UMEÅ UNIVERSITY MEDICAL DISSERTATIONS

New Series No. 1799 ISSN 0346-6612 ISBN 978-91-7601-458-5

Significance of Wilms' Tumor Gene 1 as a Biomarker in Acute Leukemia and Solid Tumors

Charlotta Andersson

Department of Medical Biosciences, Clinical Chemistry and Pathology
Umeå University, Sweden
Umeå 2016

ISBN: 978-91-7601-458-5

ISSN: 0346-6612

Printed by: Print & Media

Umeå, Sweden, 2016

In memory of Aihong Li

Table of Contents

Abstract

Wilms' tumor gene 1 (*WT1*) is a zinc finger transcriptional regulator with crucial functions in embryonic development. Originally *WT1* was described as a tumor suppressor gene, but later studies have shown oncogenic properties of *WT1* in a variety of tumors. Because of its dual functions in tumorigenesis, *WT1* has been described as a chameleon gene. In this thesis, the significance of *WT1* as a biomarker was investigated in acute myeloid leukemia (AML), clear cell renal cell carcinoma (ccRCC), ovarian carcinoma (OC) and childhood B-cell precursor acute lymphoblastic leukemia (BCP-ALL).

Previous studies have suggested that expression of *WT1* is a potential marker for detection of minimal residual disease (MRD) in AML. We aimed to define expression of *WT1* as an MRD marker in AML. In adult AML patients, we found that a reduction of *WT1* expression in bone marrow (≥ 1-log) detected less than 1 month after diagnosis was associated with an improved overall survival (OS) and freedom from relapse (FFR). In peripheral blood, a reduction of *WT1* expression (≥ 2-log) detected between 1 and 6 months after treatment initiation was associated with an improved OS and FFR.

WT1 harbor pathogenic genetic variants in a considerable proportion of AML and T-lymphoblastic leukemia (T-ALL), but mutations have not been reported in BCP-ALL. We aimed to evaluate the clinical impact of *WT1* mutations and single nucleotide polymorphisms (SNPs) in BCP-ALL. Pathogenic mutations in the *WT1* gene were rarely seen in childhood BCP-ALL. However, five *WT1* SNPs were identified. In survival analyses, *WT1* SNP rs1799925 was found to be associated with worse OS, indicating that *WT1* SNP rs1799925 may be a useful marker for clinical outcome in childhood BCP-ALL. We also explored whether *WT1* mutations and SNPs in ccRCC could be used as biomarkers for risk and treatment stratification. We therefore examined whether SNPs or mutations in *WT1* were associated with *WT1* expression and clinical outcome. Sequencing analysis revealed that none of the previously reported *WT1* mutations were found in ccRCC; however, we identified six different *WT1* SNPs. Our data suggest that pathogenic *WT1* mutations are not involved in ccRCC, and the prognostic significance of *WT1* SNPs in ccRCC is considerably weak. However, a favorable OS and disease-specific survival were found in the few cases harboring the homozygous minor allele.

OC has a poor prognosis, and early effective screening markers are lacking. Serous OCs are known to express the WT1 protein. Overexpressed oncogenic proteins can be considered potential candidate antigens for cancer vaccines and T-cell therapy. It was therefore of great interest to investigate whether anti-WT1 IgG antibody (Ab) measurements in plasma could serve as biomarkers of anti-OC response. We found limited prognostic impact, but the results indicated that anti-WT1 IgG Ab measurements in plasma and WT1 staining in tissue specimens could be potential biomarkers for patient outcome in the high-risk subtypes of OCs.

In conclusion, the results of this thesis indicate that *WT1* gene expression can provide information about MRD of patients with AML, and *WT1* SNP rs1799925 may be used as a biomarker for predicting clinical outcome in childhood BCP-ALL. In ccRCC, the prognostic significance of *WT1* SNPs is weak and limited to the subgroup of patients that are homozygous for the minor allele. In OCs anti-WT1 IgG Ab measurement in plasma and WT1 staining in tissue specimens could possibly be used as biomarkers for predicting patient outcome in the high-risk subtypes of OCs.

Abbreviations

AA	Amino Acids
Ab	Antibody
AL	Acute Leukemia
ALL	Acute Lymphoblastic Leukemia
AML	Acute Myeloid Leukemia
BCP-ALL	B-Cell Precursor Acute Lymphoblastic Leukemia
BM	Bone Marrow
ccRCC	Clear Cell Renal Cell Carcinoma
CG	Control Gene
CN-AML	Cytogenetically Normal AML
CR	Complete Remission
DDS	Denys–Drash Syndrome
DNMT3A	DNA Methyltransferase 3A
DSS	Disease Specific Survival
EFS	Event Free Survival
ELISA	Enzyme-Linked Immunosorbent Assay
ELN	European LeukemiaNet
EOC	Epithelial Ovarian Carcinomas
FAB	French-American-British
FFR	Freedom From Relapse
HGSC	High-Grade Serous Carcinoma
IgG	Immunoglobulin G
IHC	Immunohistochemistry
Kb	Kilo Base Pairs
kDa	Kilo Dalton
KTS	Lysine, Threonine and Serine
LGSC	Low-Grade Serous Carcinoma
LOH	Loss Of Heterozygosity
MAPK	Mitogen-Activated Protein Kinase
MDS	Myelodysplastic Syndrome
MRD	Minimal Residual Disease
NGS	Next-Generation Sequencing
NSCLC	Non-Small Cell Lung Cancer
OC	Ovarian Carcinoma
OS	Overall Survival
PB	Peripheral Blood
PCR	Polymerase Chain Reaction
PFS	Progression Free Survival
RCC	Renal Cell Carcinoma
RFS	Relapse Free Survival
RQ-PCR	Real-Time Quantitative PCR
SNP	Single Nucleotide Polymorphism
T-ALL	T-Acute Lymphoblastic Leukemia
TNM	Tumor-Node-Metastasis
VHL	Von Hippel-Lindau
WAGR	Wilms' Tumor, Aniridia, Genitourinary abnormalities, Mental retardation
WHO	World Health Organization
WRU	WT1 Reacting Unit
WT	Wilms' Tumor
WT1	Wilms' Tumor Gene 1
WT1	Wilms' Tumor Gene 1 (protein)

