remodeling (18,19). The activity of this pathway is tightly modulated by inhibitory SMADs, primarily SMAD7, which exerts negative feedback by competing with R-SMADs for receptor binding or recruiting E3 ubiquitin ligases to promote receptor degradation (17,18).

In the context of urological malignancies, the canonical TGF-β/Smad axis has been demonstrated to be aberrantly activated across BC, PC and RCC (20). In clear cell RCC (ccRCC), the TGF-β/SMAD pathway is hyperactivated and contributes to tumor progression through the induction of fibrogenesis-related gene expression, as evidenced by the finding that neuropilin-1 (NRP1) promotes ccRCC proliferation and migration via TGF-β/SMAD pathway engagement, with NRP1 knockdown substantially reducing p-SMAD2/3 levels and suppressing invasive capacity *in vitro* (21). Similarly, in BC, environmental carcinogens such as antimony have been shown to promote metastatic progression through enhancing SUMOylation-dependent TGF-β/SMAD2/3 axis activation, leading to increased SMAD2/3 nuclear accumulation and transcriptional activity (22). Conversely, the tumor-suppressive arm of canonical signaling has also been documented; circ_0003266 was found to suppress BC progression by upregulating SMAD7 expression via sponging miR-503-5p, thereby restraining TGF-β pathway overactivation (23). These observations underscore that the canonical Smad cascade serves as a double-edged sword, the net outcome of which depends on the specific molecular context and concurrent alterations in upstream regulators.

Non-canonical signaling branches. Beyond the Smad-dependent route, TGF-β receptors can activate multiple non-canonical signaling pathways that operate in parallel with or independently of Smad proteins to modulate cellular responses. These branches include the mitogen-activated protein kinase (MAPK) cascades (ERK1/2, p38 MAPK and c-Jun N-terminal kinase), the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway and Rho-like GTPase signaling networks, all of which contribute to TGF-β-mediated cell migration, survival and cytoskeletal reorganization (17,19). In BC, TGF-β1 has been shown to induce apoptosis through non-canonical activation of p38 MAPK and JNK/SAPK cascades, culminating in caspase-9/8/3-mediated cell death, while the pro-survival AKT pathway remains unaffected (24). In RCC, the type III TGF-β receptor (TβRIII) mediates apoptosis independently of the canonical TGF-β/Smad pathway through p38 MAPK signaling (25). Furthermore, HOOK1, a tumor suppressor downregulated in RCC, inhibits tumor growth and metastasis through both canonical TGF-β/ALK5/p-Smad3 and non-canonical TGF-β/MEK/ERK/c-Myc pathways (26). Notably, non-canonical signaling is not merely ancillary but frequently exerts critical effects that complement or override Smad-dependent transcriptional programs. For instance, in PC, TGF-β promotes cell migration through the TRAF6-mediated K63-linked ubiquitylation of the p85α regulatory subunit of PI3K, which enhances PI3K-AKT signaling in a Smad-independent manner, as demonstrated by the observation that TRAF6 knockdown abrogated TGF-β-induced AKT activation and reduced PC cell migration by more than 50% in wound-healing assays (27). Moreover, non-canonical ALK7 signaling, a less explored branch of the TGF-β superfamily, has been shown to drive pancreatic cancer metastasis through β-catenin/MMP-mediated basement membrane breakdown, suggesting that atypical TGF-β family receptors may also contribute to invasive progression in certain contexts (28).

The interplay between canonical and non-canonical pathways adds considerable complexity to TGF-β signaling outcomes. In hepatic cancer cells, the cationic surfactant cetrimonium bromide was shown to suppress cell migration and invasion through modulating both canonical (Smad2/3 phosphorylation) and non-canonical (ERK1/2 and p38 MAPK) TGF-β branches, suggesting that simultaneous inhibition of multiple signaling arms may be required for effective pathway blockade (29). More recently, PMEPA1 was identified as a critical mediator that promotes mTOR inhibitor resistance in breast cancer through a non-canonical TGF-β mechanism, highlighting that non-canonical branches can confer therapeutic resistance independent of Smad signaling (30). This integrative perspective positions non-canonical TGF-β

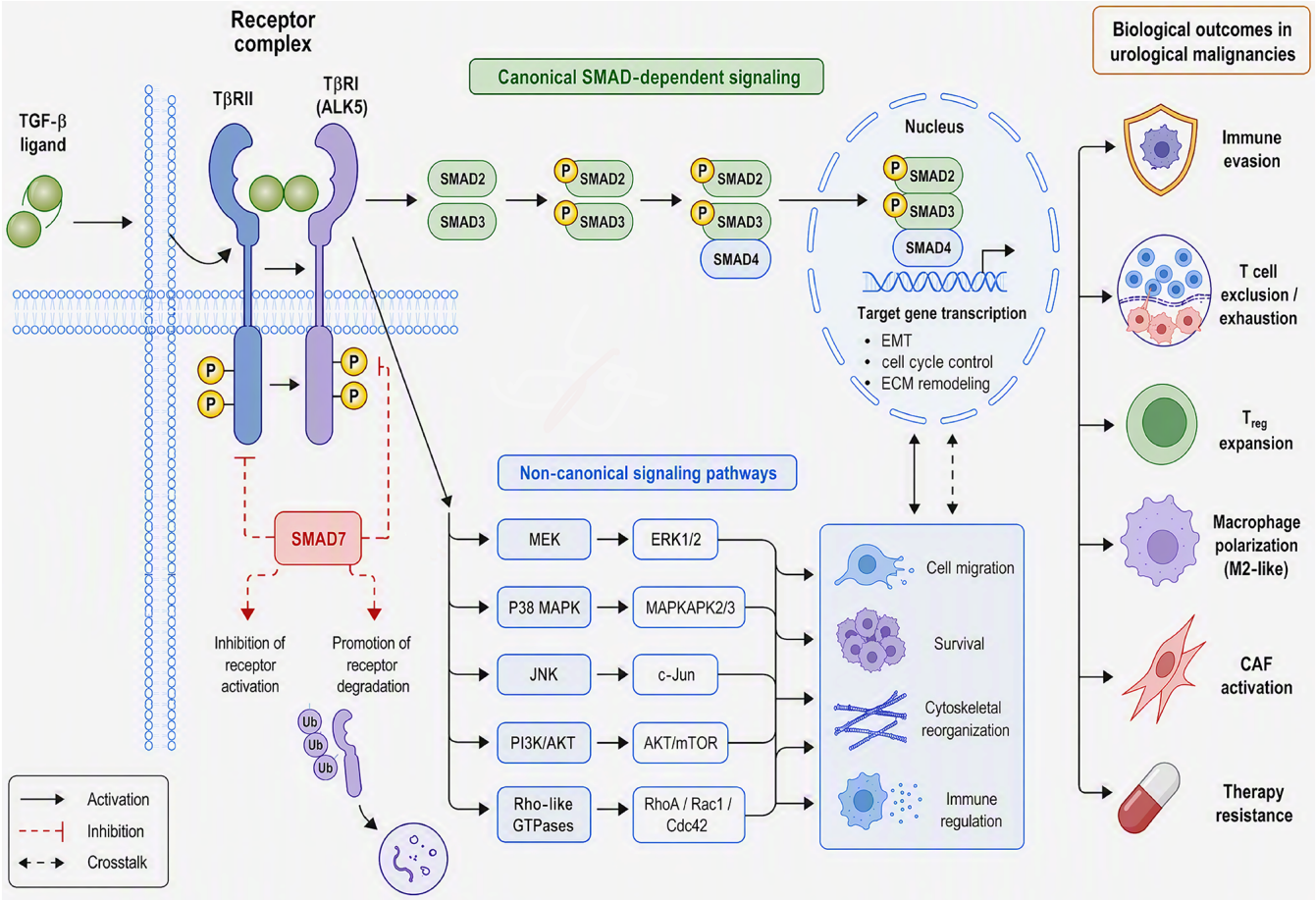

Figure 1. Molecular architecture of TGF-β signaling pathways. TGF-β ligands bind to the type II receptor (TβRII), which recruits and phosphorylates type I receptor (TβRI/ALK5). Activated TβRI phosphorylates Smad2/3, which form heteromeric complexes with Smad4 and translocate to the nucleus to regulate target gene transcription. Inhibitory Smad7 provides negative feedback by competing with R-Smads or promoting receptor degradation. Upon receptor complex activation, non-canonical branches including MAPK (ERK1/2, p38 and JNK), PI3K/AKT, and Rho-like GTPase pathways are directly initiated by activated TβR complexes, independent of SMAD7, to modulate cell migration, survival, and cytoskeletal reorganization. The net biological output of TGF-β signaling is context-dependent, exerting either tumor-suppressive or tumor-promoting functions depending on cellular and microenvironmental determinants. CAF, cancer-associated fibroblast.

cascades as indispensable components of the pathway's overall biological output, particularly in cancer cells that have acquired resistance to Smad-directed interventions.

Context-dependent dual roles in tumorigenesis. A defining feature of TGF-β signaling is its paradoxical dual functionality, wherein the pathway acts as a potent tumor suppressor in normal epithelial cells and early-stage tumors, yet switches to a tumor-promoting and pro-metastatic driver in advanced malignancies (9,31). In normal cells and precancerous lesions, TGF-β induces growth arrest through upregulation of cyclin-dependent kinase inhibitors such as p21 and p15, and promotes apoptosis via activation of pro-apoptotic effectors including BIM and DAPK (9). This tumor-suppressive activity is predominantly mediated through the canonical Smad-dependent transcriptional program. However, as tumors progress, genetic and epigenetic alterations disrupt the growth-inhibitory responses while preserving or enhancing the pro-oncogenic functions of TGF-β, including EMT induction, fibroblast activation, angiogenesis and immune evasion (12,31).

The mechanistic basis for this functional switch has been attributed to several factors, including loss-of-function mutations in Smad4 or TGF-β receptor components, hyperactivation of oncogenic pathways such as Ras and Wnt, and changes in the composition of the TME (32,33). In colorectal cancer cells, TGF-β was shown to act as a dual regulator of COX-2/PGE2-mediated tumor promotion, with its oncogenic vs. tumor-suppressive output being determined by cross-interaction with H-Ras and Wnt/β-catenin pathways; TGF-β enhanced COX-2 expression and PGE2 production specifically in cells harboring activated H-Ras or Wnt signaling, while exerting inhibitory effects in cells lacking these oncogenic inputs (34). In urological malignancies, this contextual duality manifests as differential prognostic implications of TGF-β pathway activation across tumor stages. In localized PC, elevated TGF-β signaling correlates with indolent disease and favorable outcomes, whereas in CRPC, the same pathway promotes metastasis and immune desert formation (33). Similarly, in ccRCC, TGF-β-driven fibrogenesis contributes to tumor progression through NRP1-mediated pathway engagement, yet the prognostic value of TGF-β signatures remains subtype-dependent, underscoring the need for context-specific interpretation of pathway activation (20,21). This intrinsic duality poses fundamental challenges for

TGF-β-targeted therapy, as systemic inhibition may disrupt its tumor-suppressive functions in normal tissues and early-stage lesions, thereby inadvertently promoting carcinogenesis in certain contexts (12).

A critical yet often overlooked dimension of TGF-β biology is that TGF-β is secreted as a large latent complex and requires activation to release mature TGF-β. Integrins of the αV family, particularly αVβ6 and αVβ8, are the principal physiological activators of latent TGF-β through binding to the RGD motif in LAP and exerting mechanical force. This activation mechanism is spatially restricted, providing a basis for local, context-dependent signaling that systemic inhibition cannot easily discriminate. In BC, integrin β8 (ITGB8) expressed on tumor cells plays an immunosuppressive role in BCG therapy response, with high ITGB8 expression associated with post-BCG recurrence and ITGB8 knockdown decreasing TGF-β1 secretion (35). αVβ8-expressing prostate tumors similarly activate TGF-β to suppress antitumor immunity. These observations establish integrin-mediated latent TGF-β activation as a tumor-specific mechanism of immune evasion that may be therapeutically exploited with improved safety compared with systemic pan-TGF-β inhibition (9,11,12).

3. Core mechanisms of TGF-β-mediated immune microenvironment remodeling

TGF-β signaling can orchestrate a multifaceted remodeling of the TIME through coordinated actions on adaptive and innate immunity, stromal compartments, vascular architecture and cellular metabolism. This section systematically dissects the core mechanisms by which TGF-β drives immunosuppression, beginning with its effects on T cell dysfunction and exhaustion, followed by reprogramming of innate immune cells toward protumor phenotypes, activation of CAFs with consequent ECM remodeling, induction of vascular abnormalities and immune-excluded tumor phenotypes, promotion of EMT as a crosstalk hub between metastasis and immune evasion, and finally, metabolic reprogramming that reinforces the tolerogenic microenvironment. The multifaceted immunosuppressive mechanisms orchestrated by TGF-β signaling, encompassing T cell dysfunction, innate immune cell reprogramming, CAF-mediated stromal remodeling, vascular abnormalities, EMT, and metabolic rewiring, are schematically depicted in Fig. 2, illustrating how these interconnected pathways converge to establish a hostile immune microenvironment that promotes tumor progression and therapy resistance. Evidence cited in this section is categorized as either direct evidence from urological malignancies or general tumor-biology evidence from non-urological models. Non-urological findings are explicitly identified and are not extrapolated to urological malignancies unless supported by urological data.

