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REVIEW

The tumor microenvironment and immune targeting therapy in pediatric renal tumors

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Abstract

This review highlights the role of several immunomodulating elements contributing to the tumor microenvironment of various pediatric renal tumors including Wilms tumor. The roles of innate and adaptive immune cells in renal tumors are summarized as well as immunomodulatory cytokines and other proteins. The expression and the predictive role of checkpoint modulators like PD-L1 and immunomodulating proteins like glypican-3, B7-H3, COX-2 are highlighted with a translational view toward potential therapeutic innovations. We further discuss the current state of preclinical models in advancing this field of study. Finally, examples of clinical trials of immunomodulating strategies such as monoclonal antibodies and chimeric antigen receptor T (CAR-T) cells for relapsed/refractory/progressive pediatric renal tumors are described.

KEYWORDS
immunotherapy, pediatric renal tumor, tumor microenvironment

Abbreviations: ASC, adult stem cell; CAR-T, chimeric antigen receptor T cell; CCSK, clear cell sarcoma of the kidney; COX-2, cyclooxygenase 2; GEMM, genetically engineered mouse model; GPC3, glypican-3; ICD, immunogenic cell death; ICI, immune checkpoint inhibitor; ICR, immunologic constant of rejection; IGF, insulin-like growth factor; iPSC, induced pluripotent stem cell; mDC, mature dendritic cell; MRTK, malignant rhabdoid tumor of the kidney; MSI, microsatellite instability; NK, natural killer; OS, overall survival; PD-1, programmed death-1; pDC, plasma dendritic cell; PD-L1, programmed death-ligand 1; PDX, patient-derived xenograft; PRAME, preferentially expressed antigen of melanoma; PSC, pluripotent stem cell; RCC, renal cell carcinoma; RMC, renal medullary carcinoma; SWI/SNF, SWItch/Sucrose Non-Fermentable; TAA-T, tumor-associated antigen-specific T cell; TAM, tumor-associated macrophages; TFE-RCC, TFE translocation RCC; TGFβ, transforming growth factor beta; TIL, tumor-infiltrating lymphocyte; TMB, tumor mutational burden; TME, tumor microenvironment; TNFα, tumor necrosis factor alpha; Treg, T regulatory lymphocytes; VEGF, vascular endothelial growth factor; WT, Wilms tumor; WT1, Wilms tumor 1.

Amy B. Hont and Benoit Dumont contributed equally to this work. Andrew L. Hong and Arnauld Verschuur jointly supervised this work.

1 | INTRODUCTION

Renal tumors in children represent about 5%–6% of pediatric cancers and include nephroblastoma (or Wilms tumor [WT]), clear cell sarcoma of the kidney (CCSK), malignant rhabdoid tumor of the kidney (MRTK), renal cell carcinoma (RCC), congenital mesoblastic nephroma, and other rare renal tumors. The management of WTs has benefited from technological advances, and multimodal treatment currently has resulted in improved stratification and clinical outcomes. However, some aggressive subtypes of WT with blastemal predominance, anaplastic histology, or 1q gain remain challenging to treat and confer a poorer prognosis.[1–5]

The immune response is a critical component playing a complex role in cancer development. On one hand, the immunosurveillance process leads to the elimination of tumor cells. On the other hand, immune cells drive a selective pressure on tumor cells leading to cancer immunoediting and immune escape, potentially promoting tumor development. Contrary to adult tumors, which are particularly rich in T cells, the immune infiltrate of pediatric tumors seems to be dominated by innate immune cells, essentially myeloid cells and macrophages.[6] This difference might be explained by a lower mutational burden in pediatric tumors, which in turn leads to lower antigen presentation.[7] As such, the importance of inflammation and immune response in pediatric tumors has not been deeply studied. Immune checkpoint inhibitors (ICIs) expose the tumor to the immune system and trigger a T-cell response, resulting in use of ICIs in adult oncology.[8,9]

Deciphering the immune microenvironment in pediatric renal tumors may identify novel treatment paradigms. This review describes different elements contributing to the tumor microenvironment (TME), including the role of innate (natural killer [NK] cells, dendritic cells/macrophages, granulocytes, mast cells) and adaptive (T and B lymphocytes) immune cells that may interact with immune-suppressive or -activating targets such as programmed death-1 (PD-1) and its ligand programmed death-ligand 1 (PD-L1). The role of several immune-modulating proteins in pediatric renal tumors will be described as well as the potential therapeutic opportunities.

