

# **MOLECULAR MECHANISMS ASSOCIATED WITH ACUTE MYELOID LEUKEMIA: REVIEW ARTICLE**

*Mohammed Al Asseri*

*Ahed J Al Khatib*

*A'aesha M Qasem*

Jordan University of Science and Technology, Jordan

*Abdullah Yahya Hamadi*

University of Tabuk, Saudi Arabia

*Fatima Laiche*

Mostaganem University, Algeria

---

## **Abstract**

The present review article purposes to introduce the molecular mechanisms underlying acute myeloid leukemia (AML). An overview of AML was introduced including its classification, therapeutic options and the roles of mutations in p53 and WT1 genes. The possibility of molecular targeting therapies was also discussed. The efforts of many researchers concerning AML were taken into consideration during this review.

We also pointed to the various roles of WT1 gene as suppressor or oncogene. The important relationship between p53 gene and WT1 gene was highlighted so that when WT1 gene stabilizes p53, the pathological picture concerning p53 if being mutated or not should be reconsidered again.

---

**Keywords:** AML, p53, WT1, suppressor gene, oncogene

## **Introduction**

### **Mutations Associated with Acute Myeloid Leukemia (AML)**

Several studies have recently emphasized the presence of recurring mutations in several genes with biologic and prognostic implications to be characterized in AML, particularly within the normal karyotype and/or intermediate-risk cytogenetic subset. Those genes in which mutations may affect disease classification and risk stratification schemes include *FLT3*, *NPM*, *NRAS*, *MLL*, and *CEBPA* (Ito et al., 2006; Mrozek et al., 2007).

### **Mutations Associated With WT1**

Mutations in the *WT1* gene have been included to predispose to AML (Gaidzik, Dohner, 2008; Gaidzik et al., 2009; Renneville et al., 2009). The expression of *WT1* is localized mainly in tissues of the developing genitourinary and hematopoietic systems, and mutations in *WT1* occur in both syndrome-associated and sporadic cases of nephroblastoma (Wilms tumor), the most common childhood renal malignancy (Little et al., 2004). Other studies also indicated the expression of *WT1* in CD34 hematopoietic progenitors and its overexpression in a subset of acute leukemias (Vardiman et al., 2002).

The WT1 protein comprises of a proline-glutamine-rich N-terminal transcriptional regulatory domain (exons 1-6), in addition to 4 C-terminal zinc finger domains (exons 7-10) which, in turn, facilitate DNA binding (Scharnhorst et al., 2001). Exons 5 and 9 were shown to be under the effect of alternative splicing, leading to 4 different splice isoforms. Furthermore, posttranslational modifications and alternate start codons can result in additional protein products (Phoenix et al., 2010). The WT1 protein may have various roles such as a transcriptional activator or a transcriptional repressor. The variety of roles of WT1 depends on several factors including the level of expression, the specific isoform, and the cellular context (Yang et al., 2007).

Mutations of *WT1* in relation AML were mainly reported to be in the zinc-finger domains, which lead to producing a protein that is expected to be unable to bind DNA. Most mutations have been reported to occur in exon 7. Taken together, a premature stop codon and a truncated protein lacking the C-terminal zinc fingers would be resulted (Paschka et al, 2008; Virappane et al., 2008; Renneville et al., 2009).

It has been reported that missense mutations predominate in exon 9, such mutations interrupt DNA binding capacity through affecting amino acid residues either directly involved in DNA binding or essential to the structure of the zinc-finger motif (Little et al., 2004).

### **Classification of AML**

According to the French-American-British (FAB) system, the subtypes of AML were described as M0 through M7. Another classification was adopted by the World Health Organization (WHO) in which AML was reclassified into four categories. The four categories are AML with recurrent genetic abnormalities, AML with multilineage dysplasia, therapy-related AML and myelodysplastic syndromes (Vardiman et al., 2002).