TGF-β drives T cell dysfunction and exhaustion. TGF-β exerts profound immunosuppressive effects on the adaptive immune system, particularly through direct modulation of $CD8^+$ effector T cells and regulatory T cells (Tregs). Evidence derived from human BC samples demonstrate that tumor cells suppress $CD8^+$ T cell cytotoxic function via an ICAM-1/TGFβ2-mediated pathway, leading to perforin-deficient $CD8^+$ T cells that exhibit a Tc2 phenotype with elevated programmed cell death protein 1 (PD-1) and GATA-3 expression (36). *In vitro* experiments and mouse models have further shown that tumor-derived TGFβ1 directly attenuates T cell antitumor activity through regulation of PD-1 mRNA expression (37). Multi-omics profiling of human RCC specimens has identified NNMT in tumor endothelium as a key regulator of $CD8^+$ T cell exhaustion via TGF-β signaling, with NNMT-high tumor-associated endothelial cells directly contacting exhausted $CD8^+$ T cells to form an immunosuppressive niche (38). Furthermore, studies in RCC cell lines and mouse xenograft models demonstrate that TOPK mediates immune evasion by upregulating PD-L1 expression through activation of the ERK2 and TGF-β/Smad pathways, thereby significantly suppressing $CD8^+$ T cell infiltration and function in RCC (15). These findings demonstrate that TGF-β-mediated T cell exhaustion is a mechanism supported by evidence across urological malignancies. By contrast, evidence for TGF-β-induced $Foxp3^+$ Treg expansion via the TMPRSS2-ERG fusion gene in PC and TGF-β1-mediated Treg accumulation following irradiation represents urological tumor-specific mechanisms that have not been documented in non-urological cancers.

Parallel to its effects on effector T cells, TGF-β potently promotes the differentiation, recruitment and immunosuppressive activity of Tregs. Moesin, a membrane-organizing protein translationally regulated by TGF-β, is required for optimal TGF-β signaling and efficient development of induced Tregs (iTregs) through regulation of TβRII surface abundance and stability (39). In PC, the TMPRSS2-ERG fusion gene promotes tumor growth by increasing the migration, activation and infiltration of $Foxp3^+$ Tregs, with elevated TGF-β levels accompanying this process (40). Moreover, TGF-β1 mediates the radiation response of PC, with TGF-β1 levels positively correlating with Treg accumulation following irradiation (41). In patients with metastatic CRPC, Tregs in peripheral blood mononuclear cells exhibit increased suppressive function, and TGF-β1 expression correlates with Treg accumulation in PC biopsy specimens (42). The integrin αvβ8 expressed on tumor cells provides a tumor-specific mechanism of Treg enrichment by binding to latent TGF-β presented on T cells, thereby activating TGF-β and driving immunosuppressive Treg differentiation (43). In BC, TGF-β-induced Treg expansion has been shown to suppress cytotoxic T cells in the TME, and clinical analysis of patients with bladder urothelial carcinoma revealed that peripheral blood levels of Tregs, MDSCs and TGF-β are significantly elevated and positively correlated with each other (44). In pancreatic cancer, LMO7 promotes immune evasion through Treg differentiation and chemotaxis via direct binding to Foxp1, which negatively regulates TGF-β and CCL5 expression; elevated TGF-β and CCL5 subsequently facilitate Treg enrichment (45). It should be noted that the LMO7-mediated Treg enrichment mechanism has been characterized primarily in pancreatic cancer and has not yet been directly validated in urological malignancies. These observations collectively indicate that TGF-β employs both cell-intrinsic mechanisms (moesin-mediated TβRII stabilization) and tumor-specific pathways (αvβ8-mediated TGF-β activation, TMPRSS2-ERG-driven Treg recruitment) to expand the Treg compartment and reinforce immunosuppression.

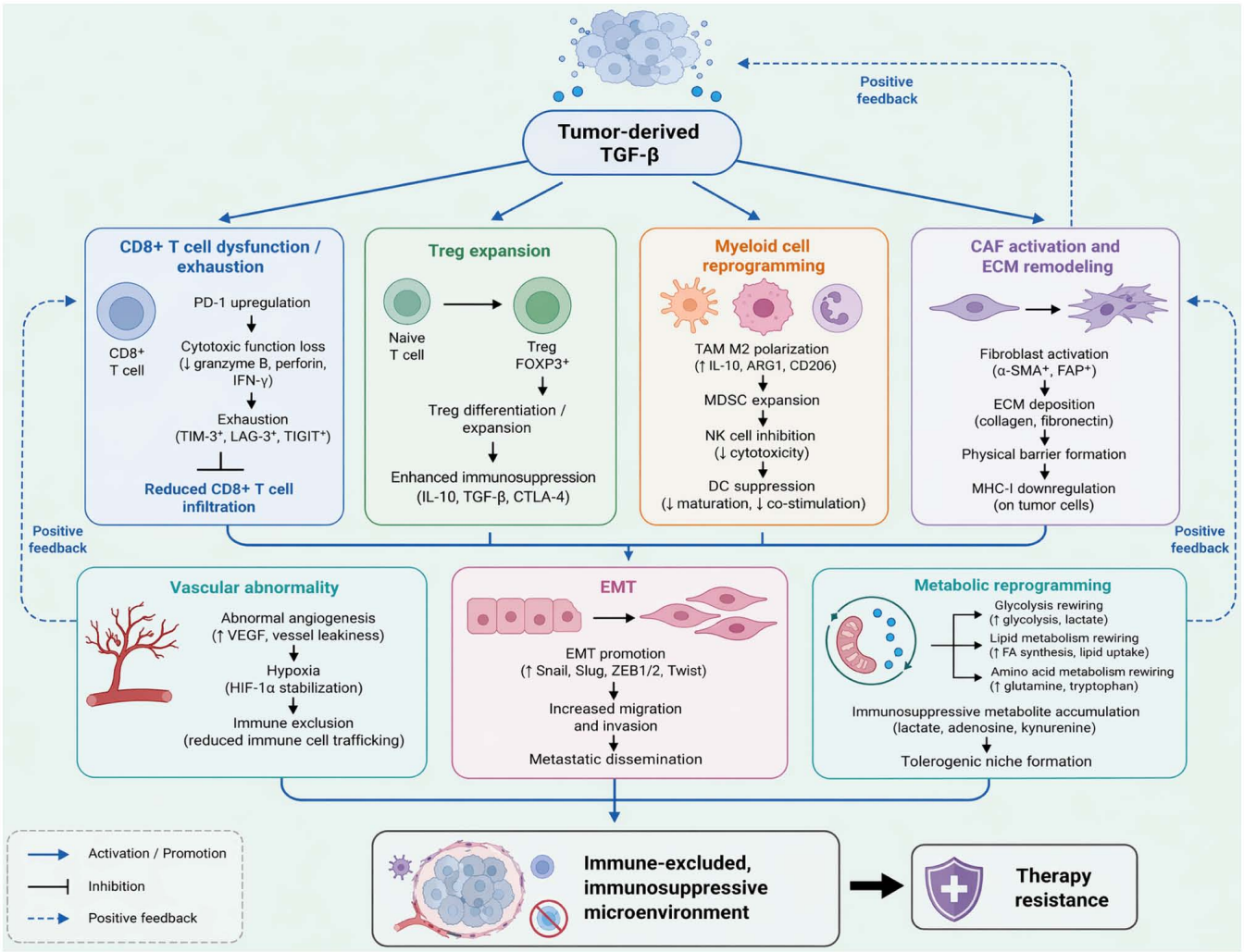

Figure 2. TGF-β signaling may drive multifaceted immune microenvironment remodeling in urological malignancies. This figure illustrates the core mechanisms by which TGF-β signaling drives immunosuppressive microenvironment remodeling in urological malignancies. TGF-β exerts profound effects on multiple cellular compartments: i) $CD8^+$ T cell exhaustion and dysfunction through direct inhibition of cytotoxic molecules and PD-1 upregulation, alongside expansion of immunosuppressive Tregs; ii) polarization of innate immune cells toward protumor phenotypes, including M2 TAMs, MDSCs, N2 tumor-associated neutrophils, and suppression of dendritic cell maturation and NK cells cytotoxicity; iii) activation of CAFs and subsequent ECM remodeling, forming physical barriers to immune infiltration; iv) induction of vascular abnormalities and immune-excluded tumor phenotypes; v) promotion of EMT linking metastasis with immune evasion; and vi) metabolic reprogramming of both tumor and immune cells, reinforcing the tolerogenic microenvironment through feed-forward circuits. Collectively, these interconnected pathways establish TGF-β as a central orchestrator of immune evasion and immunotherapy resistance across bladder cancer, prostate cancer and renal cell carcinoma. TAMs, tumor-associated macrophages; EMT, epithelial-mesenchymal transition; MDSC, myeloid-derived suppressor cell; MHC-I, major histocompatibility complex class I; ECM, extracellular matrix; DCs, dendritic cells; HIF, hypoxia-inducible factor; PD-1, programmed cell death protein 1.

Reprogramming of innate immune cells toward immunosuppressive phenotypes. TGF-β extensively reprograms the innate immune compartment, polarizing multiple myeloid lineages toward immunosuppressive and protumor phenotypes. In BC, studies using human tumor samples, cell lines and mouse models have revealed a feed-forward loop with tumor cells: BC cells reprogram TAMs toward M2 polarization through lactate-induced hypoxia-inducible factor (HIF)-1α signaling, and M2 TAMs in turn secrete TGF-β that promotes glycolysis, cancer stemness and EMT in BC cells via the TGF-β/Smad2/3 pathway, while simultaneously enhancing PD-L1 mRNA m6A methylation (46). Mechanistically, M2 TAM-derived TGF-β enhances PD-L1-mediated immune escape in BC through PKM2 dimer-STAT3 complex nuclear translocation, increasing glycolytic flux and promoting PD-L1 expression through PKM2 kinase activity (14). Phenotypic characterization of patients with BC confirms that TAM infiltration is significantly elevated in tumor tissues, with M2 phenotype correlating positively with disease severity (47). Single-nucleus RNA-seq analysis of non-muscle invasive BC has further shown that TGF-β signaling enrichment scores increase progressively from treatment-naive to post-BCG progression samples, with M2 macrophages relatively more abundant in the progression group (48). These findings underscore that TGF-β may serve as both a downstream effector and an upstream contributor of TAM-mediated immunosuppression in a self-reinforcing circuit.

TGF-β also promotes the expansion and activation of MDSCs. MicroRNA (miR)-155 and miR-21 are identified as the two most upregulated miRNAs during MDSC induction,

and TGF-β upregulates these miRNAs to promote MDSC expansion; overexpression of miR-155 and miR-21 enhances while their depletion reduces cytokine-induced MDSC frequency (49). These findings were established primarily in non-urological experimental systems and have not been directly validated in urological malignancies. In lung adenocarcinoma (LUAD), exosomal PVRL4 promotes tumor progression by enhancing MDSC-secreted TGF-β1, establishing an exosome-mediated mechanism for TGF-β-driven MDSC activation (50). This mechanism represents a conserved general finding that requires validation in urological malignancies. Furthermore, TGF-β directly inhibits DC maturation and antigen-presenting capacity; TGF-β exposure impairs the ability of DCs to present antigen, stimulate tumor-sensitized T lymphocytes, and migrate to draining lymph nodes (51). In PC, tumor stroma-derived factors including TGF-β skew monocyte-to-DC differentiation toward a suppressive $CD14^+$ $PD\text{-}L1^+$ phenotype, providing direct evidence for TGF-β-mediated DC suppression in a urological malignancy (52). In leukemia models, exosomes induce DC immunosuppression via the TGFB2-MRPL58 axis, characterized by selective suppression of CD1a and TLR2 with significant downregulation of proinflammatory cytokines (53). This mechanism has been characterized in leukemia models and has not been validated in urological malignancies.