List of Papers

This thesis is based on the following papers and manuscripts, which are referred to in the following text by their corresponding Roman numerals (I-IV):

I. **Andersson C**, Li X, Lorenz F, Golovleva I, Wahlin A, Li A. *Reduction in WT1 gene expression during early treatment predicts the outcome in patients with acute myeloid leukemia*. Diagn Mol Pathol. 2012. **21**(4): p 225-33.

II. Li X, Wang S, Sitaram RT, **Andersson C**, Ljungberg B, Li A. *Single nucleotide polymorphisms in the Wilms' tumour gene 1 in clear cell renal cell carcinoma*. PLoS One. 2013. **8**(3):e58396. doi: 10.1371/journal.pone.0058396.

III. **Andersson C**, Oji Y, Ohlson N, Wang S, Li X, Ottander U, Lundin E, Sugiyama H, Li A. *Prognostic significance of specific anti-WT1 IgG antibody level in plasma in patients with ovarian carcinoma*. Cancer Med, 2014. **3**(4): p. 909-18.

IV. Ottosson S, **Andersson C**, Li X, Wang S, Nilsson S, Li A. *Analysis of single nucleotide polymorphisms and mutational status of the Wilms' tumor gene 1 in childhood B-cell precursor acute lymphoblastic leukemia*. Manuscript.

Introduction

Wilms' tumor (WT), also called nephroblastoma, was first characterized by the German pathologist and surgeon Dr Carl Max Wilhelm Wilms (1867-1918) in 1899. His diagnosis of WT was based on clinical and histological appearance, the latter typically being a triphasic renal tumor consisting of blastemal, epithelial and stromal elements [1]. Max Wilms was unfortunately infected with diphtheria and died in May 1918. Almost 100 years after the publication of his monograph on the pathology of the childhood kidney tumors, it was discovered that a deletion of chromosome region 11p13 was linked to WT [2-4]. The gene was later isolated and named Wilms' tumor gene 1 (*WT1*). *WT1* gene was found to encode a putative zinc finger transcriptional regulator with crucial functions in embryonic development and was originally described as a tumor suppressor gene [2]. However, owing to *WT1* overexpression in a variety of solid cancers that normally do not express *WT1*, it has later been suggested that *WT1* might play an oncogenic role [5]. The WT1 protein has been demonstrated as a promising target for cancer immunotherapy. Clinical trials of WT1 peptide vaccination in patients with myeloid malignancies and several solid cancers has resulted in positive outcomes [6].

WT1 structure

The *WT1* gene spans approximately 50 kb DNA at chromosome locus 11p13. The gene consists of 10 exons and encodes an mRNA transcript of about 3 kb. The mRNA translates into a 449-amino-acid protein with a proline- and glutamine-rich amino terminus harboring defined functional domains that exert transcriptional repression, activation, self-association, DNA binding, RNA recognition and nuclear localization signals [7, 8]. The carboxy terminal domain of WT1 contains four Krüpple-like, $cysteine_2$-$histidine_2$ zinc fingers encoded by exon 7-10, which are involved in RNA and protein interactions that permit binding to DNA sequences. This DNA-binding domain of WT1 shares high homology with the zinc finger region of early growth response protein 1 [9, 10]. In addition to binding DNA and some proteins, zinc fingers can regulate RNA targets and mediate nuclear localization [8]. Besides binding to other proteins, WT1 can also self-associate, and the major domain required for this self-association has been mapped to the first 182 amino acids of WT1 [10].

In mammals, exons 5 and 9 of *WT1* pre-mRNA are alternatively spliced, giving rise to four different splice isoforms designated A, B, C and D [11, 12]. The molecular weight of the WT1 proteins has been variously reported

between 49 and 54 kDa [13]. The first alternative splicing event affects the entire exon 5 and leads to the presence or absence of 17 amino acids (AA) between the proline/glutamine-rich terminal domain and the carboxy terminal zinc finger domain of WT1. The second alternative splicing event generates either inclusion or exclusion of three AA—lysine, threonine and serine (KTS)—at the end of exon 9, affecting the conformation of zinc fingers three and four in the WT1 protein. These two isoforms (+KTS and –KTS) are conserved in all vertebrates and fish, and non-mammalian vertebrates appear to express only these two variants [14]. The mRNA isoform containing both splice inserts is the most prevalent variant in both human and mouse, whereas the least common is the transcript missing both inserts [12]. Studies have demonstrated that WT1 isoforms lacking the KTS insertion (–KTS) bind to DNA more strongly and act as transcriptional regulators [15]. The gene product that contains the insertion (+KTS) also acts as a transcriptional regulator, in addition to being associated with post-transcriptional processes. The use of an upstream CTG start codon or an internal ATG start codon at the end of exon 1 result in a truncated isoform [5]. Another WT1 isoform, AWT1, arises from the use of an alternative promoter that resides within exon 1 [16].

Also, alternative *WT1* mRNAs are generated through RNA editing at nucleotide 839 where leucine 280 is replaced by proline [17]. The *WT1* gene may thus produce different mRNA isoforms, suggesting that each isoform has a distinct contribution to the function of the *WT1* gene and that balanced expression of the isoforms is essential for proper WT1 function [7].

