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Potential Use of WT1 as a Diagnostic and Therapeutic Marker of Acute Myeloid Leukemia: A Review

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HIGHLIGHTS

- ❖ WT1 is widely overexpressed in AML, supporting its value as both a biomarker and therapeutic target
- ❖ RT-qPCR remains the primary method for WT1 detection, but emerging protein- and peptide-based assays offer more practical MRD-monitoring options across diverse clinical settings
- ❖ Combining WT1 with other molecular markers improves diagnostic accuracy and personalized AML care

ABSTRACT

Acute myeloid leukemia (AML) is a heterogeneous malignancy characterized by uncontrolled proliferation and impaired hematopoietic differentiation of myeloid lineage with rapid disease progression. Among the various biomarkers studied, Wilms' tumor 1 (WT1) is a widely expressed gene in AML, making it an attractive biomarker candidate for diagnosis and minimal residual disease (MRD) monitoring. This review evaluates the potential of WT1 as an effective diagnostic biomarker and the current detection methods, highlighting the advantages and limitations of the current gold standard, RT-qPCR, and exploring potential alternative approaches, such as ELISA and antibody-based flow cytometry, for its clinical applicability. Using public gene datasets, bioinformatic analysis further supports WT1's overexpression in AML, though with varying levels across subtypes, suggesting its potential as part of a multimodal diagnostic plan rather than a standalone marker. Beyond diagnostics, WT1 is also a promising therapeutic target, with peptide and dendritic cell vaccines, as well as TCR-engineered T cells, demonstrating encouraging clinical outcomes. Next-generation strategies, including CAR-T cells, bispecific T-cell engagers, and mRNA vaccines, are advancing preclinical and early clinical studies. Together, these findings highlight WT1 as both a biomarker and a therapeutic target, with future integration into precision medicine likely to improve AML detection, risk stratification, and treatment.

INTRODUCTION

Acute myeloid leukemia (AML) is a genetically heterogeneous malignancy of the hematopoietic system, characterized by the clonal expansion of undifferentiated myeloid progenitors (Romanova et al., 2022). Most commonly diagnosed in older adults, the disease has an increasing incidence worldwide and persistently has high mortality rates despite many advancements in chemotherapy and bone marrow transplantation (Zhou et al., 2024). Currently, the diagnostic framework of AML involves bone marrow aspirate examination, immunophenotyping by flow cytometry, and cytogenetic and molecular profiling (Ally & Chen, 2024). These approaches are often time-intensive, costly, and dependent on the presence of specific genetic mutations that aren't always present in a significant portion of AML patients. To combat this, the need for the identification of a broadly applicable and reliable biomarker for diagnosis, prognosis, and treatment becomes critically important.

One such candidate is the Wilms' Tumor 1 gene, which was originally identified as a tumor suppressor gene, but was found to act as an oncogene in hematologic malignancies such as AML, where it is frequently overexpressed in over 80% of all cases, regardless of subtype (Nian et al., 2025; Yang et al., 2007). Studies have explored monitoring WT1 mRNA levels as a tool for minimal residual disease monitoring via real-time quantitative PCR (RT-qPCR), taking advantage of the minimal expression in healthy hematopoietic cells and consistently elevated levels in leukemic blasts (Lazzarotto & Candoni, 2022). While other molecular markers such as NPM1, FLT3, and CEBPA are important for risk stratification, they are limited to specific patient subsets, highlighting the value of WT1 as a broadly applicable biomarker. Although this method has promise in identifying disease relapse and response to therapy, its ability as a primary diagnostic marker and its utility across different detection platforms remain underexplored.

This paper aims to address that gap by critically evaluating the diagnostic value of WT1 in AML across various detection methods, including mRNA-based assays and emerging peptide-based platforms such as ELISA and flow cytometry. Here, we conducted bioinformatics analysis of WT1 expression, followed by a literature review to analyze the potential use of WT1 as an AML marker. We also discussed comparisons of the practical and technical merits of different WT1-targeting technologies and explored their potential to replace or complement currently available molecular diagnostic strategies by studying expression profiles, assay feasibility, and potential diagnostic performance.

WT1 GENE AND PROTEIN FUNCTION

The Wilms' tumor 1 (WT1) gene is located on chromosome 11p13 and comprises 10 exons. It produces an approximately 3 kb mRNA transcript, which is then translated into the WT1 protein (Hastie, 2017). WT1 was originally identified as a tumor suppressor gene that was involved in the development of Wilms' tumor, a childhood kidney cancer. However, further studies into the gene found that WT1 also has oncogenic properties, particularly in leukemia and other hematologic malignancies such as acute myeloid leukemia (AML) (Nian et al., 2025). The WT1 protein contains a proline- and glutamine-rich N-terminal domain that contributes to transcriptional activation, as well as four zinc finger domains at the C-terminus that mediate DNA and RNA binding. This enables the gene to function as a transcription factor, regulating genes involved in cell cycle control, apoptosis, proliferation, and differentiation (Nishikawa et al., 2020).

The first evidence of WT1 expression in leukemia was presented by Miwa et al. (1992), who studied leukemic cells of patients with ALL and AML, and showed the involvement of this gene in the early stage of hematological cell differentiation. Menssen et al. (1995) showed that WT1 is expressed in the majority of human acute leukemias, making the WT1 gene transcript a 'pan-acute leukemic' marker. More current studies showed that WT1 is expressed at a low level in normal hematopoietic progenitor cells, and leukemic

cells are mainly generated as a result of leukemic transformation of WT-1 expressing normal hematopoietic progenitor cells, due to the impairment of WT1 downregulation that is required for normal cell differentiation (Zhou et al., 2020). In general, the expression of WT1 varies between and within different forms of human leukemia. In healthy individuals, WT1 is expressed at very low levels, mainly in $CD34^{+}$ hematopoietic stem and progenitor cells to help moderate self-renewal and differentiation (Rampal & Figueroa, 2016). Here, WT1 expression is gradually downregulated as the cells mature. In leukemogenesis, however, WT1 is frequently overexpressed and dysregulated, resulting in its malignant transformation and interfering with normal blood cell development (Giudice et al., 2021). A persistent overexpression of WT1 in leukemic cells disrupts the natural downregulation that occurs during blood cell differentiation. This results in the hematopoietic cells remaining immature and undifferentiated, the hallmark of acute leukemias (Guo et al., 2022). WT1 is upregulated across all leukemia subtypes, but its expression levels vary by leukemia type and patient age (Zhang et al., 2015). Notably, WT1 levels are usually low in the chronic phase of leukemia but are frequently elevated in the acute and blast crisis phases of the disease (Giudice et al., 2021).

