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REVIEW

WT1 gene: a potential therapeutic target for multiple cancer treatment strategies

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Abstract
The Wilms' Tumor 1 (WT1) gene has attracted significant attention in oncological research owing to its involvement in the pathogenesis of a variety of malignancies. Initially identified in association with Wilms' tumor, a pediatric renal carcinoma, WT1 is now recognized for its broader implications in the etiology of multiple cancers, including leukemia, ovarian cancer, and lung cancer. This review offers a comprehensive analysis of the historical development of WT1 research, explores its mechanisms in cancer progression, and assesses its potential as a therapeutic target, with an emphasis on its clinical implications for cancer treatment strategies.

Keywords WT1 gene · Tumor suppressor gene · Oncogene · Gene therapy · Immunotherapy

Introduction

Cancer remains one of the most significant global health challenges, contributing to high rates of morbidity and mortality. It ranks among the top three leading causes of death in individuals aged 30 to 69 years in 177 out of 183 countries [1]. According to the 2022 Global Cancer Statistics, nearly 20 million new cancer cases and approximately 10 million cancer-related deaths occurred worldwide in that year [2]. Cancer encompasses a diverse group of diseases characterized by uncontrolled and abnormal cell proliferation. These malignant cells can invade surrounding tissues and metastasize to distant organs via the blood and lymphatic systems. Metastasis is the predominant cause of cancer-related deaths [3–5]. Normal cellular development and tissue homeostasis rely on tightly regulated processes, including cell proliferation, differentiation, and apoptosis. Disruption in any of these processes can result in unchecked cell growth, contributing to cancer initiation and progression [6]. Cell growth is regulated through the coordinated actions of specific genes within each cell. Genomic instability plays a central role in the development of neoplastic diseases by promoting mutations and chromosomal alterations [7].

Two major categories of genes are implicated in cancer: tumor suppressor genes and oncogenes. A disruption or imbalance between these gene classes can drive abnormal cell proliferation. Oncogenes (OGCs) arise from mutations in proto-oncogenes—genes that normally regulate cell division and growth. When mutated, proto-oncogenes become oncogenes, leading to excessive protein production or increased activity beyond normal regulatory limits. This dysregulation enables cells to proliferate autonomously, disregarding the organism's growth control mechanisms.

Tumor suppressor genes (TSGs) are essential for maintaining normal cellular homeostasis by regulating cell division and promoting apoptosis. Under physiological conditions, TSGs prevent excessive cell proliferation and ensure the removal of damaged or abnormal cells. However, when TSGs become inactivated or dysfunctional, cells can proliferate uncontrollably, bypassing the body's regulatory mechanisms. The simultaneous activation of oncogenes (OGCs) and inactivation of TSGs create a permissive environment for unchecked cell growth and resistance to apoptosis—two fundamental hallmarks of cancer initiation and progression [8–10].

Advancing the identification, functional characterization, and mechanistic understanding of these gene classes is critical for progress in cancer research and clinical management. This knowledge underpins the discovery of

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novel biomarkers and drives the development of targeted therapies, ultimately enabling improved prevention, early detection, and personalized treatment strategies.

The WT1 gene (Wilms Tumor 1) was first identified in studies of Wilms tumor, a pediatric renal malignancy. In 1990, researchers mapped the WT1 gene to chromosome 11p13 by analyzing chromosomal abnormalities in affected patients. These findings led to the recognition of WT1 as a tumor suppressor gene, based on its frequent inactivation in Wilms tumor cases [11].

Wilms' tumor is the most common pediatric renal malignancy, accounting for approximately 7% of all childhood cancers [12]. Genetic analyses of affected patients frequently reveal mutations in several genes, including the homozygous inactivation of the WT1 tumor suppressor gene [13, 14].

Despite being initially identified and classified as a tumor suppressor, accumulating research has demonstrated that WT1 can also function as an oncogene under certain cellular contexts, earning it the designation of a "chameleon gene." [15]. The significance of WT1 extends beyond oncology; it plays critical roles in developmental biology, cancer progression, and clinical diagnostics. Recent studies, such as that by Jing et al. (2022), have shown that WT1 can inhibit the proliferation of human renal carcinoma cells and induce G2/M cell cycle arrest by upregulating IL-24 expression [16].

Moreover, WT1 is implicated in the pathogenesis of a broad spectrum of malignancies, including hematologic cancers and various solid tumors. These include leukemia, breast cancer, ovarian cancer, renal cell carcinoma, mesothelioma, melanoma, glioblastoma, and soft tissue sarcomas, among others [17–24].

Structure of WT1 gene

The human WT1 gene, located on chromosome 11p13, is a complex locus spanning approximately 50-kb and composed of 10 exons. It encodes a 3-kb mRNA transcript [25, 26].

The WT1 gene encodes a transcription factor characterized by four zinc finger motifs at its C-terminal region, each comprising two cysteine and two histidine residues (Fig. 1). These zinc finger domains enable DNA and RNA binding, direct nuclear localization, mediate protein–protein interactions and regulate gene transcription. In contrast, the N-terminal region is enriched in proline, glutamic acid, serine, and glycine residues. This region enables WT1 to self-associate and plays a crucial role in regulating both transcriptional activation and repression [27–29].The WT1 gene generates multiple transcript variants through alternative splicing at two coding exons. One splicing event involves the inclusion or exclusion of exon 5, which encodes a 17-amino acid peptide, located just N-terminal to the four zinc finger motifs. Another splicing event occurs at exon 9, resulting in the inclusion or exclusion of a three amino acid sequence lysine-threonine-serine (KTS)-positioned between the third and fourth zinc fingers [30–33]. Alternative splicing produces several WT1 isoforms, with four major variants designated A, B, C, and D. Isoform A lacks both the 17–amino acid insert and the KTS sequence. Isoform B contains the 17–amino acid insert but lacks the KTS sequence.

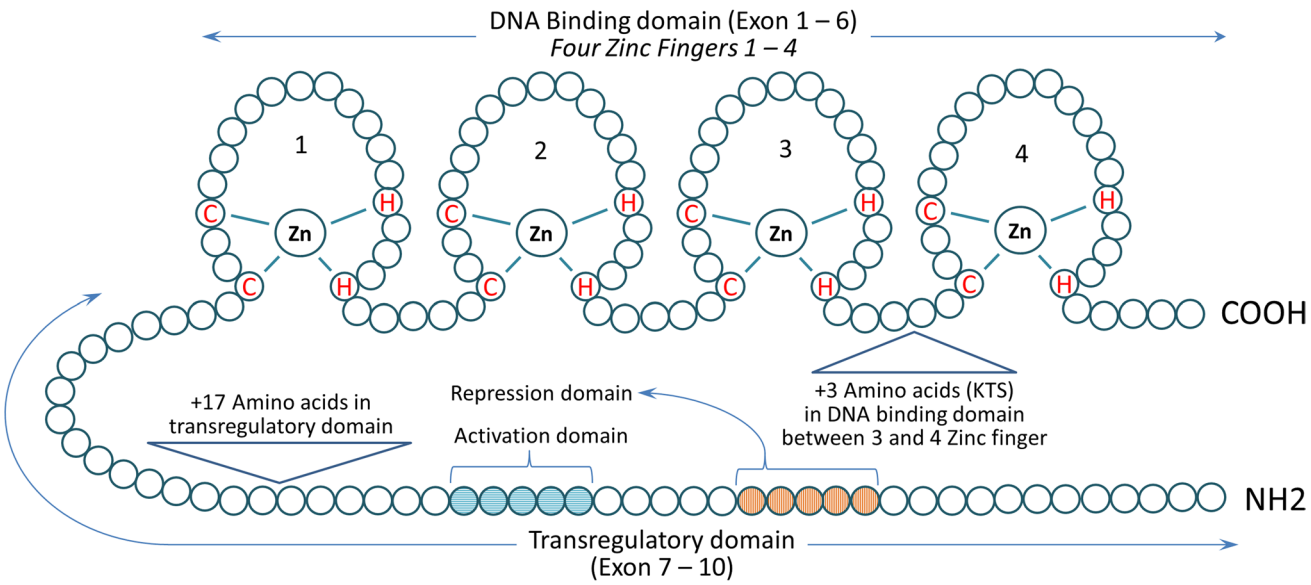

Fig. 1 Schematic Representation of the Structural Organization of the WT1 Protein. The C-terminus of the WT1 protein contains four zinc finger motifs, which are implicated in DNA and RNA binding. The N-terminus features a transregulatory domain. Brown circles represent the repression domain, while blue circles indicate the activation domain

Isoform C includes the KTS sequence but does not have the 17–amino acid insertion, while isoform D harbors both the 17–amino acid insert and the KTS inserts. These isoforms exhibit distinct functional properties and regulate specific target genes, with expression patterns that are consistent yet tissue-dependent [32, 34, 35].

Molecular function of WT1 gene

WT1 regulates a broad spectrum of target genes involved in key cellular pathways such as growth signaling, cell cycle progression, differentiation, and apoptosis. By modulating these genes, WT1 plays critical roles in physiological processes including cell proliferation, survival, programmed cell death, and the maintenance of mesenchymal–epithelial balance. Its transcriptional activity is context-dependent; in some cases, WT1 functions as an activator, while in others, it acts as a repressor. This dual role highlights WT1's versatility in regulating gene expression and cellular processes [36–42]. For instance, WT1 activates Wnt4 gene expression in the kidney to promote MET, while repressing its expression in the heart to regulate EMT. This context-dependent regulation is achieved through WT1's recruitment of different cofactors: coactivators like CBP/p300 in the kidney and corepressors like BASP1 in the heart. [43]. Multiple studies have shown that WT1's dual role as both an activator and repressor is influenced by the diversity of its isoforms, its interactions with different binding partners, and the specific cellular context in which it functions [27, 28, 44]. This functional versatility arises from the WT1 N-terminal region, which contains both a transcriptional activation domain and a suppression domain (SD), allowing it to modulate gene expression in a context-dependent manner. Importantly, a defined region within the N-terminus can inhibit WT1's own activation domain, further contributing to its regulatory complexity [45, 46]. The suppression domain of WT1 represses the activity of other transcriptional activators by recruiting the cosuppressor, brain acid-soluble protein 1 (BASP1). BASP1 serves as a crucial component of the WT1 cosuppressor complex and plays a key role in modulating WT1's transcriptional activity [47, 48].

The WT1 gene and its isoforms regulate genes critical for cellular differentiation and survival in multiple organs, including the spleen, adrenal glands, liver, diaphragm, gonads, kidneys, and urogenital system [49–51]. WT1 is also essential for heart development and regulates gene expression in cardiomyocytes to maintain cardiac homeostasis and promote repair after injury, highlighting WT1 as a promising target for cardiac regeneration therapies [52]. Quenneville et al. (2024) identified a novel WT1 isoform induced under prolonged severe hypoxia in cancer cells, which drives a non-canonical epithelial–mesenchymal transition, illustrating WT1's functional versatility through isoform-specific gene regulation in stress conditions [53]. Additionally, studies have demonstrated that loss of WT1 function impairs the regulation of key genes required for kidney development and promotes tumorigenesis, contributing to the onset of Wilms' tumor. These observations emphasize WT1's critical role in regulating cellular proliferation and differentiation [54].

WT1 also contributes significantly to RNA processing and metabolism by interacting with splicing factors. In addition, it influences mRNA transport and stability. [32, 44, 55]. WT1 participates in translation process, as the WT1 protein shuttles between the nucleus and cytoplasm [56, 57]. Moreover, it regulates mRNA turnover through interactions with the 3′ untranslated region (UTR), implicating microRNA processing pathway genes in the pathogenesis of Wilms' tumor [58].

WT1 exhibits a dual role in cancer pathogenesis

In Wilms' tumor, WT1 primarily functions as a tumor suppressor by regulating genes essential for cellular differentiation and apoptosis. It suppresses oncogenic signaling pathways while promoting differentiation and programmed cell death in tumor cells [59–61]. However, the role of WT1 in other cancers is far more complex and context-dependent, influenced by the cellular environment and its interaction with key pathways such as p53. For instance, Yao et al. (2021) demonstrated that WT1 suppresses acute myeloid leukemia cell proliferation in a p53-dependent manner, highlighting its tumor-suppressive function when p53 is active [62]. Additionally, Jing et al. (2022) reported that WT1 inhibits the proliferation of renal carcinoma cells and induces G2/M arrest by upregulating pro-apoptotic genes IL-24, reinforcing its tumor-suppressive activity in renal cell carcinoma (RCC) [16].