## **Treatment of AML**

There are two approaches to treat AML based on cytarabine and anthracyclines. The outcomes associated with AML are still remaining poor, especially for those patients who are older or carry higher-risk disease. New updates in AML therapy have led to the development and study of novel agents that target AML by diverse and varied mechanisms among which are targeted therapeutics such as kinase inhibitors and oligonucleotide constructs. Other choices include inhibitors of histone deacetylase, which cause growth arrest and apoptosis through histone acetylation and resultant conformational changes (Amir et al., 2010).

Other studies discussed that that the majority of patients under age 60 accomplish a complete remission (CR) with traditional anthracycline and cytarabine based induction regimens. But the problem encountered is due to poor long-term survival rates approximately 30–40% (Lowenberg et al., 1999). Furthermore, it has been shown to have poorer prognosis for those with high-risk AML, such as those who are older, who had preceding myelodysplastic syndromes (MDS) or myeloproliferative disorders (MPD), or those with secondary AML from environmental exposures or prior chemotherapy. A complete remission is achieved in less than 40% of cases, with survival rates of less than 10% (Bao et al., 2005).

New class of therapies are concerned with developing agents which target cell signaling and cycling, as well as those which interrupt DNA repair and replication. Some of these therapies are still in early phases of development and study, while others have shown promise in preclinical and clinical investigation (Amir et al, 2010).

## **The Effect of WT1 on Apoptosis**

There is a considerable evidence indicating that one of the functions of WT1 is regulate apoptosis, but there is a debate arising from conflicting reports whether the role of WT1 is pro- or anti-apoptotic. Thorough revision of literature indicates that the apparent contradictions probably reflect cell lineage and isoform-specific differences in WT1 function. Furthermore, expression of WT1 inhibits programmed cell death in some cell types but promotes it in others. It has also been indicated that different WT1 isoforms can exert distinct effects as well. A relationship between the mechanisms by which WT1 regulates apoptosis and bcl-2 family members has been established and showed to either direct or indirect WT1 target genes, including bcl-2 itself, the pro-apoptotic family members Bak and Bax, and the anti-apoptotic family member Bfl-1/A1. It has also been shown that the specific bcl-2 family members regulated by WT1 expression vary by cell type and by isoform. Thus, a complete understanding of the role of WT1 in

apoptosis should take into account that both lineage- and isoform-specific effects of WT1 at both the cellular and molecular levels (Loeb, 2006).

### **The Role of P53 in Hematological Cancer**

It has been shown through literature that the inactivation of the wild-type p53 gene (TP53) attributed by various genetic alterations to be a major event in human carcinogenesis. Furthermore, it has been estimated that more than 60% of human primary tumors have a mutation in the p53 gene. On the other hand, genetic alterations in the p53 gene have low incidence (10–20%). However, epidemiological studies of the hematological malignancies have indicated that the prognosis of patients with a mutation in the p53 gene is worse than those expressing the wild-type p53 protein (Peller and Rotter, 2003).

Several studies reported that p53 mutations as an independent factor for short survival, mainly in elderly patients with unfavorable karyotype (Nakano et al., 2000; Stirewalt et al., 2001). However, it has been reported that in patients with AML previously treated with alkylating agents, the incidence of mutations increased to 27% (Christiansen et al., 2001). It has also been found that a high incidence of mutations in AML patients to be associated with defective DNA mismatch repair and complex karyotype (Zhu et al., 1999). The previous findings have also been confirmed a meta-analysis of a number of studies which indicated a low incidence of p53 mutations (5%) on infant acute lymphatic leukemia (ALL) (Krug et al., 2000).

Studies on human leukemia-lymphoma cell lines revealed mutations on p53 gene (Prokocimer et al., 1998). Another interesting observation points to the alterations in the p53 gene. It has been recently observed that p53 gene alterations often emerge in cell lines although the original tumor cells had wild-type p53 (Peller and Rotter, 2003). Furthermore, evidence was shown that p53 alteration in a minor clone may confer a survival advantage to malignant cells in vitro and presumably also in vivo (Drexler et al., 2000).