2 | THE TUMOR MICROENVIRONMENT IN WILMS TUMOR

WT is the most common pediatric renal malignancy, accounting for 6% of pediatric cancers, with an incidence of seven children per million. WT is an embryonal tumor, consisting of coexisting components: stroma, epithelium, sometimes rhabdomyomatous, and immature blastema tissue. These cell types resemble the different stages of embryonal renal development and serve as a good model for the study of intra-tumoral heterogeneity,[10] given that tumors respond to therapy differently depending on the predominant tissue.[5] This allows for better representation for in vivo models.

In pediatric solid tumors studied so far, the immune infiltrate is mainly constituted by innate immune cells and particularly macrophages.[6] Immunohistochemistry studies have shown that WT is infiltrated by both adaptive and innate immune cells (Figure 1).[11] In a cohort of 61 patients with WT, the proportion of M2-type macrophages (associated with wound healing and tissue repair rather than M1 that elicit an immune response) significantly increased with the tumor stage (I–III). High density of M2-type macrophages correlated with shorter overall survival and nonfavorable histology.[12] Interestingly, while adaptive immune cells are mostly localized to tumor stroma, innate immune cells are also present in other regions of the tumor. Furthermore, tumor-associated macrophages (TAM) are more abundant in intratumoral regions than in peritumoral tissue. The inflammatory mediators cyclooxygenase 2 (COX-2), hypoxia-inducible factor 1 alpha (HIF-1α), vascular endothelial growth factor (VEGF), interleukin 6/phosphorylated signal transducer and activator of transcription 3 (IL-6/pSTAT3), and phosphorylated extracellular signal-regulated kinases (p-ERK) also play a major role in the WT microenvironment and biology. These factors also co-localize with the TAM in tumor stroma, which suggests a functional association.[13] Holl et al. showed an elevated level of NK cells in WT.[14]

In WT, immune infiltrate analysis shows that CD8 and CD4 T cells are present in the tumor and exhibit an activated phenotype.[14] Interestingly, infiltration by immune cells is independent of chemotherapy status, with similar T and NK cell infiltration in tumor samples, both chemotherapy-naive and chemotherapy-exposed samples.[14] Furthermore, tumor-infiltrating lymphocytes (TILs) in WT displayed an activated phenotype compared with peripheral blood immune cells. The presence of these tumor-infiltrating CD8 T cells correlates with positive outcomes. These data suggest that WT is an immune-engaged tumor. However, in a mouse model of WT, the TME includes immunosuppressive cells like T regulatory (Treg) cells and increased plasmacytoid dendritic cells (pDC).[13] Cytokines are an important player in immunomodulation. In WT, immunosuppressive cytokines such as IL-10, transforming growth factor beta (TGFβ), and tumor necrosis factor alpha (TNFα) are secreted in the TME.[13] TGFβ expression is correlated to invasion/metastasis and disease progression in WT.[15] TGF-β1 expression status was associated with disease-free survival (50 vs. 75 months, $p = .022$) but not OS (62 vs. 76 months, $p = .14$). Further, cytokine-activated NK cells isolated from peripheral blood mononuclear cells of healthy donors are capable of killing WT primary cultures in vitro.[16]

Tumor cells and associated stromal cells express cell-surface proteins that can directly inhibit immune responses. One of the most studied inhibitory pathways involves PD-L1 and PD-L2, B7 family members and ligands for the inhibitory receptor PD-1 expressed by activated T cells.[17,18] Measurements of PD-L1 have been evaluated as a putative biomarker to inform the response to ICIs, but levels of expression are not always correlated with clinical responses.[19] Different studies found that between 14% and 29% of WT show tumor upregulation of PD-L1.[20–22] Zhang et al. showed PD-L1 expression in 29% of 77 primary WT tumors, and 35% in metastatic WT.[21] This PD-L1 expression was correlated with late stage, unfavorable histology, progressive disease, and predicted a poor prognosis in univariate analysis. Other authors showed tumor PD-L1 expression in 14% of 81 patients with WT.[22] This expression was significantly more frequent in