Furthermore, other larger and smaller WT1 isoforms have been identified. Additional isoforms of WT1 can arise from alternative translation start points. Translation initiated at a CUG upstream of the initiator AUG [18] results in WT1 protein isoforms with molecular masses of 60-62 kDa. An evolutionary conserved internal translation initiation site at the second in-frame AUG (AUG127) of the *WT1* mRNA results in amino terminally truncated WT1 isoforms, with molecular masses of 36-38 kDa [19]. Both larger and smaller WT1 isoforms can be detected in different mammalian tissues. WT1 has also been reported to be post-translationally modified by phosphorylation, ubiquitylation and sumoylation [20-22]. The existence of other WT1 posttranslational modifications, such as acetylation or methylation, has yet to be determined [23].

Hence, the *WT1* gene encodes multiple protein isoforms, which are generated by a combination of alternative splicing of DNA, alternative translation start sites, alternative RNA splicing and RNA editing. The

number of isoforms generates a considerable potential for diverse functions of WT1 proteins.

WT1 target genes

Many functions have been ascribed to the multiple WT1 protein isoforms over the last decades. The most well-documented function for WT1 protein is that of a transcription factor. WT1 promotes gene activation or repression depending on cellular and promoter context [24]. Many genes have been identified by various approaches to be regulated, either positively or negatively, by WT1. The identification of WT1 target genes is an ongoing and difficult process due to the isoform- and tissue-context-specific roles of WT1. An extensive number of WT1 target genes have been identified and thematically grouped [7, 23].

Proposed target genes transcriptionally regulated by WT1 include:

Genes involved in growth and development

Many WT1 target genes underscore the growth-regulatory effect of WT1. Examples include many genes encoding for growth factors and their receptors, cell-cycle-regulation and development. WT1 exerts activation of genes encoding for insulin-like growth factor 2 [25], amphiregulin [26], erytropoetin [27], erytropoetin receptor [28], Dax-1 [29], Sry [30], anti-Müllerian hormone type 2 receptor [31], Sprouty 1 [32], nestin [33], class IV POU-domain factor [34], taurine transporter gene [35], WT1-induced inhibitor of Dishevelled [36] and TrkB neurotrophin receptor [37]. The syndecan-1 protein, which has been found to have a role in the mesenchymal–epithelial transition, is trancriptionally activated by WT1 [38] and the developmental regulatory gene *paired box 2* is repressed by WT1 during normal kidney development [39].

Furthermore, genes encoding for connective tissue growth factor, platelet-derived growth factor A, colony-stimulating factor 1, transforming growth factor-beta, insulin receptor, insulin-like growth factor 1 receptor, androgen receptor, estrogen receptor A and epidermal growth factor receptor are reported to be repressed by WT1 [40-48].

Genes involved in differentiation, cytoskeleton organization and cell adhesion

WT1 plays a role in the control of differentiation. Genes of cell cycle regulating proteins such as p21 [49] and retinoblastoma suppressor associated protein 46 are activated by WT1 [50], but cyclin E [51] and ornithine decarboxylase are repressed [52]. Hartwig *et al.* performed

genomic characterization of WT1 targets in nephron progenitor cells during kidney development [53]. They identified several WT1 target genes, including genes involved in actin cytoskeleton organization and biogenesis, cell adhesion and cell–cell signaling [53].

Genes involved in WNT signaling and MAPK signaling

A previous genome-wide screening study performed by Kim *et al.* identified genes directly regulated by WT1 and functionally grouped them into MAPK signaling, axon guidance and WNT pathways [54]. Among genes directly bound and regulated by WT1, nine were identified in the WNT signaling pathway, suggesting that WT1 modulates a subset of WNT components and responsive genes by direct binding [54].

Genes involved in apoptosis

Several genes that regulate apoptosis are WT1 target genes. Genes upregulated by WT1 are the *Bcl-2 proto-oncogene* [55], *A1/BFL1* [56] and pro apoptotic Bcl-2 family member *Bak* [57]. The proto-oncogene *JunB* is repressed by WT1 [58]. The anti-apoptotic function of WT1 is also evident in development. WT1-null mice display an increase in apoptosis of metanephric mesenchyme, which leads to complete agenesis of the kidneys [59].

Genes involved in epigenetic regulation

Several studies have shown that the gene products of WT1 targeted genes are involved in epigenetic regulation of gene expression. Szemes *et al.* have demonstrated that WT1 transcriptionally regulates the de novo DNA methyltransferase 3A (DNMT3A) and that cellular WT1 levels can influence DNA methylation of gene promoters genome-wide. Furthermore, they also demonstrated elevated DNMT3A at hypermethylated genes in WT cells, including a region of long-range epigenetic silencing. They also demonstrated that depletion of WT1 in WT cells resulted in reactivation of gene expression from methylated promoters, such as Transforming Growth Factor Beta 2, known as a key modulator of epithelial-mesenchymal transitions [60].

Other target genes

Other WT1 target genes that can be transcriptionally activated include the genes encoding for vitamin D receptor [61], E-cadherin [62] and SMAD3 [63]. WT1 has also been shown to suppress expression of the gene encoding for human telomerase reverse transcriptase [63].

WT1 protein partners

In addition to target genes, WT1 also interacts with protein partners. WT1 is known to bind to several proteins in analogy with the transcriptional regulatory role of WT1. Many of these proteins are also transcription factors that regulate *WT1* [7]. Identification of protein partners may reveal novel information on how *WT1* is involved in cellular proliferation and differentiation. The interaction partners of WT1 DNA-binding proteins can be separated into five categories [23]. WT1 interacting partners in the first two categories are involved in transcriptional regulation (DNA-binding transcription factors and transcriptional co-regulators), whereas members of the last three categories are involved in post-transcriptional regulation, proteolysis of WT1 and epigenetic regulation.