WT1 exhibits a dual role in tumorigenesis, acting as both a tumor suppressor and an oncogene. This functional dichotomy is primarily driven by specific WT1 isoforms, which exert distinct and often opposing effects on key biological processes. Isoform expression levels and activity critically influence tumor progression and clinical outcomes, emphasizing the need for isoform-specific regulation in cancer biology [59, 63]. WT1's oncogenic or tumor-suppressive function also depends on factors such as developmental stage, cell type, differentiation status, and the timing of its expression or mutation. Additionally, its activity is modulated by binding partners, co-occurring genetic mutations, and features of the tumor microenvironment [15, 25, 27, 28, 44].

WT1 predominantly acts as an oncogene in many adult cancers by modulating apoptotic pathway in a complex, context-specific manner. It promotes cell survival by upregulating anti-apoptotic genes and suppressing pro-apoptotic pathways. This oncogenic activity is particularly evident in

leukemic cells, where WT1 fosters an anti-apoptotic environment by enhancing the expression of survival genes and repressing apoptosis, thereby contributing to its oncogenic role [64–67].Furthermore, mutations in the WT1 gene or epigenetic modifications, such as promoter methylation, can alter its transcriptional activity and shifting its role from tumor suppressive to oncogenic. The epigenetic landscape of the tumor microenvironment further modulates WT1 expression and activity, reinforcing its dual and context-dependent role in cancer pathogenesis [59, 63].

Table 1 presents the context-dependent cellular and tissue-specific functions of the WT1 gene. Originally identified as a tumor suppressor, WT1 also functions as an oncogene in various malignancies. In cancers such as acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), non-small-cell lung cancer (NSCLC), and glioblastoma, WT1 promotes cell proliferation and survival by upregulating oncogenic targets, including BCL-2, MYC, VEGF, and EGFR [65, 66, 68, 69]. In these contexts, WT1 serves as a therapeutic target, particularly through immunotherapeutic strategies such as WT1 peptide vaccines. Conversely, in cancers like Wilms' tumor, mesothelioma, and breast cancer, WT1 acts as a tumor suppressor. Loss-of-function mutations in WT1 in these cases are associated with impaired differentiation, reduced apoptosis, and deregulated cell cycle control. Under normal conditions, WT1 supports differentiation, apoptosis, and cell cycle arrest by activating targets like p21, p53, and IGF-binding proteins [15, 70]. These dual and often contradictory roles of WT1 in cancer underscore the importance of context-specific analysis in both diagnostic assessment and therapeutic development.

WT1 interactions with other tumor suppressors

WT1 engages in complex and context-dependent interactions with other tumor suppressor genes, thereby regulating critical cellular processes such as apoptosis, cell cycle progression, and genomic stability. Recent studies demonstrate that WT1 directly interacts with members of the p53 protein family including p53, p63, and p73—modulating their transcriptional activities and influencing cellular outcomes related to apoptosis, cell cycle control, and tumorigenesis (Tables 2 and 3).

P53:

WT1 physically interacts with p53 through its zinc finger domains, particularly zinc fingers 1 and 2 [71]**.** This interaction stabilizes p53 by extending its half-life and shielding it from proteasomal degradation. As a consequence, WT1 enhances p53's transcriptional activity, promoting the expression of genes involved in cell cycle arrest and apoptosis [42, 44]**.** Despite this activation, WT1 paradoxically inhibits p53-induced apoptosis, highlighting a context-dependent regulatory complexity in their interaction [44, 71]. Bordin et al. (2018) further confirmed this intricate relationship by showing that WT1 loss impairs p53-mediated DNA damage responses in T-cell acute lymphoblastic leukemia, reducing transcription of pro-apoptotic genes such as BAX, BBC3, and GADD45A [72]. Additionally, another study provided evidence that p53 can transcriptionally repress WT1 target genes such as podocalyxin, revealing reciprocal regulation between these tumor suppressors [73]. Collectively, these studies illustrate that the WT1-p53 interaction establishes a dynamic regulatory balance that controls cell proliferation and apoptosis, influencing both cellular homeostasis and tumorigenesis.

P73 and p63:

P63 and p73, homologs of p53, play critical roles in epithelial development and contribute to various cancers, though their functions remain less defined compared to p53. These proteins act as tumor suppressors or oncogenes depending on the cellular context and isoform expression [74]. WT1 interacts with all four isoforms of p73 and p63 through its zinc finger domains, modulating their transcriptional activities in a context-dependent manner. These interactions inhibit the

Table 1 WT1 as Oncogene vs Tumor Suppressor

Feature	WT1 as Oncogene	WT1 as Tumor Suppressor
Cancer types	AML, MDS, NSCLC, glioblastoma, ovarian, pancreatic	Wilms' tumor (pediatric kidney cancer), mesothelioma, breast cancer
Expression status	Overexpressed	Loss of function or deletion
Mechanism	Promotes proliferation, inhibits apoptosis	Promotes differentiation, cell cycle arrest, apoptosis
Downstream targets	↑ BCL-2, ↑ VEGF, ↑ MYC, ↑ EGFR	↑ p21, ↑ p53, ↑ IGF-BP, ↑ nephrin, ↑ podocalyxin
Mutation type	Gain-of-function, overexpression	Frameshift, nonsense, or dominant-negative mutations
Therapeutic approach	Target inhibition: vaccines, TCR-T, mAbs, CRISPR	Gene restoration or functional activation
Prognostic value	Poor prognosis if overexpressed	Poor prognosis if inactivated (esp. in syndromic Wilms')
Mouse model phenotype	Overexpression → tumor formation	Knockout → lethal kidney defects

Table 2 WT1 interactions with other cancer genes

Aspect	Wilms Tumor 1 (WT1) [25, 112]	TP53 [72, 73]	KRAS [143]	HER2 (ERBB2)[114]	BCR-ABL[194, 205]
Gene Role	Tumor suppressor (normal) & oncogene (in cancer)	Tumor suppressor	Oncogene	Oncogene	Fusion oncogene
Cancer Types	Leukemia, Wilms tumor, mesothelioma, glioblastoma	Most solid tumors	Pancreatic, lung, colon	Breast, gastric	CML, ALL
Mechanism in Cancer	Overexpressed in many cancers; affects differentiation & proliferation	Loss-of-function (LOF) mutations → loss of cell cycle control	Gain of function (GOF) mutations → uncontrolled growth	Gene amplification → overactive signaling	Fusion protein → constant kinase activation
Targeting Strategies	Vaccines, CAR-T, peptide immunotherapy, antisense, siRNA	Gene therapy, synthetic lethality, reactivating mutants	Direct inhibitors (difficult), KRAS G12C inhibitors	Monoclonal antibodies (trastuzumab), TKIs	TKIs (imatinib, dasatinib)
Therapeutic Challenges	WT1 has both oncogenic and tumor suppressor roles; nuclear localization limits druggability	Difficult to replace tumor suppressor; reactivation should be safe	Targeting mutant protein selectively without affecting normal	Resistance, cardiotoxicity	Resistance mutations
Clinical Trials	Active trials for vaccines (e.g., galinpepimut-S) & CAR-T	Trials on p53 reactivation & gene delivery	KRAS G12C inhibitors (e.g., sotorasib) approved	Many FDA-approved drugs	Highly successful targeted therapy model
Biomarker Use	Yes, for prognosis & MRD in leukemia	Yes, TP53 status affects treatment decisions	Yes, KRAS mutation testing is routine	HER2 testing standard in breast cancer	BCR-ABL monitoring essential

Table 3 WT1 Gene Involvement In Various Type Cancers

Cancer Type	WT1 Involvement	Additional Notes
Wilms' Tumor [12, 27]	Germline and somatic mutations; classic tumor suppressor role	WT1 mutations observed in about 20% of cases; associated with syndromes like WAGR and Denys–Drash
Acute Myeloid Leukemia (AML) [17]	Overexpression; mutations; prognostic marker	High WT1 expression linked to poor prognosis; used as a minimal residual disease marker
Desmoplastic Small Round Cell Tumor [145–147]	EWS-WT1 gene fusion resulting from t(11;22)(p13;q12) translocation	Pathognomonic EWS-WT1 fusion protein drives tumorigenesis
Glioblastoma and Other Gliomas [22, 140–142]	Hyper-expression correlates with tumor grade and proliferation	WT1 expression associated with higher MIB-1 index, indicating increased proliferation
Osteosarcoma [204]	Downregulation of the PI3K/AKT pathway & increased Bax/Bcl2 ratio	WT1 overexpression correlates with aggressive metastatic behavior
Lung Cancer [91, 125]	Overexpression in both small-cell and non-small-cell lung cancers	WT1 expression observed in various lung cancer cell lines and tissues
Colon Cancer [133, 134]	Overexpression in tumor cell lines; variable expression in tissues	WT1 expression detected in colon cancer cell lines; tissue expression varies
Breast Cancer [110–112]	WT1 is shown to influence breast cancer by regulating other genes	Acts as a tumor suppressor or oncogene and affects cancer progression and therapy
Ovarian Cancer [126–128]	Overexpression, particularly in serous carcinoma subtypes	WT1 serves as a diagnostic marker; expression varies among subtypes
Pancreatic Cancer [136–138]	Overexpression in ductal adenocarcinoma	WT1 expression observed in pancreatic cancer tissues
Esophageal Cancer [218]	Overexpression in tumor tissue, enhanced cell migration and invasion	WT1 expression detected in esophageal cancer specimens
Thyroid Cancer [236]	Overexpression in primary thyroid cancers	WT1 expression observed in various thyroid cancer types
Head and Neck Squamous Cell Carcinoma [76]	WT1 regulates p63; promotes proliferation	WT1 involvement in cell proliferation and tumor progression, indicating potential targets for immunotherapy in HNSCC

DNA binding and transcriptional activation abilities of p73 and p63, thereby influencing cell differentiation, apoptosis, and tumor suppression [75]**.** The complex interplay between WT1 and these p53 family members highlights the intricate regulation of cellular transcriptional networks.

In squamous cell carcinoma of the head and neck (SCCHN), WT1 directly regulates p63 expression and promotes cell proliferation. Silencing WT1 in FaDu cells reduces p63 levels and decreases proliferation, demonstrating that WT1 acts as a positive transcriptional regulator of p63 in this cancer model. Chromatin immunoprecipitation (ChIP) and quantitative PCR analyses confirmed that WT1 binds directly to the promoters of both TAp63 and ΔNp63 isoforms, further supporting its role in transcriptional regulation of p63 [76]**.**

These findings collectively underscore the dynamic regulatory network between WT1 and p53 family proteins in controlling key cellular processes and maintaining genomic integrity. Disruptions in these interactions contribute to tumorigenesis, emphasizing the therapeutic potential of targeting this regulatory axis. Understanding the mechanisms underlying interactions between WT1 and p53 family proteins opens avenues for potential targeted therapies. Modulating these interactions could restore normal transcriptional regulation, offering potential strategies for treating cancers associated with WT1 and p53 dysfunction.

PTEN

WT1 regulates PTEN expression through multiple mechanisms. In non-small-cell lung cancer (NSCLC), the long non-coding RNA WT1-AS—transcribed from the antisense strand of WT1, acts as a molecular sponge for miR-494-3p. This interaction prevents miR-494-3p-mediated repression of PTEN, thereby inhibiting the PI3K/AKT signaling pathway and suppressing cell proliferation, survival, migration, and invasion. [77]. In Wilms' tumor, bioinformatics analysis identified miR-21-5p as a potential regulator that targets and suppresses PTEN, thereby activating PI3K/AKT pathway and promoting tumorigenesis, including enhanced tumor cell migration and invasion. This finding suggests that altered regulation of PTEN by miRNAs like miR-21-5p could contribute to the pathogenesis of Wilms' tumor [78]. Similarly, increased levels of miR-19b suppress PTEN expression, leading to heightened PI3K/AKT pathway activity and promoting proliferation and apoptosis resistance, while

inhibition of miR-19b reverses these effects [79].These findings emphasize the multifaceted regulation of PTEN by WT1 and its downstream mediators. Understanding these interactions could offer novel therapeutic opportunities for cancers involving PI3K/AKT dysregulation.

BRCA1

Researchers have not reported any direct interaction between Wilms' Tumor 1 (WT1) and BRCA1. Although both genes function as crucial tumor suppressors involved in DNA repair and cancer progression, their direct interaction remains speculative. Recent studies have further clarified BRCA1's role in chromatin remodeling and DNA damage repair. For example, Witus et al. (2023) demonstrated that intrinsically disordered regions in BRCA1/BARD1 mediate histone H2A ubiquitylation and chromatin recruitment, key processes in the DNA damage response [80]. Wegert et al. (2025) analyzed cases of bilateral Wilms tumor and identified germline variants in WT1, TRIM28, and BRCA2, suggesting possible shared predisposition pathways. However, this study did not implicate BRCA1 in direct functional association with WT1 [81].