### **TP53 and Apoptosis**

It has been found that p53 has the ability to act as a substrate for jun N-terminal kinase (JNK) activity in JNK signaling-induced apoptosis. In this process, JNK can act as either to destabilize p53 through binding, promoting ubiquitin-mediated degradation, or to stabilize p53 by phosphorylation, and by thus it inhibits its degradation. p53-dependent apoptosis includes parallel or sequential activation of various genes leading to cell death response. Through this pathway involves, there is an activation of the mitochondrial Apaf-1/caspase pathway, death receptor signaling, CD95, and cleavage of downstream caspases (Herr and Debatin, 2001).

Patients with hematological malignancies are subjected to various therapeutic options among which are cytotoxic agents including DNA damaging drugs, antimetabolites, mitotic inhibitors, purine analogues, or inhibitors of topoisomerase. All these treatments lead to the induction of cellular stress, which, in turn, elicit apoptosis. p53-induced apoptosis in various hematological tumors is often impaired and thus p53-independent pathways may take over. As an example, in pediatric acute lymphoblastic leukemia, primary leukemic cells and derived cell lines presented Fas(CD95)-mediated apoptosis with high levels of Fas expression in p53 mutant cells but not in p53 wild-type cells (Zhou et al., 1998).

In AML patients, there are various types of leukemic cells that can be detected by their high- or low-uptake of daunorubicin. The high uptake cells leads to induction of apoptosis. This intra clonal heterogeneity in drug uptake is thought to contribute to relapse in AML patients (Palucka et al., 1999).

### **WT1 and Leukemia: dual roles, a tumor suppressor or an oncogene?**

There has been a debate in literature if WT1 works as a tumor suppressor or as an oncogene. Furthermore, the difficulty in understanding the biology of WT1 is attributed to the existence of different splice isoforms, which have different functions and are differentially expressed as well as WT1's controversial role in leukaemogenesis. What makes the picture more complicated is that although WT1 has been firstly identified in Wilms' tumors due to mutations, about 10% of the Wilms' tumors actually have mutations in the gene (Haber et al., 1990, 1993; Gessler et al., 1994). Accordingly, it is believed that other loci may be involved, e.g., *FWT1* (Rahman et al., 1996). An interesting observation has been found in which Wilms' tumor patients develop leukemia as a second primary tumor (Moss et al., 1989; Pritchard., 1994).

Furthermore, it has been reported that leukaemias are common in relatives of children with Wilms' tumor. It has also been observed that WT1 is involved during early haematopoiesis as well as its very high expression in more undifferentiated progenitor CD34+/CD33- cells (100-fold more than in differentiated precursors CD33+) leads to the question on whether WT1 is also implicated in leukaemogenesis (Maurer et al., 1997).

Research trials to introduce the appropriate answer has been shown to be contradictory because WT1 has been found to be mutated in sporadic leukaemia at a rate of 10%, which is comparable to Wilms' tumors and at a rate of 15% in AML patients (King-Underwood et al., 1996). It is worth mentioning that most of the mutations identified led to zinc-finger loss and a C-terminally truncated WT1 protein would lose its DNA binding ability but would retain its protein-protein interaction capacity, having, however, its usual function altered. It is also worth mentioning that even heterozygous

mutations are sufficient to contribute to leukaemogenesis (King-Underwood et al., 1998). This finding is explained depending on the resulting mutation so that if the result of the mutation is a truncated protein that can still self-associate, which, in turn, alters the subnuclear localization of the wild-type protein and produce WT1 proteins that act in a dominant negative manner (Englert et al., 1995). Taken together, these characteristics give WT1 a tumor suppressor function, which leads to leukaemogenesis when lost due to mutation during early haematopoiesis. Other studies revealed oncogenic role for WT1 based on its overexpression in acute leukaemic cell lines and acute leukaemias of myeloid and lymphoid origin (Menssen et al., 1995; Inoue et al., 1997).