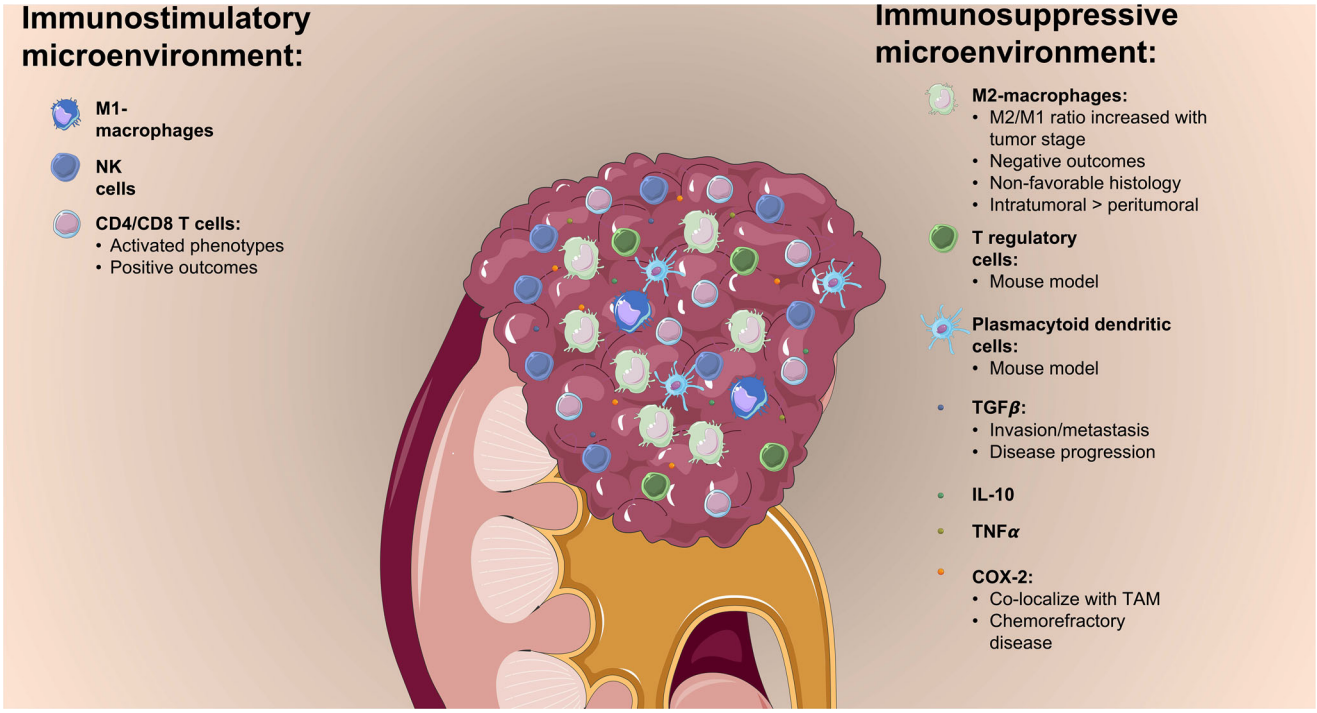

FIGURE 1 Immune tumor microenvironment of Wilms tumor (WT). The immune microenvironment of WT is constituted by immunostimulatory and immunosuppressive components. Some of these elements have been described in WT of patients and others in mouse models. Although the immunosuppressive context plays an important role in WT, this figure shows that the immune system may be engaged in a tumor response.

anaplastic WT. Interestingly, PD-L1 expression in favorable histology WT was associated with an increased risk of recurrence, independent of tumor stage.[20]

Recently, genomic analyses were performed in WT as compared with neuroblastoma and adult cancers to assess for neoantigen gene expression.[23] WT expressed much lower levels of neoantigen gene expression as compared to neuroblastoma and adult tumors. However, subgroups with significantly higher levels of neoantigens included TP53-mutated WT. In an independent cohort of 30 WT, PD-L1 expression correlated with presence of TILs.[24] Given the complexity of tumor immune responses, PD-L1 expression and TIL infiltration may not be sufficient to accurately predict clinical response to ICIs.

3 | THE TUMOR MICROENVIRONMENT IN NON-WILMS RENAL TUMORS

3.1 | Malignant rhabdoid tumor of the kidney and renal medullary carcinoma

Malignant rhabdoid tumors of the kidney are known to be aggressive cancers, with patients often presenting at an advanced stage and having dismal outcomes. MRTK are characterized by biallelic loss of *SMARCB1* (or rarely *SMARCA4*), a member of the SWItch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex.