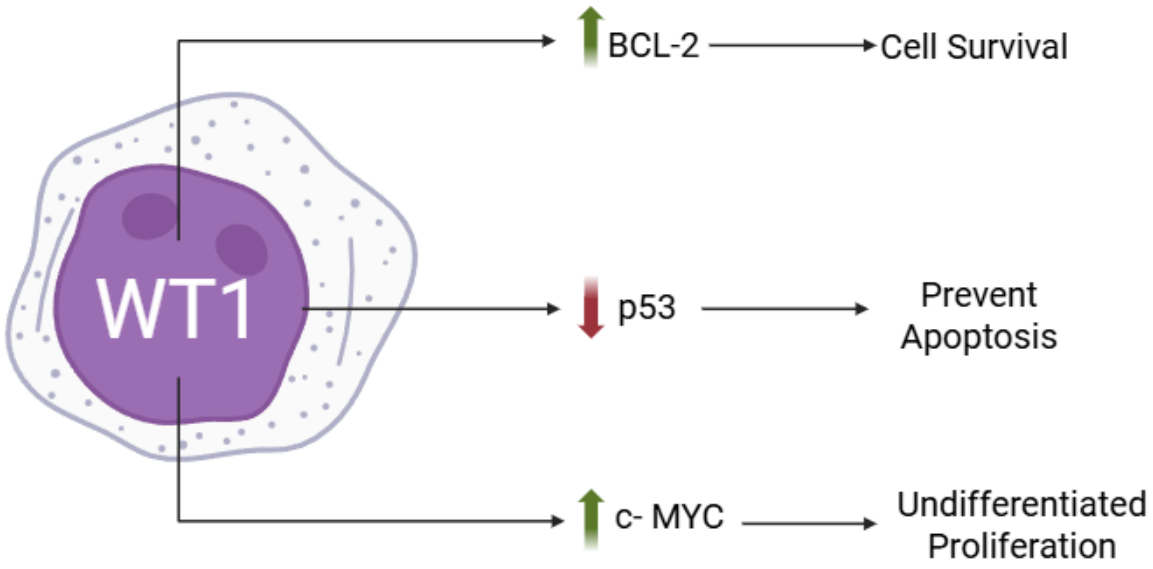

Figure 1. WT1-mediated survival signaling in AML

Additionally, WT1 is known to influence the expression of key regulatory genes such as BCL-2, p53, and c-MYC, prioritizing cell survival over apoptosis (Musalli et al., 2019; Zhou et al., 2020) as seen in **Figure 1.** Through these mechanisms, WT1 actively contributes to leukemic transformation and maintenance, making it not only a marker of disease but also a potential target for diagnostic and therapeutic strategies in AML.

EXPRESSION PATTERNS OF WT1 IN AML

To investigate the relevance of WT1 as a diagnostic biomarker in AML, its mRNA expression profile was evaluated across various AML subtypes and stages of normal hematopoietic differentiation via the "Normal hematopoiesis with AMLs" dataset from the FoBiN (Functional Omics in Blood Informatics) database (FoBinf, 2024). FoBiN is a web platform that provides access to curated hematopoietic transcriptomic datasets from the BloodSpot database, a publicly available, peer-reviewed resource that incorporates bulk gene expression data from normal and malignant hematopoiesis, gathered from public

repositories such as GEO, ArrayExpress, and The Cancer Genome Atlas. The WT1 expression data come mainly from Affymetrix microarray studies, where the raw signals are cleaned and scaled using standard statistical methods (RMA) and adjusted to remove technical differences between studies (ComBat) to allow fair comparison of WT1 expression between AML samples and normal blood cell populations. The figure was generated by searching WT1 in the 'gene symbol or alias' search bar and selecting the 'Normal hematopoiesis with AMLs' dataset. Expression levels are then shown as normalized $\log_2$ values.