WT1 in normal and abnormal development

The WT1 protein is indispensable for normal development of the genitourinary system. The metanephric kidney is formed through reciprocal inductive signals between the mesodermal mesenchyme and the ureteric bud, an outgrowth of the Wolffian duct [64, 65]. Initially, the proliferating mesenchyme condenses around the ureteric bud and, by unknown signals, induces bud branching necessary for nephrogenesis. Consistent with a prominent role for WT1 in the differentiation of the metanephric mesenchyme, it has been demonstrated that WT1 can induce features of renal epithelial differentiation in mesenchymal fibroblasts [66]. During subsequent nephrogenesis, WT1 continues to be expressed in the posterior part of the nephron, while in the mature nephron WT1 protein expression is restricted to the podocytes [67-69]. Development of several other organs and tissues also requires WT1. WT1-null mice are embryonic lethal with complete agenesis of the kidneys, gonads, heart, diaphragm, spleen and adrenal glands, and they die of heart failure caused by thinning of the epicardium [59, 70, 71]. Later studies have also determined an important role of WT1 in the development of neuronal tissue, olfactory epithelia, retina ganglia and peripheral taste system [72-74].

The cloning of the *WT1* gene has been facilitated by the mapping of deletions in chromosome 11p13 of patients with WAGR syndrome (WT, aniridia, genitourinary abnormalities, mental retardation) [3, 4, 75, 76]. Two other syndromes are also associated with germline mutations in the *WT1* gene (Table 1): Denys-Drash syndrome (DDS) [77] and Fraiser syndrome. DDS is similar to WAGR and includes a predisposition to the development of WT. However, in contrast to WAGR, these patients have much more severe genitourinary abnormalities, including pseudohermaphroditism and streak gonads, a form of gonadal aplasia. DDS patients also have the additional finding of glomerulonephropathy. The majority of germline heterozygous

point mutations associated with DDS occurred in the zinc finger 2 or 3 region and were shown to disrupt DNA binding by WT1 [78]. The other congenital syndrome related to alterations in the *WT1* gene is Frasier syndrome which is caused by nucleotide variants in the *WT1* intron 9 donor splice site [79, 80].

Table 1. Congenital syndromes associated with mutations in *WT1* (Adapted from Scharnhorst *et al.*, 2001 [7])

Syndrome	***WT1* status**	**Phenotype**
WAGR	Heterozygous deletion at chromosome 11p13	Wilms' tumor; Aniridia; Genitourinary abnormalities; Retardation Infrequent gonadoblastoma
DDS	Heterozygous point mutations (in zinc fingers)	Diffuse mesangial sclerosis causes glomerular nephropathy Often Wilms' tumor Females: normal gonads Males: phenotype of gonads varies, appearing as streak gonads, female internal and external genitalia, or mild hermaphroditism
Frasier	Heterozygous point mutation in splice donor site in intron 9; occasional mutation within exon 9	Glomerulopathy characterized by unspecific focal and segmental glomerular sclerosis; one case of Wilms' tumor reported; gonads: male-to-female sex reversal (female external genitalia, streak gonads, XY karyotype), frequently gonadoblastoma

Abbreviations: DDS: Denys-Drash Syndrome, WAGR: Wilms' tumor, Aniridia, Genitourinary abnormalities, Mental retardation.

Hematopoiesis and *WT1*

Hematopoiesis is the process of production of all blood cells from a few hematopoietic stem cells, which occurs in the bone marrow (BM) [81]. Expression of *WT1* in human cells of hematopoietic origin has suggested a role for *WT1* in control of proliferation and differentiation of hematopoietic cells. In human hematopoiesis, few (~1%) of the CD34+ multipotent progenitor cells in both the uncommitted quiescent (CD38-) fraction and the committed (CD38+) fraction in the BM express *WT1*. During differentiation the expression is rapidly downregulated [82-85]. The WT1 protein is highly expressed in the majority of patients with leukemia and in leukemic cell lines [86-89]. It has been a matter of debate whether *WT1* is overexpressed in leukemic cells compared to normal hematopoietic progenitors. A report using single cell analysis of *WT1* expression shows that *WT1* expression is normally restricted to a small subset of hematopoietic progenitor cells and that the expression levels per cell among these progenitors are quite similar to those in leukemic cells. Thus, the results could indicate that *WT1* expressing CD34+ BM cells were normal counterparts of leukemia cells and that *WT1* expression correlates to immaturity rather than a malignant phenotype [83].

***WT1* as a tumor suppressor gene**

WT1 was initially discovered as a tumor suppressor gene in WT and *WT1* has been proven to function as a classic tumor suppressor of WT growth in multiple genetic and experimental studies, as reviewed by Scharnhorst *et al.*, 2001 [7]. WT is known to be genetically heterogeneous, and *WT1* mutations are present in only about 20% of WT [90, 91]. The ability of *WT1* to induce growth suppression and suppress tumorigenesis in mice also highlights its potential role as a tumor suppressor [92-94]. *WT1* has also been found to induce programmed cell death in osteosarcoma cell lines [48] and induce apoptosis in the Saos-2 cell line and B16F10 murine melanoma cell line [95]. *WT1* can also downregulate growth factor receptors such as the epidermal growth factor receptor and the insulin receptor, altering the balance of survival signals toward death [96].