Inactivation of *SMARCB1* is the major genetic abnormality in MRTK, though recent studies have revealed additional recurrent and complex genetic mutations.[25,26] Pediatric tumors harbor 14 times lower tumor mutational burden (TMB) compared to adult tumors.[7] Although low TMB generally correlates with limited tumor antigens and immunogenicity, for *SMARCB1*-mutant tumors including MRTK enhanced antitumor, immune function is seen including unexpectedly elevated levels of immune cytolytic activity similar to adult cancers.[27,28] Interestingly, there is an inverse correlation of mRNA levels of *SMARCB1* and PD-L1.[29] These results suggest that additional genetic or epigenetic mechanisms may contribute to immunogenicity of MRTK.

The tumor immune microenvironment of MRTK is predominantly composed of $CD68^+$ monocytes and macrophages and $CD8^+$ cytotoxic T cells. Indicative of both pro- and antitumor immune functions, overexpression of genes mediating T-cell function and activation as well as T-cell inhibition are observed.[27,28] The presence of TILs and expression of PD-1 and PD-L1 have been observed to be associated with potential efficacy of ICIs in MRTK.[30] In a genetically engineered mouse model (GEMM) of MRTK, ICIs resulted in tumor regression and subsequent tumor immunity upon re-challenge.[28] Clinically, there are anecdotal reports of response to single-agent atezolizumab (anti-PD-L1) in patients with MRTK, as well as in a patient with a metastatic tumor with rhabdoid features when combined with conventional chemotherapy.[31–33] Ongoing studies are investigating nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4) in pediatric patients with *SMARCB1*-deficient tumors (NCT: 04416568). Combination of ICI therapy with additional agents has the potential for improved efficacy.[34] For example, inhibition of EZH2 methyltransferase, which is a core

enzymatic subunit of PRC2 and can antagonize the gene expression activity of the SWI/SNF complex, is being studied as a therapeutic strategy in *SMARCB1*-deficient tumors.[35] In addition, inhibition of EZH2 leads to increased tumor antigen MHC presentation and PD-L1 expression and potent antitumor immunity via reprogramming of regulatory T cells, making it an attractive combination with ICIs.[34,36,37]

In addition to ICIs, the apparent immunogenicity of MRTK may be amenable to adaptive cellular therapies. For example, chimeric antigen receptor T (CAR-T) cells against glypican-3 (GPC3), a surface protein expressed on the surface of many pediatric solid tumors including 43%–65% of MRTK (and 30%–77% of WT),[38–40] are highly cytotoxic against malignant rhabdoid tumor cell lines and are undergoing clinical evaluation in liver tumors including malignant rhabdoid tumor of the liver.[38,41,42]

Another rare renal tumor, renal medullary carcinoma (RMC), also has loss of *SMARCB1*, primarily through balanced chromosomal translocations.[43] RMC is primarily seen in adolescents and young adults with African ancestry who have sickle cell trait.[44] CheckMate920 and Alliance A031702 clinical trials included patients with non-clear cell RCC such as RMC to receive treatment with ICIs. However, due to relatively small number of patients treated, it is currently difficult to discern the efficacy of this therapeutic strategy. Recent studies have identified activation of the innate immune pathway through cyclic GMP-AMP synthase interferon genes (cGAS-STING). To this end, as therapy for patients with RMC is not standardized, several patients have been treated with ICIs with mixed results.[45]

3.2 | Translocation RCC

Of children and adolescents with RCC, the most common (~42%) is translocation RCC.[46] This is characterized primarily by the transcription factor E3 (*TFE3*) translocation. PD-L1 expression has been seen in patients with TFE translocation RCC (TFE-RCC).[47] Recent studies have identified $CD8^+$ T cells infiltrating translocation RCC tumors, but they have different mechanisms of apparent T-cell exhaustion as compared to RCCs.[48] Currently, the Children's Oncology Group is assessing whether ICI (nivolumab) in addition to axitinib (a second-generation VEGFR tyrosine kinase inhibitor) has activity in TFE-RCC (NCT03595124).

3.3 | Clear cell sarcoma of the kidney

CCSK is an aggressive malignancy that typically presents in young children with a propensity to metastasize to the bones and brain. Histopathology can show numerous variant patterns, but CCSK can be identified by internal tandem duplications in BCL-6 co-receptor (BCOR) and translocation t(10;17)(q22;p13) leading to the fusion YWHAE-NUTM2B/E.[49] CCSK are treated with intensive chemotherapy regimens and would benefit from more focused investigation of targeted or immunotherapeutic strategies for CCSK. One potential marker may be the immunologic constant of rejection (ICR), a gene signature reflective of Th1 activation, expression of cytolytic immune proteins, and immune regulation,[50] and may be a predictor of response to immunotherapy.[51,52] Sherif et al. investigated the application of this model in CCSK, as well as MRTK and WT.[53] While the ICR score was low in CCSK, the specific gene signature was consistent with an inflammatory subtype also seen in many of the neuroblastoma specimens, and characterized by Th1 enrichment.[53] The number of CCSK samples was low, precluding significant association with outcomes.