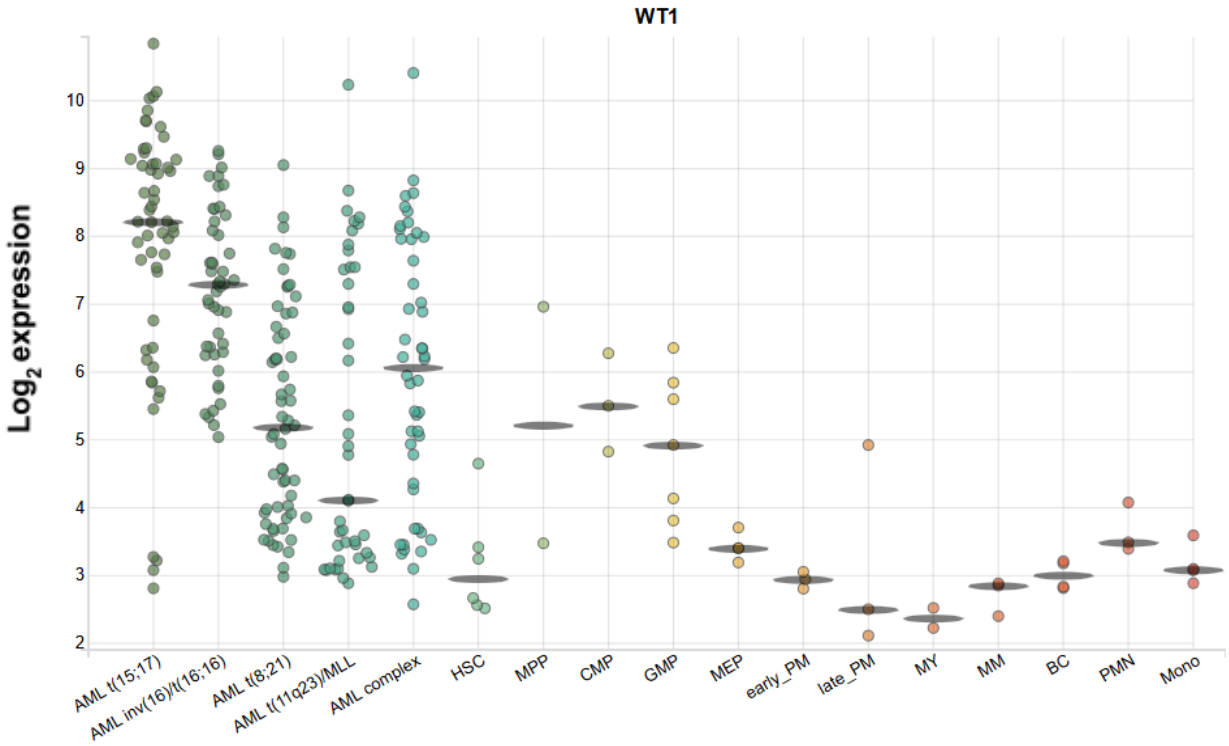

Figure 2. WT1 gene expression across AML subtypes and normal hematopoietic lineages (FoBiN)

Figure 2 features a violin plot featuring WT1 mRNA $\log_2$ expression levels in AML subtypes (left) and healthy hematopoietic cell populations (right). This includes data on HSC (hematopoietic stem cell), MPP (multipotent progenitor), CMP (common myeloid progenitor), GMP (granulocyte-monocyte progenitor), MEP (megakaryocyte-erythroid progenitor), and terminal myeloid lineages, where the black bars indicate median expression.

As shown in the figure, WT1 expression is noticeably elevated across all AML subtypes, compared to healthy hematopoietic populations. AML samples, such as t(15;17), inv(16), t(8;21), and MLL-rearranged leukemias, show higher WT1 levels, consistently above a median $\log_2$ of 6. On the other hand, hematopoietic stem and progenitor populations such as HSCs, MPPs, CMPs, and downstream myeloid lineages, including PMNs, monocytes, and erythroid progenitors (MEPs), displayed significantly lower WT1 expression, at around $\log_2$ values of 2 to 5.

This data highlights the difference in WT1 expression during normal hematopoietic development and highlights its abnormal upregulation as a hallmark of AML. The consistent overexpression in multiple cytogenetic subtypes reinforces the potential of WT1 as a pan-AML biomarker, but may have differing levels of effectiveness across subtypes. In those with lower or overlapping baselines with the norm, WT1 alone may not be sufficient for sensitive detection.

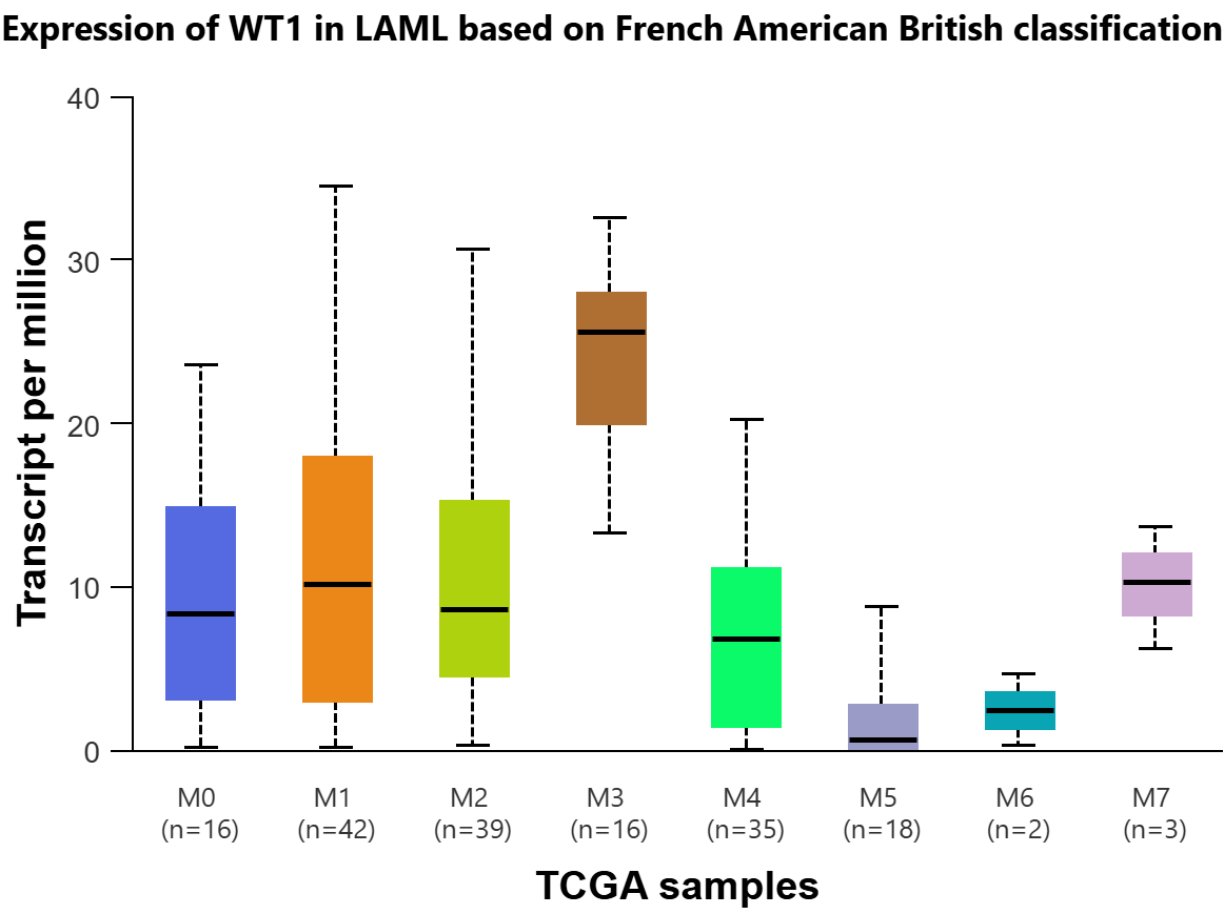

Figure 3. WT1 expression in AML subtypes (TCGA - FAB Classification)