***WT1* as an oncogene or a chameleon gene**

Whereas *WT1* behaves as a tumor suppressor gene in WT, a wealth of data on the overexpression of *WT1* in a variety of human cancers of both hematological and non-hematological origin suggests *WT1* plays a role as an oncogene. Overexpression of WT1 has been demonstrated in carcinomas from a variety of origins, including lung cancer [97, 98], breast cancer [99], colon cancer [100], pancreatic cancer [101], ovarian cancer [102, 103], primary astrocytic tumors [104], sarcomas [105], malignant melanoma [106], mesothelioma [107] and other tumors. *WT1* has been shown to

regulate the cell cycle, by contributing to the pro-proliferative effect. WT1 protein also exerts transcriptional regulation of growth factors and growth factor receptors and has the potential to increase apoptosis, which contributes to WT1-regulated cell survival. In breast cancer cells, ablation of the WT1 protein led to increased apoptosis and cell cycle arrest at G1 [108]. Studies have also revealed a role of WT1 in angiogenesis and vascularization, endothelial cell proliferation and migration, which are important steps for tumor growth [109]. Regarding tumor treatment, peptide vaccines against WT1 are showing promising results in suppressing tumor growth in patients with leukemia, breast and lung cancer [110]. In addition, the treatment of tumor cell lines with cytotoxic drugs leads to proteolysis of WT1 by the serine protease HtrA2 [111]. This process could potentially be exploited to target malignancies in which *WT1* acts as an oncogene.

The oncogenic role of *WT1* in leukemia has been extensively studied, and the role of *WT1* in leukemia appears to be complex and also contradictory. Overexpression of *WT1* has been reported in acute myeloid leukemia (AML), chronic myeloid leukemia, acute lymphoblastic leukemia (ALL) and myelodysplastic syndrome (MDS) [89, 112, 113]. Collectively, these studies strongly suggest a tumor-promoting or oncogenic role of *WT1* in leukaemogenesis. However, some findings support a tumor suppressor role of *WT1* in leukemia. A considerable proportion of AML and precursor T-cell lymphoblastic leukemia (T-ALL) shows *WT1* mutations. The first report of somatic *WT1* mutations associated with development of AML was published in 1994 by Pritchard-Jones *et al.* [114]. Since then, large cohort studies of cytogenetically normal AML (CN-AML) cases have confirmed the frequency of about 10% mutated *WT1* in adult AML [115-117]. The incidence of *WT1* mutations in T-ALL is reported in the same interval as AML, and most of the detected T-ALL mutations are similar to those observed in AML, resulting in truncation of the zinc-finger domain of WT1 [118]. The majority of mutations involve insertions, deletions and missense mutations such as those observed in patients with DDS [119]. These data suggest that as in WT, *WT1* has a tumor suppressor function in leukemia. The oncogenic or tumor-suppressive effect of *WT1* alterations is likely to be a result of how a cell at a particular stage of development responds to perturbations in the expression of those genes [119]. In 2011 Huff V. suggested retirement of the labels “tumor suppressor” and “oncogene” to describe the *WT1* function and introduced the ingenious label “chameleon gene” for *WT1* [119].

Acute leukemia (AL) and *WT1* in AL

The word “leukemia” originates from the Greek word *leukos*, meaning white, and *haima*, meaning blood. Leukemia is a malignant disease of hematopoietic tissues. Patients suffering from leukemia often have high

amounts of white blood cells because of accumulation of immature dysfunctional white blood cells in the BM and peripheral blood (PB). Patients may have symptoms of fatigue, sweating, emaciation, bleeding and infections. The transition of a normal cell to a leukemic cell depends on genetic changes, which lead to disturbed gene and protein functions in cells. Today, we have a large but yet incomplete knowledge about the pathogenic genetic and epigenetic changes involved in the development of leukemia. Worldwide, the overall incidence of AL is approximately 4/100,000 population per year, with 70% of these cases being AML. ALL is predominantly a disease of children, with 75% of cases occurring in patients under 6 years of age. The vast majority of cases of AML occur in adults with a median age of 60 years [120]. Possible etiological factors associated with leukemia include viruses, ionizing radiation, cytotoxic chemotherapy and benzene [121]. Leukemia is classified as myeloid or lymphoid depending on the differentiation status of the cells and then further divided into chronic or acute leukemia.

There are two major systems that are used to classify leukemia. The first generally accepted uniform classification system, the French-American-British (FAB), was published in 1976 [122]. The classification was based on the morphological characteristics of the leukemic blasts in association with cytochemical reactivity patterns. The FAB-classification was revised in 1985 [123] and was used until 2001, when the World Health Organization (WHO) introduced a new classification [120] that also took into account medical history and cytogenetic and immunophenotypic findings. The WHO classification was updated in 2008 [124]. According to the FAB criteria AL was separated into myeloid and non-myeloid leukemia. The myeloid leukemia group contained eight types, FAB M0-M7, and three lymphoblastic types of leukemia, FAB L1-L3. All forms of AML requested more than 30% blasts in the BM. In the WHO classification, the blast threshold for diagnosis of AML was reduced from 30% to 20% blasts in the PB or BM. However, patients with the recurrent cytogenetic abnormalities t(8;21)(q22;q22), inv(16)(p13q22) or t(16;16)(p13;q22), and t(15;17)(q22;q12) should be considered to have AML regardless of blast counts. AML was divided into four main groups: AML with recurrent genetic abnormalities, AML with multilineage dysplasia, therapy-related AML and AML not otherwise categorized. The former AML M3 (defined as AML t(15;17)(q22;q12)) was renamed acute promyelocytic leukemia.