In summary, while WT1 and BRCA1 both play significant roles in tumor suppression and DNA repair, future studies are needed to investigate whether any functional relationship exists between WT1 and BRCA1 in cancer biology.

CDC73 (Parafibromin)

WT1 directly represses the tumor suppressor CDC73 in oral squamous cell carcinoma (OSCC) by binding to its promoter and inhibiting its transcription. This repression reduces CDC73 expression, leading to increased cell proliferation and decreased apoptosis. These findings suggest that WT1 functions as an oncogene in this context by actively suppressing the expression of CDC73, a key regulator of transcription and cell cycle control [82].

Bcl-2 family proteins

WT1 directly regulates both pro-apoptotic and anti-apoptotic members of the Bcl-2 family in a context-dependent manner. In some settings, WT1 upregulates anti-apoptotic proteins such as Bcl-2, thereby enhancing cell survival and resistance to apoptosis [83]. In contrast, under different conditions, WT1 activates the expression of pro-apoptotic genes like Bak and Bik, promoting apoptosis [84]. Another study demonstrates that WT1, with Par-4, binds the Bcl-2 promoter and represses its expression, promoting apoptosis [85]. These findings underscore WT1's dual regulatory role in apoptosis, demonstrating that it can either promote or inhibit cell death by modulating Bcl-2 family proteins depending on the cellular environment.

MAP kinase phosphatase 3 (MKP3)

WT1 induces the expression of MAP kinase phosphatase 3 (MKP3), also known as DUSP6, a negative regulator of the Ras/MAPK signaling pathway. By upregulating MKP3, WT1 decreases ERK phosphorylation, thereby suppressing cell proliferation. This regulation reveals a novel mechanism through which WT1 exerts growth-inhibitory effects by modulating MAPK pathway signaling [86].

β-Catenin

In breast cancer cells, WT1 reduces β-catenin protein levels and impairs β-catenin/TCF transcriptional activity, thereby suppressing Wnt signaling. This destabilization of β-catenin is associated with WT1-mediated inhibition of breast cancer cell growth [87].

These findings underscore the multifaceted role of WT1 in regulating tumor suppressor pathways. The functional outcomes of WT1 interactions vary across cell types and tumor contexts, reflecting its highly context-dependent nature. Further research is needed to fully elucidate the complex network of WT1 interactions with other tumor suppressors and their implications in cancer biology. Despite it initially classified as a tumor suppressor, WT1 also exhibits oncogenic properties in several malignancies, making it a promising target for cancer research and immunotherapy. Therapeutic strategies, such as intracellular antibodies targeting WT1, have shown potential in preclinical studies [88].

Why WT1 is a promising cancer target

Widespread overexpression in cancers

Wilms' Tumor 1 (WT1) serves as a compelling target for cancer immunotherapy due to its widespread overexpression across various hematologic and solid malignancies including leukemia, lung cancer, ovarian cancer, pancreatic cancer, and thymic tumors, while maintaining limited expression in normal adult tissues. Notably, researchers have reported WT1 overexpression in approximately 20% of intrahepatic cholangiocarcinoma (CCA) cases [89, 90].

In non-small-cell lung cancer (NSCLC), Li et al. (2023) demonstrated that WT1 expression is significantly elevated in tumor tissues and cell lines. They further observed that higher WT1 levels correlate with poor prognosis and increased immune cell infiltration, implicating WT1 in NSCLC progression [91]. Collectively, these studies highlight WT1 as a universal tumor-associated antigen. Its aberrant overexpression, association with aggressive disease

features, and limited normal tissue expression underscore its value as a target for therapeutic interventions across a broad spectrum of cancers.

Intracellular localization and immunogenicity

WT1 is an intracellular protein that undergoes antigen processing, allowing tumor cells to present WT1-derived peptides via HLA class I molecules. This presentation enables cytotoxic $CD8^+$ T cells to specifically recognize and eliminate WT1-expressing tumor cells. Immunotherapies, including peptide vaccines, T cell therapies, and CAR-T cells, exploit this mechanism to target WT1-positive cancers. Nishida et al. (2022) showed that WT1 peptide vaccination induces strong cellular and humoral immunity in ovarian cancer, improving clinical outcomes [92]. Liu et al. (2016) showed that WT1 peptide vaccination in myeloid leukemia elicited WT1-specific T-cell responses with TCR clonal enrichment [93]. Further, Liu et al. (2018) demonstrated WT1-specific T-cell responses with TCR clonal enrichment in myeloid leukemia, with Montanide adjuvant enhancing vaccine efficacy [94]. These studies collectively confirm that antigen processing and HLA presentation expose intracellular WT1 to immune recognition, establishing WT1 as a robust and versatile target for cancer immunotherapy.

Functional role in tumorigenesis

WT1 promotes tumorigenesis by regulating genes involved in proliferation, survival, and metastasis. In non-small-cell lung cancer (NSCLC), WT1 enhances malignancy by co-expressing with oncogenic factors such as MYC and matrix metalloproteinase 2 (MMP2), which drive cancer progression. Li et al. (2023) demonstrated that microRNA-498-5p suppresses WT1 expression, thereby reducing NSCLC aggressiveness and suggesting a regulatory axis with therapeutic potential [91].

Integrating population studies and WT1-targeted therapies

Integrating population studies with WT1-targeted therapies offers a robust strategy to understand and treat cancers driven by WT1 dysregulation. WT1 functions as either a tumor suppressor or an oncogene, depending on the cancer type and patient-specific genetic context, thereby shaping therapeutic decisions.

Population-based studies have defined the clinical relevance of specific WT1 mutations in determining cancer risk and prognosis. Nagano and Nozu (2025) analyze genotype–phenotype correlations in WT1-related disorders, including Wilms tumor. Their review highlights that truncating mutations in WT1 associate with a higher Wilms tumor risk compared to missense mutations and frequently result in Denys–Drash syndrome, a condition marked by early onset nephropathy and a high tumor incidence. In contrast, missense mutations, particularly those involving the DNA-binding domain, often lead to Frasier syndrome, which presents with focal segmental glomerulosclerosis and a comparatively lower risk of tumor development. [95].

The study emphasizes the importance of understanding these genetic variations to guide personalized treatment strategies. By elucidating genotype–phenotype relationships, they promote the development of mutation-specific therapeutic approaches. Clinicians can use this mutation-specific information to predict disease progression and tailor interventions accordingly. Previous study analyzed WT1 exon 8 and 9 missense mutations in the DNA-binding domain and demonstrated that these mutations produce diverse phenotypes, ranging from mild nephropathy to varying tumor risks. The findings confirmed that missense mutations generally cause milder clinical symptoms than truncating mutations and underscore the importance of detailed genetic analysis to guide personalized treatment of WT1-related disorders [96]**.** Similarly, Lipska et al. (2014) investigated 64 patients with WT1-related glomerulopathy and identified clear genotype–phenotype correlations. They found that dominant-negative missense mutations in the zinc finger domains typically led to Frasier syndrome, which involves slow-progressing kidney disease and gonadal dysgenesis. In contrast, truncating mutations or those outside the zinc finger domains often resulted in Denys–Drash syndrome, which presents with early onset nephrotic syndrome and increased Wilms tumor risk. Their study confirmed that the type and location of WT1 mutations significantly influence disease severity and progression [97]. Liu et al. (2023) analyzed WT1 mutations in Chinese children with nephropathy and further supported the genotype–phenotype correlations described by Nagano and Nozu. They found that truncating mutations cause severe early onset nephropathy and increase tumor risk, while missense mutations lead to milder phenotypes. This population-specific data highlight the importance of integrating genetic insights to guide personalized treatment approaches for WT1-related disorders [98].

In conclusion, integrating comprehensive population-level genetic data with emerging WT1-targeted therapeutic approaches provides a powerful framework to enhance our understanding of WT1's dual role as a tumor suppressor and oncogene across diverse cancer types. Insights from genotype–phenotype correlation studies enable precise identification of mutation-specific risks and clinical outcomes, which are critical for tailoring personalized treatment strategies. Building on this foundation, innovative therapies such as antisense oligonucleotides (ASOs) [99] and small interfering RNAs (siRNAs) [100–102] offer promising tools to selectively inhibit oncogenic WT1 isoforms in both hematologic

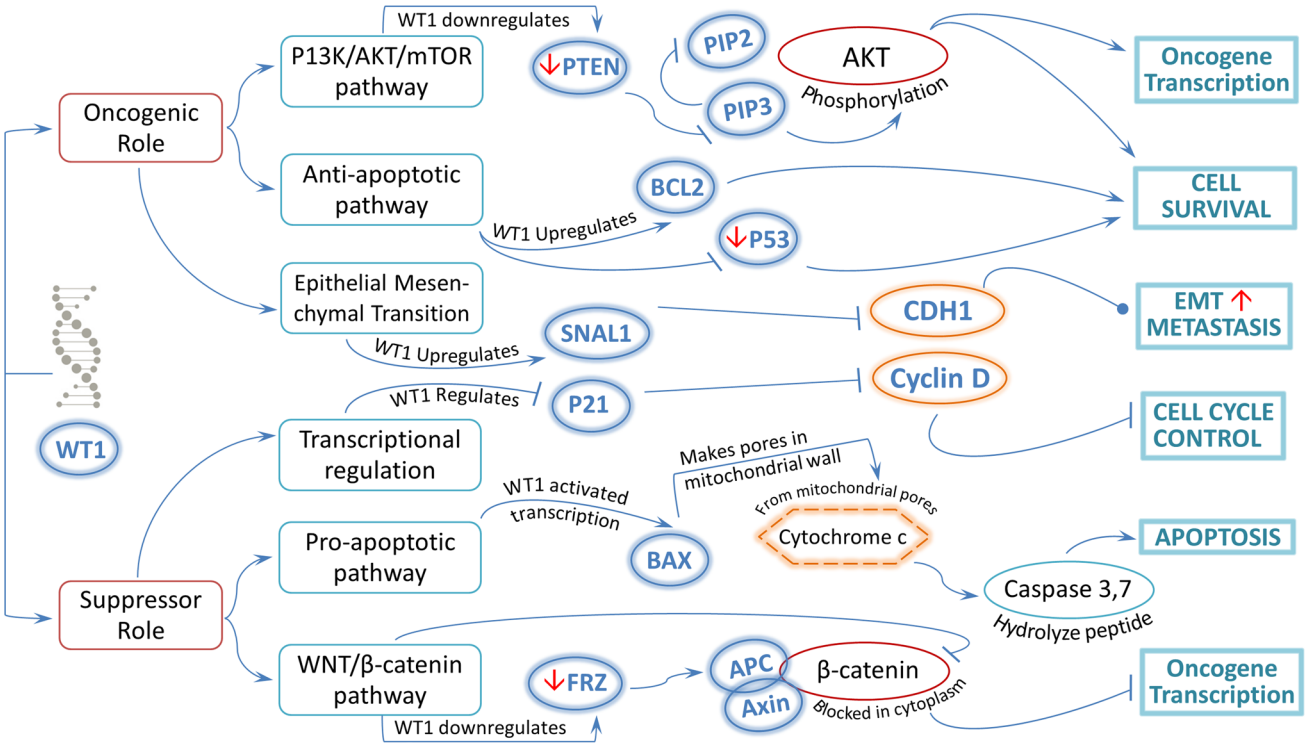

Fig. 2 The Role of WT1 in Cancer Pathogenesis through the Regulation of Target Genes: The Wilms tumor 1 (WT1) gene is primarily recognized as a tumor suppressor, acting as a transcriptional repressor; however, in certain cancers, it can function as an oncogene by serving as a transcriptional activator [42]. The biological effects of WT1 expression are context-dependent, influenced by the specific cell type and the surrounding regulatory microenvironment, which collectively determine whether WT1 promotes cellular proliferation or exerts growth-inhibitory effects. As a tumor suppressor, WT1 regulates transcriptional pathways such as the activation of p21, which inhibits Cyclin D and controls cell cycle progression. WT1 also promotes apoptosis through the activation of the pro-apoptotic protein BAX. Additionally, WT1 suppresses the Wnt/β-catenin signaling pathway, further contributing to its tumor suppressive functions [84, 87]. Conversely, when WT1 acts as an oncogene, it promotes tumor invasion and metastasis through epithelial–mesenchymal transition (EMT), partly by repressing E-cadherin expression [127]. WT1 also enhances the transcription of Bcl-2, an anti-apoptotic protein that supports cell survival [83]. Furthermore, WT1 downregulates the tumor suppressor gene PTEN, inhibiting its phosphatase activity and preventing the dephosphorylation of PIP3, thus contributing to cellular growth and survival [78]

malignancies and solid tumors. Concurrently, immunotherapeutic interventions including WT1 peptide vaccines are being actively developed and clinically evaluated to stimulate targeted anti-tumor immune responses [103, 104]. By leveraging population genetics alongside molecularly informed WT1-targeted treatments, clinicians and researchers can advance mutation-guided precision oncology that maximizes therapeutic efficacy while minimizing adverse effects, ultimately improving prognosis and quality of life for patients with WT1-associated cancers.