Other researchers found that high expression of WT1 to be correlated with less-differentiated phenotypes (Pritchard-Jones and King-Underwood, 1997). In another study, it was found that overexpression is thought to be isoform-dependent as it has been associated with an excess of the exon-5 containing WT1 variant (Renshaw et al., 1997).

### **WT1 monitoring in core binding factor AML**

In view of the fact that minimal residual disease may help to establish clinical decisions in patients with AML. Lasa (2009) reported that WT1 offers the possibility to analyze those cases without currently known underlying genetic abnormalities. Lasa tried to compare the value of chimeric specific quantitative PCR with WT1 PCR in CBF acute leukemia. Accordingly, 445 samples from 96 AML (49 AML1-ETO+ and 47 CBF-MYH11+) cases were included in the study. AML1-ETO or CBF-MYH11 levels were obtained using the conditions of the BIOMED group and compared with the results of WT1 levels using sensitive primers and conditions. Simultaneously, normal range expression of WT1 was established using RNA obtained from eight healthy donors. WT1 mutations were also investigated both at RNA and at the genomic level. The majority of CBF samples showed rises in WT1 levels (88.7%) at diagnosis. However, 18% of AML1- ETO showed WT1 levels below 250 copies in contrast with 5% CBF-MYH11 cases. WT1 mutation was not detected in any case (70 diagnostic samples). There was a correlation between WT1 levels at diagnosis and the CD34 blast population estimated by flow cytometry in CBF-MYH11+ cases. It was also found that no association between WT1 levels and clinical outcome. A high concordance between chimeric transcript analysis and WT1 levels in CR patients was also found. Concordance was also high in relapsed patients (78% in AML1-ETO and 98% in CBF-MYH11+ cases). Both WT1 and specific chimeric transcript identified and rescued false negative results of the other test. It was recommended that additional studies are needed to determine whether the rare discrepancies are

a reflection of the cooperative nature of WT1 overexpression or a consequence of the uneven distribution in the leukemic population. Lasa concluded that WT1 is a powerful MRD tool even in cases with currently available molecular targets.

In a study conducted by Gu (2010), real-time quantitative reverse transcriptase polymerase chain reaction method was established for detecting the expression levels of WT1 gene and WT1+17AA isoforms in 226 AML bone marrow (BM) cells. It was found that WT1 gene was 2–3 logarithms expressed more in AML BM cells at initial diagnosis or relapse than in normal BM cells ( $p < 0.001$ ), with predominant WT1+17AA isoforms expression (the ratio of WT1+17AA/WT1 more than 0.50). It is worth mentioning that the ratio of WT1+17AA/WT1 was statistically higher in relapsed AMLs than in initially diagnosed ( $p = 0.01$ ), indicating that WT1+17AA isoforms might participate in AML relapse.

Gray (2012) conducted a study and showed that WT1 levels may be a useful predictor of leukemia free survival (LFS) following treatment of acute myeloid leukemia (AML). A retrospective study in which levels of WT1 expression from patients with de novo AML were measured from bone marrow and peripheral blood at diagnosis, post-induction, postconsolidation and relapse was reported. It was demonstrated that higher levels of WT1 in peripheral blood at diagnosis are associated with poorer LFS independent of age and cytogenetic risk-group ( $n = 85$ ,  $p = 0.028$ ). When measured at post-consolidation, the presence of detectable WT1 was shown to be associated with poorer LFS in univariate analysis of both peripheral blood ( $p = 0.024$ ) and bone marrow ( $p = 0.019$ ). In a multivariate analysis including age and cytogenetic risk, the association remained significant for bone marrow ( $p = 0.016$ ) with a trend observed for peripheral blood ( $p = 0.06$ ).