According to the WHO classification, ALL FAB L1 and L2 were subdivided into Precursor B-cell ALL and Precursor T-cell ALL. ALL FAB L3 corresponds to Burkitt leukemia. The blast threshold in the WHO-classification for ALL is 25%; if lower blasts and signs of a mass lesion are present, they should instead be considered lymphomas. The current WHO classification is presented in Table 2.

Table 2. AML and related precursor neoplasms and precursor lymphoid neoplasms (Adapted from WHO 2008 [124])

ACUTE MYELOID LEUKEMIA
Acute myeloid leukemia with recurrent genetic abnormalities
- AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFC-MYH11*
- Acute promyelocytic leukemia with t(15;17)(q22;q12); *PML-RARA*
- AML with t(9;11)(p22;q23); *MLLT3-MLL*
- AML with t(6;9)(p23;q34); *DEK-NUP214*
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q25.2); *RPN1-EVI1*
- AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1*
- AML with mutated *NPM1*
- AML with mutated *CEBPA*

Acute myeloid leukemia with myelodysplasia-related changes
Therapy-related myeloid neoplasms
Acute myeloid leukemia, not otherwise specified
- AML with minimal differentiation
- AML without maturation
- AML with maturation
- Acute myelomonocytic leukemia
- Acute monoblastic and monocytic leukemia
- Acute erythroid leukemia
- Acute megakaryoblastic leukemia
- Acute basophilic leukemia
- Acute panmyleosis with myelofibrosis

Myeloid sarcoma
Myeloid proliferations related to Down syndrome
- Transient abnormal myelopoiesis
- Myeloid leukemia associated with Down syndrome

Blastic plasmacytoid dendritic cell neoplasm

ACUTE LEUKEMIA OF AMBIGUOUS LINEAGE
Acute undifferentiated leukemia
Mixed phenotype acute leukemia with t(9;22)(q34;q11.2); *BCR-ABL1*
Mixed phenotype acute leukemia with t(v;11q23); *MLL* rearranged
Mixed phenotype acute leukemia, B/myeloid, not otherwise specified
Mixed phenotype acute leukemia, T/myeloid, not otherwise specified
Mixed phenotype acute leukemia, not otherwise specified – rare types
Other ambiguous lineage leukemia
- Natural killer (NK) cell lymphoblastic leukemia/lymphoma

PRECURSOR LYMPHOID NEOPLASMS
B lymphoblastic leukemia/lymphoma not otherwise specified
B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
- B lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2); *BCR-ABL1*
- B lymphoblastic leukemia/lymphoma with t(v;11q23); *MLL* rearranged
- B lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22); *TEL-AML1 (ETV6-RUNX1)*
- B lymphoblastic leukemia/lymphoma with hyperdiploidy
- B lymphoblastic leukemia/lymphoma with hypodiploidy (hypodiploid ALL)
- B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32); *IL3-IGH*
- B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3); *E2A-PBX1 (TCF3-PBX1)*

T lymphoblastic leukemia/lymphoma

Prognostic factors in AML may be subdivided into those related to patient characteristics and general health condition and those related to characteristics of the AML clone. Patient-related prognostic factors are age, comorbidities, and previous existence of prior MDS or previous cytotoxic therapy for another disorder. Clone-related prognostic factors are chromosome and molecular abnormalities [125]. Genetic abnormalities are the strongest known prognostic factor. A version of risk groups for adult patients with AML are given in Table 3.

Table 3. Cytogenetic/molecular risk groups for adult patients with AML (Adapted from Döhner *et al.*, 2010 [125])

Favorable
t(8;21)(q22;q22); *RUNX1-RUNX1T1*
inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
Mutated *NPM1* without *FLT3*-ITD (normal karyotype)
Mutated *CEBPA* (normal karyotype)

Intermediate-I
Mutated *NPM1* and *FLT3*-ITD (normal karyotype)
Wild-type *NPM1* and *FLT3*-ITD (normal karyotype)
Wild-type *NPM1* without *FLT3*-ITD (normal karyotype)

Intermediate-II
t(9;11)(p22;q23); *MLLT3-MLL*
Cytogenetic abnormalities not classified as favorable or adverse

Adverse
inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
t(6;9)(p23;q34); *DEK-NUP214*
t(v;11)(v;q23); *MLL* rearranged
-5 or del(5q); -7 or del(7q); del(17p); complex karyotype

In ALL, current risk factor estimation is based on a number of criteria, including age, leukocyte count and cytogenetics. Hyperdiploidy and t(12;21) (p13;q22) are associated with favorable prognosis, while hypodiploidy and presence of the Philadelphia chromosome, t(9;22) (q34;q11.2), confers an adverse prognostic effect [126]. Furthermore, 11q23/MLL rearrangements are found in approximately 80% of infants with childhood B-cell precursor acute lymphoblastic leukemia (BCP-ALL) and are associated with a highly dismal prognosis [127]. Despite the improved 5-year prognosis, ALL remains the main cause of disease-related death in children, and current prognostic factors cannot entirely answer for clinical outcome [128, 129]. Inter-patient variation in treatment responses has been suggested as a possible result of germline genetic variations of the host. For example, low-hypodiploid ALL (with 32-39 chromosomes) is associated with germ-line alterations in *TP53* [130].

Another prognostic factor in AL is minimal residual disease (MRD). Current treatment protocols are based on these prognostic factors, which contribute to individualized therapy and risk-adapted intensification [125]. Studies have shown that detection of MRD in AML is prognostically relevant [131-133]. Recently Grimwade and Freeman [134] presented a rationale for detection of MRD in AML. Given the heterogeneity of AML in terms of genetic and immunophenotypic profile they concluded that a "one size fits all" approach should be considered unrealistic. Achieving standardization of each methodology is clinically important. The monitoring of MRD as determined by RT-PCR detecting leukemia-specific targets (eg, gene fusions, gene mutations, overexpressed genes), by multiparameter flow cytometry or newer molecular technologies identifying leukemia-associated aberrant phenotypes remains an active and important field of investigation.