NK cells cytotoxicity is likewise suppressed by TGF-β. In complex 3D leukemia microenvironment models, TGF-β significantly reduces NK cells-mediated cytotoxicity in a time-dependent manner and impairs NK cells infiltration into tumor spheroids by altering key gene pathways related to cytotoxicity, adhesion and migration (54). Genetic ablation of SMAD4 in human NK cells confers resistance to TGF-β and activin A suppression, preserving cytotoxic function, cytokine secretion and IL-2/IL-15-driven proliferation, with SMAD4-knockout NK cells demonstrating enhanced tumor penetration and tumor growth control (55). This establishes SMAD4 as a critical node through which TGF-β inhibits NK cells function. Finally, TGF-β induces N2 polarization of tumor-associated neutrophil. Smad3 is essential for N2 TAN polarization in non-small cell lung carcinoma, with Smad3-knockout mice showing a shift from N2 to N1 TAN states accompanied by enhanced neutrophil infiltration and tumor regression (56). The miR-941/FOXN4/TGF-β feedback loop similarly drives N2 TAN polarization in LUAD, indicating that TGF-β-mediated TAN polarization represents a conserved mechanism of innate immune reprogramming across tumor types (57).

Activation of CAFs and ECM remodeling. CAFs represent a key stromal cell population through which TGF-β orchestrates immune evasion. In BC, cancer cell-derived exosomes are internalized by fibroblasts and trigger their differentiation into CAFs through exosome-mediated TGF-β transfer and SMAD pathway activation (58). Once activated, CAFs regulate the secretion of ECM components under the influence of TGF-β, contributing to the formation of an immunosuppressive physical barrier; notably, CAF-associated gene signatures have been shown to predict resistance to immune checkpoint blockade (ICB) (59). TGF-β1 dominates stromal fibroblast-mediated EMT in BC cells through the FAP/VCAN axis, establishing TGF-β1 as the critical mediator of crosstalk between stromal fibroblasts and tumor cells (60). Single-cell and spatial transcriptomic analyses in ovarian cancer reveal that TGF-β-driven fibroblast activation and ECM production form physical barriers that exclude T cells from the TME, while TGF-β also reduces major histocompatibility complex class I (MHC-I) expression on tumor cells (61). This CAF-ECM-T cell exclusion axis has been most extensively characterized in ovarian cancer; however, emerging evidence from urological malignancies supports its relevance. In ccRCC, tumor-derived TGF-β1 activates CAFs via SMAD signaling, inducing POSTN expression and recruiting $APOE^+$ TAMs through integrin and chemokine axes, thereby forming an immune-exclusion niche that drives immunotherapy resistance (62). Similarly, in ccRCC, hypoxia and TGF-β/STAT3 signaling drive CAFs to mature into ECM-dominant $POSTN^+$ phenotypes, which secrete periostin to recruit and polarize $CCL3^+$ macrophages, establishing a spatially organized stromal-immune mechanism of immune exclusion (63). This CAF-ECM-T cell exclusion axis represents a critical mechanism by which TGF-β creates immune-desert phenotypes, operating through both physical (ECM deposition) and molecular (MHC-I downregulation) mechanisms to establish an immune-excluded tumor phenotype.

TGF-β-induced vascular abnormality and immune-excluded tumor phenotype. Beyond stromal remodeling, TGF-β signaling in endothelial cells critically influences tumor vasculature and immune cell trafficking. Endothelial cell-specific deletion of TβRII results in increased tumor angiogenesis accompanied by fragile neo-vessels, enhanced hemorrhage and hypoxia, which collectively contribute to an immune-excluded phenotype by impairing effective T cell delivery to tumor sites (64). This endothelial TβRII deletion study was conducted in a genetically engineered mouse model and has not been directly validated in urological malignancies. Conversely, TGF-β3 promotes vascular normalization in PC, upregulating endothelial adhesion molecules and enhancing $CD8^+$ T cell infiltration, thereby potentiating immunotherapy and chemotherapy responses (65). This dichotomy, wherein TGF-β1 generally promotes abnormal vascularization while TGF-β3 induces vascular normalization, highlights the context-dependent and isoform-specific effects of TGF-β family members on tumor vasculature and immune exclusion. The net effect in most advanced urological malignancies, however, favors TGF-β-driven vascular abnormalities that contribute to immune exclusion and therapeutic resistance.

TGF-β-induced EMT: A crosstalk hub between metastasis and immune evasion. EMT serves as a critical nexus linking TGF-β-driven metastasis and immune evasion. In BC, TCF7 functions as a prognostic biomarker by strengthening EMT and stemness through TGF-β/SMAD3 signaling (66). In upper tract urothelial carcinoma (UTUC), TGFB1I1 expression regulates cell proliferation and viability and induces EMT to facilitate cell migration, playing an essential role in promoting tumor aggressiveness (67). During PC progression, advanced cancer cells acquire resistance to TGF-β-mediated growth inhibition,

and TGF-β instead drives cancer progression through EMT, cell migration, invasion and immunosuppression (68). This functional switch from tumor suppression to tumor promotion is a hallmark of TGF-β biology in urological malignancies, with EMT representing a central mechanism through which TGF-β simultaneously promotes metastatic dissemination and establishes an immunosuppressive microenvironment. The TCF7/TGF-β/SMAD3 axis in BC exemplifies how EMT-regulatory transcription factors are directly integrated with TGF-β signaling to coordinate these dual pro-tumorigenic programs.

TGF-β-mediated metabolic reprogramming in tumor and immune cells. TGF-β may function as a metabolic contributor to the tolerogenic crosstalk within the TME, establishing a host-tolerant environment that facilitates immune evasion across multiple cancer types (69). TGF-β signaling regulates cancer metabolic reprogramming through multiple interconnected pathways, modulating glycolysis, lipid metabolism, and amino acid metabolism in both tumor and immune cells (70). It should be noted that the evidence for TGF-β-mediated metabolic reprogramming has been derived primarily from non-urological tumor models, and direct validation in urological malignancies remains limited. In BC, the feed-forward loop between M2 TAMs and tumor cells is fundamentally driven by aerobic glycolysis, with M2 TAM-derived TGF-β promoting tumor cell glycolysis while tumor cell-derived lactate reinforces M2 polarization through HIF-1α (46). The integration of TGF-β signaling with metabolic pathways, including the PKM2 dimer-STAT3 axis that links glycolysis to PD-L1 expression, positions TGF-β as a potential node connecting cellular metabolism, immune evasion and therapeutic resistance (14). Recent studies have further demonstrated that tumor-derived TGFβ3 triggers mitochondrial rewiring and succinate accumulation in bladder smooth muscle, reprogramming it into metastasis-supporting vascular niches through a TGFβ3-SMAD2-SQOR-succinate axis (46,60). Understanding these metabolic dimensions of TGF-β-mediated immune remodeling may reveal novel therapeutic vulnerabilities, particularly given that metabolic interventions could potentially disrupt the feed-forward circuits that sustain TGF-β-driven immunosuppression.

In summary, the core mechanisms of TGF-β-mediated immune microenvironment remodeling described in this section exhibit varying degrees of urological-specific validation. $CD8^+$ T cell exhaustion, Treg expansion, M2 macrophage polarization, CAF activation with ECM remodeling and metabolic reprogramming have all been directly demonstrated in at least one urological malignancy, with BC and RCC contributing the most robust evidence. By contrast, TGF-β-mediated MDSC expansion, DC suppression, and vascular abnormality mechanisms have been less extensively characterized in urological malignancies and are, in part, inferred from non-urological tumor models. These mechanisms should therefore be regarded as conserved general findings that warrant dedicated investigation in bladder, prostate and renal cancers.

4. TGF-β-mediated immune remodeling in specific urological malignancies

It should be emphasized that the clinical context of immunotherapy is not equivalent across these tumors. BC and RCC are generally ICI-responsive diseases in which primary or acquired resistance can emerge, whereas PC displays intrinsic low sensitivity to ICIs rather than typical acquired resistance (3-7). In BC, TGF-β pathway activation has been implicated in primary resistance to ICB and failure of BCG immunotherapy. In PC, TGF-β signaling intersects with androgen receptor (AR) pathways and drives neuroendocrine differentiation (NED) while fostering an immune-excluded bone metastatic niche. In RCC, the crosstalk between TGF-β and HIFs creates a uniquely immunosuppressive metabolic environment. In addition, rare urological malignancies, including UTUC, testicular cancer, and penile cancer, are briefly considered, because TGF-β pathway activation and miR-mediated TGF-β regulation have been implicated in their progression, although dedicated evidence in these tumors remains limited (67,71). A comparative overview of these tumor type-specific mechanisms is provided in Fig. 3, highlighting the key molecular drivers and affected immune cell populations in bladder, prostate and renal cancers.

BC

Expression, prognostic value and pathway activation signatures. In BC, aberrant TGF-β pathway activation has been extensively documented, with pathway signatures demonstrating both prognostic and microenvironmental implications. A novel TGF-β risk score, constructed from a 12-gene signature, was shown to independently predict OS in patients with BC, with high-risk patients exhibiting significantly worse prognosis and increased sensitivity to chemotherapeutic agents including cisplatin, gemcitabine and doxorubicin (72). This risk score further stratified tumors by immune phenotype: High-risk tumors displayed elevated M2 macrophage infiltration and enhanced TGF-β pathway activation, whereas low-risk tumors were characterized by stronger cytolytic activity and $CD8^+$ T cell infiltration (72). These observations establish TGF-β pathway activation as a determinant of both clinical outcomes and immune contexture in BC.

Beyond gene expression signatures, specific molecular drivers of TGF-β-mediated immune evasion have been identified. ELN (tropoelastin), secreted by CAFs, was recently shown to function as a TGFBR2-binding partner that triggers SMAD2/3-dependent TGF-β1 autocrine signaling, establishing a feed-forward loop that drives metastasis and immunosuppressive niche formation (73). Mechanistically, ELN upregulation in BC cells promoted PD-L1 expression and induced an immunosuppressive microenvironment characterized by reduced $CD8^+$ T cell infiltration and increased MDSC accumulation, with ELN expression serving as an independent poor prognostic factor (73). Similarly, TBX3 was found to shape an immunosuppressive microenvironment in BC by transcriptionally regulating multiple immune-related genes, with TBX3 overexpression correlating with reduced $CD8^+$ T cell infiltration and increased resistance to anti-PD-L1 therapy (74). These findings collectively establish that TGF-β pathway activation in BC is driven by both tumor-intrinsic (TBX3) and stromal-derived (ELN and CAFs) factors that converge on immune exclusion.

TGF-β-driven immunosuppression and ICI resistance in MIBC. In MIBC, TGF-β signaling has emerged as a critical mediator of primary resistance to ICB. Integrative analysis of