4 | POTENTIAL PRECLINICAL MODELS

Many preclinical models (e.g., orthotopic xenograft models) used for exploring immune-targeting compounds on solid tumors are not adequate because of the lack of normal host immunological conditions. WT (and other less common renal tumor) cell lines are limited and can be difficult to generate. Cell lines facilitate in vitro studies, while patient-derived xenograft (PDX) models may be able to better recapitulate biological heterogeneity for in vivo studies. However, these models are also challenged by the potential for clonal selection or tumor evolution that may occur during engraftment.[54] Syngeneic (immunocompetent) models or GEMM have only been developed on a limited scale. One relevant GEMM of WT exists having a combination of pathogenic genetic alterations (Wilms tumor 1 [*WT1*] and *IGF2*) for WT. This mouse model should provide a powerful tool to study the biology of WT initiation and investigate therapeutic strategies.[55]

While cancer cell lines have yielded important scientific insights, successful clinical implementation of preclinical findings remains limited. In the last decade, advances in 3D culture technologies, such as organoid technology, have yielded new culture protocols, allowing for the efficient establishment of cell models that can be cultured over prolonged periods of time while retaining characteristics of the tissues from which they were derived. Organoids can be derived from pluripotent stem cells (PSCs; derived from embryonic stem cells or by dedifferentiation of committed cells [induced PSCs: iPSCs]) or organ-restricted adult stem cells (ASCs).[56]

In the context of the kidney, Takasato et al. described a protocol to differentiate iPSC to kidney organoids.[57] These kidney organoids are composed of nephrons segmented into glomeruli as well as all tubule compartments, from proximal tubule to collecting duct, an endothelial network and renal interstitium. Several other studies have reported the generation of kidney organoids from iPSCs with even additional layers of cellular complexity.[58] Generally, iPSC-derived kidney organoids recapitulate many aspects of nephrogenesis. Considering that at least some pediatric renal tumor entities arise because of derailed nephrogenesis,[59–61] iPSC-derived kidney organoids may give the opportunity to study tumor initiation in the context of a developing organ-like structure, as well as for studying the intricate interactions of tumor cells with their environment. Following such a strategy, Waehle et al. demonstrated that ablation of *WT1* in human iPSC-derived kidney organoids induces a gene expression signature similar to WTs.[62]

Similar approaches can be used to study the contribution of other genetic alterations in pediatric renal tumor entities.

ASC-derived organoid technology allows for the establishment of organoid cultures directly from primary patient material. As such, organoids were developed from many different healthy as well as tumor tissues, including gut,[63] colon,[64] breast,[65] ovaries,[66] pancreas,[67] and liver.[68] These organoids were demonstrated to have predictive value for the patient's tumor drug response.[69–72] Although organoid technology has so far been primarily applied to adult malignancies, several recent studies described the use of organoids for childhood malignancies including renal tumors. This includes protocols to grow organoids from normal kidney epithelium, primarily resembling tubular segments.[73] Using a similar approach, recently organoids have been derived from different renal tumor entities, including WT, MRTK, and RCC.[74] These pediatric kidney cancer organoids were generated from tumors in a vast majority of cases, with consistent success in chemotherapy-naïve specimen, and were observed to retain many characteristics of primary patient tumors such as phenotypic, genetic, and gene expression signatures, and therefore provide representative models of native tumors. The ability to maintain key characteristics over serial culturing and xenograft transfer provides reliable models for co-culture experiments, gene editing, and high-throughput drug test.[74] In addition to organoids, spheroid WT models have also given rise to xenograft tumors in mice and successfully cultured again without loss of blastemal characteristics.[75]

However, the TME is typically not captured in organoid cultures, which limits their use for the development of immunotherapies and studies into the TME. The development of tumor organoid co-cultures with, for instance, immune cells,[76] as well as organoid-on-a-chip approaches[77] should add additional layers of complexity to organoid cultures and broaden its applicability in immunotherapy development.

