



The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Wilms' Tumor 1 Functions As a Tumor Suppressor to Suppress FLT3-STAT Signaling and Epigenetic Remodeling in Acute Myeloid Leukemia (CALGB 8461, 9665 and 20202; Alliance)

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The Wilms' Tumor 1 (*WT1*) gene is a transcription factor that is recurrently mutated or commonly overexpressed in several cancer types. In acute myeloid leukemia (AML), frequent overexpression of *WT1* and poor patient outcomes associated with *WT1* mutations highlight its importance in the disease; however, there are no tailored treatments for these patients. Furthermore, *WT1*'s fundamental role as either an oncogene or tumor-suppressor remains unresolved. Here we examine the roles of both wild-type and mutant *WT1* in AML through epigenetic and mechanistic studies. Using our findings we propose a personalized treatment strategy.

WT1 mutations are enriched in the *NPM1*^{mut} subset of AML; thus we first focused our study on a cohort of 581 patients with *de novo* AML and *NPM1* mutations enrolled on Alliance for Clinical Trials in Oncology studies. Transcriptomic analyses revealed that *WT1*^{mut} patients phenocopied a distinct, aberrant gene expression signature associated with *FLT3* internal tandem duplications (ITD), a mutation known to activate STAT signaling and associated with poor outcome in AML. We observed that *WT1* expression levels were remarkably elevated in *FLT3*-ITD patients and were driven by *STAT5A* binding to the *WT1* promoter. *STAT5A* binding and subsequent *WT1* upregulation were blocked by small molecule *FLT3* inhibitors.

These findings linking *FLT3* activity with *WT1* expression raise two possibilities: either *WT1* is an oncogene cooperating with the *FLT3*-*STAT* pathway, or *WT1* naturally functions to suppress *FLT3* signaling in a negative feedback loop subverted by *WT1*

mutations. As WT1 interacts with TET2 to facilitate epigenetic remodeling and DNA binding, we performed genome-wide DNA methylation analysis on *WT1*^{mut} AML patients and observed selective hypermethylation of WT1 binding motifs consistent with loss of function. We confirmed that *WT1* mutations in AML commonly cause truncation of the C-terminal DNA binding domain in our patient cohort. Using co-immunoprecipitation, ChIP-sequencing and luciferase reporter assays, we found that mutant WT1 functions as a dominant-negative, inhibiting WT1 derived from the wild-type allele. Investigation of WT1 target genes by intersecting transcriptomic and DNA methylation profiles identified hypermethylation and downregulation of miR-193a, leading to a significant downregulation of miR-193a-3p in *WT1*^{mut} AML patients. Overexpression of wild-type, but not mutated, WT1 rescued miR-193a expression in AML cell lines. Enforced expression of miR-193a significantly delayed AML onset *in vivo*. In addition, overexpression of miR-193a in multiple human AML cell lines and primary patient samples impaired AML cell growth and colony-forming capacity while promoting monocytic differentiation, underscoring its role as potent tumor suppressor. Therapeutic modulation of miRNA levels in cancer patients has been limited by inefficient delivery and tissue enrichment. To overcome this, we tested a novel lipid-nanoparticle (LNP) formulation of miR-193a-3p (INT-1B3), currently being investigated in a phase I clinical trial (NCT04675996). Biweekly i.v. treatments of INT-1B3 in the immunocompetent Hoxa9/Meis1 (H9M)-transduced model system prevented AML formation, highlighting the potent anti-leukemic activity of this miRNA based therapeutic. Overexpression of miR-193a-loaded LNPs downregulated FLT3 expression and suppressed STAT signaling in primary AML samples. Finally, treatment of primary AML cells with FLT3 inhibitors revealed enhanced sensitivity of *WT1*^{mut} cells in the absence of *FLT3*-ITD, highlighting the role of wild-type FLT3 in *WT1*^{mut} cells.

In summary, we uncovered a critical negative-feedback loop maintained by WT1 to suppress FLT3 activity. Loss-of-function *WT1* mutations subvert the tumor suppressor function of WT1 via failure to maintain miR-193a expression, leading to increased FLT3 expression and STAT5 signaling, subsequently impairing differentiation, increasing proliferation and disease aggressiveness in AML (Figure 1). Our findings advocate for use of FLT3 inhibition and miR-193a supplementation for treatment of *WT1*^{mut} patients, a subgroup with poor outcomes and no targeted treatment options.