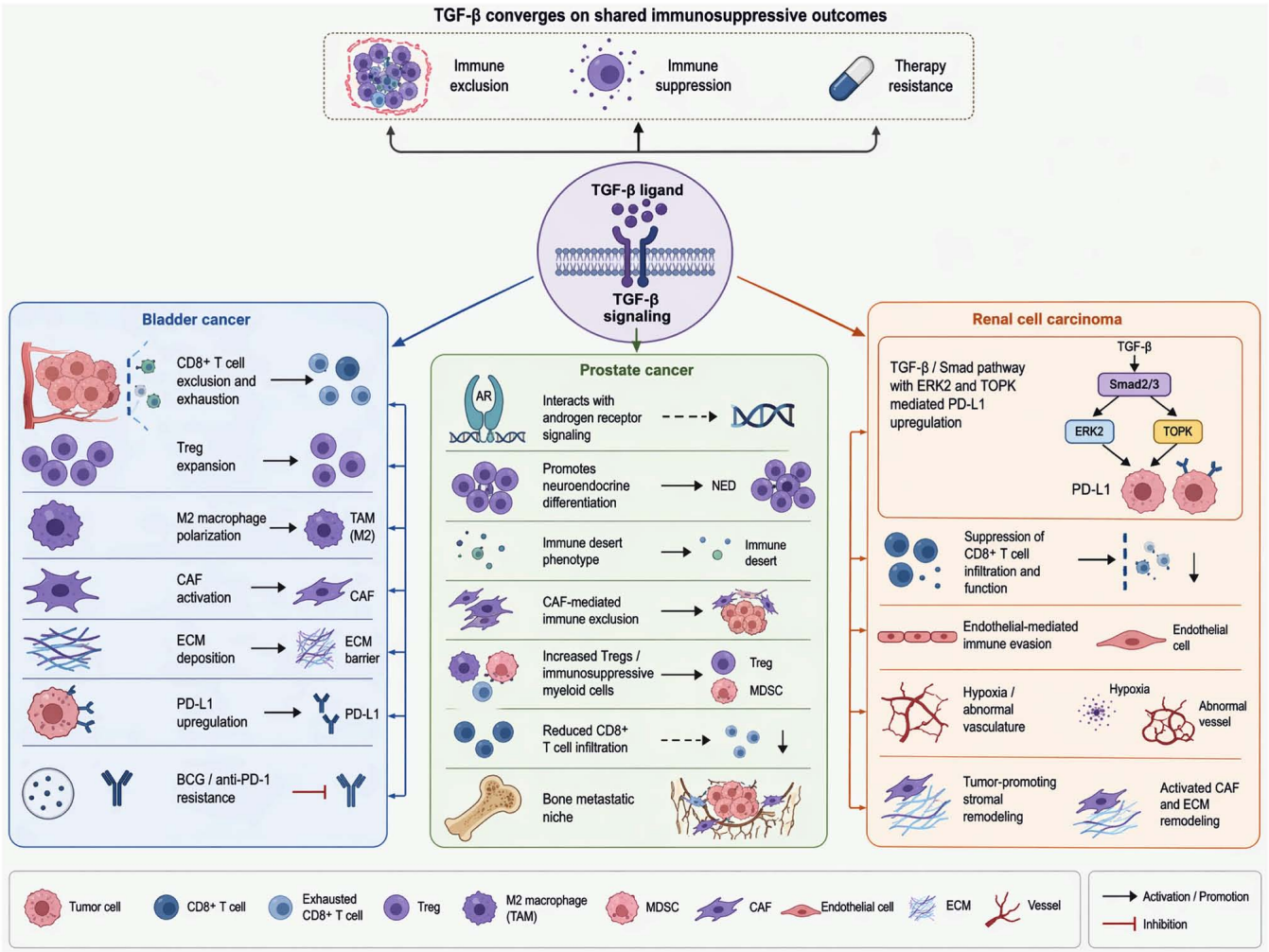

Figure 3. Tumor type-specific mechanisms of TGF-β-mediated immune remodeling in urological malignancies. In bladder cancer, TGF-β signaling may drives an immunosuppressive niche through CAF- and tumor cell-derived factors (ELN, TBX3) that promote immune exclusion and limit NK cell and CD8+ T cell cytotoxicity, contributing to BCG and ICI resistance. In prostate cancer, TGF-β signaling promotes NED, activates CAFs to establish an immune-desert microenvironment, and suppresses Th1 responses in the bone metastatic niche, contributing to immunotherapy refractoriness in CRPC. In renal cell carcinoma, TGF-β pathway activation, crosstalk with hypoxia-inducible factor 2α, and stromal-immune interactions (for example, POSTN+ CAFs and CCL3+ macrophages) drive immune evasion and resistance to both anti-angiogenic agents and ICIs. This figure summarizes the shared pathways and tumor type-specific adaptations of TGF-β-driven immune remodeling across major urological malignancies. CAF, cancer-associated fibroblast; NED, neuroendocrine differentiation; ECM, extracellular matrix; TAMs, tumor-associated macrophages; PD-1, programmed cell death protein 1; Treg, regulatory T-cells; ICI, immune checkpoint inhibitor; MDSC, myeloid-derived suppressor cells.

human MIBC cohorts incorporating angiogenesis signatures and TMB revealed that a patient subgroup resistant to ICB was characterized by high angiogenesis signature, low TMB, and activation of TGF-β signaling with downregulated T-cell cytolytic signatures (75). This TGF-β-high/angiogenesis-high phenotype identified patients with significantly worse PFS following ICB therapy, suggesting that concurrent targeting of TGF-β and angiogenic pathways may be necessary to overcome resistance in this subset (75).

The cellular mechanisms underlying TGF-β-driven ICI resistance in MIBC extend to innate immune suppression. TGF-β signaling was shown to limit the effector function capacity of NK cells in human BC, with TGF-β-rich tumors inhibiting NK cells-mediated antibody-dependent cellular cytotoxicity through downregulation of CD16 expression (13). Importantly, TGF-β inhibition restored NK cells tumoricidal capacity, revealing a previously unappreciated mechanism of innate immune suppression that may complement T cell-directed immunotherapies (13). Furthermore, platinum-based chemotherapy, a standard neoadjuvant regimen in MIBC, was found to induce opposing effects on immunotherapy response-related spatial and stromal biomarkers, with chemotherapy increasing fibroblast-based TGF-β signaling and increasing distances from immune cells to cancer cells (76). This chemotherapy-induced TGF-β pathway activation may paradoxically inhibit antitumor immune responses, providing a rationale for combining TGF-β inhibitors with chemoradiotherapy in the neoadjuvant setting (76).

TGF-β blockade in sensitizing BC to immunotherapy. Preclinical evidence supports the strategy of TGF-β blockade to sensitize BC to immunotherapy. Multi-omics analysis revealed that NUDT10 expression, a gene involved in m7G methylation, may regulate TGF-β signaling and is associated with poor response to anti-PD-L1 therapy, with differentially methylated genes enriched in the TGF-β pathway correlating with immunotherapy resistance (77). The mechanistic link

between TBX3 and immunotherapy resistance was further validated *in vivo*, where TBX3 knockdown sensitized BC tumors to anti-PD-1 therapy through enhanced $CD8^+$ T cell infiltration and function (74). These findings suggest that TGF-β pathway activation represents a therapeutically actionable vulnerability in a subset of patients with BC who are otherwise refractory to ICB monotherapy.

TGF-β signaling in non-muscle invasive BC and BCG therapy resistance. The role of TGF-β signaling extends beyond muscle-invasive disease to non-muscle invasive BC (NMIBC), particularly in the context of BCG immunotherapy resistance. Longitudinal single-nucleus RNA-seq analysis of NMIBC cases revealed that TGF-β signaling emerges as a key pathway showing gradual enrichment from pre-treatment to post-progression samples following BCG therapy (48). The aforementioned study identified distinct TME subtypes linked to BCG resistance, including $LAMP3^+$ DCs and inflammatory CAFs (iCAFs), with the latter exhibiting TGF-β pathway activation (48). Complementary to these findings, ITGB8 was shown to play an immunosuppressive role in BCG therapy response; high ITGB8 expression was significantly associated with post-BCG recurrence ($P<0.001$), and ITGB8 knockdown markedly decreased TGF-β1 secretion ($P<0.01$) (35). These observations indicate that ITGB8-mediated TGF-β1 activation represents a tumor-specific mechanism of BCG therapy failure, positioning TGF-β inhibition as a potential strategy to enhance BCG efficacy in NMIBC.

PC

TGF-β signaling in PC progression and NED. Unlike BC and RCC, in which primary or acquired ICI resistance can develop, PC is intrinsically insensitive to ICIs (6,7). In PC, TGF-β signaling undergoes a functional transition from tumor suppression in early disease to tumor promotion in advanced stages, a switch that is intimately linked to NED. IL-6 was shown to activate the TGF-β-SMAD2 axis and promote NED of PC cells under androgen-depleted conditions through p38MAPK activation (78). This finding establishes the TGF-β-SMAD2-p38MAPK axis as a driver of treatment-emergent neuroendocrine PC (NEPC), a lethal subtype characterized by resistance to AR signaling inhibitors (78). Complementing this mechanism, endothelial-mesenchymal transition (EndoMT) in the TME was identified as a contributor to NED; IL-1β and TGF-β2 induced EndoMT in vascular endothelial cells, and EndoMTed endothelial cells promoted neuroendocrine features in PC cells via GM-CSF secretion (79). Importantly, enzalutamide treatment increased IL-1β and TGF-β2 secretion, creating a reciprocal loop that promotes NEPC and may underlie acquired resistance to AR-targeted therapies (79).

TGF-β-mediated immune desert microenvironment in CRPC. CRPC is characterized by an immune desert phenotype, and TGF-β signaling may play an important role in establishing this immunosuppressive contexture. Single-cell sequencing of CRPC tissues revealed that TGF-β signaling and ECM remodeling pathways are remarkably upregulated in CRPC-associated fibroblasts, with high CRPC-CAF abundance correlating with immunosuppression and immunotherapy insensitivity (6). Importantly, anti-TGF-β therapy was shown to potentiate anti-PD-1 immunotherapy efficacy in PC models, suggesting that CAF-derived TGF-β represents a major barrier to T cell infiltration and function in CRPC (6). The immune desert nature of PC has been further contextualized as a category characterized by few intratumor effector T cells, low TMB, high density of immunosuppressive myeloid cells, and suppressive cytokines including TGF-β (7). These features collectively render CRPC largely refractory to current immunotherapies, highlighting the need for TGF-β-targeted strategies to remodel the immune microenvironment.

Crosstalk between TGF-β and AR signaling in immune regulation. The interplay between TGF-β and AR signaling adds additional complexity to immune regulation in PC. CTHRC1, expressed predominantly in myofibroblast-like CAFs, was shown to be associated with resistance to anti-androgen therapy; TGF-β signaling was activated in CTHRC1-positive myofibroblastic CAFs (myCAFs) and involved in TAM polarization (80). Mechanistically, CTHRC1 may regulate AR signaling through the CCN2/CAV1/AR pathway, with high CTHRC1 expression correlating with reduced sensitivity to anti-androgen drugs (80). Conversely, AR inhibition was found to paradoxically suppress antitumor neutrophil responses against bone metastatic PC via regulation of TβRI expression, revealing that androgen-mediated TGF-β expression suppresses PMN cytotoxicity and that androgen deprivation therapy (ADT) can inadvertently promote TGF-β pathway activity in the bone microenvironment (81). These bidirectional interactions between AR and TGF-β signaling underscore the importance of understanding context-dependent effects when combining hormonal therapies with immunotherapies.

TGF-β-driven immunosuppressive bone marrow microenvironment in metastatic CRPC. Bone metastasis represents a particularly challenging site of CRPC progression, and TGF-β may play a prominent role in creating an immunosuppressive bone marrow microenvironment. Mechanistic studies have demonstrated that tumors in the bone promote osteoclast-mediated bone resorption, which releases TGF-β that restrains Th1 lineage development (82). Blocking TGF-β along with immune checkpoint therapy increased Th1 subsets, promoted clonal expansion of $CD8^+$ T cells, and induced regression of bone CRPC with improved survival (82). Single-cell RNA sequencing further identified that TGF-β, MIF and CXCL pathways are enriched in the bone metastasis microenvironment of PC, aligning with observations in human CRPC bone metastasis (83). These findings establish TGF-β as a candidate node in the immunosuppressive bone metastatic niche, where it suppresses T helper polarization and limits the efficacy of ICB.

RCC

TGF-β pathway alterations across RCC subtypes. In RCC, TGF-β pathway alterations exhibit subtype-dependent prognostic implications. SMAD4, the principal mediator of TGF-β-induced EMT, was shown to correlate with cancer stem cell features and tumor aggressiveness in RCC, establishing SMAD4 expression as a prognostic marker associated with poor clinical outcomes (84). Comprehensive genomic and transcriptomic analysis of ccRCC identified mutations and gene expression changes in the TGF-β pathway, revealing that OS of patients with ccRCC with high TGF-β scores was

unexpectedly improved compared with those with low TGF-β scores, with TGF-β score correlating with expression of key ccRCC and deacetylation genes (85). This paradoxical finding highlights the context-dependent nature of TGF-β signaling in RCC, where pathway activation may reflect distinct tumor biology rather than simply indicating poor prognosis.

TGF-β-related immune evasion and resistance to anti-angiogenic agents. In ccRCC, fibrogenesis-driven tumor progression is mediated through NRP1 engagement of the TGF-β/SMAD pathway, with NRP1 upregulation paradoxically associated with favorable prognosis in TCGA due to stromal enrichment (21). This observation further underscores the subtype-dependent prognostic implications of TGF-β pathway activation in RCC (21). Resistance to ICB combined with TKIs, a standard first-line regimen in advanced RCC, has been linked to TGF-β pathway activation; transcriptomic analysis revealed that resistance involves TGF-β-TGFBR signaling leading to upregulation of CEACAM1 on neutrophils (86). These findings position TGF-β pathway activation as a resistance mechanism to both anti-angiogenic agents and immunotherapies in RCC.

Prognostic and predictive value of TGF-β gene signatures in RCC immunotherapy. Efforts to stratify patients with RCC for immunotherapy have incorporated TGF-β signaling-related gene signatures. A novel TGF-β signaling-related gene signature was developed to predict clinical prognosis and immunotherapy responses in patients with ccRCC, with high-risk patients showing immunosuppressive phenotypes and reduced benefit from PD-1 blockade (87). The TME-driven drug resistance mechanisms in urologic cancers, including RCC, have been shown to involve TGF-β-mediated T cell exclusion, regulatory T cell expansion, and adaptive checkpoint upregulation, with hypoxia and metabolic reprogramming further promoting immune suppression through HIF-2α activation (11).