Identification of Wilms' tumor 1 (WT1) target genes and its role in cancer pathogenesis through the regulation of other genes

WT1 regulates numerous genes involved in critical cellular processes, including proliferation, differentiation, apoptosis, survival, and metastasis. Acting as a transcriptional regulator, WT1 either activates or represses these target genes, thereby driving diverse biological outcomes such as cell growth, differentiation, and apoptosis (Fig. 2). Researchers have identified a wide array of WT1 target genes through microarray analyses [105–107]. These target genes include AREG, CDKN1A (p21/CIP1), DAX1, colony-stimulating factor 1 (CSF-1), Müllerian-inhibiting substance (MIS), SF1, sex-determining region Y (SRY), CDH1 (E-cadherin), podocalyxin, nephrin (NPH1), BAK, and NTRK2. Together, they mediate the diverse regulatory functions of WT1 in essential cellular processes [84, 108, 109].

A genome-wide expression profiling study identified several direct target genes of WT1, including members of the EGF family of growth factors such as AREG, EREG, and HB-EGF, along with the chemokine CX3CL1 and the cytokine IL-11. Additional analyses using qRT-PCR, chromatin immunoprecipitation (ChIP), and immunohistochemistry further confirmed other potential direct targets of WT1, including IL1RAP, SLUG, JUNB, SLC20A1, and TIMP3 [40].

We present a concise overview of the role of WT1 in various cancers, emphasizing its regulatory impact on gene expression and underscoring its clinical relevance as both a prognostic marker and a potential therapeutic target across multiple cancer types.

Breast cancer

Elevated WT1 expression is commonly observed in breast cancer cells; high WT1 mRNA expression is associated with poorer disease-free survival. While no significant correlation was found between WT1 levels and clinicopathological parameters such as tumor size or lymph node status, the prognostic value of WT1 expression suggests its potential utility as an independent prognostic biomarker [110]**.** Recent study reports that WT1 is overexpressed in triple-negative breast cancer (TNBC), correlates with poor prognosis, and drives metastasis by transcriptionally activating PFKFB4 to enhance glycolysis. Co-expression of WT1 and PFKFB4 is linked to worse clinical outcomes, highlighting WT1's oncogenic and prognostic significance in TNBC [111].WT1 regulates genes involved in proliferation, migration, apoptosis, and survival, influencing key oncogenic and tumor suppressor pathways such as p53, EphA2, HER2, ER-α, and TPα. It controls caspase family members that mediate apoptosis and affect tumor progression. In hormone-responsive breast cancer, WT1 modulates estrogen receptor signaling by regulating genes in estrogen biosynthesis and cellular response to estrogen. These roles establish WT1 as a critical factor in breast cancer pathophysiology and a promising prognostic biomarker and therapeutic target [112]**.**

Previous studies have suggested that WT1 may function as a tumor suppressor gene in breast cancer by inhibiting cell proliferation. For instance, one study reported that WT1 suppresses breast cancer cell growth by destabilizing β-catenin, thereby inhibiting the Wnt/β-catenin signaling pathway, which promotes tumor progression [87]. Additionally, WT1 plays a complex and context-dependent role through its interactions with estrogen receptor alpha (ERα) and growth factor signaling pathways. Reizner et al. (2005) demonstrated that WT1 interacts with estrogen receptor alpha (ERα) and inhibits its transactivation of the insulin-like growth factor 1 receptor (IGF-1R) gene in breast cancer cells. This inhibition reduces IGF-1R expression and subsequently suppresses cell proliferation, further supporting WT1's tumor-suppressive function in specific breast cancer contexts. [113]**.**

Conversely, Nasomyon et al. (2014) reported that WT1 promotes breast cancer progression by upregulating the expression of ERα, human epidermal growth factor receptor 2 (HER2), and epidermal growth factor receptor (EGFR), suggesting an oncogenic role for WT1 in certain cellular contexts [114]. By enhancing ERα and HER2 expression, which are key drivers of breast tumor growth and aggressiveness—WT1 may facilitate tumor progression. These findings, in contrast to its tumor-suppressive functions, underscore the context-dependent nature of WT1 activity. Understanding this duality is crucial for elucidating WT1's potential as a therapeutic target in breast cancer. Further supporting its regulatory complexity, Müller et al. (2017) demonstrated that WT1 stimulates transcription of IGFBP5, a gene encoding insulin-like growth factor-binding protein 5. By increasing IGFBP5 expression, WT1 can modulate insulin-like growth factor (IGF) signaling by sequestering IGF ligands and altering IGF receptor activity, thereby influencing breast cancer cell proliferation and survival [115]. A recent study defines the complex, isoform-dependent roles of WT1 in breast cancer and emphasizes the need for individualized molecular profiling of WT1 isoforms in breast cancer patients. The researchers reported that downregulation of specific WT1 isoforms, particularly WT1-A ($EX5^-$/KTS^-), correlates with improved survival outcomes, suggesting a potential tumor-suppressive function for this variant. Conversely, other isoforms may exert oncogenic effects, highlighting WT1's dual role in tumor biology [59]. One study investigates the role of WT1 isoforms in triple-negative breast cancer (TNBC). It shows that specific isoforms (WT1-B and WT1-C) enhance vasculogenic mimicry and metastatic potential. The findings highlight the isoform-dependent effects of WT1 on TNBC aggressiveness [116]. WT1 regulates epithelial-to-mesenchymal transition (EMT) by modulating key transcription factors such as Slug, which controls cellular plasticity essential for EMT. This study highlights pivotal role of WT1 in controlling EMT dynamics via Slug and suggesting a broader influence on tumor cell plasticity and metastatic potential [117]. Another report demonstrated that elevated WT1 expression in breast cancer cells disrupts the epithelial mesenchymal balance, promoting a mesenchymal phenotype linked to increased tumor aggressiveness. WT1 also alters cellular metabolism and modulates sensitivity to the chemotherapeutic agent docetaxel. These results highlight WT1's multifaceted role in regulating EMT, tumor metabolism, and treatment response, thereby contributing to cancer progression and therapy resistance [118].

Acute myeloid leukemia (AML)

In acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS), WT1 is frequently overexpressed and occasionally mutated, highlighting its role in leukemogenesis and potential as a therapeutic target. Approximately 70–90% of AML patients show WT1 overexpression, while 6–15% carries WT1 mutations [119]. These mutations frequently occur in conjunction with other genetic alterations, such as FLT3-ITD and NPM1 mutations, contributing to disease aggressiveness and promote leukemogenesis. WT1 also modulates

several molecular pathways in leukemic cells, supporting the survival and self-renewal of leukemia-initiating cells (LICs) [120–122]. It also interacts with epigenetic regulators like DNMT3A, further promoting cancer cell growth and survival in AML patient [123].

Myelodysplastic SYNDROMES (MDS)

WT1 is frequently overexpressed in MDS and correlates with poor overall survival (OS). However, emerging evidence suggests that the prognostic relevance of WT1 expression in myelodysplastic syndromes (MDS) is modulated by the presence of specific genetic mutations, notably alterations in TP53. These findings underscore the multifaceted role of WT1 in MDS pathogenesis and highlight the necessity of integrating mutational profiling particularly TP53 status into prognostic stratification frameworks. [124].

Non-Small-Cell lung cancer (NSCLC)

WT1 is upregulated in NSCLC tissues and cell lines and correlates with poor prognosis and increased immune cell infiltration. Functional assays show that WT1 promotes cell proliferation, survival, and invasion. Survival analyses indicate that elevated WT1 expressions correlate with decreased overall and progression-free survival, supporting its role as a prognostic biomarker and therapeutic target [91]. MicroRNA-498-5p acts as a natural inhibitor of WT1, indicating a potential therapeutic strategy to suppress WT1 expression in NSCLC. Xu et al. (2013) further demonstrated that WT1 is overexpressed in NSCLC tissues relative to adjacent normal tissues and its expression positively correlates with cell proliferation in both cell lines and a xenograft mouse model. This study revealed that WT1 enhances cell proliferation by upregulating Cyclin D1 and phosphorylated retinoblastoma protein (p-pRb), facilitating S-phase entry and cell cycle progression. These findings establish WT1 as an oncogenic driver in NSCLC through modulation of cell cycle regulators [125].

Ovarian carcinoma

WT1 plays a significant role in ovarian cancer. Liu et al. (2014) reported that WT1 overexpression correlates with aggressive clinical features such as advanced stage, poor differentiation, and reduced overall survival, suggesting WT1 as a biomarker for tumor aggressiveness and prognosis. [19]. In contrast, Taube et al. (2016) found that WT1 is commonly expressed in high-grade serous ovarian carcinoma (HGSC) and associates with improved overall survival (OS) and progression-free survival (PFS), independent of age, stage, and residual tumor status [126]. Han et al. (2020) demonstrated that WT1 promotes ovarian cancer progression by regulating E-cadherin and ERK1/2 signaling. WT1 directly binds the E-cadherin promoter, suppressing its expression and inducing epithelial-to-mesenchymal transition (EMT) via ERK1/2 signaling pathway activation, thus driving tumor invasiveness. Targeting WT1 could inhibit these oncogenic pathways and offer therapeutic benefits [127]. Van Amerongen et al. (2022) identified novel WT1-derived peptides and engineered WT1-specific T cell receptors (TCRs) that selectively recognize and target WT1-expressing tumor cells. These TCRs exhibit potent antitumor activity against acute myeloid leukemia (AML) and ovarian carcinoma in preclinical models, supporting WT1-targeted TCR immunotherapy as a promising treatment for WT1-positive malignancies [128].

Kidney Wilms tumor

WT1 regulates several genes implicated in Wilms tumor (WT) and fetal kidney development. Zeng et al. (2024) identified EMCN and CCNA1 as key hub genes, demonstrating that EMCN overexpression and CCNA1 knockdown significantly reduced Wilms tumor cell viability, proliferation, migration, and invasion, indicating their roles as potential prognostic biomarkers and therapeutic targets. [129]. WT1 also governs genes involved in mesenchymal-to-epithelial transition (MET), a process essential for nephrogenesis. In WT1-mutant Wilms tumors, concurrent CTNNB1 mutations frequently activate Wnt/β-catenin signaling, which disrupts epithelial differentiation and promotes abnormal stromal cell proliferation. This molecular interplay impairs kidney architecture and promotes tumorigenesis [130]. Royer-Pokora et al. (2023) further demonstrated that WT1-mutant Wilms tumors exhibit gene expression profiles aligned with early renal stem cell states, supporting their origin from undifferentiated nephron progenitors [131]. In parallel, Rialdi et al. (2023) showed that WNTinib, a selective multi-kinase inhibitor, effectively blocks Wnt/β-catenin signaling in β-catenin-mutant tumors. Given the frequent activation of β-catenin in WT1-mutant Wilms tumors, WNTinib may offer a promising targeted therapeutic strategy in this context [132]. These findings support WT1 as a diagnostic and prognostic marker and emphasize the therapeutic potential of targeting the Wnt/β-catenin pathway in Wilms tumor.

Renal cell carcinoma (RCC)

WT1 exhibits tumor-suppressive activity in renal cell carcinoma (RCC). Jing et al. (2022) demonstrated that WT1 overexpression in human RCC A498 cells significantly

upregulates interleukin-24 (IL-24), a cytokine with anti-tumor properties. This induction leads to G2/M cell cycle arrest and marked inhibition of cell proliferation, indicating a direct role of the WT1/IL-24 axis in tumor suppression. Furthermore, analysis of The Cancer Genome Atlas (TCGA) data revealed that WT1-regulated genes such as TXNIP and GADD45A correlate inversely with tumor stage and histological grade. Elevated expression of these genes associates with favorable prognoses, reinforcing WT1's protective function in RCC progression [16].

These findings underscore WT1's context-dependent role in tumor biology and support the WT1/IL-24 signaling pathway as a promising therapeutic target, particularly in RCC cases, especially for those resistant to conventional therapies [133].