5 | IMMUNE TARGETING THERAPY

5.1 | Potential targets and rationale

Studies investigating immunologic and molecular targeted therapies for patients with WT and other pediatric renal tumors are still in early phase (Table 1). Studies of the TME, as detailed above, demonstrate variable infiltration of activated T cells and low level of checkpoint expression.[14,78–81] This evidence of an endogenous immunologic response in WT, as well as preliminary results from ongoing clinical trials, support continued investigation of immunotherapy for these patients. The following reviews potential targets, preclinical and clinical investigations, and future directions.

5.1.1 | Immune checkpoints

Expression of immune checkpoints provides a potentially potent mechanism for immunotherapy. As described in the above section "The tumor microenvironment in Wilms tumor," PD-L1 expression on WTilms tumor is variable, more commonly found in anaplastic histology, and associated with more aggressive disease and relapse.[20–22] Additionally, TILs in WT have been found to have higher expression of PD-1 compared to circulating T cells,[14] though Pinto et al. found PD-1 expression in WT samples to be low.[82] In non-Wilms renal tumors, tumor-infiltrating $CD8^+$ T cells expressing PD-1 have been identified in MRTK and TFE-RCC, and PD-L1 expression has been identified in these tumors as well, supporting the potential efficacy of ICI therapy.[28,30,47,48] CTLA4 expression has been less frequently studied in these patients, though ICI targeting CTLA4 has been used clinically for groups with advanced pediatric solid tumors, including renal tumors.[83]

5.1.2 | B7-H3

B7-H3 (CD276) has a complex function in the immune system. Recent evidence points to its predominant role as an inhibitor of the innate and adaptive immune system,[84] perhaps partly via decreased secretion of inflammatory cytokines interferon gamma and TNFα.[85–87] With low expression in healthy organs, high B7-H3 expression has been seen in many cancers, including RCC[81,88] and WT.[89] B7-H3 expression has been correlated with metastasis and invasion, angiogenesis, and support of the epithelial–mesenchymal transition.[85,88] Expression has also been associated with higher grade disease and worse patient outcomes.[81,85,88]

Preclinical studies and clinical studies utilizing B7-H3 targeted therapy are promising, including B7-H3 CAR-T in solid tumor murine models.[89] Similarly, B7-H3 monoclonal antibodies have demonstrated promising results in animal models of RCC.[90] Preliminary results of B7-H3 monoclonal antibodies to treat pediatric and adult patients with cancer showed potential antitumor activity as evidenced by disease stabilization and clinical responses.[91–93] These studies have demonstrated favorable safety profiles thus far.[89]

5.1.3 | COX-2

COX-2 is an enzyme upregulated in response to inflammatory signals (e.g., TNFα) and is responsible for the synthesis of prostanoids and chemokines that generate a proinflammatory response.[94–96] COX-2 expression is low to minimal in healthy tissues[95] and has been identified in many adult malignancies.[97–104] Moreover, expression has been associated with chemorefractory and aggressive disease and poor prognosis.[98,102,105,106] COX-2 expression in RCC[107] has been correlated with decreased progression-free survival.[108] Tumors with *WT1* gene mutations demonstrate upregulated COX-2 pathway, leading to increased pDCs, Tregs, and immunosuppressive cytokines and hormones (IL-10, TNFa, and TGFb).[14] Consequently, despite its proinflammatory nature, COX-2 has been identified as an important immunosuppressive marker in the TME of WT, functioning to support tumor growth and neo-angiogenesis and recruit TAMs in addition to other cells of the innate and adaptive immune system.[12] Analysis