In his study, Ishikawa (2011) showed that overexpression of the Wilms' tumor gene 1 (WT1) to be observed in most leukemia cells. In addition to four major isoforms of WT1, an N-terminally truncated isoforms (sWT1) has been identified. The research group quantified the transcript levels of sWT1 and full-length WT1 (fWT1) in 237 patients with acute myeloid leukemia (AML). sWT1 expression was observed in 45 of 237 (19.0%) AML patients, particularly in acute promyelocytic leukemia (59.3%). Although sWT1 expression was not associated with other genetic mutations and prognosis, fWT1 expression level in sWT1-expressing AML was significantly higher than that in un-expressing AML. The previous findings led to propose a possible cooperation of sWT1 and fWT1 in the pathophysiology of AML.

Zhao (2010) conducted a study taking into consideration that the p53 tumor suppressor can limit proliferation in response to cellular stress through several mechanisms. Here, the research team test if the recently described

ability of p53 to limit stem cell self-renewal suppresses tumorigenesis in acute myeloid leukemia (AML) which is an aggressive cancer in which p53 mutations have been reported to associate with drug resistance and adverse outcome. The research team adopted an approach combined mosaic mouse models, Cre-lox technology, and in vivo RNAi to disable p53 and simultaneously activate endogenous Kras G12D a common AML lesion that promotes proliferation but not self-renewal. Study data showed that p53 inactivation strongly cooperates with oncogenic Kras G12D to induce aggressive AML, while both lesions on their own induce T-cell malignancies with long latency. This synergy is thought to be mediated by a pivotal role of p53 in limiting aberrant self-renewal of myeloid progenitor cells, such that loss of p53 counters the deleterious effects of oncogenic Kras on these cells and enables them to self-renew indefinitely. As a result, myeloid progenitor cells expressing oncogenic Kras and lacking p53 become leukemia-initiating cells, resembling cancer stem cells capable of maintaining AML in vivo. Taken together, these findings establish an efficient new strategy for interrogating oncogene cooperation, and provide strong evidence that the ability of p53 to limit aberrant self renewal contributes to its tumor suppressor activity.

Paydas (1995) investigated the expression of p53 protein by immunohistochemistry in 58 patients with leukemia. The study showed positive expression of p53 in 7 of 24 cases with acute myeloid leukemia (AML), 3 of 15 cases with chronic lymphocytic leukemia (CLL), one of 11 cases with chronic myeloid leukemia (CML) and 4 of 8 cases with acute lymphoid leukemia (ALL). Further analysis revealed that of patients having p53 expression, one case with AML had refractory anemia with excess blasts-transformation (RAEB/t), one case with CLL had Richter's syndrome and another one with CML was in accelerated phase. Taken together, 26% of leukemia cases had p53 protein expression. Finally, it was concluded that p53 protein abnormalities may have an important role in leukomogenesis and in the development of more malignant clones in chronic leukemias.

Cell cycle control is a crucial event in normal hematopoiesis, and abnormalities of regulatory cell cycle genes have been found by a study conducted by Zolota (2007) to contribute to the development of many hematologic malignancies. The present study investigates the immunohistochemical expression of seven essential cell cycle proteins (p21, p27, p14, p16, p53, mdm2, and cyclin E) in paraffin-embedded sections from 42 bone marrow biopsies obtained from an equal number of patients with newly diagnosed acute myeloid leukemia (AML). This study revealed (i) a high frequency of p53+/mdm2-/p21-phenotype, which is probably a result of p53 gene mutation and/or inhibition of mdm2 action by p14(ARF); (ii) expression of p27+/cyclinE-phenotype in most cases, suggesting that p27

may act as a potent cyclin-dependent kinase inhibitor; (iii) expression of p16 only in very few cases; and (iv) no relationship between the expression of any of the above proteins and survival as well as histologic subtype.

### References:

Adriana Lasa, Maite Carricondo, Camino Estivill, Elena Bussaglia, Ignasi Gich, Salut Brunet, Anna Aventin, Jorge Sierra, Josep F. Nomdedeu (2009). WT1 monitoring in core binding factor AML: Comparison with specific chimeric products. *Leukemia Research* 33, 1643–1649.