Endometrial carcinoma

In endometrial carcinoma, especially the serous and clear cell subtypes, elevated WT1 expression correlates with poorer overall survival and disease-free survival. WT1 levels associate positively with advanced tumor stage and higher histological grade, highlighting its potential as a prognostic marker [134]. However, studies have yet to establish WT1 as an independent prognostic factor, and further validation is required. Current studies actively explore WT1-targeted therapies to develop personalized treatment strategies in endometrial carcinoma [133].

Uterine serous carcinoma (USC)

WT1 plays a potential diagnostic and prognostic role in uterine serous carcinoma (USC), where its expression may influence treatment outcomes. WT1 expression is detected in over 20% of uterine serous carcinoma (USC) cases [135]. Notably, WT1-positive tumors exhibit significantly greater chemosensitivity, which correlates with a trend toward improved progression-free survival [135]. Another study concluded that WT1 expression correlates with poor prognosis in certain histotypes and may serve as both a diagnostic and prognostic marker, particularly in high-grade subtypes like USC. Evaluating WT1 status in USC could help predict chemotherapy response, stratify patients by risk and guide personalized treatment strategies [134].

Pancreatic ductal adenocarcinoma (PDA)

In pancreatic ductal adenocarcinoma (PDA), WT1 is predominantly expressed in the cytoplasm of tumor cells and within tumor-associated vasculature. Higher cytoplasmic WT1 expression correlates with shorter overall survival (OS) and disease-free survival (DFS). Multivariate analysis has identified WT1 overexpression as an independent predictor of poor prognosis, highlighting its potential as a therapeutic target [136]. A systematic review and meta-analysis of 29 studies involving 4,090 patients further confirmed that WT1 positivity is significantly associated with worse OS and DFS/RFS/PFS across solid tumors, including PDA [137]. These findings support WT1 as a prognostic biomarker, potentially useful in identifying high-risk PDA patients who may benefit from more aggressive or tailored treatment strategies.

In addition, WT1-targeted immunotherapy, such as peptide-loaded dendritic cell vaccines, has shown promise in PDA, especially when combined with standard chemotherapy, indicating WT1's relevance as a target for novel immunotherapeutic interventions [138].

Soft tissue sarcomas (sts)

WT1 is frequently expressed in several soft tissue sarcoma (STS) subtypes, including rhabdomyosarcoma and malignant peripheral nerve sheath tumors. Interestingly, in high-grade STS, strong WT1 expression correlates with improved survival outcomes. This paradoxical finding underscores its context-dependent function in tumor biology and highlights the need for further research to understand its prognostic significance and potential for immunotherapy. [139].

Glioblastoma and other glial tumors

WT1 protein is expressed in nearly all glioblastomas and anaplastic glial tumors. Studies show a significant positive correlation between WT1 expression and cellular proliferation markers, such as the MIB-1 index, indicating WT1's role in promoting tumor cell proliferation. These findings support WT1 as a potential therapeutic target in malignant glial tumors [140–142].

KRAS-driven cancers

In KRAS-driven cancers, WT1 modulates oncogenic signaling and cellular senescence. WT1 interacts with KRAS, a key regulator of proliferation and survival, and exhibits context-dependent roles—either promoting tumor growth or inducing senescence. In KRAS-driven lung cancer models, WT1 loss induces senescence, suggesting a tumor-suppressive function [143]. This effect is mediated by WT1's regulation of metabolic and oncogenic pathways, including MYC and glutamine metabolism, which are critical for tumor maintenance.

Disruption of WT1 impairs these pathways, reducing cell viability in KRAS-mutant tumors. Additionally, WT1 serves as a potential predictive biomarker for immunotherapy

response in non-small-cell lung cancer (NSCLC), linking its role to immune modulation and prognosis [144]. These findings position WT1 as a dual-function therapeutic target in KRAS-driven malignancies—either to enhance senescence or suppress oncogenic activity.

Desmoplastic small round cell tumor (dsrct)

The WT1 gene, particularly as part of the EWSR1::WT1 fusion oncogene, drives pathogenesis of desmoplastic small round cell tumor (DSRCT) by regulating key pathways, including upregulating CCND1, which promotes tumor proliferation via the cyclin D–CDK4/6–RB axis. Molecular profiling of DSRCTs also reveals overexpression of growth factor receptors such as FGFR4, IGF2, and ERBB2, identifying additional therapeutic targets. These molecular insights highlight CDK4/6 inhibitors and other targeted therapies as promising strategies for desmoplastic small round cell tumors [145–147].

Collectively, the evidence underscores WT1 as a pivotal regulator of tumor biology across a wide spectrum of malignancies. Its regulatory roles span key oncogenic processes, including gene transcription, apoptosis, cell proliferation, epithelial-to-mesenchymal transition (EMT), immune evasion, and metabolic reprogramming.

WT1 overexpression is consistently associated with adverse clinical outcomes in several cancers such as breast cancer, AML, NSCLC, and pancreatic ductal adenocarcinoma, highlighting its utility as a negative prognostic biomarker. Conversely, in select tumors like renal cell carcinoma and certain soft tissue sarcomas, WT1 exhibits tumor-suppressive activity, further reinforcing its functional complexity. Isoform-specific effects in cancers such as breast cancer and Wilms tumor emphasize the importance of precise molecular characterization of WT1 variants for prognostic and therapeutic purposes.

Wilms' tumor 1 (WT1) gene as a marker for minimal residual disease (MRD)

Minimal residual disease (MRD) comprises small populations of malignant cells that persist after treatment and evade detection by conventional diagnostics such as imaging and standard laboratory tests. These residual cells can lead to disease relapse, particularly in hematologic malignancies including leukemia, lymphoma, and multiple myeloma. Recent studies underscore the prognostic and therapeutic significance of MRD monitoring, which complements baseline clinical, cytogenetic, and molecular data. MRD assessment enables early relapse prediction and informs treatment decisions, especially in post-transplant settings. Its utility is well established in acute lymphoblastic leukemia (ALL), acute promyelocytic leukemia (APL), and chronic myeloid leukemia (CML) [148–151].

The Wilms' Tumor 1 (WT1) gene has emerged as a useful molecular marker for measurable residual disease (MRD) monitoring in acute myeloid leukemia (AML), particularly in patients lacking specific genetic aberrations [152]. Although WT1 is not AML-specific and is classified as a pan-leukemic marker, it is overexpressed in various leukemias and occasionally detected in normal bone marrow [153–155]. Its expression in peripheral blood allows for non-invasive monitoring of disease burden [156]. Cilloni et al., in collaboration with the European LeukemiaNet (ELN), developed a standardized qRT-PCR assay for reproducible quantification of WT1 transcripts in bone marrow and peripheral blood samples [157, 158]. The ELN recommends WT1 thresholds of 250 copies per 10^4 ABL copies in bone marrow and 50 copies per 10^4 ABL copies in peripheral blood to distinguish residual disease from normal expression, optimizing sensitivity and specificity [159].

Although WT1 is widely used as an MRD marker, its expression also occurs in healthy individuals, which can complicate interpretation. To reduce false positives, clinicians should interpret WT1 results alongside clinical data and complementary MRD tools like flow cytometry and genomic analyses. Advances in PCR methodologies and standardized guidelines have enhanced WT1 MRD detection reliability. Next-generation sequencing (NGS) represents a promising, though still evolving, MRD surveillance tool [160].

As a diagnostic and prognostic marker

The Wilms' Tumor 1 (WT1) gene is overexpressed in over 80% of acute myeloid leukemia (AML) cases, making it a broadly applicable marker for measurable residual disease (MRD) detection, particularly in patients lacking mutations such as NPM1 or fusion genes like RUNX1-RUNX1T1 [159, 161]. The WT1 overexpression is utilized as a diagnostic biomarker, while WT1 expression levels predict outcomes and fulfilling a prognostic marker rule. Studies have consistently demonstrated significantly elevated WT1 expression in AML patients compared to healthy controls, supporting its role in MRD monitoring and outcome prediction, and revealing that WT1 mRNA as a reliable prognostic marker for treatment response and relapse risk in AML [151, 162] The paper by Ugale et al. (2024) investigates the prognostic impact of WT1 gene mutations in acute myeloid leukemia (AML). It demonstrates that WT1 mutations are associated with poor risk stratification and reduced overall survival. The study supports incorporating WT1 mutation status into AML prognostic models for better clinical outcomes. [163]. One study examines the expression of Wilms tumor gene 1 (WT1) in high-grade uterine sarcomas. It demonstrates

that WT1 is frequently expressed in these tumors and is associated with poor prognosis. The study suggests WT1 as a potential prognostic biomarker and therapeutic target in high-grade uterine sarcoma [164]. In non-small-cell lung cancer (NSCLC), WT1 promotes tumor malignancy and serves as a novel prognostic biomarker. Inhibition of WT1 by microRNA-498-5p reduces tumor aggressiveness, indicating WT1 as a potential therapeutic target [91]. These findings establish WT1 as a key prognostic marker for guiding personalized cancer treatment.

Post-Treatment monitoring

Multiple studies demonstrate that WT1 plays a critical role in post-treatment monitoring of AML by tracking remission status and predicting relapse. Detectable WT1 mRNA in patients who achieve complete remission correlates with a higher relapse risk. WT1's high sensitivity allows detection of residual leukemic cells even when cytogenetic or other molecular markers are undetectable [158, 159, 162].

Prognostic marker for Post-Hematopoietic stem cell transplantation (HSCT)

WT1 serves as a critical prognostic marker after hematopoietic stem cell transplantation (HSCT), with elevated WT1 levels early post-transplant strongly predicting disease relapse. In such cases, clinicians may initiate donor lymphocyte infusion or preemptive chemotherapy to mitigate relapse risk [158, 165].

In allogeneic stem cell transplantation (allo-SCT) patients, increased WT1 expression correlates with higher relapse incidence. Combining WT1 monitoring with other MRD detection methods, such as flow cytometry, improves relapse prediction and guides post-transplant treatment decisions [166].

WT1: A therapeutic target for different approaches of cancer treatment

WT1 as a Tumor-associated antigen

Wilms' tumor 1 (WT1) is overexpressed in numerous hematologic and solid malignancies and has been identified as a prominent tumor-associated antigen (TAA). Its selective expression in cancer cells compared to normal tissues makes WT1 an ideal target for immunotherapy. WT1-derived peptides induce robust cytotoxic T lymphocyte (CTL) and helper T cell responses that specifically eliminate WT1-expressing tumor cells while sparing healthy cells [103, 104]. Current therapeutic approaches include peptide vaccines, T-cell therapies, and gene-silencing techniques designed to maximize antitumor efficacy and minimize off-target toxicity (Fig. 3). Advances in these strategies have enhanced their specificity, safety, and clinical effectiveness. Ongoing preclinical and clinical studies continue to refine WT1-targeted therapies, supporting their role in personalized cancer immunotherapy. Below is an overview of the latest strategies and directions in this field, supported by recent clinical and preclinical studies.

Potential strategies in WT1-directed therapies

wt1-based immunotherapeutic vaccines

WT1 peptide vaccines activate the immune system to specifically recognize and eliminate WT1-expressing tumor cells. These vaccines deliver WT1-derived peptides, which antigen-presenting cells present via major histocompatibility complex (MHC) molecules, thereby stimulating WT1-specific cytotoxic T lymphocytes (CTLs) to target and destroy tumor cells expressing WT1 (Fig. 4). Clinical studies demonstrate that WT1 vaccines safely induce strong immune responses across various tumor types. Combining HLA class I and II peptides enhances these responses, correlating with improved clinical outcomes and prolonged survival, especially in hematologic malignancies [103, 104, 167–170]. A randomized Phase II trial in elderly AML patients showed that immunoreactivity to the WT1 peptide vaccine (OCV-501) correlated with improved prognosis, supporting its therapeutic potential in this patient population [171]. In pediatric oncology, WT1-targeted vaccination showed clinical benefit in children with relapsed rhabdomyosarcoma and diffuse midline glioma, leading to stable disease and improved outcomes. These findings underscore the potential of WT1-targeted therapies in pediatric refractory cancers [172]. A Phase I clinical study investigated a cocktail vaccine of WT1 HLA class I and II peptides for recurrent malignant glioma. The study demonstrated the feasibility and safety of this approach, with indications of immune responses, highlighting its potential as a therapeutic strategy for glioma patients [173]. Recent advancements include WT1 peptide-pulsed dendritic cell (WT1-DC) vaccines and WT1 mRNA-electroporated dendritic cell (WT1 mRNA-DC) vaccines, both of which significantly boost antitumor immunity in diverse cancers [104].