Crosstalk between TGF-β and hypoxia/HIF pathways in ccRCC immune remodeling. The integration of TGF-β signaling with HIF pathways represents a defining feature of ccRCC immune remodeling. In patients with ccRCC, HIF-2α induces SCF expression, which recruits mast cells that impair antitumor immunity partly by secreting IL-10 and TGF-β, establishing a direct link between HIF-2α-driven oncogenesis and TGF-β-mediated immune suppression (88). Mechanistically, HIF-1α and HIF-2α exert divergent functions in ccRCC despite both being stabilized by VHL loss. HIF-1α is mainly linked to acute/severe hypoxic adaptation, glycolytic metabolism, and context-dependent tumor-restraining or apoptotic responses, whereas HIF-2α preferentially drives pseudohypoxic oncogenic programs, including angiogenesis, proliferation and immune evasion, and is considered the dominant oncogenic HIF isoform in ccRCC. This functional divergence explains the opposite correlation patterns of HIF-1α and HIF-2α with TGF-β signaling and tumor-promoting biomarkers, in which TGF-β signaling and tumor-promoting biomarkers associate positively with HIF-2α but negatively with HIF-1α (85,88). This crosstalk suggests that targeting both HIF-2α and TGF-β pathways may be necessary to overcome the immunosuppressive microenvironment in ccRCC. The integration of TGF-β and HIF pathways is further supported by findings that most tumor-promoting biomarkers in ccRCC are positively associated with TGF-β signaling and HIF-2α, and negatively associated with pVHL and HIF-1α (85).

Comparative synthesis: Shared core mechanisms and tumor type-specific adaptations of TGF-β-driven immune remodeling. Across BC, PC and RCC, TGF-β signaling converges on a core immunosuppressive program that transcends anatomical location and represents the fundamental basis for considering TGF-β as a central therapeutic target. This shared mechanistic core encompasses $CD8^+$ T cell exhaustion and exclusion, regulatory T cell expansion, M2 macrophage polarization, CAF activation with consequent ECM remodeling, and metabolic reprogramming that collectively establishes a tolerogenic niche and drives immunotherapy resistance (10,11). However, superimposed upon this common framework are three tumor type-specific adaptations that distinguish TGF-β biology in each malignancy (Table I).

In PC, TGF-β signaling is uniquely integrated with AR pathways, creating bidirectional crosstalk that adds considerable complexity to immune regulation. IL-6 activates the TGF-β-SMAD2-p38MAPK axis to drive NED of PC cells under androgen-depleted conditions, promoting treatment-emergent NEPC, a lethal subtype characterized by resistance to AR signaling inhibitors (78). In the bone metastatic niche, TGF-β released from osteoclast-mediated bone resorption restrains Th1 lineage development, and blocking TGF-β along with immune checkpoint therapy increases Th1 subsets, promotes clonal expansion of $CD8^+$ T cells, and induces regression of bone CRPC (82). Furthermore, single-cell sequencing has revealed that TGF-β signaling and ECM remodeling pathways are remarkably upregulated in CRPC-associated fibroblasts, with high CAF abundance correlating with immunosuppression and diminished RFS (6).

In RCC, the defining feature of TGF-β-mediated immune remodeling is its integration with HIF pathways, a consequence of the VHL inactivation that fundamentally shapes clear cell RCC biology. HIF-2α induces SCF expression, which recruits mast cells that impair antitumor immunity partly by secreting IL-10 and TGF-β, establishing a direct link between HIF-2α-driven oncogenesis and TGF-β-mediated immune suppression (88). The prognostic value of TGF-β pathway activation is subtype-dependent; comprehensive genomic and transcriptomic analysis has revealed that OS of patients with ccRCC with high TGF-β scores was unexpectedly improved compared with those with low TGF-β scores, highlighting the context-dependent nature of TGF-β signaling in RCC (85). Resistance to ICB combined with TKIs has been linked to TGF-β-TGFBR signaling leading to upregulation of CEACAM1 on neutrophils (86).

In BC, TGF-β pathway activation is intricately linked to primary resistance to both BCG immunotherapy and ICB through tumor-specific mechanisms. Integrin β8 (ITGB8), expressed on tumor cells, plays an immunosuppressive role in BCG therapy response; high ITGB8 expression is significantly associated with post-BCG recurrence, and ITGB8 knockdown markedly decreases TGF-β1 secretion (35). ELN (tropoelastin), secreted by CAFs, functions as a TGFBR2-binding partner that triggers SMAD2/3-dependent TGF-β1 autocrine signaling, establishing a feed-forward loop that drives metastasis and immunosuppressive niche formation characterized

Table I. Comparative overview of TGF-β-mediated immune remodeling across urological malignancies.

Feature	Bladder cancer	Prostate cancer	Renal cell carcinoma
Shared core mechanisms	$CD8^+$ T cell exhaustion, Treg expansion, M2 macrophage polarization, CAF activation, ECM remodeling, metabolic reprogramming.	$CD8^+$ T cell exhaustion, Treg expansion, M2 macrophage polarization, CAF activation, ECM remodeling, metabolic reprogramming.	$CD8^+$ T cell exhaustion, Treg expansion, M2 macrophage polarization, CAF activation, ECM remodeling, metabolic reprogramming.
Disease-specific drivers	ITGB8 (tumor cell-derived TGF-β1 activation), ELN (CAF-derived TGFBR2-binding partner), TBX3 (transcriptional regulator).	AR-TGF-β bidirectional crosstalk, neuroendocrine differentiation (TGF-β-SMAD2-p38MAPK axis), bone metastasis niche (Th1 suppression).	HIF-2α-TGF-β crosstalk, VHL inactivation context, mast cell recruitment (SCF-dependent).
Unique immune context	BCG therapy resistance (NMIBC); NK cell dysfunction via CD16 downregulation.	Immune desert phenotype in CRPC; bone marrow immunosuppressive niche.	Pseudohypoxia-driven metabolic reprogramming; $POSTN^+$ $CAF\text{-}CCL3^+$ macrophage spatial axis.
Therapeutic implications	TGF-β inhibition to sensitize to BCG and ICB; targeting ITGB8 or ELN-TGFBR2 axis.	Combined TGF-β + AR targeting; TGF-β-resistant CAR T cells; TGF-β blockade + ICB in bone metastases.	Combined TGF-β + HIF-2α targeting; TGF-β inhibition to overcome TKI/ICI resistance.
Prognostic significance	TGF-β risk score predicts OS and immune phenotype.	Stage-dependent: tumor suppression in early disease, tumor promotion in CRPC.	Subtype-dependent: high TGF-β scores may paradoxically associate with improved OS in ccRCC.

AR, androgen receptor; BCG, Bacillus Calmette-Guérin; CAF, cancer-associated fibroblast; ccRCC, clear cell renal cell carcinoma; CRPC, castration-resistant prostate cancer; ECM, extracellular matrix; ICB, immune checkpoint blockade; ICI, immune checkpoint inhibitor; NMIBC, non-muscle-invasive bladder cancer; OS, overall survival; TKI, tyrosine kinase inhibitor.

by reduced $CD8^+$ T cell infiltration and increased MDSC accumulation (73). In MIBC, integrative analysis has revealed that a patient subgroup resistant to ICB is characterized by high angiogenesis signature, low TMB, and activation of TGF-β signaling with downregulated T-cell cytolytic signatures (75).

Collectively, these observations suggest that while TGF-β signaling may operate through a conserved core immunosuppressive program across urological malignancies, the specific molecular drivers and tissue context, including AR crosstalk in PC, HIF-2α integration in RCC, and BCG resistance-associated factors in BC, confer disease-specific adaptations that must be considered in the rational design of TGF-β-targeted therapeutic strategies.

TGF-β pathway in rare urological malignancies. In UTUC, TGF-β1-induced transcript 1 (TGFB1I1) was identified as a regulator of cell proliferation and migration (67). TGFB1I1 expression was found to regulate urothelial carcinoma cell proliferation and viability and induce EMT to facilitate cell migration, playing an essential role in promoting tumor aggressiveness in both UTUC and urinary bladder urothelial carcinoma (67). These observations suggest that TGF-β pathway activation may similarly promote immune evasion and progression in UTUC, though dedicated studies in this rare malignancy remain limited.

Comprehensive analysis of TGF-β and miR interplay in genitourinary cancers has documented elevated TGF-β expression in tumor tissue relative to normal tissue for testicular cancer, along with summarizing the complex interplay between miRs and TGF-β signaling across genitourinary malignancies including penile cancer (71). The miR-mediated regulation of TGF-β signaling in these rare cancers may represent a therapeutic vulnerability, though the limited incidence of these malignancies presents challenges for clinical investigation.

5. Therapeutic targeting of TGF-β pathway in urological malignancies

The multifaceted immunosuppressive functions of TGF-β signaling in urological malignancies have spurred intensive efforts to develop therapeutic strategies that block this pathway. Despite robust preclinical rationale, the clinical development of TGF-β-targeting agents has been consistently hampered by two principal challenges: On-target toxicity, which arises from the pleiotropic physiological functions of TGF-β in normal tissues and manifests as cardiovascular, bleeding and dermatologic adverse events that have necessitated dose reductions or intermittent schedules (89-91), and limited single-agent antitumor efficacy, as illustrated by the absence of objective

responses with fresolimumab monotherapy in RCC (92) and the modest response rates observed in early-phase combination trials (93). A diverse array of agents, including ligand-neutralizing antibodies, receptor kinase inhibitors and TGF-β-resistant cellular immunotherapies, has been evaluated in preclinical models and early-phase clinical trials. However, the translation of these approaches from bench to bedside has proven challenging, with several promising agents failing to demonstrate meaningful clinical benefit in later-stage trials. This section provides a critical assessment of the clinical evidence for each therapeutic modality, highlighting both successes and limitations. Table II provides a comprehensive overview of these therapeutic modalities. Clinical and preclinical evidence is separately summarized in Table II, with clinical studies detailing study phase, sample size, prior ICI exposure, efficacy, safety and trial status.

Classes of TGF-β-targeted therapeutic agents. Fresolimumab (GC1008), a human monoclonal antibody capable of neutralizing all three TGF-β isoforms, was evaluated in a Phase I study involving patients with advanced RCC or malignant melanoma. Among 28 patients with RCC, no objective responses were observed, although several patients experienced prolonged stable disease (92). The absence of objective responses in this unselected population, despite evidence of target engagement, raised early concerns about whether pan-TGF-β neutralization alone provides sufficient antitumor activity in RCC, and highlighted the likely need for combination strategies. However, the small sample size and lack of a biomarker-driven design limit the interpretability of these negative findings. More recently, linavonkibart (SRK-181), a selective antibody targeting latent TGF-β1, was combined with pembrolizumab in the Phase 1 DRAGON trial for patients with ICI-resistant advanced solid tumors. In anti-PD-1-resistant ccRCC, the confirmed objective response rate (ORR) reached 20.0%, while patients ϛιτη urothelial cancer achieved a 9.1% ORR (93). Although these responses are encouraging in the context of ICI-resistant disease, the modest and highly variable response rates across tumor types underscore the heterogeneity of TGF-β-driven resistance mechanisms and suggest that TGF-β1 blockade may benefit only a subset of patients. The lack of a biomarker-stratified design in this Phase I trial limits the ability to determine whether responses were confined to patients with baseline TGF-β pathway activation.

Small-molecule inhibitors targeting type I TGF-β receptor/activin receptor-like kinase 5 (TβRI/ALK5) constitute the most extensively investigated class of TGF-β-directed therapeutics. Galunisertib (LY2157299), a first-in-class TβRI inhibitor, demonstrated potent antitumor activity in preclinical models (94). However, Phase II clinical trials in both newly diagnosed and recurrent glioblastoma, as well as in refractory metastatic pancreatic cancer, failed to demonstrate meaningful clinical benefit, with no improvement in primary survival endpoints. These negative results raise important questions about the adequacy of target inhibition achieved with this agent in the TME and the appropriateness of patient selection in these trials. PF-06952229, another selective TβRI inhibitor, was evaluated in a first-in-human Phase I dose-escalation study in advanced solid tumors; among treated patients, one patient with PC achieved a confirmed partial response lasting 31 months (89). While this single durable response in PC is noteworthy, the overall response rate across the study population was low (with no other objective responses reported), and dose-limiting toxicities occurred in 8.6% of patients, underscoring the narrow therapeutic index of this class. The study was not designed to test efficacy in a biomarker-enriched population, limiting the generalizability of the findings. The anti-TGF-β monoclonal antibody SAR439459, assessed as monotherapy and in combination with cemiplimab in a Phase 1/1b study, demonstrated an 8.4% ORR across tumor types in Part 2B. However, the study was ultimately discontinued due to unclear efficacy signals and bleeding risk (95). The discontinuation of SAR439459 development exemplifies a recurring pattern in the field: Early-phase studies demonstrating manageable safety and modest activity are followed by termination due to insufficient efficacy signals or unacceptable toxicity. This highlights the high attrition rate in TGF-β inhibitor development and the critical need for more robust preclinical models that can better predict clinical outcomes. Furthermore, A Pluronic F-127-based microsphere delivery system encapsulating a TGF-β1 inhibitor was developed to enhance the therapeutic efficacy of gemcitabine in BC, representing a promising strategy to improve drug targeting and reduce systemic toxicity (96). However, this approach remains at a preclinical stage, and its clinical feasibility and ability to overcome the fundamental challenges of TGF-β targeting, including on-target toxicity and pathway duality, remain to be demonstrated.