Disclosures Abdelbaky: Roche: Ended employment in the past 24 months. **Blachly:** AbbVie: Consultancy; AstraZeneca: Consultancy; *Epigenetic classification of leukemia*: Patents & Royalties: PCT conversion filed; *Leukemia Diagnostic Device*: Patents & Royalties: Being prosecuted; *Astellas*: Consultancy. **Baer:** Abbvie (Inst): Research Funding; *Ascentage Pharma (Inst)*: Research Funding; *FORMA Therapeutics (Inst)*: Research Funding; *Kite, a Gilead company (Inst)*: Research Funding; *Kura Oncology (Inst)*: Research Funding; *Takeda (Inst)*: Research Funding. **Klusmann:** Boehringer Ingelheim: Consultancy; *Jazz Pharmaceuticals*: Consultancy. **Eisfeld:** *Karyopharm Therapeutics*: Other: spouse employment; *Astra Zeneca*: Honoraria, Other: CEI Advisory Board; *OncLive*: Honoraria.

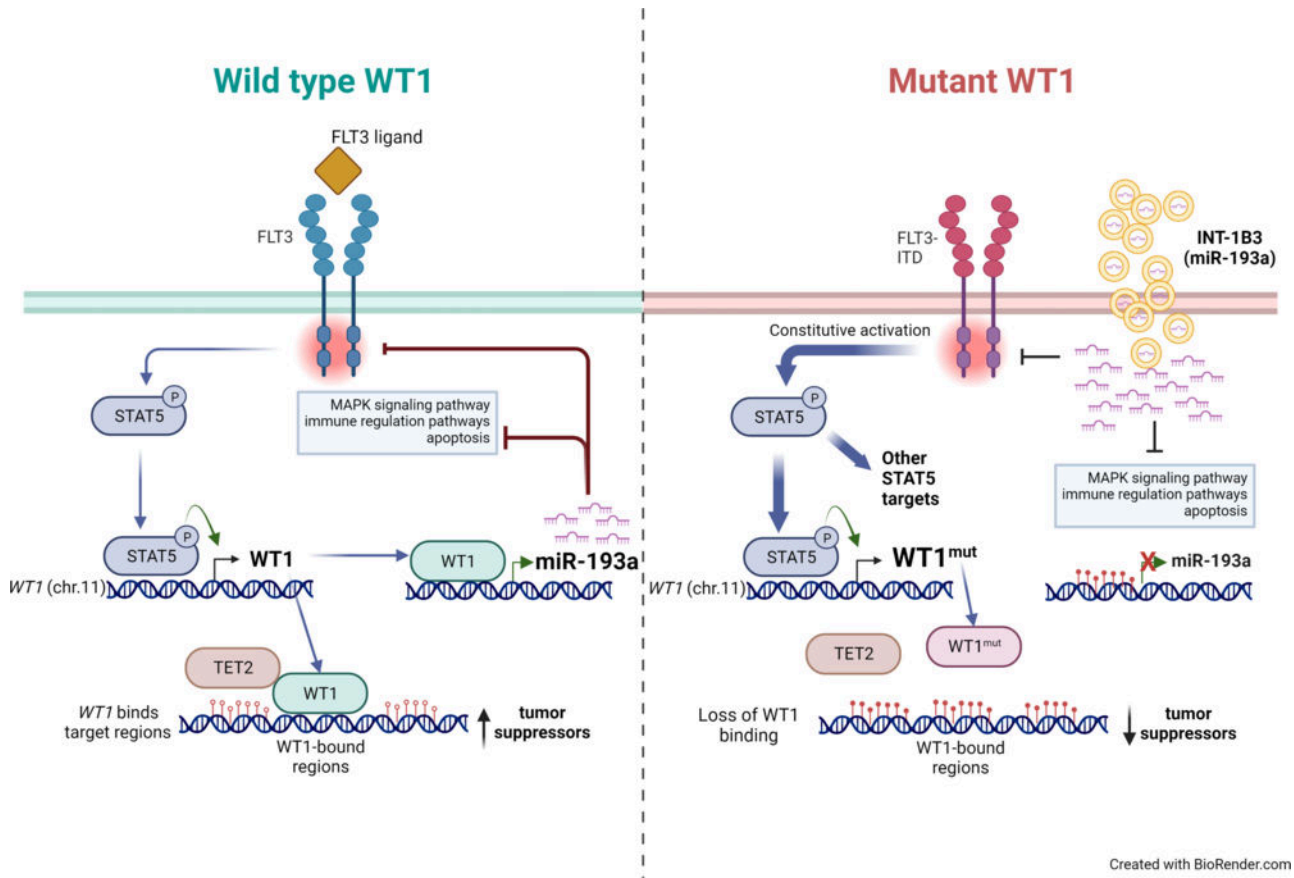


Figure 1

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