Preclinical and Clinical Evidence Supporting WT1 Vaccine Efficacy

Wilms' tumor 1 (WT1) is a well-characterized tumor-associated antigen widely expressed in hematologic and solid malignancies. WT1-based immunotherapeutic vaccines have shown promising preclinical and clinical outcomes.

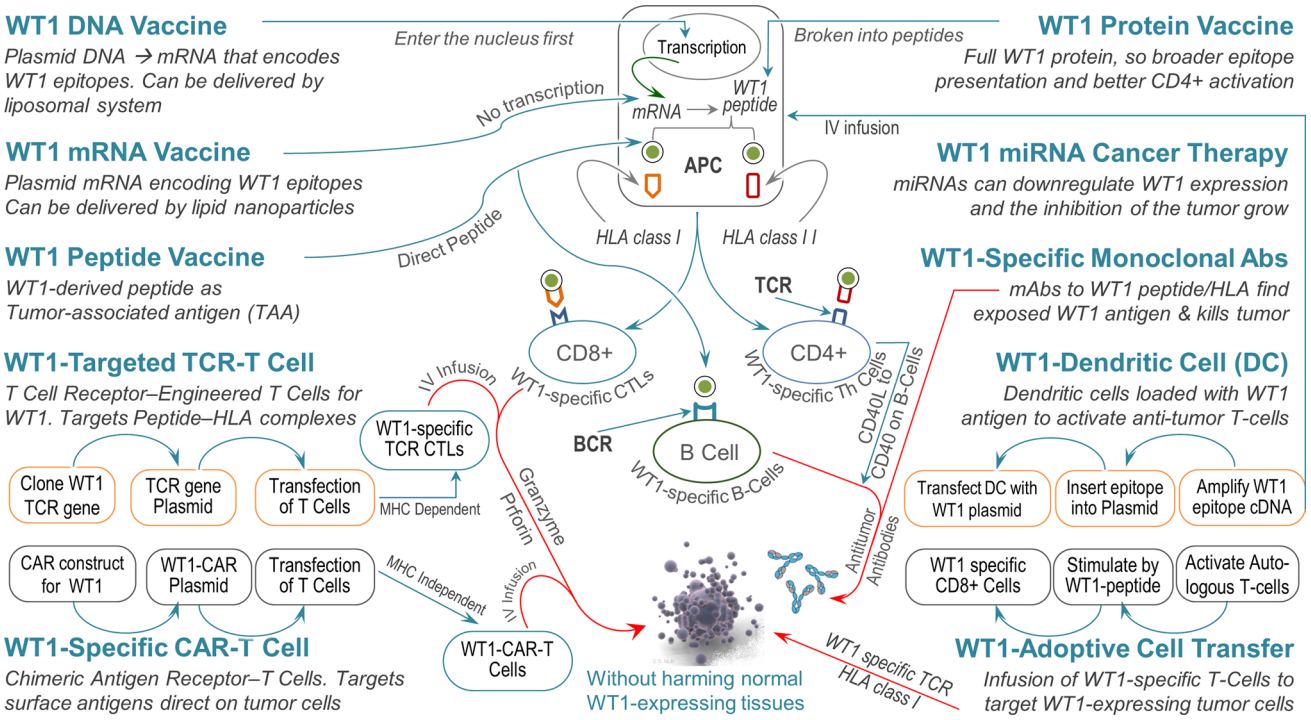

Fig. 3 This figure presents an integrated summary of thirteen representative therapeutic strategies targeting the WT1 gene in cancer, emphasizing its dual role as an oncogene and an immunogenic target. Each approach is described at the molecular or cellular level, beginning with WT1 DNA vaccines [190, 191], which activate cytotoxic T lymphocytes against WT1-expressing tumors and stimulate helper T cell responses. Cellular immunotherapies, including dendritic cell (DC) vaccines [68] and T cell receptor-engineered T cell (TCR-T) therapies [200], enhance tumor-specific immune activation. Monoclonal antibodies [104, 194] and bispecific T cell engagers [196, 197] either disrupt WT1-associated signaling pathways or redirect immune effector cells to tumor sites. The figure also presents RNA-based therapies, such as WT1-targeting siRNAs [205, 206] and antisense oligonucleotides, which suppress WT1 expression at the transcriptional or post-transcriptional level. Additionally, CRISPR/Cas9 gene editing [202] offers permanent genomic disruption of oncogenic WT1 alleles. Small molecule inhibitors target downstream effectors of WT1, while epigenetic modulators reverse aberrant gene expression patterns driven by WT1. Collectively, these strategies illustrate how WT1's biological functions are being leveraged across diverse therapeutic platforms to treat both hematologic and solid tumors

A systematic review published in Cureus highlighted the application of the WT1 vaccine in advanced pancreatic cancer, emphasizing its potential to improve clinical outcomes by inducing specific immune responses targeting tumor cells [174].

A significant Phase I/II study evaluated the use of a WT1 recombinant protein vaccine in elderly patients with acute myeloid leukemia (AML) in remission. The study demonstrated the vaccine's ability to stimulate both humoral and cellular immune responses, with detailed analysis of minimal residual disease (MRD) and WT1 expression providing further insights into its therapeutic efficacy [175].

Suwabe et al. (2024) reported durable WT1-specific $CD8^+$ CTL responses lasting up to ten years following WT1 peptide vaccination, underscoring the vaccine's role in sustaining long-term remission and preventing cancer recurrence [176].

Dendritic cell-based WT1 vaccines and immune activation Dendritic cells (DCs) loaded with WT1 peptides or mRNA function as potent antigen-presenting cells that elicit strong immune responses by bypassing HLA restrictions and activating both $CD4^+$ helper and $CD8^+$ cytotoxic T cells [177].

Recent studies demonstrate that WT1-mRNA-loaded DC vaccines induce type 1 $CD4^+$ and $CD8^+$ T-cell responses, achieving disease control and prolonged survival in patients with advanced solid tumors such as glioblastoma, metastatic breast cancer, and malignant pleural mesothelioma. These advances emphasize WT1-targeted immunotherapy's growing role, particularly in relapsed or refractory acute myeloid leukemia [177, 178].

Berneman et al. showed WT1-mRNA dendritic cell vaccines elicit strong type 1 T-cell responses in solid tumor patients, correlating with improved clinical outcomes. The study supports the therapeutic potential of WT1-targeted dendritic cell vaccines in eliciting antitumor immunity [68]. Despite their promise, DC vaccines require complex manufacturing and often predominantly stimulate $CD4^+$ T cells, which may limit effective cytotoxic T lymphocyte (CTL)

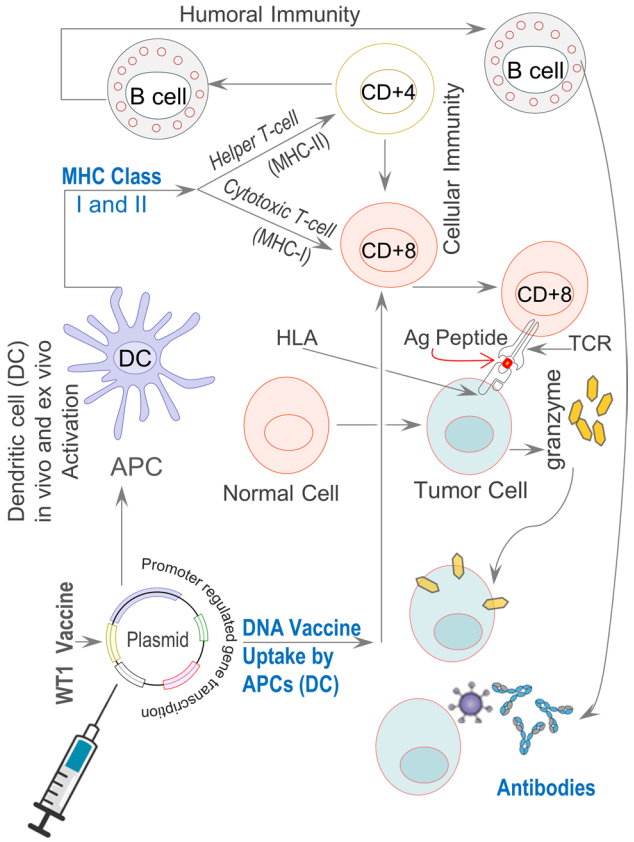

Fig. 4 The WT1 vaccine initiates an immune response that results in the destruction of tumor cells: Oncogenic factors continuously drive the malignant transformation of cells, but the immune system typically identifies and eliminates most of these transformed cells. DNA vaccines, such as the WT1 vaccine, stimulate innate immune cells—including dendritic cells (DCs), natural killer (NK) cells, and natural killer T (NKT) cells—that are capable of recognizing and eliminating transformed cells. Antigens captured by antigen-presenting cells (APCs), such as dendritic cells, presented to other immune cells, initiating adaptive immune responses mediated by the WT1 vaccine to target and attack cancer cells. The WT1 vaccine enhances the immune system's ability to recognize tumor-specific antigens and activates both T cells and B cells to mount a targeted immune response against the cancer, without inducing significant autoimmune side effects, thereby facilitating a successful and effective clinical outcome [104, 190]

responses. IL-15-transfected DCs improve T-cell activation and facilitate scalable batch production, offering a significant advancement in vaccine design [179].

Combination therapies to enhance WT1-directed immunotherapy

Recent advancements have concentrated on improving the immunogenicity and therapeutic efficacy of WT1-directed immunotherapies through the use of other treatment modalities such as adjuvants and combination therapies. Combining WT1-directed immunotherapies with other treatment modalities holds promise for enhancing therapeutic outcomes. For example, combining WT1 peptide vaccines with standard chemotherapy agents, such as S-1, has been investigated in clinical trials for patients with advanced pancreatic cancer. These combination strategies aim to synergistically modulate the tumor microenvironment and improve the efficacy of immune-based therapies [180].

Preclinical studies demonstrate that combining WT1 vaccines with therapies such as checkpoint inhibitors and chemotherapy synergistically enhances immune responses and overcomes tumor immune evasion. Manning-Geist et al. (2023) conducted a Phase I trial evaluating the multivalent WT1 peptide vaccine galinpepimut-S combined with the checkpoint inhibitor nivolumab in patients with WT1-expressing ovarian cancer in second or third remission. The combination induced WT1-specific T-cell responses in 91% of patients and achieved a 70% progression-free survival rate at one year, highlighting its potential to augment antitumor immunity and improve clinical outcomes [181]. The study P4.06 reports that combining WT1 peptide vaccination with imatinib therapy in a patient with chronic myeloid leukemia (CML) led to a sustained long-term molecular remission. This remission correlated with an increased frequency of WT1-specific cytotoxic T lymphocytes (CTLs) even after stopping the WT1 vaccine, suggesting the combination enhances durable immune control of the disease [182].

A phase I trial investigated the use of granulocyte–macrophage colony-stimulating factor (GM-CSF) or CpG oligodeoxynucleotides (CpG-ODN) as immunoenhancement adjuvants in Wilms' Tumor 1 (WT1) peptide vaccine therapy for patients with solid malignancy. The study found that the addition of CpG-ODN improved the effectiveness of the vaccine, with a disease control rate of 60% in the CpG-ODN group [183]. The choice of adjuvants plays a critical role to boost immune responses to WT1 peptide vaccines. For instance, the combination of Montanide ISA-51 and CpG7909 has been used to enhance immune responses. However, studies have shown that suboptimal concentrations of these adjuvants can impair the activation of antigen-presenting cells and the subsequent T-cell responses. Therefore, optimizing adjuvant formulations is essential for improving vaccine outcomes [184]. One notable study investigated an oral WT1 protein vaccine using Bifidobacterium longum 420, to deliver an oral WT1 protein vaccine in leukemia-bearing mice, which induced robust WT1-specific cytotoxic T lymphocyte (CTL) responses and activated helper T cells. This vaccine elicited intestinal immunity, enhancing antitumor activity via WT1-specific $CD8^+$ T cells supported by $CD4^+$ T cell help with reduced tumor volume correlating to increased CTL frequency, demonstrating potential for systemic and local immune activation against WT1-expressing tumors [185].

Minagawa et al. (2023) developed a combination oral WT1 vaccine using B. longum 420 and 2656, containing a helper epitope. This formulation amplified $CD4^+$ T cell-mediated immune responses and enhanced antitumor efficacy in the intestinal tract of leukemia models by promoting WT1-specific immune cell activation [186].

Another study developed a WT1-pulsed dendritic cell vaccine using human $CD141^+$ DCs matured with zoledronate, enhancing vaccine efficacy. This approach robustly activated $CD8^+$ T cells and elicited strong cytotoxic T lymphocyte (CTL) responses against solid tumors [187].