TABLE 1 Current therapeutic targets and active or completed clinical trials

Target	Therapeutic	Diagnosis	Clinical experience
B7-H3	MGA271 (B7-H3 monoclonal antibody)	Refractory solid tumors	Disease stabilization and partial response; favorable safety profile[92]
	MGA271 (B7-H3 monoclonal antibody)	Pediatric relapsed/refractory solid tumors	Completed, awaiting results (NCT02982941)
	B7-H3 CAR-T	Pediatric relapsed/refractory solid tumors	Ongoing (NCT04483778)
	B7-H3 CAR-T	Pediatric relapsed/refractory solid tumors	Ongoing (NCT04897321)
	MGC018 (B7-H3 monoclonal antibody)	Refractory solid tumors	Ongoing (NCT05293496)
COX-2	Celecoxib (COX-2 inhibitor)	Pediatric relapsed/refractory solid tumors	Ongoing (NCT02574728)
	Celecoxib (COX-2 inhibitor)	Stage IV renal cell carcinoma	PR ($n = 3$) and SD ($n = 5$) of 17 patients treated in combination with interferon alfa-2b (NCT01158534)
GPC3	GPC3 peptide vaccine	Pediatric relapsed/refractory solid tumors	66.6% Response rate (CR + PR + SD)[40]
	GPC3 CAR-T	Pediatric relapsed/refractory solid tumors	Ongoing (NCT04377932)
	GPC3 CAR-T	Pediatric relapsed/refractory solid tumors	Ongoing (NCT04715191)
	Codrituzumab (GPC3 monoclonal antibody)	Pediatric relapsed/refractory solid tumors	Ongoing (NCT04928677)
TAA	Autologous TAA-T (WT1, preferentially expressed antigen of melanoma [PRAME], survivin specific)	Pediatric relapsed/refractory solid tumors	73% Response rate (CR + PR + SD)[116]
	Autologous TAA-T (NY-ESO-1, PRAME, survivin, MAGEA4, SSX specific)	Pediatric relapsed/refractory solid tumors	Completed, awaiting results (NCT02239861)
	Third party TAA-T (WT1, PRAME, survivin specific)	Pediatric relapsed/refractory solid tumors	Ongoing (NCT05238792)
PD-L1	Pembrolizumab	Pediatric relapsed/refractory solid tumors	Ongoing; objective response 5.9% in patients with solid tumors/lymphoma preliminarily (0 of 3 with WT, 1 of 2 with MRTK)[132]
	Atezolizumab	Relapsed/refractory solid tumors	PFS: 1.2 months in WT cohort (NCT02541604)
PD-1/CTLA-4	Nivolumab/Ipilimumab	Relapsed/refractory INI-negative tumors	Ongoing (NCT04416568)
PD-1	Nivolumab (with axitinib)	Metastatic/unresectable TFE translocation RCC (TFE-RCC)	Ongoing (NCT03595124; AREN1721)
TNFa	Recombinant TNFa	Pediatric relapsed/refractory Wilms tumors	42.1% Response rate (CR + PR + SD)[133]

of WT specimens demonstrate frequent expression of COX-2,[96,97,109] including tumors with anaplastic and favorable histology, primary and metastatic tumors, chemorefractory disease, and xenografts.[96,97] Notably, there is an absence of expression in nests of nephroblastomatosis, putative precursors to WT development.[97] In preclinical and mouse models (neuroblastoma, osteosarcoma, Ewing sarcoma), COX-2 inhibition suppressed tumor growth, supporting its further investigation in WT and pediatric renal tumor models.[109–112]

5.1.4 | GPC3

GPC3 is a cell-surface glycoprotein involved in cell growth and development. The inactivation of *GPC3* leads to Simpson–Golabi–Behmel overgrowth syndrome, which is associated with higher risk of development of solid tumors including WT. Both germline (SGB syndrome) and somatic mutations have been identified.[113] Overexpression is linked to renal tumors via hyperactivation of the Hedgehog signaling pathway,[42,114] and GPC3 leads to increased expression of a multidrug resistance-associated protein.[42] In WT samples, GPC3 expression was identified in >30% of samples in one study[39,115]; in another, 77% of primary WT samples and 93% of metastatic WT samples expressed GPC3.[40] GPC3 expression has also been detected in MRTK.[42]

Clinical trials implementing GPC3 targeted therapies (including vaccine, monoclonal antibodies, bispecific antibodies, antibody–drug conjugates, and adoptive cell therapy) show potential efficacy evidenced by prolonged disease stabilization.[42] To date, only one

study, investigating a GPC3 peptide vaccine, has included pediatric patients.[40] This therapeutic target warrants further investigation in patients with renal tumors.

5.1.5 | Tumor-associated antigen-specific T cells

Tumor-associated antigen-specific T-cell (TAA-T) products are ex vivo expanded T cells specific for multiple tumor-associated antigens, typically via stimulation with peptide-pulsed antigen presenting cells. This approach may overcome the challenge of tumor antigen heterogeneity and immune escape via antigen loss through the targeting of multiple antigens simultaneously and recapitulation of the physiologic antitumor T-cell response in vivo. A study investigating TAA-T products targeting the antigens WT1, preferentially expressed antigen of melanoma (PRAME), and survivin treated 15 pediatric and adult patients with high-risk solid tumors, seven of whom had WT.[116] These antigens have documented expression on WT.[117–121] In the published results, there were no dose limiting toxicities, and a majority of these patients experienced prolonged disease stabilization, consistent with in vivo efficacy of these agents.[116] The study is currently investigating the addition of lymphodepleting chemotherapy administered prior to TAA-T cell infusion to enhance T-cell persistence and function in vivo (NCT02789228), focusing on patients with WT given encouraging preliminary results.