Figure 3 further supports this claim, presenting WT1 expression data extracted from The Cancer Genome Atlas (TCGA) with a total of 171 AML cases stratified by the French-American-British (FAB) classification system (M0–M7), where it shows WT1 overexpression clearly among most AML subtypes (Chandrashekar et al., 2017, 2022; Vakiti et al., 2024). This data is accessible via the UALCAN analysis page by searching WT1 on the TCGA tab, then choosing expression. AML subtypes such as M2 (acute myeloblastic leukemia with maturation) and M3 (acute promyelocytic leukemia) display a largely elevated WT1 expression, while other subtypes such as M5 (monocytic), M6 (erythroid), and M7 (megakaryoblastic) show more modest WT1 levels.

This heterogeneity shows notable consideration for clinical use. While overall WT1 overexpression supports the proposed potential of WT1 being a general biomarker for AML diagnosis or monitoring, its effectiveness may differ across subtypes. In those with lower or overlapping baselines with the norm, WT1 alone may not be sufficient for sensitive detection. Therefore, while WT1 shows promise as a pan-AML biomarker, its diagnostic accuracy may need to be enhanced by combining it with additional molecular markers, particularly in subtypes with weaker or more heterogeneous expression profiles.

A common limitation when it comes to data from the TCGA is that the cohort is demographically skewed, with the TCGA AML cohort coming mainly from Caucasian patients (n = 156), and fewer samples from African-American (n = 13) and Asian (n = 2) populations. As a result, ethnicity-specific interpretations of WT1 expression cannot be strongly assessed with this dataset, and it is primarily used to illustrate WT1 expression variability across AML subtypes, rather than any conclusions about racial or ethnic differences in WT1 regulation.

WT1 MUTATION IN AML

Other than overexpression of WT1 found in around 80% AML cases, WT1 was also found to be mutated in small subsets of AML, in approximately 6-15% cases. Several different WT1 mutations have been described in AML, which occur primarily in exons 7 and 9, that include base substitutions, deletions, and insertions (Goel et al., 2020; Rampal & Figueroa, 2016). The vast majority of mutations result in the creation of stop codons and reading frame shifts, resulting in loss-of-function and expression of a truncated protein lacking the zinc-finger domain.

The mutation of WT1 in AML mostly correlates with epigenetic dysregulation that leads to AML leukemogenesis. WT1 mutations in AML define a distinct epigenetic subtype characterized by loss of TET-dependent DNA hydroxymethylation and consequent CpG hypermethylation, particularly at polycomb repressive complex 2 (PRC2) target genes. Mutant WT1 is mutually exclusive with TET2 and IDH1/2 mutations and produces a highly overlapping DNA hypermethylation signature, with globally reduced 5-hydroxymethylcytosine levels comparable to TET2- and IDH-mutant AML, indicating that WT1 normally acts as a cofactor that recruits TET2/TET3 to specific genomic loci to maintain active DNA demethylation (Sinha et al., 2015). Disruption of the WT1–TET interaction by WT1 mutation results in stable silencing of differentiation-associated and PRC2-marked genes. These findings, together with increased EZH2 expression, support a model in which aberrant DNA and histone methylation cooperate to enforce a leukemogenic differentiation block in WT1-mutant AML (Rampal & Figueroa, 2016).

WT1 overexpression occurs in the majority of AML patients, yet the WT1 protein is also mutated—typically with loss-of-function—in some cases, creating a paradox about its role in the disease. Interestingly, multiple studies reveal no significant difference in WT1 expression levels between WT1 wild-type and mutant AML, confirming that WT1 transcript quantification remains a reliable biomarker for AML regardless of mutation status (Rostami et al., 2021).

WT1 AS A BIOMARKER IN AML

Following the observation made in **Figure 2**, WT1 is shown as a potential biomarker candidate for AML due to its consistent overexpression across a broad subset of AML patients. Unlike mutation-specific markers such as RUNX1-RUNX1T1 or FLT3, which are common targets but limited to specific genetic subtypes, WT1 is expressed in the majority of AML cases, offering the potential for more universal coverage (Romanova et al., 2022). Due to this, its role as a biomarker becomes particularly valuable in cases where fusion or mutated genes are not available or undetectable. WT1 now acts as an important biomarker that would fill that diagnostic gap, enabling clinicians to follow patients lacking molecular markers for MRD monitoring. Furthermore, due to its elevated expression during active disease and rapid decline after, WT1 is well-suited for long-term surveillance for treatment response and early relapse detection (Lazzarotto & Candoni, 2022).

Although WT1 is not entirely leukemia-specific, as low levels can be observed in regenerating or inflamed tissue, its overall expression in AML is largely distinct from healthy controls. When measured via highly sensitive techniques such as RT-qPCR, WT1 transcript levels have been shown to demonstrate a strong prognostic value and a correlation with the disease burden (Malagola et al., 2016). Therefore, WT1 presents as a promising pan-subtype biomarker for AML that complements existing molecular markers. Its broad applicability and high expression in malignant but not healthy tissue support its growing utility in both diagnosis and MRD tracking, particularly for patients lacking clearer molecular abnormalities.