Rationale and clinical evidence of TGF-β inhibition combined with ICIs. Preclinical evidence strongly supports the combination of TGF-β blockade with ICIs. In BC models, TGF-β1 inhibition enhanced the therapeutic efficacy of gemcitabine when delivered via microspheres (96). A proposed mechanistic basis for this synergy involves capacity of TGF-β to exclude $CD8^+$ T cells from the TME and promote regulatory T cell accumulation; pathway inhibition reverses these effects, thereby potentiating ICI activity. Despite this robust preclinical rationale, the clinical translation of TGF-β/ICI combinations has been fraught with difficulties. A critical review of the literature reveals that while preclinical studies exploring combined TGF-β and immune checkpoint inhibition have demonstrated near-uniform positive results across tumor types, landmark clinical studies have reinforced the challenges of translation (97). This discrepancy may stem from the complexity of the TME in patients relative to preclinical models, as well as the absence of robust predictive biomarkers to identify patients most likely to benefit from TGF-β-targeted interventions.

The DRAGON trial of linavonkibart plus pembrolizumab provided the most direct clinical evidence to date for TGF-β/ICI combination therapy in urological malignancies. In ICI-resistant ccRCC, the 20.0% ORR compares favorably to historical outcomes with ICI monotherapy in the second-line setting. However, the modest responses in urothelial cancer (9.1%) highlight the heterogeneity of TGF-β-driven resistance mechanisms across tumor types (93). It is important to note that this was an open-label, single-arm Phase I study without a control arm, and the results require confirmation in larger, randomized trials.

Table II. TGF-β-targeted therapeutic strategies in urological malignancies: Agents, mechanisms and clinical evidence.

First author/s, year	Agent/Strategy	Target/Mechanism	Tumor type(s)	Study phase/Evidence level	Key findings	(Refs.)
Morris *et al*, 2014	Fresolimumab (GC1008)	Pan-TGF-β (isoforms 1, 2, 3)	RCC, melanoma	Phase I clinical trial	Well tolerated; no objective responses in RCC (n=28); several patients achieved prolonged stable disease	(92)
Yap *et al*, 2026	Linavonkibart (SRK-181) + pembrolizumab	Latent TGF-β1 + PD-1	ICI-resistant ccRCC, urothelial cancer, other solid tumors	Phase I clinical trial (open-label, single-arm)	Confirmed ORR: 20.0% in ccRCC, 9.1% in urothelial cancer; manageable safety profile; proof of mechanism established	(93)
Yingling *et al*, 2018	Galunisertib (LY2157299)	TβRI/ALK5 kinase	Preclinical models (various)	Preclinical study (*in vitro* and *in vivo* models)	Potent antitumor activity; reverses TGF-β-mediated suppression of T cell proliferation; inhibits TGF-β-induced EMT and cell migration	(94)
Yap *et al*, 2024	PF-06952229	TβRI/ALK5 kinase	Advanced solid tumors, mCRPC	Phase I clinical trial	One prostate cancer patient achieved confirmed PR lasting 31 months; DLTs in 8.6% (grade 3 anemia, intracranial hemorrhage); dose-proportional exposure with pSMAD2/3 modulation	(89)
Baranda *et al*, 2024	SAR439459 ± cemiplimab	TGF-β (monoclonal antibody) + PD-1	Advanced solid tumors, HCC	Phase I/1b clinical trial	ORR 8.4% across tumor types (Part 2B); study discontinued due to unclear efficacy and bleeding risk (21.4% fatal bleeding in HCC)	(95)
Xiong *et al*, 2025	TGF-β1 inhibitor + gemcitabine (Pluronic F-127 microsphere)	TGF-β1 + DNA synthesis	Bladder cancer	Preclinical study (experimental bladder cancer models)	Enhanced therapeutic efficacy of gemcitabine in bladder cancer models; improved drug targeting and reduced systemic toxicity	(96)
Brossa *et al*, 2018	TRC105 + sunitinib	Endoglin (TGF-β co-receptor) + VEGFR	RCC	Preclinical *in vitro* study (renal tumor-derived endothelial cells)	TRC105 reduced renal tumor-derived endothelial cell viability and migration; combination with sunitinib showed enhanced antiangiogenic effects	(98)
Shobaki *et al*, 2025	dnTGFβRII-armored CAR T cells (STEAP2-targeting, αPSMA x CD28 bispecific)	Dominant-negative TGF-β receptor II	mCRPC	Preclinical study (CAR T-cell functional assays)	TGF-β-resistant CAR T cells maintain cytotoxic function in TGF-β-rich microenvironments where conventional CAR T cells become dysfunctional	(100)

Table II. TContinued.

First author/s, year	Agent/Strategy	Target/Mechanism	Tumor type(s)	Study phase/Evidence level	Key findings	(Refs.)
Zhou *et al*, 2019	SB431542 + radiofrequency ablation	TGF-β/Smad pathway	Bladder cancer	Preclinical study (*in vitro* and *in vivo* models)	SB431542 reversed RFA-induced TGF-β signaling activation and enhanced apoptosis, potentiating antitumor effect of RFA	(101)

ccRCC, clear cell renal cell carcinoma; DLT, dose-limiting toxicity; dnTGFβRII, dominant-negative TGF-β receptor type II; EMT, epithelial-mesenchymal transition; HCC, hepatocellular carcinoma; ICI, immune checkpoint inhibitor; mCRPC, metastatic castration-resistant prostate cancer; ORR, objective response rate; PD-1, programmed cell death protein 1; PR, partial response; RCC, renal cell carcinoma; RFA, radiofrequency ablation; TβRI, TGF-β receptor type I; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor.

Combination strategies beyond ICI co-administration. TGF-β and VEGF signaling pathways exhibit extensive crosstalk in promoting abnormal tumor vasculature and immune exclusion. In RCC, the monoclonal antibody TRC105, which targets endoglin (a TGF-β co-receptor), was evaluated in combination with sunitinib. TRC105 treatment reduced renal tumor-derived endothelial cell viability and migration, and the combination with sunitinib showed enhanced antiangiogenic effects (98). However, clinical validation of this combination remains limited; a Phase II trial of TRC105 plus sunitinib in advanced RCC did not meet its primary endpoint, and the development of TRC105 was subsequently discontinued. This underscores the challenge of translating preclinical synergy into clinical benefit, even when a strong biological rationale exists.

The intersection of TGF-β and AR signaling creates both opportunities and challenges for combination therapy in PC. In bone metastatic PC, AR inhibition was found to paradoxically suppress antitumor neutrophil responses via regulation of TβRI expression, indicating that ADT can inadvertently promote TGF-β pathway activity in the bone microenvironment (81). This finding underscores the importance of carefully timed TGF-β inhibition in the context of hormonal therapy. The Phase 2 trial of galunisertib plus enzalutamide in mCRPC represents the most direct clinical evaluation of this combination strategy (NCT02452008). In this multicenter, randomized Phase II trial, 61 chemotherapy-naïve mCRPC patients with disease progression on abiraterone were randomized 3:2 to receive galunisertib plus enzalutamide (n=37) or enzalutamide alone (n=23). The addition of galunisertib did not improve radiographic PFS (rPFS) compared with enzalutamide alone (median rPFS 5.6 vs. 7.3 months; HR, 1.23; 95% CI, 0.72-2.11; P=0.44). OS was also similar between the two arms (median OS, 21.6 vs. 24 months; HR, 1.00; 95% CI, 0.55-1.83; P=1.00). PSA30 response rates were 22% in the combination arm vs. 35% in the enzalutamide arm (P=0.30), and PSA50 responses were 19% vs. 26%, respectively (P=0.50). Grade 3-4 treatment-related adverse events occurred in 11% of patients in the combination arm vs. 13% in the enzalutamide arm. These findings do not support further development of galunisertib plus enzalutamide in this patient population, and the negative outcomes of several galunisertib combination trials in other tumor types, caution against overinterpreting the preclinical rationale. Until robust clinical data are available, this combination remains experimental.

Engineering resistance to TGF-β-mediated suppression represents a frontier in cellular immunotherapy. Disrupting the SKI-SMAD2/3 axis has been shown to enhance adoptive T cell therapy by counteracting TGF-β-mediated immunosuppression (99). In PC, an all-in-one CAR T cell platform incorporating dominant-negative TGF-β receptor II armoring, STEAP2 targeting, and αPSMAxCD28 bispecific secretion has been developed for mCRPC. These TGF-β-resistant CAR T cells maintain cytotoxic function in TGF-β-rich microenvironments where conventional CAR T cells become dysfunctional, representing a promising strategy to overcome a major barrier to cellular immunotherapy efficacy in PC and other TGF-β-rich solid tumors (100). While this approach is conceptually elegant, it remains at a preclinical stage, and the translation to clinical practice will require demonstration of safety and efficacy in patients. The potential for enhanced

CAR-T cell activity to precipitate cytokine release syndrome or other immune-related adverse events in the context of TGF-β pathway blockade must also be carefully evaluated.

Radiotherapy induces TGF-β pathway activation in the TME, potentially limiting its antitumor efficacy. The TGF-β/Smad pathway inhibitor SB431542 was shown to enhance the antitumor effect of radiofrequency ablation on BC cells. Mechanistically, SB431542 reversed radiofrequency ablation-induced TGF-β signaling activation and enhanced apoptosis, suggesting that TGF-β inhibition may potentiate locoregional therapies (101). However, this finding is based solely on *in vitro* and *in vivo* models, and no clinical data exist to support this combination. The preclinical nature of these findings underscores the substantial gap between mechanistic demonstration and therapeutic application.

Beyond summarizing individual clinical outcomes, several recurring themes emerge from the clinical development of TGF-β-targeted agents including galunisertib, fresolimumab, bintrafusp alfa and SAR439459 that explain the frequent disconnect between preclinical promise and clinical failure (97). First, on-target toxicity, particularly cardiovascular effects, bleeding and dermatologic toxicity, has repeatedly narrowed the therapeutic window, necessitating dose reductions or intermittent schedules that may compromise efficacy (89-91). Isoform-selective inhibition targeting TGFβ1, while sparing TGFβ2 and TGFβ3, has been proposed to mitigate systemic toxicity while preserving antitumor activity (102). Second, the absence of validated predictive biomarkers has been a consistent limitation across all major trials; without biomarker-driven patient selection, efficacy signals in TGF-β-activated subsets are diluted in unselected populations. Third, pathway redundancy and the context-dependent dual functionality of TGF-β, tumor suppressor in early-stage disease vs. tumor promoter in advanced malignancies, limit the efficacy of single-agent blockade and may require rational combinations with immunotherapies or anti-angiogenic agents (9). Fourth, the striking discordance between near-uniform preclinical success and consistent clinical failure highlights the inadequacy of current preclinical models in recapitulating the complexity of the human TME, including stromal heterogeneity, immune contexture and spatial TGF-β signaling organization (97). Collectively, these lessons argue for a shift toward biomarker-driven, mechanism-based trial designs with rigorous patient selection and rational combination strategies.

6. Predictive biomarkers for TGF-β-targeted immunotherapy

The heterogeneous clinical outcomes of TGF-β-targeted immunotherapies underscore the urgent need for reliable predictive biomarkers to guide patient selection. Ideal biomarkers should capture TGF-β pathway activation status, reflect the resultant immunosuppressive contexture, and associate with resistance or sensitivity to pathway inhibition. However, candidate biomarkers differ substantially in their level of clinical validation. To provide a clinically actionable framework, this section categorizes biomarkers into three tiers: i) clinically validated biomarkers that have been evaluated in prospective trials or rigorously validated in independent cohorts with demonstrated association with immunotherapy outcomes; ii) biomarkers supported by promising prospective clinical data, including those with retrospective validation in multiple independent cohorts or those evaluated in the context of specific TGF-β-targeted therapeutic trials; and iii) exploratory biomarkers that have shown association in single retrospective studies but require further validation before clinical application. Candidate biomarkers span multiple analytical levels, including tissue-based molecular signatures, stromal and immune cellular correlates, circulating analytes, radiomic features and spatial multi-omica readouts. To provide a clinically actionable framework, this section categorizes biomarkers into these evidence-based roles rather than treating all candidates as predictive biomarkers. A comprehensive overview of these biomarker categories is provided in Table III, summarizing representative examples from each tumor type alongside their clinical context and key limitations.