WT1 is highly expressed in the majority of AML patients, and there has been interest as to whether *WT1* could provide prognostic information and function as a universal molecular MRD marker. The majority of AML patients express *WT1* at diagnosis, as it appears in 73-93% of patients [135]. Several authors have reported that expression of the *WT1* gene at diagnosis may be predictive of outcome [87, 136, 137]. However, some studies have found no prognostic relevance for the level of *WT1* expression at diagnosis [131, 133, 138]. Some reports didn't show any significant association between *WT1* expression and different FAB subtypes [139], while other reports have indicated higher *WT1* expression in M3 AML [140] and fewer *WT1* transcripts in M5 AML [136, 137, 140].

In addition to high *WT1* expression, mutations in the *WT1* gene have also been found in leukemia. *WT1* mutations and single nucleotide polymorphisms (SNPs) have also been suggested as prognostic factors. *WT1* mutations are more frequent in CN-AML or in AML with mutations in the *FLT3* gene [115, 141]. *WT1* mutations found in AML are mostly heterozygous, with a remaining *WT1* wild-type allele. They are missense mutations, deletions and insertions that result in a truncated WT1 protein with loss of the DNA-binding domain [117]. The mutational "hotspots" are in exon 7 and 9. Heterozygous mutations could also lead to haploinsufficiency of the *WT1* function. The reported homozygous *WT1* mutations in AML are biallelic through loss of heterozygosity (LOH) due to somatic acquired uniparental disomy, which implies that mutation precedes mitotic recombination, which acts as a "second hit" responsible for removal of the remaining wild-type allele [142]. The incidence of *WT1* mutations in T-ALL is similar to the incidence in AML. Regarding *WT1* mutations in BCP-ALL, no data are available in the literature. Haploinsufficency of *WT1* as a result of *WT1* mutations contributing to leukemic disease may seem in agreement with

WT1's role as a tumor suppressor gene. Other studies strongly suggest an oncogenic role for *WT1* in leukemogenesis [143]. If *WT1* can act as an oncogene, mutated *WT1* may show a gain of function, becoming more oncogenic as compared with wild-type *WT1*. The coexistence of *WT1*-mutations and FLT3-ITD in CN-AML patients may indicate a synergistic effect in leukemogenesis [144].

Unlike DNA mutations, synonymous SNPs encode a substitution in the DNA sequence without altering the resultant proteins [145]. It is still much disputed whether synonymous SNPs are a gene variation with a functional role. However, "silent" SNPs have been found to be associated with over 50 diseases [146]. The mechanisms of this effect have not yet been fully understood. It is suggested that synonymous SNPs have the ability to alternate mRNA splicing, stability and expression, as well as protein folding [147, 148]. Recently, clinical interest has been raised regarding the prognostic impact of SNP rs16754 in *WT1* exon 7 for patients with leukemia. This exon has two alleles that can harbor the nucleotide adenine (A) or guanine (G); the result is a homozygous ($WT1^{AA}$ or $WT1^{GG}$) or heterozygous ($WT1^{AG}$) genotype. The minor allele frequency of *WT1* SNP rs16754 ($WT1^{AG}$ or $WT1^{GG}$) has been reported in approximately 27% of patients with AML [149-152]. In a German study, patients with CN-AML who were carrying rs16754 ($WT1^{AG}$) and rs16754 ($WT1^{AA}$) genotypes were found to have a better outcome compared to patients with the rs16754 ($WT1^{GG}$) genotype [149]. However, in a large Cancer and Leukemia Group study, patients with CN-AML who had the rs16754 ($WT1^{GG}$) genotype had a more favorable outcome among a subset of patients with *FLT3*-ITD [152]. Nevertheless, in a Korean cohort, the different genotypes of rs16754 did not have any significant impact on clinical outcome in CN-AML [153].

Renal cell carcinoma (RCC) and *WT1* in RCC

RCC accounts for about 2-3% of all adult malignancies [154]. In Sweden, around 1000 RCC cases are diagnosed every year. Epidemiological studies have identified several risk factors for RCC, including tobacco smoking, exposure to carcinogenic arsenic compounds, overweight and blood hypertension [155]. The roles of other described risk factors like analgesics; exposure to asbestos, gasoline or trichloroethylene; and protective effects of alcohol, fruit and vegetables are less clear [156]. Most RCCs are sporadic, but they can also occur in hereditary forms. Approximately 3-4% can be explained by genetic predisposition [157].

Malignant parenchymal neoplasm of the kidney is classified into five subtypes according to the Heidelberg criteria [158]. The five most common RCC types are:

1. Clear cell RCC (ccRCC)
2. Papillary RCC
3. Chromophobe RCC
4. Collecting duct RCC
5. Renal cell carcinoma, unclassified

RCC is classified according to the current WHO classification of RCC, which summarizes the achievements and contributions of previous classifications [155]. ccRCC is the most common subtype of RCC, constituting 80-90% of RCC cases [158]. ccRCC is architecturally diverse, with solid, alveolar, trabecular and acinar patterns. The carcinomas typically contain a network of small thin-walled blood vessels. The cytoplasm is commonly filled with lipids and glycogen, which are dissolved in routine histologic procession, creating a clear cytoplasm surrounded by a distinct cell membrane. Sarcomatoid and fibromyxoid changes, ossification and calcifications occur in small proportions of ccRCC.