Amir T. Fathi, Steven Grant, Judith E. Karp (2010). Exploiting cellular pathways to develop new treatment strategies for AML. *Cancer Treatment Reviews* 36, 142–150.

Bao T, Smith BD, Karp JE (2005). New agents in the treatment of acute myeloid leukemia: a snapshot of signal transduction modulation. *Clin Adv Hematol Oncol*, 3:287–96.

Christiansen DH, Andersen MK, Pedersen-Bjergaard J. (2001). Mutations with loss of heterozygosity of p53 are common in therapy-related myelodysplasia and acute myeloid leukemia after exposure to alkylating agents and significantly associated with deletion or loss of 5q, a complex karyotype, and a poor prognosis. *J Clin Oncol* 19:1405–1413.

David M. Loeb (2006). WT1 Influences Apoptosis through Transcriptional Regulation of Bcl-2 Family Members. *Cell Cycle*, 5(12): 1249-1253.

Drexler HG, Fombonne S, Matsuo Y, Hu ZB, Hamaguchi H, Uphoff CC (2000). P53 alterations in human leukemialymphoma cell lines: in vitro artifact or prerequisite for cell immortalization? *Leukemia* 14:198 -206.

Englert, C., M. Vidal, S. Maheswaran, et al. (1995). "Truncated WT1 mutants alter the subnuclear localization of the wild-type protein." *Proc Natl Acad Sci U S A* 92(26):11960-4.

Gaidzik V, Dohner K (2008). Prognostic implications of gene mutations in acute myeloid leukemia with normal cytogenetics. *Semin Oncol*, 35(4): 346-355.

Gaidzik VI, Schlenk RF, Moschny S, et al (2009). Prognostic impact of WT1 mutations in cytogenetically normal acute myeloid leukemia: a study of the German-Austrian AML Study Group. *Blood*, 113(19):4505-4511.

Gessler, M., A. Konig, K. Arden, *et al.* (1994). "Infrequent mutation of the WT1 gene in 77 Wilms' Tumors." *Hum Mutat* 3(3): 212-22.

Haber, D. A., A. J. Buckler, T. Glaser, *et al.* (1990). "An internal deletion within an 11p13 zinc finger gene contributes to the development of Wilms' tumor." *Cell* 61(7): 1257-69.

Haber, M. J., G. J. Liefers, P. Paul, *et al.* (1993). "Homozygous somatic Wt1 point mutations in sporadic unilateral Wilms tumor." *Proc Natl Acad Sci U S A* 90(4): 1416-9.