Tumor tissue-based biomarkers. Tissue-based biomarkers provide direct assessment of TGF-β pathway activity within the TME. In BC, a 12-gene TGF-β risk score was developed to independently predict OS, with high-risk patients demonstrating significantly worse prognosis and elevated M2 macrophage infiltration, whereas low-risk tumors exhibited stronger cytolytic activity and $CD8^+$ T cell infiltration (72). This risk score is primarily a prognostic and immune-contexture marker; its utility for selecting patients for TGF-β-directed therapies remains unproven. In ccRCC, a TGF-β signaling-related gene signature was constructed as an independent prognostic factor; high-risk patients showed immunosuppressive phenotypes and reduced benefit from PD-1 blockade, supporting its role as a treatment-response-associated marker rather than a validated predictive biomarker for TGF-β-targeted therapy (87). However, the prognostic value of TGF-β signatures in RCC remains subtype-dependent, underscoring the need for tumor type-specific validation.

Single-gene biomarkers have also been investigated as surrogate indicators of TGF-β pathway activation. SMAD4, the principal mediator of TGF-β-induced EMT, correlates with cancer stem cell features and poor clinical outcomes in RCC, establishing its expression as a prognostic marker associated with tumor aggressiveness (84). In BC, TBX3 overexpression was found to increase TGFβ1 secretion and promote CAF infiltration; importantly, TBX3 knockdown sensitized tumors to anti-PD-1 therapy through enhanced $CD8^+$ T cell infiltration and function (74). Furthermore, multi-omics analysis revealed that m7G methylation-related genes may be involved in TGF-β signaling-mediated anti-PD-L1 response in BC. Differential methylation patterns in this pathway correlated with immunotherapy resistance, and integrative analysis identified NUDT10 as one of the key genes associated with poor prognosis and reduced response to anti-PD-L1 therapy (77). Despite these promising findings, most tissue-based biomarkers have been derived from retrospective cohorts and require prospective validation to establish their predictive value for TGF-β-targeted interventions. From a translational perspective, the 12-gene TGF-β risk score in BC and the seven-gene TGF-β signaling-related signature in ccRCC represent the most extensively validated tissue-based prognostic biomarkers in multiple cohorts (72). Single-gene candidates such as SMAD4, TBX3 and NUDT10, while

Table III. Candidate biomarkers for TGF-β-targeted immunotherapy in urological malignancies: Prognostic, treatment-response-associated and predictive categories.

First author/s, year	Biomarker category	Biomarker/Platform	Tumor type	Clinical context	Key finding	(Refs.)
Liu *et al*, 2021	Tissue-based (gene signature)	12-gene TGF-β risk score	Bladder cancer	Prognosis and immune phenotyping	High-risk score predicts worse OS, elevated M2 macrophage infiltration, and enhanced TGF-β pathway activation; low-risk tumors show stronger cytolytic activity and $CD8^+$ T cell infiltration	(72)
Wu *et al*, 2023	Tissue-based (gene signature)	TGF-β signaling-related gene signature	ccRCC	Prognosis and immunotherapy response	High-risk patients show immunosuppressive phenotypes and reduced benefit from PD-1 blockade	(87)
Rasti *et al*, 2021	Tissue-based (single gene)	SMAD4 expression	RCC	Prognosis	SMAD4 correlates with cancer stem cell features, tumor aggressiveness, and poor clinical outcomes	(84)
Liu *et al*, 2025	Tissue-based (single gene)	TBX3 expression	Bladder cancer	Immunotherapy resistance	TBX3 overexpression increases TGFβ1 secretion and CAF infiltration; knockdown sensitizes tumors to anti-PD-1 therapy	(74)
Liang *et al*, 2026	Tissue-based (multi-omics)	NUDT10/m7G methylation-related genes	Bladder cancer	Anti-PD-L1 response	NUDT10 regulates TGF-β signaling; differential methylation patterns correlate with immunotherapy resistance	(77)
Qiu *et al*, 2025	Stromal/immune	CAF abundance (single-cell sequencing)	CRPC	Immunotherapy response	High CRPC-CAF abundance correlates with immunosuppression and diminished RFS; anti-TGF-β therapy potentiates anti-PD-1 efficacy	(6)
Hou, 2025	Stromal/immune	TGFβ-activated myofibroblastic CAFs	Solid tumors (general)	T-cell exclusion	CAFs drive T-cell exclusion via physical barriers and downstream molecules (βig-h3, Endo180, LRRC15, NOX4)	(103)
Song *et al*, 2026	Stromal/immune	SUSD2 (single-cell transcriptomics)	Bladder cancer	Microenvironment and prognosis	SUSD2 identified as key immunosuppressive molecule; TGF-β receptor inhibition reverses its effects on tumor proliferation and migration	(104)
Shariat *et al*, 2001	Circulating (cytokine)	Plasma TGF-β1	Bladder cancer	Prognostic/pathway-activity marker; not a validated predictive biomarker for TGF-β-targeted therapy	Preoperative plasma TGF-β1 levels strongly predict clinical outcome, including disease recurrence and disease-specific mortality	(105)
Rijnders *et al*, 2023	Circulating (immune metric)	MNTR	Metastatic urothelial cancer	Pembrolizumab resistance	High MNTR predicts poorer OS (2.2 vs. 8.9 months, HR 6.6) and PFS (1.5 vs. 5.2 months, HR 5.6); surpasses NLR and PD-L1 CPS	(109)

Table III. Continued.

First author/s, year	Biomarker category	Biomarker/Platform	Tumor type	Clinical context	Key finding	(Refs.)
Hanusek *et al*, 2022	Circulating (cytokine)	Plasma TGF-β1	RCC	Detection and monitoring	Higher plasma TGF-β1 in metastatic vs. localized RCC; may aid in early recurrence detection and treatment monitoring	(107)
Xia *et al*, 2017	Circulating (EV-based)	Tumor-derived exosomal TGF-β1	ccRCC	NK cell dysfunction	Exosomes preferentially enriched with TGF-β1 mediate NK cell dysfunction via TGF-β/SMAD signaling; TGF-β knockdown abrogates this effect	(108)
Ringuette Goulet *et al*, 2018	Circulating (EV-based)	Exosomal TGF-β (cancer cell-derived)	Bladder cancer	CAF differentiation	Exosome-mediated TGF-β transfer triggers fibroblast-to-CAF differentiation via SMAD pathway; exosomal TGF-β accounts for 53.4-86.3% of total supernatant TGF-β	(58)
Liu *et al*, 2023	Radiomic/imaging	MRI radiomics-based LASSO model	Bladder cancer	Prognostic stratification	Ten-radiomics-feature model achieved AUC of 0.839 (training) and 0.810 (validation) for prognostic stratification	(111)
Huang *et al*, 2026	Radiomic/imaging	MRI radiomic phenotypes	Very high-risk NMIBC	Immunotherapy response	Radiomic phenotypes stratify treatment response and define immune states in patients receiving tislelizumab plus nab-paclitaxel	(112)
Ku *et al*, 2025	Radiomic/imaging	Radiogenomic TGF-β	Prostate cancer activity signature	Biochemical recurrence post-radiotherapy	TGF-β activity signature correlates with imaging phenotypes and predicts biochemical failure after EBRT + ADT; retains prognostic capacity in independent cohorts	(110)
Yu *et al*, 2026	Spatial multi-omics	Integrated single-cell + spatial transcriptomics (n=127 samples)	Prostate cancer	Immune niche identification	FOLR2$^+$/CX3CR1$^+$ TAMs upregulate TGF-β signaling; CXCL12$^+$ iCAFs and ACTA2$^+$ myCAFs maintain spatial co-localization facilitating immune cell recruitment	(113)
Wang *et al*, 2026	Spatial multi-omics	POSTN$^+$ CAF-CCL3$^+$ macrophage axis	ccRCC	Immune exclusion and immunotherapy resistance	Spatially organized stromal-immune axis at invasive front drives immune exclusion through reciprocal TGF-β signaling	(63)

Table III. Continued.

First author/s, year	Biomarker category	Biomarker/Platform	Tumor type	Clinical context	Key finding	(Refs.)
Hu *et al*, 2026	Spatial multi-omics	iCAF-CD4 tissue-resident memory T cell axis	ccRCC	Immune microenvironment and therapy	Spatial arrangement dictates patient outcomes; iCAF-CD4 TRM interactions offer potential therapeutic targets	(114)

ADT, androgen deprivation therapy; AUC, area under the curve; CAF, cancer-associated fibroblast; ccRCC, clear cell renal cell carcinoma; CPS, combined positivity score; CRPC, castration-resistant prostate cancer; EBRT, external beam radiotherapy; EV, extracellular vesicle; iCAF, inflammatory cancer-associated fibroblast; LASSO, least absolute shrinkage and selection operator; MNTR, mature neutrophil-to-T-cell ratio; MRI, magnetic resonance imaging; myCAF, myofibroblastic cancer-associated fibroblast; NMIBC, non-muscle-invasive bladder cancer; NLR, neutrophil-to-lymphocyte ratio; NK, natural killer; OS, overall survival; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; RCC, renal cell carcinoma; RFS, recurrence-free survival; TAM, tumor-associated macrophage; TRM, tissue-resident memory.

mechanistically informative, remain exploratory prognostic, pathway-activity, or treatment-response-associated markers requiring prospective evaluation as candidate predictive markers in biomarker-driven trials (74,77).

Stromal and immune biomarkers. The cellular composition of the TME, particularly the abundance and activation state of CAFs, serves as a functional readout of TGF-β-associated immune exclusion. In CRPC, single-cell sequencing revealed that TGF-β signaling and ECM remodeling pathways were remarkably upregulated in CRPC-associated fibroblasts, with high CAF abundance correlating with immunosuppression and diminished RFS (6). Importantly, anti-TGF-β therapy potentiated anti-PD-1 immunotherapy efficacy, suggesting that CAF-derived TGF-β represents a major barrier to T cell infiltration and function (6). TGFβ-activated myCAFs drive T-cell exclusion through multiple mechanisms, including physical barrier formation and the expression of downstream molecules such as βig-h3, Endo180, LRRC15 and NOX4, which emerge as promising therapeutic targets to restore immunotherapy sensitivity (103).

Beyond CAF density, specific CAF subtypes and their driver genes have been identified as potential biomarkers. In BC, single-cell transcriptomics identified SUSD2 as a key molecule within the immunosuppressive TME; treatment with the TGF-β receptor inhibitor SB-431542 reversed the facilitative effects of SUSD2 overexpression on tumor cell proliferation and migration, highlighting SUSD2 as a potential biomarker and therapeutic target linked to TGF-β pathway activity (104). The clinical utility of these stromal biomarkers lies in their ability to capture the functional state of TGF-β signaling in the TME, though standardized assays for CAF subtyping remain to be established. Among stromal biomarkers, CAF abundance and activation status, particularly as assessed by single-cell sequencing in CRPC, are treatment-response-associated markers for immunotherapy resistance and candidate predictive markers for anti-TGF-β combination therapy; however, clinical predictive validation is lacking. By contrast, individual CAF-associated molecules such as SUDS2, β-h3 and LRRC15 remain exploratory pathway-activity or stromal markers and require prospective validation as candidate predictive biomarkers in larger cohorts (103,104).

Circulating biomarkers. Liquid biopsy approaches offer the advantage of non-invasive, serial monitoring of TGF-β pathway activity. Among circulating analytes, TGF-β has been investigated as a pathway-activity and prognostic marker, not as a validated predictive biomarker, in BC. Preoperative plasma TGF-β1 levels have been shown to strongly predict clinical outcome in patients with bladder carcinoma, including disease recurrence and disease-specific mortality (105). However, circulating TGF-β measurements should be interpreted cautiously because the choice of blood matrix and pre-analytical handling, including *ex vivo* clotting and platelet activation, can substantially affect measured TGF-β levels. Platelets are a major reservoir of latent TGF-β1, and substantial platelet-derived TGF-β1 can be released into serum, potentially confounding serum-based readouts (106). Therefore, serum TGF-β1 may largely reflect total releasable TGF-β1 rather than tumor-derived circulating TGF-β1, and serum and

plasma are not interchangeable. Standardized pre-analytical protocols, such as platelet-depleted plasma, platelet count adjustment and assay validation, are required before either matrix can be recommended for TGF-β1 measurement in urothelial carcinoma (105).

In RCC, plasma TGF-β1 levels have been examined as a prognostic or monitoring marker. Patients with metastatic disease demonstrated significantly higher TGF-β1 plasma levels compared with those with localized RCC, suggesting that TGF-β1 may be useful in early detection of RCC recurrence or for monitoring the success of immunochemotherapy, but they are not validated predictive biomarkers for TGF-β-targeted therapy (107). Furthermore, quantitative assessment of active TGF-β activity in serum has been proposed to guide treatment selection in RCC, as high TGF-β activity contributes to poor responses to TKIs relative to ICIs.