A Phase II randomized study evaluated the combination of gemcitabine chemotherapy and WT1 peptide vaccination in patients with advanced pancreatic ductal adenocarcinoma (PDAC). The combination significantly improved progression-free survival, particularly in patients who mounted a strong WT1-specific cytotoxic T lymphocyte response. These findings support the potential of combining chemotherapy with WT1-targeted immunotherapy to enhance clinical outcomes in PDAC [188] A Phase I study evaluated the safety and immunogenicity of recombinant WT1 protein combined with the immunostimulant AS15 as neoadjuvant therapy in WT1-positive breast cancer patients. The study found that the combination was well-tolerated and induced WT1-specific antibodies, particularly in patients receiving neoadjuvant aromatase inhibitors [189].

Collectively, these studies suggest that WT1-targeted vaccines, especially when combined with other immunotherapies, hold substantial promise for the treatment of various cancers by effectively stimulating the immune system to recognize and eliminate WT1-expressing tumor cells.

Personalized neoantigen vaccines

Because WT1 functions as a self-antigen, the immune system tightly regulates its expression to maintain tolerance. To overcome this, researchers now design personalized vaccines that target tumor-specific neoantigens—mutated self-antigens that the immune system can recognize as foreign. These vaccines use next-generation sequencing to identify neoantigen targets, including WT1-derived peptides, and trigger strong, antigen-specific T cell responses. Zhang et al. confirmed the safety and immunogenicity of DNA-based neoantigen vaccines in triple-negative breast cancer, demonstrating clonal T cell expansion through single-cell TCR sequencing. This clinical evidence supports the use of neoantigen-targeted immunotherapy as a promising approach in cancer treatment [190, 191].

WT1-Specific antibodies/wt1-specific antibody-based therapies

Monoclonal antibodies

Monoclonal antibodies are engineered proteins that recognize and bind specific antigens on target cells. When targeting WT1, they selectively deliver cytotoxic agents to WT1-expressing tumor cells, minimizing off-target toxicity. However, still in early stage development, this strategy offers a promising approach for precise cancer therapy [104].

WT1-Specific T cell engagers (e.g., WT1-TOPAbody)

Recent advancements in the use of WT1-specific antibodies in cancer therapy include the WT1-specific T cell engagers, such as WT1-TOPAbody, which exhibit high affinity and specificity for the WT1/HLA-A*02:01 complex. This tri-specific construct activates T cells through CD3 and 4-1BB signaling, inducing potent anti-tumor responses in both hematologic malignancies and solid tumors. Preclinical studies have demonstrated its efficacy in WT1/HLA-A02 + cancers, including leukemia, ovarian, and breast cancers [192, 193].

TCR-Mimic (TCRm) antibodies

TCR-mimic antibodies developed using Biocytogen's platform target intracellular WT1 peptides presented by MHC class I molecules. These antibodies bind with high specificity to the WT1/HLA complex, enabling selective recognition and destruction of WT1-expressing tumor cells. Preclinical models have confirmed their therapeutic potential in acute myeloid leukemia (AML), ovarian cancer, and other WT1-positive tumors [100, 193, 194]. Another study reports TCR-mimic antibody–drug conjugates targeting WT1/HLA-A*02, showing selective killing of WT1-positive tumor cells and strong antitumor effects in models [195].

Bispecific antibodies (e.g., WT1-TCB)

Novel bispecific antibodies, such as the RMF-peptide-MHC-specific T-cell bispecific antibody (WT1-TCB), have been developed to redirect T cells to WT1-expressing tumor cells. In preclinical models, WT1-TCB induced dose-dependent antitumor activity, effectively targeting leukemia stem cells and solid tumors, highlighting its potential as a therapeutic agent [196].

WT1-targeted immunotherapies—including monoclonal antibodies, T cell engagers, TCR-mimic antibodies, and bispecific antibodies—demonstrate strong preclinical efficacy and specificity. These advances support their

continued development as targeted therapies for WT1-positive malignancies.

Chimeric antigen receptor (CAR) T-cell therapy targeting WT1

Traditional CAR-T cells target surface antigens; however, WT1 is an intracellular protein. Researchers have engineered CAR-T cells to recognize WT1 peptides presented on MHC class I molecules, overcoming the challenge of WT1's intracellular localization. These engineered T cells demonstrated antitumor activity in xenograft models, with enhanced efficacy when combined with dendritic cell (DC) vaccination, suggesting a synergistic approach to boost CAR-T cell expansion and activation [69, 197].

T-cell Receptor (TCR) gene therapy

Zhang and Li (2019) review WT1-specific TCR gene therapy, where T cells are engineered to express high-affinity TCRs that selectively recognize WT1, a protein overexpressed in cancer cells but limited in normal tissues. This strategy enhances immune targeting and elimination of WT1-positive tumors. Notably, $CD4^+$ T cells engineered with TCRs against the WT1_332 peptide exhibit potent anti-leukemic activity in vitro, highlighting their potential for treating hematologic malignancies [198].

Gielis S et al. analyze the WT1-specific T-cell receptor repertoire in AML patients, revealing greater TCR diversity in remission compared to relapse. These findings highlight the therapeutic potential of diverse WT1-specific TCRs for adoptive T cell therapy. Enhanced TCR diversity may contribute to improved immune control and durable remission in AML [199].

Researchers have engineered high-affinity TCRs that selectively recognize WT1 peptides presented by MHC molecules on tumor cells, enhancing binding affinity and specificity, which in turn improves anti-tumor activity [200, 201]. Preclinical models demonstrate significant tumor regression following WT1-specific TCR-T cell therapy, and early phase clinical trials report prolonged disease-free survival in some patients [201].

Overall, WT1-specific TCR gene therapy represents a promising targeted immunotherapy, with ongoing studies essential to optimize treatment safety, efficacy, and applicability across diverse WT1-expressing malignancies.

crispr-cas9-enhanced TCR gene editing

The integration of CRISPR-Cas9 technology into TCR gene editing has enabled the development of high-avidity, WT1-specific TCRs with improved antitumor efficacy. Researchers have utilized CRISPR-Cas9 technology to enhance WT1-specific TCR gene editing by integrating TCRs into the TCR α constant (TRAC) locus and disrupting the TCR β constant (TRBC) locus. This approach prevents mispairing with endogenous TCRs, improving T cell specificity and antitumor function. Engineered T cells demonstrate potent cytotoxicity against WT1-expressing tumor cells from AML, ALL, and glioblastoma in vitro and in vivo, while minimizing off-target toxicity. Together, these findings highlight the promise of CRISPR-enhanced WT1 TCR gene therapy as a targeted and effective treatment strategy for WT1-positive malignancies [202].

Gene silencing approaches

WT1 is a promising tumor-associated antigen and therapeutic target in cancers such as leukemia, breast, and lung cancer. Gene silencing of the WT1 (Wilms' Tumor 1) gene has shown considerable potential in cancer treatment. RNA interference (RNAi) strategies effectively reduce WT1 expression, suppressing tumor cell proliferation and inducing apoptosis. WT1-specific small interfering RNA (siRNA) inhibits leukemic cell growth, supporting WT1-targeted RNA-based therapies in oncology [104, 193, 194, 203].

In breast cancer, WT1 silencing reduces tumor growth and metastasis. Aerosol delivery of PEI-RNAi complexes significantly inhibits lung metastases in preclinical models [101]; similarly, respiratory administration of neutral DOPC liposomal-siRNA (L-siRNA) enhances survival and suppresses pulmonary tumor growth [102]. Recent studies demonstrate that combining WT1 gene silencing with chemotherapy overcomes drug resistance and improves treatment efficacy. A comprehensive review highlights the potential of integrating RNAi with conventional therapies to enhance treatment outcomes [203, 204, 205, 206]. Collectively, WT1-targeted gene silencing holds strong potential to improve clinical outcomes in WT1-expressing malignancies.

Table 4 presents a concise yet comprehensive overview of therapeutic strategies targeting WT1, particularly in its oncogenic role. The "Therapy" column identifies each approach by its core design, while the "Mechanism" column describes the biological pathways modulated by the strategy. The "Development" column indicates the current stage of progress, ranging from preclinical research to Phase I/II clinical trials. Where available, the specific treatment agent or molecule is included. Abbreviations used in the table include Ab (antibody), mAbs (monoclonal antibodies), DCs (dendritic cells), and CTLC (cytotoxic T lymphocytes).

Table 4 WT1 Targeted Therapeutic Strategies

Therapy Name	Mechanism	Development	Agent/Example
WT1 Peptide Vaccine [181, 207]	Induces WT1-specific T-cell responses via synthetic peptides	Phase 2 Clinical Trials	Galinpepimut-S
WT1 mRNA Vaccine [208, 209]	Delivers WT1 mRNA to dendritic cells to stimulate anti-tumor immunity	Phase 1/2 Clinical Trials	WT1 mRNA-transfected DCs
WT1 Protein Vaccine [175]	Uses recombinant WT1 protein to elicit immune response	Phase 1 Clinical Trials	Recombinant WT1 protein
WT1 DNA Vaccine [190, 191]	Plasmid DNA encoding WT1 to induce immune response	Preclinical Studies	WT1 DNA plasmid
WT1 Dendritic Cell Vaccine [68]	Dendritic cells pulsed with WT1 peptides or mRNA to activate CTC	Phase 1/2 Clinical Trials	WT1 peptide-pulsed DCs
WT1 TCR-T Cell Therapy [200]	T cells engineered with WT1-specific T-cell receptors	Phase 1 Clinical Trials	WT1-specific TCR-transduced T cells
WT1 CAR-T Cell Therapy [69]	T cells engineered with chimeric antigen receptors targeting WT1	Preclinical Studies	WT1/HLA-A*24:02-specific CAR-T cells
WT1 mAbs Therapy [210]	Antibodies targeting WT1 peptide-HLA complexes	Preclinical Studies	ESKM (TCR-mimic antibody)
WT1 siRNA Therapy [102, 211]	Small interfering RNA to silence WT1 gene expression	Preclinical Studies	WT1-targeted siRNA
WT1 shRNA Therapy [212]	Short hairpin RNA for stable WT1 gene silencing	Preclinical Studies	WT1-targeted shRNA
WT1 CRISPR Gene Editing p [202]	CRISPR-Cas9-mediated knockout of WT1 gene	Preclinical Studies	CRISPR-Cas9 targeting WT1
WT1 Bispecific Ab Therapy [196]	Antibodies binding WT1 and T-cell receptors to direct cytotoxicity	Preclinical Studies	WT1-specific bispecific antibodies

Challenges and future directions

Current clinical trials are evaluating the efficacy and safety of WT1-targeted vaccines and other therapeutic strategies across multiple cancer types. These investigations are crucial for determining the clinical applicability of WT1-based treatments in diverse oncological settings.

However, several challenges hinder the development of WT1-targeted therapies. These include the complex and context-dependent biology of WT1, difficulties in drug design, and challenges in clinical translation.

This review highlights the current challenges and complexities associated with WT1-targeted therapies and underscores the need for continued innovation in their clinical application.

WT1 as a context-dependent regulator in cancer

WT1 functions as either a tumor suppressor or an oncogene depending on the cellular and tissue context, complicating the understanding of its role in tumorigenesis and challenging the development of universal WT1-targeted therapies. To avoid unintended oncogenic or tumor-suppressive effects, therapeutic strategies must consider WT1's specific role within each cancer type. As shown in previous studies [67, 213], this complexity requires a nuanced, cancer-specific approach to therapy design. Continued mechanistic research is essential to unravel the regulatory networks governing WT1's dual roles and to translate this knowledge into safe and effective clinical applications [213].

Isoform diversity and functional complexity

WT1 isoforms exhibit distinct, context-dependent biological functions, with some promoting tumorigenesis and others acting as tumor suppressors. In ovarian epithelial cells, both WT1 (+KTS) and (−KTS) isoforms enhance proliferation and migration, with the (+KTS) isoform exerting a stronger effect on migration [214].

Another study investigated the isoform-specific roles of WT1 in acute myeloid leukemia (AML). The authors reported that the (+KTS) isoform promotes proliferation and inhibits apoptosis, while the (−KTS) isoform suppresses cell growth and induces apoptosis through activation of Caspase 9 [215]. Goel et al. further demonstrated that (−KTS) isoforms are associated with tumor-suppressive functions, including induction of apoptosis via the p53–Bax–Caspase 9 pathway and promotion of differentiation, while (+KTS) isoforms drive oncogenesis by enhancing proliferation and blocking apoptosis [154].