- Herr I, Debatin KM. 2001. Cellular stress response and apoptosis in cancer therapy. *Blood* 98:2603–2614.
- Inoue, K., H. Ogawa, Y. Sonoda, *et al.* (1997). "Aberrant overexpression of the Wilms tumor gene (WT1) in human leukemia." *Blood* 89(4): 1405-12.
- Ito K, Oji Y, Tatsumi N, Shimizu S, Kanai Y, Nakazawa T, Asada M, Jomgeow T, Aoyagi S, Nakano Y, Tamaki H, Sakaguchi N, Shirakata T, Nishida S, Kawakami M, Tsuboi A, Oka Y, Tsujimoto Y, Sugiyama H (2006). Antiapoptotic function of 17AA(+)WT1 (Wilms' tumor gene) isoforms on the intrinsic apoptosis pathway. *Oncogene*, 25(30):4217-29.
- James X. Gray, Lyle McMillen, Peter Mollee, Sanjoy Paul, Steven Lan, Robert Bird, Devinder Gill, Russell Saal, Paula Marlton. WT1 expression as a marker of minimal residual disease predicts outcome in acute myeloid leukemia when measured post-consolidation. *Leukemia Research* 36 (2012) 453– 458.
- King-Underwood, L. and K. Pritchard-Jones (1998). "Wilms' tumor (WT1) gene mutations occur mainly in acute myeloid leukemia and may confer drug resistance." *Blood* 91(8): 2961-8.
- King-Underwood, L., J. Renshaw and K. Pritchard-Jones (1996). "Mutations in the Wilms' tumor gene WT1 in leukemias." *Blood* 87(6): 2171-9.
- Krug U, Ganser A, Koefler HP (2002). Tumor suppressor genes in normal and malignant hematopoiesis. *Oncogene* 21:3475–3495.
- Little SE, Hanks SP, King-Underwood L, *et al* (2004). Frequency and heritability of WT1 mutations in nonsyndromic Wilms' tumor patients: a UK Children's Cancer Study Group study. *J Clin Oncol*, 22(20):4140-4146.
- Lowenberg B, Downing JR, Burnett A (1999). Acute myeloid leukemia. *N Engl J Med*, 34:1051–62.
- Maurer, U., J. Brieger, E. Weidmann, *et al.* (1997). The Wilms' tumor gene is expressed in a subset of CD34+ progenitors and downregulated early in the course of differentiation in vitro. *Exp Hematol* 25(9): 945-50.
- Menssen, H. D., H. J. Renkl, U. Rodeck, *et al.* (1995). "Presence of Wilms' tumor gene (wt1) transcripts and the WT1 nuclear protein in the majority of human acute leukemias." *Leukemia* 9(6): 1060-7.
- Moss, T. J., L. C. Strauss, L. Das, *et al.* (1989). "Secondary leukemia following successful treatment of Wilms' tumor." *Am J Pediatr Hematol Oncol* 11(2): 158-61.
- Mrozek K, Dohner H, Bloomfield CD (2007). Influence of new molecular prognostic markers in patients with karyotypically normal acute myeloid leukemia: recent advances. *Curr Opin Hematol.*, 14(2):106-114.
- Nakano Y, Naoe T, Kiyoi H, Kitamura K, Minami S, Miyawaki S, Asou N, Kuriyama K, Kusumoto S, Shimazaki C, Akiyama H, Saito K, Nishimura M, Motoji T, Shinagawa K, Saito H, Ohno R (2000). Prognostic value of p53

gene mutations and the product expression in de novo acute myeloid leukemia. *Eur J Haematol* 65:23–31.

Palucka KA, Knaust E, Xu D, Macnamara B, Porwit Macdonald A, Gruber A, Peterson C, Bjorkholm M, Pisa P (1999). Intraclonal heterogeneity in the in vitro daunorubicin-induced apoptosis in acute myeloid leukemia. *Leuk Lymphoma* 32:309–316.

Paschka P, Marcucci G, Ruppert AS, et al (2008). Wilms' tumor 1 gene mutations independently predict poor outcome in adults with cytogenetically normal acute myeloid leukemia: a cancer and leukemia group B study. *J Clin Oncol*, 26(28): 4595-4602.

Paydas. S (1995). p53 protein expression in leukemias. *Acta Oncol.*, 34(1):23-6.

Phoenix A. Ho, Rong Zeng, Todd A. Alonzo, Robert B. Gerbing, Kristen L. Miller, Jessica A. Pollard, Derek L. Stirewalt, Nyla A. Heerema, Susana C. Raimondi, Betsy Hirsch, Janet L. Franklin, Beverly. Lange and Soheil Meshinchi (2010). Prevalence and prognostic implications of WT1 mutations in pediatric acute myeloid leukemia (AML): a report from the Children's Oncology Group. *Blood*, 116: 702-710.

Pritchard-Jones, K. and L. King-Underwood (1997). "The Wilms tumour gene WT1 in leukaemia." *Leuk Lymphoma* 27(3-4): 207-20.

Pritchard-Jones, K., J. Renshaw and L. King-Underwood (1994). "The Wilms tumour (WT1) gene is mutated in a secondary leukaemia in a WAGR patient." *Hum Mol Genet* 3(9): 1633-7.