5.2 | Chemotherapeutic modulations of the immune response

Conventional chemotherapy is well known for its direct cytostatic or cytotoxic effects, but growing evidence also indicates important effects on the immune system. By increasing the immunogenicity of malignant cells or by modulating the immune response especially with the inhibition of immunosuppressive mechanisms, cytotoxic drugs are able to reactivate the tumor immune response and restore the immunosurveillance of cancer cells.[122]

Vincristine is an important drug in the treatment of WT. There are few studies available to assess the impact of vinca-alkaloids on the immune system, but vincristine is capable of inhibiting T-cell proliferative response induced by mature dendritic cells (mDC) pretreated by vincristine. Vincristine-treated mDCs decrease their production of IL-12 and increase their production of IL-10.[123] Moreover, vinorelbine in association with cisplatin induces an increase of the effector T-cell and Treg ratio but also a reduction activity of Treg cells suggesting a potential reduction of the immunosuppressive response.[124]

By inducing immunogenic cell death (ICD), conventional chemotherapy may also modify the tumor immune response. Doxorubicin is a cytotoxic drug used in the first-line treatment of many stage 3 and 4 WT for example. It promotes ICD by a caspase-dependent mechanism.[125] Metronomic therapy consists of low doses of chemotherapy and other drugs (such as celecoxib) administered at regular intervals. For example, metronomic cyclophosphamide depletes Treg cells in humans, associated with the restoration of T and NK cell functions.[126] A metronomic treatment scheme containing vincristine, oral cyclophosphamide, and methotrexate showed prolonged disease stabilization in patients with relapsed WT.[127]

6 | DISCUSSION

Challenges to successful immunotherapy for pediatric renal tumors are common with other solid tumors, including the immunosuppressive TME, the high level of intra- and intertumoral heterogeneity in WT, and variability in tumor biology depending upon histology, molecular findings, and staging. While an absence in activity of checkpoint inhibitors, difficulty identifying immunogenic antigens, and lack of microsatellite instability (MSI) pose potential obstacles for immunotherapy in pediatric renal tumors, there is evidence of immune activation and infiltration in WT[12,15,80] to support investigation of immunotherapeutic strategies.

Beyond these identified obstacles to the efficacy of immunotherapy and targeted strategies, their application is hindered by the heterogeneity and small number of patients included in clinical trials for pediatric renal tumors, delaying the interpretation of the clinical efficacy of new therapies. Related is the challenge of identifying effective combination strategies and the ideal timing of immunotherapy into established therapeutic practices. Reliable predictors of response to immunotherapy specific to WT or pediatric renal tumors, such as MSI or immune checkpoint expression, and more sensitive disease monitoring (e.g., monitoring circulating tumor DNA)[116,128–130] will also aid the application of these therapies in a rational manner. Also, insight in occurrence and severity of related toxicity, as documented in adults, need further exploration.[131] International collaboration, as in HARMONICA, is of value to advance clinical and translational research through systematic reviews and collaborative clinical and biologic studies.

The use of immunotherapy and targeted agents has made its way into clinical trials for children with renal tumors in recent years (Table 1). While there are challenges, we are optimistic that advances are forthcoming in the identification of immunogenic targets, improved understanding of the TME, and improved rational combination therapeutic strategies in clinical trials. Potential advancements in the near future will likely include the utilization of other components of the antitumor immune response, such as innate immune cells (e.g., NK cells), and combination treatment strategies that incorporate targeted or immunologic treatments into standard therapy or other targeted treatments. International efforts to take this forward will contribute to the larger knowledge of the antitumor immune response and biology of WT and pediatric renal tumors, while continuing to improve the outcomes of patients and minimizing toxicities.

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CONFLICT OF INTEREST

Alex Kentsis is a consultant to Novartis, Rgenta, and Blueprint Medicines. John Anderson has founder shares in Autolus Ltd and is a consultant for Roche. The remaining authors do not have any conflicts of interest to declare.

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