Compared with other molecular markers used in AML, such as NPM1, FLT3-ITD, CEBPA, and RUNX1, which have well-defined prognostic value and in some cases guide targeted therapy, WT1 stands out

because it is expressed in the majority of AML cases. NPM1 mutations are a reliable marker for minimal residual disease monitoring but are only present in about one-third of AML patients (Falini & Dillon, 2024). FLT3-ITD is associated with a high relapse risk and directs the use of FLT3 inhibitors, but its prognostic use is limited only to mutation-positive cases. FLT3 inhibitors, yet their prognostic utility is limited to mutation-positive cases (Daver et al., 2019). CEBPA mutations, when present in both copies of the gene (biallelic), are linked to a more favorable prognosis in AML, whereas RUNX1 mutations are associated with a higher risk of treatment failure and poorer overall outcomes (Romanova et al., 2022). While these markers are valuable, they are restricted to specific patient subsets. WT1, by contrast, is present across most AML cases, including those without mutations. Although it lacks the high specificity of mutation-based markers and can sometimes be elevated in non-malignant conditions, it remains a practical universal marker for measurable residual disease.

CURRENT STANDARD: WT1 mRNA DETECTION BY RT-PCR

The most widely used method for detecting WT1 expression in AML is RT-qPCR, which measures WT1 mRNA transcript levels to monitor MRD, as WT1 mRNA is expressed at low levels in normal hematopoietic tissue but is markedly elevated in most AML cases (Malagola et al., 2016). Due to its sensitivity and reproducibility, RT-qPCR has become a gold standard for both diagnosis and prognostic evaluation, especially in patients without leukemia-specific genetic mutations such as RUNX1-RUNX1T1 or FLT3-ITD (Panuzzo et al., 2022).

European LeukemiaNet (ELN) researchers validated a quantitative WT1 assay and established reference ranges for WT1 expression in PB and BM analyzing a large number of control samples, to allow transcript levels indicative of residual leukemia to be distinguished from normal background levels; that includes exons 1 and 2, which are less prone to mutation than exons 7 and 9 (to reduce false-negative results). The upper normal values were set at 250 WT1 copies/10^4 Abelson (ABL) for BM and at 50 WT1 copies/10^4 ABL for PB, with a sensitivity of 10^{-4} to 10^{-5}. The ELN WT1 assay offers a widely applicable, standardized tool for MRD assessment in around 90% of AML cases lacking leukemia-specific markers, supporting its integration into risk scores and trials for therapy decisions like transplantation. Limitations include modest overexpression in some patients, restricting deep sequential monitoring, and rare mutations (9.4% in low-expressors), potentially causing false negatives (Cilloni et al., 2009).

In a diagnostic setting, RT-qPCR is performed on bone marrow aspirates or the peripheral blood, where the total RNA is extracted, then transcribed and amplified via WT1-specific primers, allowing for serial quantification during treatment and enabling real-time MRD monitoring for faster relapse response times (Antoniou et al., 2024). Despite its strengths, RT-qPCR is technically demanding, requiring strict RNA quality control and specialized equipment, which can be cost-intensive, especially in settings with limited resources. Furthermore, because WT1 expression is not entirely leukemia-specific, it may transiently occur due to WT1 upregulation from regenerating and inflamed tissues, allowing false positives to occur (Alikian et al., 2017). Its expression also varies among AML subtypes, further complicating the matter (Chandrashekar et al., 2017, 2022; Vakiti et al., 2024). These factors highlight the need for complementary or alternative detection methods that retain sensitivity while also improving accessibility, cost-effectiveness, and diagnostic specificity.

ALTERNATIVE WT1 DETECTION METHODS AS IMMUNOGENIC ANTIGEN

While RT-qPCR remains the gold-standard method for detecting WT1 transcript levels in AML, protein- and peptide-based detection methods have emerged as promising tools due to their ease of use,

reduced invasiveness, and potential cost-effectiveness in the clinical setting. These protein- and peptide-based WT1 detection relies on the fact that WT1 works as leukemia-associated antigens (LAAs), which can be recognized by the immune system, thus we can evaluate immune response to WT1-derived antigens.

Functional WT1-specific Cytotoxic T-Lymphocytes (CTLs) were detected in 15-25% AML patients, suggesting the existence of T cells for the WT1 antigen in the bone marrow, and might contribute to the antileukemic response. Both the activity of WT1-specific T cells in terms of IFNg release, as well as the frequency of WT1-tetramer1 CD81 T cells at the time of diagnosis of AML, could be detected at high levels. High frequencies of WT1-specific T cells were observed by flow cytometry in the peripheral blood of patients after chemotherapy. IFN-gamma and granzyme B ELISPOT assays revealed a WT1-specific functionality of these CTLs. However, the WT1-specific T-cell population was lost over time. This may explain at least in part the relapse of the patients. These findings suggest that immune response detection of WT1 might give additional benefit in AML patients to predict relapse and immune response towards the WT1 antigen (Casalegno-Garduño, 2016).

ELISA is a well-established method of protein quantification that can be adapted for WT1 detection. WT1 protein is one of the oncogenic antigens, and IgG and IgM antibodies against WT1 were produced at higher levels in patients with AML and CML. In this context, Oji et al (2024) have developed an ELISA to detect and quantify serum levels of IgG and IgM antibodies against the WT1 cytotoxic T-lymphocyte epitope peptide. In this context, synthetic WT1-derived peptides, such as WT1_126–134 or WT1_235–243, are immobilized on assay plates to capture anti-WT1 antibodies or detect WT1-specific T-cell responses in patient sera (Oji, 2024). Alzaaqi et al. (2022) demonstrated that AML patients exhibit measurable IgG and IgM responses to these peptides, highlighting their potential as markers for disease detection and monitoring. Although these methods are still in the development phase and clinical trials are needed to validate their use in clinical settings, ELISA can be a promising alternative as an indirect evaluation of WT1 expression and immunogenicity. ELISA's ease of use and compatibility with serum samples make it a great choice for settings where maintaining RNA quality or bone marrow access is limited, and importantly, to assess the T-cell or humoral immune response after WT1-based immunotherapy.