These findings underscore the functional complexity of WT1 isoforms and their divergent roles across cancer types.

Table 5 WT1 Gene-Related Therapeutic Agents, their mechanism, and status

Therapy Name	Mechanism	Tumors Targeted	Company/Study Name	Status
WT1 Peptide Vaccine (Single Epitope) [216]	Elicits WT1-specific $CD8^+$ cytotoxic T lymphocyte (CTL) response	AML, MDS, NSCLC	Multiple academic consortia	Phase I/II Completed
Galinpepimut-S [170]	Multi-epitope WT1 peptide vaccine stimulating T-cell response	Mesothelioma, Ovarian, AML	SELLAS Life Sciences	Phase II ongoing
OCV-501 [217, 171]	WT1-332 helper peptide vaccine for $CD4^+$ T-cell activation	AML	OncoTherapy Science	Phase I Completed
WT1-Pulsed Dendritic Cell Vaccine [218, 219]	Dendritic cells presenting WT1 epitopes to activate T cells	Pancreatic, Esophageal, Glioma	National Cancer Center Japan	Pilot/early trials
WT1 CAR T Cells (TCR/CAR-based) [69]	T cells engineered with WT1-targeting CARs (HLA-restricted)	Leukemia (AML, ALL), Sarcoma	City of Hope/MSKCC	Preclinical/Early phase
Oral WT1 Vaccine (B. longum vector) [220]	Recombinant *B. longum* displaying WT1 for mucosal immunization	Colorectal (mouse model)	Japanese research consortia	Preclinical (mouse)
WT1 Vaccine + Gemcitabine [188, 221]	Combination of WT1 peptide vaccination with chemo-induced immunomodulation	Pancreatic cancer	Osaka University	Phase I/II Completed
WT1 Vaccine in Pediatric Glioma [222]	WT1 peptide administered in children for glioma/rhabdomyosarcoma	Pediatric diffuse midline glioma, RMS	Osaka University/Japan Pediatric Study	Case series/feasibility
WT1 $CD4^+$ T Cell Therapy [170]	HLA-class II-restricted helper T cells targeting WT1	AML	Stanford University	Experimental/Preclinical
WT1 Vaccination Post-allo-HSCT[223]	Enhancing GvL effect via post-transplant WT1 immunotherapy	AML, MDS	Osaka University Hospital	Phase I/II
WT1 Long Peptide Vaccine[176, 181]	Long peptides induce both $CD4^+$ and $CD8^+$ responses	Solid tumors, Gliomas	National Cancer Center Japan	Phase I
WT1 TriMix mRNA Vaccine[224, 225]	WT1 mRNA electroporated into DCs + costimulatory signals (CD40L, CD70, TLR4)	AML, MDS	VUB/UZ Brussels	Phase I (early reports)

Selectively targeting oncogenic isoforms while preserving tumor-suppressive variants remains a key therapeutic challenge. Further investigation is required to elucidate the regulatory mechanisms governing WT1 isoform expression and function in cancer (Table 5).

Challenges in drug development

Therapeutic targeting of WT1 remains challenging due to its classification as an "undruggable" transcription factor. Its DNA-binding domain and protein–protein interaction interfaces are poorly accessible to small-molecule inhibitors. Moreover, WT1's nuclear localization complicates intracellular drug delivery. Recent advances in nanotechnology have introduced promising strategies, such as nucleus-targeting WT1 antagonistic peptides encapsulated in polymeric nanomicelles. While these approaches show potential, they remain in early stages of development [226].

Limited understanding of WT1's role in tumor microenvironment

The role of WT1 in the tumor microenvironment, especially its interactions with immune cells and tumor-associated factors, remains poorly characterized. This knowledge gap hampers the development of targeted therapies that effectively modulate the tumor microenvironment to improve outcomes. Immunosuppression within the tumor microenvironment significantly limits WT1 vaccine efficacy by suppressing antitumor immune responses. To enhance vaccine performance, researchers are investigating adjuvants, combination therapies, and genetic modifications that enhance antigen

presentation. Recent studies also highlight nanoparticle-based vaccines as a promising approach to overcome tumor-induced immunosuppression and improve therapeutic success in solid tumors [227].

Heterogeneity in WT1 expression across tumor types

Significant intra- and inter-tumoral heterogeneity in WT1 expression limits its utility as a universal biomarker and therapeutic target, complicating standardized WT1-targeted treatment development, particularly in tumors with low or transient WT1 levels. Van Amerongen et al. identified novel immunogenic WT1-derived peptides and engineered high-affinity T-cell receptors (TCRs) that enhance antitumor responses in acute myeloid leukemia and ovarian carcinoma [128]. Recent studies focus on enhancing the delivery and precision of WT1-targeted therapies. Researchers apply RNA interference (RNAi) and small interfering RNA (siRNA) to suppress WT1 expression, thereby inhibiting tumor growth and improving combination therapy efficacy. Additionally, CRISPR-based gene editing and WT1 peptide vaccines are under investigation to elicit targeted immune responses against WT1-expressing tumor cells. These approaches aim to overcome WT1 expression heterogeneity and enable more effective, personalized WT1-directed immunotherapies [103].

Immunogenicity and safety concerns

WT1-targeted vaccines have demonstrated safety and immunogenicity; however, eliciting durable and robust immune responses remains a challenge. In a first-in-human study, Kreutmair et al. (2022) administered WT1 recombinant protein vaccines to elderly AML patients in remission. Immune responses varied: Some patients maintained durable remissions, while others relapsed, often with clonal evolution, indicating the need for improved vaccine strategies [175].

Although early phase trials rarely report autoimmune events, emerging data suggest immune-related risks under certain conditions. Suzuki et al. (2019) documented new-onset autoimmune disorders in patients with refractory thymic epithelial malignancies receiving WT1 peptide immunotherapy. These findings highlight the necessity for stringent patient selection and close monitoring during WT1-targeted treatment [228].

The spontaneous clonal expansion of WT1-specific cytotoxic T lymphocytes in patients with WT1-expressing tumors highlights the strong immunogenicity of the WT1 antigen, with no evidence of systemic autoimmunity, supporting its potential as a safe and effective target for immunotherapy [229].

Inhibition of the immunoproteasome with ONX-0914 enhances processing and presentation of the WT1_235 epitope in mesothelioma cells, leading to increased recognition by WT1-specific cytotoxic T lymphocytes and thereby boosting tumor immunogenicity [230].

Variability in clinical responses and challenges in monitoring treatment efficacy

Clinical trials investigating WT1-targeted therapies have shown mixed results. For example, Suzuki et al. (2019) reported that only 46.2% of patients with refractory thymic epithelial malignancies developed WT1-specific IgG antibodies after peptide-based immunotherapy, with no significant tumor shrinkage observed. These findings highlight the need for more potent and broadly effective WT1-targeted approaches [228].

To address such limitations, Lahman et al. (2022) designed an alternative WT1 peptide that bypasses immunoproteasome dependency, aiming to enhance antigen presentation and potentially improve immune responses [231].

Monitoring WT1 expression levels to assess treatment response is complicated by variability across malignancies and patient populations. Deng et al. (2021) highlighted the challenges in monitoring WT1 expression levels for assessing treatment response in pediatric acute myeloid leukemia (AML), noting that WT1 expression can fluctuate, complicating its use as a reliable biomarker for minimal residual disease detection or relapse prediction [232].

These findings underscore the variability in clinical responses to WT1-targeted therapies and the challenges in monitoring treatment efficacy, emphasizing the need for improved strategies and biomarkers.

On-target, off-tumor toxicity

WT1-targeted immunotherapies pose a risk of on-target, off-tumor toxicity due to WT1's low-level expression in normal tissues such as the kidneys and gonads. WT1-specific CAR-T and TCR-engineered T cells can recognize and damage both malignant and healthy WT1-expressing cells, leading to unintended cytotoxicity. Clinical studies have documented such risks in patients with AML and MDS treated with WT1-specific TCR-transduced lymphocytes. Akahori et al. further demonstrated that WT1-targeted CAR-T cells can exhibit potent antitumor effects, but emphasized the need for enhanced specificity to mitigate off-tumor toxicity. These findings underscore the need for refined targeting strategies that maintain antitumor efficacy while minimizing collateral damage to normal WT1-expressing cells [69, 200].

Challenges in biomarker development

Reliable biomarkers for monitoring WT1-targeted therapies in Wilms tumor remain insufficiently developed. Liquid biopsy methods, including circulating tumor DNA and exosomes, demonstrate potential for early detection and disease monitoring but face limited clinical implementation. Existing studies report inconsistent findings and lack of comprehensive validation of their sensitivity and specificity. This highlights the urgent need for comprehensive research and rigorous clinical validation to establish their accuracy. Advancing biomarker development is critical to enable personalized treatment and improve outcomes in Wilms tumor patients. Despite their promise, liquid biopsies require further validation before routine clinical use [169].

Inadequate clinical trial infrastructure

Preclinical studies, case reports, and early phase trials of WT1-targeted therapies have demonstrated safety and potential efficacy; however, large-scale multicenter clinical trials remain lacking. This evidentiary gap limits the clinical integration of WT1-targeted treatments.

Most existing studies are retrospective with small cohorts, restricting result generalizability. For instance, Boublikova et al. (2006) observed substantial variability in WT1 expression in childhood acute lymphoblastic leukemia (ALL), but the limited sample size prevented definitive prognostic conclusions. This highlights the need for larger, well-designed trials to better understand WT1's clinical significance [233].

A recent large-scale analysis of 533 pediatric B-cell precursors ALL (BCP-ALL) patients reported a broad range of WT1 transcript levels (median 0.26%) and identified WT1 overexpression with poorer overall and event-free survival. However, WT1 overexpression did not independently predict outcomes across the entire cohort, highlighting the complexity and variability in WT1's prognostic significance across different patient subgroups [234].

These findings emphasize the necessity for large-scale, multicenter clinical trials to clarify WT1's prognostic role in childhood ALL and to standardize its clinical application. Strengthening clinical trial infrastructure is essential to validate WT1's significance and guide therapeutic development.

HLA Restriction in WT1-peptide-based vaccines

WT1 peptide-based vaccines exhibit immunogenicity; however, their clinical applicability is constrained across diverse populations by interindividual variability in human leukocyte antigen (HLA) expression. This variability can influence the effectiveness of WT1 peptide vaccines, as the immunogenicity of these vaccines depends on the patient's ability to present the WT1-derived peptides via their HLA molecules. For instance, immunogenic peptides such as WT1_126–134 and WT1_235–243 predominantly elicit responses in individuals with HLA-A*02:01, reducing vaccine efficacy across genetically diverse populations [177]**.** Moreover, immunogenicity is further compromised by the presence of tolerogenic epitopes within conserved domains of the WT1 protein—particularly the C-terminal zinc finger region—which can dampen the activation of effective antitumor immune responses [235].

To overcome HLA restriction and tolerance, novel strategies are being explored. For instance, next-generation DNA vaccine platforms encoding truncated or modified WT1 sequences. These constructs enhance epitope presentation, break immune tolerance, and induce neoantigen-specific T cell responses, thereby broadening patient eligibility independent of HLA subtype [177, 190, 191, 235, 236]**.** These findings underscore the need to optimize WT1-based vaccines by integrating strategies that expand HLA coverage and overcome intrinsic immune tolerance mechanisms.

Advancements in genomics and personalized medicine offer the opportunity to develop individualized WT1 treatment strategies of each patient. Further research is needed to elucidate the molecular mechanisms underlying the dual functions of WT1 and to identify biomarkers that can predict patient response to WT1-targeted therapies. A deeper understanding of these mechanisms will facilitate the development of more effective and personalized cancer treatment strategies.

Conclusion

The Wilms' Tumor 1 (WT1) gene plays a pivotal role in cancer biology, acting as both a tumor suppressor and oncogene depending on cellular context. Its regulation of key molecular pathways, interaction with tumor suppressors, and impact on gene expression highlight its significance in cancer pathogenesis. Aberrant WT1 expression in malignancies such as leukemia, lung, and ovarian cancers establishes it as a prognostic marker and a promising therapeutic target. WT1-directed strategies, including vaccines and molecular therapies, show potential to enhance treatment efficacy. Advances in immunotherapy and molecular research continue to refine these approaches. Ongoing clinical trials and focused research are essential to overcoming current challenges and maximizing the therapeutic value of WT1 in cancer management.

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supervised project administration. A.Q. contributed to the literature review and creation of illustrations. S.Q. and R.Q2 offered valuable insights into the data presentation. All authors have read and approved the final version of the manuscript.

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Declarations

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