Beyond free circulating cytokines, extracellular vesicles represent an emerging source of circulating biomarkers. In ccRCC, tumor-derived exosomes are preferentially enriched with TGF-β1, which acts as a key mediator of NK cell dysfunction through activation of the TGF-β/SMAD signaling pathway; knockdown of TGF-β abrogated this exosome-induced NK cells dysfunction (108). In BC, exosome-mediated TGF-β transfer from cancer cells to fibroblasts triggers their differentiation into CAFs via SMAD pathway activation, with exosomal TGF-β accounting for 53.4 to 86.3% of the total TGF-β present in the cancer cell supernatant (58). Despite these promising findings, several limitations warrant consideration. The inconsistent results between serum and plasma TGF-β1 measurements underscore the need for standardized pre-analytical and analytical protocols. Moreover, most circulating biomarker studies in urological malignancies have been retrospective and conducted in relatively small cohorts, requiring prospective validation in larger, well-controlled trials. The integration of multiple circulating analytes, including EV-associated TGF-β, soluble TGF-β isoforms, and immune cell ratios, into composite biomarker panels may ultimately provide greater predictive accuracy than any single analyte alone. From a validation perspective, the MNTR represents the most clinically promising circulating treatment-response-associated biomarker, having demonstrated strong predictive value for pembrolizumab resistance, but it is not a predictive biomarker for TGF-β-targeted therapy in metastatic urothelial cancer with hazard ratios surpassing those of conventional markers (109). Serum and plasma TGF-β1, while consistently elevated in patients with cancer, have not been prospectively validated as predictive biomarkers for TGF-β-targeted therapy and remain exploratory (105,107). Exosomal TGF-β, though mechanistically compelling as a candidate pathway-activity marker, requires further standardization and validation before clinical application as a predictive biomarker (58,108).

Radiomic and imaging-based biomarkers. Radiomics offers a non-invasive approach to capture tumor heterogeneity and microenvironmental features that may reflect TGF-β pathway activity (110). In BC, MRI radiomics-based models have been developed to predict molecular-subtype-associated immune prognostic signatures, supporting their role as prognostic markers, with a LASSO (least absolute shrinkage and selection operator) model incorporating ten radiomics features achieving area under the curve values of 0.839 in the training set and 0.810 in the validation set for prognostic stratification (111). Furthermore, MRI radiomic phenotypes have been shown to stratify treatment response and define immune states, supporting their role as treatment-response-associated markers in patients with very high-risk NMIBC receiving immunotherapy, suggesting that imaging features may reflect underlying immune contexture (112). In PC, radiogenomic profiling has identified a transcriptomic signature of TGF-β activity that correlates with imaging phenotypes and predicts biochemical recurrence, supporting its role as a prognostic marker after external beam radiotherapy with androgen deprivation therapy (110). This TGF-β activity signature discriminated patients destined to experience biochemical failure from those who did not and retained prognostic capacity in independent cohorts. Despite these promising findings, the field remains exploratory; most studies are retrospective with limited sample sizes, and standardized radiomic pipelines are lacking. Prospective validation and integration with molecular biomarkers will be essential to establish radiomic signatures as clinically actionable tools for TGF-β-targeted therapy selection. No radiomic marker has yet been validated as a true predictive biomarker for TGF-β-targeted therapy in urological malignancies.

Spatial multi-omics signatures. Spatial multi-omics technologies provide unprecedented resolution of TGF-β-driven immune exclusion by preserving tissue architecture (113). In BC, single-cell and spatial transcriptomics have identified NUDT10 co-localized with the TGF-β activator LRRC32, and both genes were associated with poor response to anti-PD-L1 therapy (77). In PC, integrated single-cell and spatial transcriptomic profiling of 127 samples spanning the disease continuum revealed that $FOLR2^+$ and $CX3CR1^+$ TAMs upregulate TGF-β signaling to establish immunosuppressive niches, while $CXCL12^+$ iCAFs and $ACTA2^+$ myCAFs maintain spatial co-localization facilitating immune cell recruitment (113). In ccRCC, spatial analyses have defined a spatially organized stromal-immune signaling axis, $POSTN^+$ CAFs colocalizing with $CCL3^+$ macrophages at the invasive front, which drives immune exclusion and immunotherapy resistance through reciprocal TGF-β signaling (63). The spatial arrangement of immune and stromal cells, not just their abundance, is associated with patient outcomes, with specific interactions such as the iCAF-CD4 tissue-resident memory T cell axis offering potential therapeutic targets (114). Collectively, these spatial signatures identify TGF-β-driven immune exclusion and represent candidate predictive markers for TGF-β-targeted therapies; however, true predictive validation in prospective biomarker-stratified trials is still lacking. However, spatial multi-omics approaches currently remain in the exploratory phase, with most findings derived from relatively small cohort studies. Their clinical translation will require validation in larger, prospective cohorts, standardization of analytical pipelines, and demonstration of cost-effectiveness relative to simpler tissue-based or circulating assays.

7. Challenges and future perspectives

Despite considerable progress in elucidating TGF-β-mediated immune evasion mechanisms and advancing pathway-targeted

therapeutics, several formidable challenges impede the successful clinical translation of TGF-β inhibition strategies in urological malignancies. These challenges span safety and toxicity limitations, tumor heterogeneity and the context-dependent dual functionality of TGF-β signaling, biomarker-driven patient selection barriers, and a spectrum of emerging research frontiers that hold promise for overcoming current obstacles.

Systemic TGF-β pathway inhibition presents substantial safety concerns that have limited the clinical development of numerous agents. The development of TGF-β receptor inhibitors has been historically hindered by cardiac valvulopathy and arteriopathy findings in non-clinical toxicology studies (90). For instance, PF-06952229, a selective TGF-β-R1 inhibitor, demonstrated dose-limiting toxicities in 8.6% of patients, including grade 3 anemia and intracranial tumor hemorrhage, although an optimized intermittent dosing schedule mitigated cardiac-related adverse findings (89). Similarly, the bifunctional fusion protein bintrafusp alfa, which co-targets TGF-β and PD-L1, exhibited bleeding events and anemia in 36.4 and 31.1% of patients respectively, compared with 11.8% each with pembrolizumab alone (91). These observations highlight that pan-TGF-β blockade may be inherently limited by on-target toxicity in normal tissues. In this context, isoform-selective inhibition has emerged as a promising alternative; TGFβ1 is the dominant isoform in tumors, while TGFβ2 and TGFβ3 play more critical roles in cardiovascular development. Selective neutralization of TGFβ1 has demonstrated therapeutic efficacy without cardiovascular toxicity, suggesting that isoform-specific targeting may preserve the tumor-suppressive functions of other isoforms while mitigating systemic adverse effects (102).

A second major challenge arises from the intrinsic duality of TGF-β signaling, which acts as a tumor suppressor in early-stage disease yet promotes metastasis and immune evasion in advanced malignancies (9). This functional switch complicates therapeutic intervention because systemic inhibition may inadvertently disrupt tumor-suppressive functions in normal tissues and early-stage lesions, potentially promoting carcinogenesis in certain contexts. In urological cancers, the prognostic implications of TGF-β pathway activation are subtype-dependent, further complicating patient selection. The heterogeneity of the TME adds another layer of complexity; TGFβ-activated myCAFs drive T-cell exclusion through multiple mechanisms, including physical barrier formation and the expression of downstream molecules such as βig-h3, Endo180, LRRC15 and NOX4 (103). This functional redundancy suggests that targeting TGF-β alone may be insufficient to fully reverse CAF-mediated immunosuppression, necessitating combination approaches that address multiple arms of the immunosuppressive network.

The translation of preclinical success to clinical benefit has proven particularly challenging. Preclinical studies exploring combined TGF-β and immune checkpoint inhibition have demonstrated near-uniform positive results across tumor types, yet landmark clinical studies have reinforced the difficulties of translation (97). This discrepancy may stem from the complexity of the TME in patients relative to preclinical models, as well as the absence of robust predictive biomarkers to identify patients most likely to benefit from TGF-β-targeted interventions. Recent efforts have identified a conserved biomarker comprising combined TGF-β, DNA repair, and immune context signatures that can prospectively identify patients whose tumors may be converted from cold to hot with appropriate therapy; importantly, inhibiting TGF-β activity converted high-βAlt cold tumors into ICB-responsive tumors via NK cells (115). However, the clinical application of such composite biomarkers remains limited by technical complexity and the need for prospective validation. In BC, multi-omics analysis has revealed that m7G methylation-related genes, particularly NUDT10, may be involved in TGF-β signaling-mediated anti-PD-L1 response, offering potential avenues for biomarker development; differential methylation patterns in the TGF-β pathway correlated with immunotherapy resistance, and integrative analysis identified NUDT10 as a key gene associated with poor prognosis and reduced response to anti-PD-L1 therapy (77).

Looking forward, several emerging research frontiers offer opportunities to address these challenges. The emerging strategies discussed below are largely derived from non-urological tumor models and require direct validation in urological malignancies. The intersection of TGF-β signaling with immunogenic cell death and innate immune activation represents a promising therapeutic avenue. Co-targeting TGF-β and PD-L1 has been shown to sensitize tumors to immunogenic chemotherapy, with translational studies revealing the unfavorable prognostic effect of the TGF-β pathway on immune responses in human tumors (116). Furthermore, metabolic reprogramming mediated by TGF-β has emerged as a novel therapeutic vulnerability. While TGF-β promotes malignant traits in advanced cancers, numerous cancer cells remain sensitive to its potent cytostatic effects; co-targeting this metabolic vulnerability with a metabolic inhibitor alongside an anti-TGF-β agent significantly enhances therapeutic efficacy (117). M2 macrophage-derived TGF-β regulates glycolysis and lymphatic metastasis via a PKM2-centered axis, suggesting that metabolic interventions could disrupt the feed-forward circuits sustaining TGF-β-driven immunosuppression (118). The formation of tertiary lymphoid structures represents another frontier. TGF-β-mediated silencing of the genomic organizer SATB1 promotes Tfh cell differentiation and drives intratumoral tertiary lymphoid structure formation, resulting in reduced tumor growth in preclinical models (119). The formation of these structures relies on cytokine signaling networks between heterogeneous cell populations, including stromal cells, lymphocytes and cancer cells, suggesting that TGF-β modulation could be harnessed to promote tertiary lymphoid structures formation and enhance antitumor immunity (120).

Finally, advanced delivery systems offer the potential for tumor-specific TGF-β blockade. Red blood cell membrane-camouflaged nanoparticles co-encapsulating sonosensitizers and TGF-β inhibitors have demonstrated targeted delivery and improved therapeutic outcomes (121). Similarly, ROS-responsive MnO_2 nanozymes co-delivering cGAMP and TGF-β inhibitors have shown synergistic activation of STING signaling and remodeling of the immunosuppressive microenvironment, illustrating how nanomedicine can overcome the limitations of systemic pathway inhibition (122). Collectively, these emerging approaches underscore the importance of

context-specific targeting strategies that balance efficacy with safety, address tumor heterogeneity through combinatorial regimens, and leverage advances in drug delivery to achieve localized pathway modulation. The future of TGF-β-targeted therapy in urological malignancies will likely depend on integrating these innovations with robust biomarker-driven patient selection to realize the full therapeutic potential of this critical pathway. Key lessons from clinical translation have emerged from the failure of several TGF-β-targeted agents in later-stage trials (galunisertib, SAR439459, bintrafusp alfa). Systemic pan-TGF-β inhibition has a narrow therapeutic index due to on-target toxicity (112,113); isoform-selective inhibition (TGFβ1) and integrin-targeted approaches may offer improved safety (63); and the absence of biomarker-driven patient selection likely obscured benefits in unselected populations (97). Future trials must incorporate predictive biomarkers from the outset to identify the subset of patients most likely to benefit, rather than assuming that TGF-β inhibition is universally beneficial.

8. Conclusions

TGF-β signaling drives immune evasion and immunotherapy resistance across urological malignancies, with histological/subtype-dependent heterogeneity. It orchestrates T cell exhaustion, macrophage polarization, CAF activation, ECM remodeling and metabolic reprogramming. Despite preclinical promise, clinical translation remains limited by on-target toxicity, pathway duality and unvalidated biomarkers. The preclinical-to-clinical gap reflects inadequate models, absent predictive biomarkers and pathway redundancy. Future strategies should integrate isoform-selective inhibitors, combinatorial regimens, predictive biomarkers, advanced drug-delivery/nanomedicine platforms and tertiary lymphoid structure-directed immunomodulation, with biomarker-driven trials to bridge this gap.

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Authors' contributions

LY and XS contributed to the conception and design of the study, performed the literature search and data extraction, and drafted the initial manuscript. PS participated in the conception and design of the review, provided critical revision of the manuscript for important intellectual content, supervised the overall framework and interpretation of the included evidence, and gave final approval of the version to be published. All authors reviewed and edited the manuscript, approved the final version of the manuscript, and agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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