CLINICAL INTEGRATION OF WT1 DETECTION: UTILITY, LIMITATIONS, AND FUTURE POTENTIAL

While various techniques for WT1 expression in AML exist, their clinical integration depends not only on their analytical performance but also on their logistical, economic, and structural considerations. RT-qPCR remains the gold standard due to its high sensitivity and reliability in MRD surveillance; however, its use is greatly limited by the need for fresh, high-quality RNA, specialized equipment, and technical expertise. These issues are particularly felt in low-resource environments, where delays in sample processing and limited laboratory infrastructure pose significant challenges to timely and accurate quantification. In such settings, the high cost of RT-qPCR reagents, equipment maintenance, and quality control restricts its routine use. These constraints emphasize the need for alternative diagnostic strategies that are less dependent on such specialized faculties. Some of the aforementioned protein- and peptide-based platforms, like ELISA or WT1-loaded tetramer assays, require simpler workflows and lower operational costs, making them easier to integrate into routine clinical practice. Unlike RT-qPCR, which demands strict RNA handling and expensive thermal cyclers, these assays can be carried out by simple standard laboratory equipment and peripheral blood samples, reducing both the technical barriers that such sophisticated equipment requires and the patient's burden.

Despite their great promise, these methods currently lack large-scale clinical validation and use, making wide-scale adoption difficult. Furthermore, their lower sensitivity compared to nucleic acid assays

raises questions about their use in minimal disease detection versus broader relapse surveillance. Perhaps a more optimal pathway is to use WT1-based assays with other molecular markers such as NPM1 or FLT3-ITD, or immunophenotypic markers such as CD123 or leukemia-associated immunophenotypes (LAIPs), to categorize risk better and create a more personalized monitoring strategy, depending on the individual (Ivey et al., 2016; Schuurhuis et al., 2018).

The integration of WT1 monitoring into clinical practice has direct implications for patient management. The regular assessment of WT1 levels allows clinicians to detect impending relapse earlier than conventional hematologic methods. This allows for the timely identification and therapeutic intervention necessary to aid the patient, via chemotherapy or transplantation. Additionally, the easier tracking of WT1 can help adjust the intensity of follow-up visits and treatment plans, minimizing the use of unnecessary procedures for patients in stable remission while focusing resources on those at higher risk. This patient-centered approach revolutionizes the current approach to AML treatment by demonstrating how WT1 detection not only improves prognostic evaluation but also actively affects clinical decision-making to improve treatment outcomes. As the field moves toward precision hematology, WT1 detection presents a promising target for developing a cheaper and easier AML detection method.

CURRENT WT1-TARGETED IMMUNOTHERAPIES IN AML

While WT1 has gained considerable attention as a diagnostic and prognostic biomarker in AML, its role as a therapeutic target has become an emerging solution in hematological oncology. The high expression in leukemic blasts has allowed it to be identified as a top-ranking tumor-associated antigen by the National Cancer Institute and allowed for the development of WT1-targeted immunotherapies, including peptide vaccines, T-cell receptor (TCR)-engineered adoptive T cells, and dendritic cell (DC)-based vaccines, all of which aim to empower the immune system and selectively eliminate WT1-expressing leukemic cells or prevent relapse.

WT1 is an intracellular protein that will be degraded by the proteasome. The short WT1 peptides are transported into the endoplasmic reticulum, loaded onto MHC class I, and presented at the cell surface. Generating WT1-specific cytotoxic $CD8^+$ T cell (CTL) responses that recognize peptides presented on the cell surface by MHC class I molecules has been a major goal for WT1-targeted therapy (Dao et al., 2017). Several WT1-derived class I epitopes bind common HLA alleles and can stimulate $CD8^+$ cytotoxic T lymphocytes (CTLs) that recognize and kill $WT1^+$ leukemia and solid tumor cells. Longer WT1 peptides contain class II epitopes that activate $CD4^+$ helper T cells; these $CD4^+$ responses support robust and durable $CD8^+$ CTL and antibody responses against WT1-expressing tumors (May et al., 2007). WT1 peptide vaccines induce immunogenicity because short WT1 sequences are bound and presented by specific HLA molecules on antigen-presenting cells (APCs), which then activate HLA-restricted $CD8^+$ and $CD4^+$ T cells that recognize WT1-expressing tumor cells. Each immunogenic WT1 peptide has a motif that fits only certain HLA class I or class II alleles, so T-cell response occurs only in individuals carrying those HLA types (Asemissen et al., 2006). The number of immunogenic WT-1 peptide antigens previously identified and reported is very limited and largely confined to a set of peptides presented by the HLA alleles A*0201, A*2402, and DRB1*0401 (Doubrovina et al., 2012).

One of the most well-studied therapeutic approaches when targeting WT1 is the use of WT1 peptide vaccines. These vaccines use short immunodominant WT1-derived peptides, specifically WT1_235–243 and WT1_126–134, which are presented on the patient's HLA-A*2402 and HLA-A*0201 molecules, respectively, as employed in the multivalent WT1 vaccine galinpepimut-S, which has displayed safety and immunogenicity in AML patients (Maslak et al., 2018). Through antigen-presenting cells, the

peptides are displayed and processed, activating cytotoxic $CD8^+$ T lymphocytes that specifically recognize and destroy WT1-overexpressing AML cells (Kreutmair et al., 2022).

Several clinical trials after 2015 were conducted to confirm the safety and immunogenicity of WT1 peptide vaccines in AML patients. In a Phase II study by Maslak et al. (2018), a multivalent WT1 peptide vaccine by the name of galinpepimut-S demonstrated the ability to induce a robust WT1-specific immune response, achieving a prolonged relapse-free state for some of the participating patients. On a similar vein, Kreutmair et al. (2022) evaluated a recombinant WT1 protein vaccine combined with the AS01_B adjuvant in elderly patients with AML in remission. There, they found that the vaccine successfully induced $CD4^+$ T cell and humoral responses in their patients, with one achieving long-term molecular remission, which lasted for over five years. Despite this, several vaccinated patients who developed humoral responses still experienced relapse and passed during their follow-up, highlighting that simply activating antibody responses alone is insufficient for AML control, and an effective disease suppression likely requires a more robust and persistent $CD8^+$ T cell-mediated response. While WT1 peptide-based vaccines have demonstrated good tolerability and the ability to stimulate immune recognition of WT1-positive AML cells, their therapeutic impact is severely limited by factors such as HLA restriction, immune suppression in heavily treated patients, and the potential for leukemic immune escape.