Prokocimer M, Unger R, Rennert HS, Rotter V, Rennert G (1998). Pooled analysis of p53 mutations in hematological malignancies. *Hum Mutat* 12:4–18.

Rahman, N., L. Arbour, P. Tonin, *et al.* (1996). "Evidence for a familial Wilms' tumour gene (FWT1) on chromosome 17q12-q21." *Nat Genet* 13(4): 461-3.

Renneville A, Boissel N, Zurawski V, et al (2009). Wilms tumor 1 gene mutations are associated with a higher risk of recurrence in young adults with acute myeloid leukemia: a study from the Acute Leukemia French Association. *Cancer*, 115(16):3719-3727.

Renshaw, J., L. King-Underwood and K. Pritchard-Jones (1997). "Differential splicing of exon 5 of the Wilms tumour (WT1) gene." *Genes Chromosomes Cancer* 19(4): 256-66.

Scharnhorst V, van der Eb AJ, Jochemsen AG (2001). WT1 proteins: functions in growth and differentiation. *Gene*, 273(2):141-161.

Shoshana Peller and Varda Rotter (2003). TP53 in Hematological Cancer: Low Incidence of Mutations With Significant Clinical Relevance. *Human mutation* 21:277-284.

- Stirewalt DL, Kenneth J, Meshinchi KS, Appelbaum FR, Slovak ML, Willman CL, Radich JP (2001). FLT3, RAS, and TP53 mutations in elderly patients with acute myeloid leukemia. *Blood* 97:3589–3595.
- Vardiman, J. W., N. L. Harris and R. D. Brunning (2002). The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood* 100(7): 2292-302.
- Vassiliki Zolota, Chaido Sirinian, Maria Melachrinou, Argyrios Symeonidis, Dionysios S Bonikos (2007). Expression of the regulatory cell cycle proteins p21, p27, p14, p16, p53, mdm2, and cyclin E in bone marrow biopsies with acute myeloid leukemia. Correlation with patients' survival. *Pathology – Research and Practice* 203, 199–207.
- Virappane P, Gale R, Hills R, et al (2008). Mutation of the Wilms' tumor 1 gene is a poor prognostic factor associated with chemotherapy resistance in normal karyotype acute myeloid leukemia: the United Kingdom Medical Research Council Adult Leukaemia working party. *J Clin Oncol*, 26(33):5429-5435.
- Weiyang Gu, Shaoyan Hub, Zixing Chen, Guoqiang Qiu, Jiannong Cen, Bai He, Jun He, WeiWu (2010). High expression of WT1 gene in acute myeloid leukemias with more predominant WT1+17AA isoforms at relapse. *Leukemia Research* 34, 46–49.
- Yang L, Han Y, Suarez Saiz F, et al (2007). A tumor suppressor and oncogene: the WT1 story. *Leukemia*, 21(5):868-876.
- Yuichi Ishikawa, Hitoshi Kiyoi, Tomoki Naoe (2011). Prevalence and clinical characteristics of N-terminally truncated WT1 expression in acute myeloid leukemia. *Leukemia Research* 35, 685–688.
- Zhen Zhao, Johannes Zuber, Ernesto Diaz-Flores, Laura Lintault, Scott C. Kogan, Kevin Shannon, and Scott W. Lowe (2010). p53 loss promotes acute myeloid leukemia by enabling aberrant self-renewal. *Genes & Development*, 24:1389–1402.
- Zhou M, Gu L, Yeager AM, Findley HW (1998). Sensitivity to Fas-mediated apoptosis in pediatric acute lymphoblastic leukemia is associated with a mutant p53 phenotype and absence of Bcl-2 expression. *Leukemia* 12:1756–1763.
- Zhu YM, Das-Gupta EP, Russell NH (1999). Microsatellite instability and p53 mutations are associated with abnormal expression of the MSH2 gene in adult acute leukemia. *Blood* 94:733–740.