Another therapeutic approach involves the use of WT1-specific T cell receptor (TCR)-engineered adoptive T cell therapy (TCR-T), where, unlike vaccines which depend on the patient's immune system to generate WT1-specific responses, this method involves harvesting patient-derived T cells and then genetically modifying them to express a high-affinity TCR that recognizes WT1 specifically when presented on HLA molecules (Ruggiero et al., 2020). These cells are expanded *in vitro* and then reintroduced into the patient to target the WT1-expressing leukemic cells. This method bypasses the need for any endogenous priming and delivers a ready-made population of WT1-specific killer T cells, capable of targeting leukemic blasts with precision.

Most clinical studies on this topic have been conducted in the context of hematopoietic stem cell transplantation (HSCT), which is a standard treatment for high-risk or relapsed AML, in which the patient's diseased bone marrow is replaced with healthy stem cells either from the patient themselves or from a donor, however, relapse remains a big challenge even after transplant (Gielis et al., 2024). TCR-T therapy is typically administered after HSCT as an adjunct immunotherapy used to reinforce adjunct immunotherapy. One such trial was done by Chapuis et al. (2017) in a landmark Phase I trial where WT1-specific TCT-T cells were infused into AML patients who had undergone HSCT. The engineered T cells successfully persisted in the long term, where they migrated to the bone marrow and maintained cytotoxic activity against WT1-expressing AML cells. It was found that several patients remained MRD negative for extended periods. Follow-up studies by Chapuis et al. (2019) revealed that these WT1-specific WT1-specific TCR-T cells retained memory-like characteristics, allowing them to provide long-term immune surveillance and prevent relapse without inducing significant graft-versus-host disease (GvHD) or other off-target toxicities.

Another trial comes from another Phase I study by Tawara et al. (2017), which evaluated the safety and persistence of WT1-specific TCR gene-transduced lymphocytes in patients with AML and high-risk myelodysplastic syndrome. Here, the engineered T cells were well tolerated, found no evidence of toxicity in normal tissues, and persisted in peripheral blood for up to eight weeks or longer in most patients. In most cases, the TCT-T cells remained detectable and retained peptide-specific reactivity, further supporting the safety and efficacy of WT1-specific TCR-T therapy. Despite the promising results, several challenges remain. The therapy is currently restricted only to patients expressing certain HLA types, namely HLA-A*0201, limiting its applicability to a subset of AML patients. The complex manufacturing process also leads to high

costs, creating a barrier for widespread implementation. Not to mention, there is still a potential of immune escape, where leukemic cells reduce WT1 expression to avoid detection (Kang et al., 2022).

Distinct from peptide vaccines is another WT1-targeted immunotherapeutic strategy that involves the use of dendritic cell (DC) vaccines, where patient-derived monocytes are collected and differentiated *ex vivo* into dendritic cells and are then loaded with WT1-derived peptides, mRNA, or whole WT1 protein (van de Loosdrecht et al., 2018). The antigen-loaded DCs are reinfused into the patient to stimulate both $CD4^+$ helper and $CD8^+$ cytotoxic T cells, generating a broader immune response than peptide vaccines on their own via long-term immune memory. This allows the approach to overcome the impaired immune priming often seen in AML patients after chemotherapy, as DCs are potent stimulators of naïve T cells (Palomares et al., 2024).

A clinical evaluation of WT1 DC vaccines was conducted by Anguille et al. (2017), where, in a phase II clinical trial, they treated AML patients in post-remission with WT1 mRNA-electroporated DCs. The vaccine induced robust WT1-specific $CD8^+$ T-cell responses, where 43% of the patients achieved an antileukemic immune response, and nine of them reached molecular remission, several of which were stable beyond five years with no adverse effects reported. Palomares et al. (2024) had further developed this strategy by altering the WT1 DC vaccines to include multi-antigen formulations, such as combined WT1/PRAME-loaded DCs, achieving sustained remission in over half of treated patients during long-term follow-up.

Despite the success, DC vaccines face several limitations. Their production is labour-intensive, requiring *ex vivo* manipulation under strict GMP conditions, and the functionality of autologous DCs may be impaired in heavily treated patients. Moreover, AML cells can evade vaccine-induced immunity through mechanisms such as HLA downregulation, expression of immune checkpoint ligands (e.g., PD-L1), and secretion of immunosuppressive cytokines (TGF-β, IL-10), which dampen T-cell activity (Kang et al., 2022). Although WT1 dendritic cell vaccines have shown great promise in their immune response and durable remissions in some AML patients, the challenges, such as complex manufacturing, patient-specific variability, and immune escape, limit their ability for broader clinical use. These limitations result in the exploration of novel WT1-targeted approaches that aim to combat these barriers and improve treatment outcomes.

FUTURE DIRECTIONS IN WT1-BASED THERAPEUTIC STRATEGIES FOR AML

While significant progress has been made in the development of WT1-targeted immunotherapies through peptide vaccines, TCR-engineered T cells, and dendritic cell vaccines, these approaches are still limited by factors such as HLA restriction, manufacturing complexity, and immune evasion by leukemic cells. These challenges have driven the development of next-generation WT1-based therapies designed to broaden patient applicability, durability of immune responses, and integration with other therapeutic strategies.

One such promising approach is the development of WT1-directed chimeric antigen receptor (CAR) T cells. It was found that in preclinical studies by Rafiq et al. (2016), T-cell receptor-mimic CAR T cells specific for WT1 successfully eliminated WT1-positive AML cells and demonstrated strong antileukemic activity in xenograft models, and that the addition of interleukin-12 secretion to these engineered cells further enhanced their persistence, cytokine production, and antitumor potency. This study highlights that CAR T-cell platforms can be adapted to intracellular targets like WT1 and improved through cytokine co-expression.

CAR-T therapy represents a paradigm shift in immunotherapy by engineering patient T cells to

express chimeric antigen receptors (CARs) that precisely target tumor antigens, leading to potent, persistent tumor killing independent of MHC restriction. CAR-T cells bind surface tumor antigens via the CAR's extracellular domain, triggering intracellular signals that activate cytotoxicity, proliferation, and memory formation without needing HLA presentation (Akahori et al., 2018). This MHC-independent action overcomes tumor immune escape via HLA downregulation, a common resistance mechanism in AML, and enables serial killing of multiple tumor cells by a single CAR-T. In this context, WT1 is considered a promising but not yet ideal target for CAR-T therapy due to its overexpression in AML and many solid tumors, low normal tissue expression, and role in cancer cell survival, though its intracellular nature poses technical challenges that can be overcome mainly by targeting surface WT1 peptide–HLA complexes. WT1 is an intracellular transcription factor, not a surface antigen like CD19, so standard scFv-based CARs cannot bind it directly; most WT1 CARs use scFv antibodies that recognize WT1 peptide (e.g., WT1 235–243) presented by HLA-A*24:02 on the tumor cell surface. These HLA restriction limits applicability of WT1-based CAR-T therapy, e.g., HLA-A*24:02 CARs work only in patients/tumors expressing that allele (common in Asians but ~15–20% in Caucasians), creating a need for multi-HLA or promiscuous designs (Wang et al., 2025). A preclinical study using murine xenograft model showed that Preclinical WT1/HLA-A24:02 CAR-T cells kill HLA-A24$^+$ WT1$^+$ leukemia and solid tumor lines *in vitro* and regress xenografts *in vivo*, with enhanced efficacy when combined with DC vaccines (Akahori et al., 2018).

Similarly, bispecific T cell engagers (BiTEs) that redirect endogenous T cells toward WT1-expressing cells are under preclinical investigation. These engagers redirect the patient's T cells to kill WT1-expressing leukemic cells and are engineered with antigen-binding domains: one attaches to CD3 on T cells, while the other recognizes WT1 peptides presented on HLA molecules of AML cells (Dao et al., 2015). By becoming physical bridges for T-cells to their target, they promote the release of cytotoxic mediators, leading to efficient tumor cell destruction. Preclinical studies by Tian et al. (2021) using a WT1-CD3 bispecific antibody derived from the ESK1 TCR-mimic antibody demonstrated strong, selective killing of WT1-positive AML cells both *in vitro* and in mouse models. This approach bypasses the need for *ex vivo* T-cell manipulation and offers a scalable and highly specific immunotherapeutic strategy that could complement or enhance existing AML treatments.

Combination therapies are also being explored to enhance the efficacy of WT1-targeted immunotherapies. Hypomethylating agents such as azacitidine can increase WT1 expression on leukemic cells and enhance their recognition via immune effectors; however, this also leads to the upregulation of inhibitory ligands that suppress T-cell activity. This dual effect led to the combination of these agents with checkpoint inhibitors, which block pathways like PD-1 and CTLA-4 to restore immune function and improve antileukemic responses (Daver et al., 2018). Beyond these combinations, targeting innate immune checkpoints such as TIM-3 or CD47 is being explored as another way to overcome immune evasion. Agents directed at these pathways, including magrolimab and sabatolimab, have shown promising activity in early studies, supporting their potential integration with WT1-targeted therapies to achieve stronger and more durable responses (Abaza & Zeidan, 2022).

Advances in RNA vaccine technology are also contributing to the future of WT1-targeted therapies. Unlike mRNA-electroporated dendritic cell vaccines, direct mRNA formulations use lipid nanoparticles or similar carriers to deliver WT1-encoding RNA *in vivo*, allowing the patient's own cells to produce and present the antigen (Fu et al., 2025). This approach eliminates the need for *ex vivo* cell manipulation, enabling scalable, off-the-shelf production while still inducing WT1-specific immune responses, serving as a potent and versatile alternative to cell-based approaches (Palomares et al., 2024).

Ongoing clinical trials are expected to clarify the safety, efficacy, and long-term benefits of these

next-generation strategies. As these novel approaches develop, integrating WT1-targeted immunotherapies with existing AML treatments may provide a path to reduce relapse and achieve more durable remissions. Despite these promising developments, several challenges still remain. Most of the next-generation therapies, such as WT1-directed CAR-T cells and mRNA vaccines, are still in early-phase trials with limited patient numbers. Questions about how long the immune responses would last, whether side effects may emerge, or concerns about the high cost of manufacturing must still be answered. Addressing these issues will require long-term study and a coordinated front to refine protocols, optimize dosing, and identify which patients stand to benefit the most from these innovations. These advances would solidify WT1 as not only a biomarker but also a central therapeutic target in the evolving landscape of AML therapy.

CONCLUSION

The Wilms' Tumor 1 (WT1) gene remains a compelling biomarker for acute myeloid leukemia (AML) due to its consistent overexpression across most AML subtypes and role in both disease pathogenesis and immune recognition. Bioinformatics analysis further supports the relevance of WT1 expression in AML, showing uniform dysregulation compared to normal hematopoiesis. However, despite WT1's general overexpression in AML, subtype-specific variability and overlap with normal expression levels may limit its individual diagnostic power. As a biomarker, WT1 supports measurable residual disease monitoring and early relapse detection, with RT-qPCR remaining as the clinical gold standard for WT1-based diagnostics despite its technical and logistical limitations. However, alternative approaches, such as ELISA and ELISPOT, can be used by integrating the characteristic of WT1 as an immunoantigen. Beyond diagnostics, WT1 has emerged as a therapeutic target, with peptide vaccines, TCR-engineered T cells, and dendritic cell vaccines showing promising clinical results, while CAR-T cells, bispecific T cell engagers, and mRNA vaccines represent the next wave of innovation, although it is limited by the presentation of WT1 peptide by selective HLA type. Future integration of WT1 detection with other molecular markers and targeted therapies may enable a more personalized, effective, and accessible approach to AML management. As a whole, these advances establish WT1 not only as a biomarker but also as a central part of precision hematology in shaping the future of AML care.

AUTHOR CONTRIBUTIONS

AR: Conceptualization, Writing, Supervision. **YOH**: Writing, Data curation.

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COMPETING INTERESTS

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