Tumor stage is the most important prognostic factor for predicting the survival of RCC patients. Tumor stage describes the anatomical extension of the disease and is assessed according to the TNM-system, UICC, 2009, Table 4 [159].

Table 4. TNM classification and stage grouping (UICC 2009, 7th edition [159])

Stage	TNM	Definition
I	T1 N0 M0	≤7 cm tumor diameter, confined to the kidney
		T1a: ≤4 cm tumor diameter
		T1b: >4 cm tumor diameter
II	T2 N0 M0	>7 cm tumor diameter, limited to the kidney
		T2a: tumor >7 to 10 cm
		T2b: tumor >10 cm
III	T3 N0-1 M0	Tumor extension into major veins or perinephritic tissues but not into the ipsilateral adrenal gland and not beyond Gerota fascia
		T3a: extends into the renal vein, perinephric fat
		T3b: extends into vena cava below the diaphragm
		T3c: extends into vena cava above the diaphragm
IV	T4 N0-1 M0-1	Tumor extension to other organs, beyond Gerota fascia

Abbreviations: Primary tumor (T), Regional lymph nodes (N), Distant metastasis (M).

After stage, nuclear grade is the most important prognostic feature of ccRCC. The 4-tiered nuclear grading system according to Fuhrman [160] is presented in Table 5.

Table 5. The 4-tiered nuclear grading system according to Fuhrman (Adapted from Eble *et al.* [155]).

Grade	Definition using the 10× objective
I	Small hyperchromatic nuclei (resembling mature lymphocytes) with no visible nucleoli and little detail in the chromatin
II	Finely granular "open" chromatin but inconspicuous nucleoli
III	The nucleoli must be easily unequivocally recognizable
IV	Nuclear pleomorphism, hyperchromasia and single to multiple nucleoli

Previous studies have demonstrated genetic abnormalities in ccRCC, of which inactivation of the tumor suppressor gene von Hippel-Lindau (*VHL*) plays a role in the pathogenesis [161]. Inactivation of the *VHL* gene can occur through hypermethylation or mutations, including deletions, insertions, missense, nonsense and splice junction alterations [162]. Up to 70% of sporadic ccRCC cases have a *VHL* somatic mutation and LOH [163, 164]. Only a few studies have investigated *WT1* in human RCC. In 1998, Campbell *et al.* demonstrated aberrant *WT1* expression in four out of five ccRCC samples and in several cell lines, which contraindicated *WT1* as a tumor suppressor [165]. However, other studies have demonstrated lower *WT1* expression in RCCs compared to the tumor-free kidney cortex [63, 166, 167], indicating that *WT1* acts as a tumor suppressor in ccRCC.

Ovarian carcinoma (OC) and *WT1* in OC

OC is the seventh most common cancer diagnosis among women worldwide and the fifth most common cancer diagnosis among women in higher-resource regions [168]. The world rate is estimated to be 6.3/100,000 women and is highest in high-resource countries (9.3/100,000 women) [168]. The incidence and mortality rates of OC have declined in the Nordic countries from the mid-1980s [169]. However, the prognosis is still poor, with 5-year relative survival around 40% in Sweden [169]. The use of oral contraceptives [170], parity and breastfeeding [171] are thought to have a protective effect against developing any subtype of OC because of decreased numbers of ovulation cycles (theory of incessant ovulation). The use of menopausal estrogen treatment increases the risk [172]. Genetic factors, like Breast Cancer 1 and Breast Cancer 2 gene (*BRCA1* or *BRCA2)* mutations

increase the risk for developing OC and breast cancer [173]. Approximately 90% of OCs are epithelial ovarian carcinomas (EOC). Based on histopathology, immunohistochemistry (IHC) and molecular genetic analysis, they are classified into different subtypes according to WHO histopathological standards, including serous, endometrioid, clear cell, mucinous and undifferentiated tumors [174]. Improved understanding of the molecular alterations involved in carcinogenesis has contributed to the conclusion that EOC is no longer considered a single entity. EOCs are nowadays considered different disease processes with each subtype having distinct genetic risk factors, underlying molecular events during oncogenesis, stages at diagnosis, and responses to chemotherapy, as reviewed by Gurung *et al.* [175]. Five subtypes of OC are described as sufficiently distinct and well characterized to be considered different diseases. An additional classification, based on molecular genetics, divides EOC into two categories, designated Type I and II [176, 177]. Type I tumors comprise low-grade serous carcinomas (LGSC), low-grade endometrioid carcinomas, clear cell carcinomas, mucinous carcinomas and Brenner tumors. They are generally indolent, present in TNM stage I (tumor confined to the ovary) and are characterized by specific mutations, including *KRAS*, *BRAF*, *ERBB2*, *CTNNB1*, *PTEN, PIK3CA*, *ARID1A* and *PPPR1A*, which target specific cell signaling pathways. Type I tumors rarely harbor *TP53* mutations and are relatively stable genetically [176]. Type II tumors are high-grade serous carcinomas (HGSC), high-grade endometrioid carcinomas, carcinosarcomas and undifferentiated carcinomas. They are aggressive, present in advanced TNM stage, and have a very high frequency of *TP53* mutations but only rarely harbor the mutations detected in Type I tumors. In addition, Type II tumors have molecular alterations that perturb expression of *BRCA* either by mutation of the gene or by promoter methylation. A hallmark of these tumors is that they are genetically highly unstable [176]. Serous carcinomas account for about 75% of EOC and are further divided into HGSC and LGSC, with distinct clinical and molecular features. Almost all serous tumors are HGSC.

HGSC is the most prevalent (70%) and most aggressive histological subtype and may arise in the fallopian tube epithelium, either directly from a carcinoma in the fallopian tube or from tubal epithelium implanted in the ovary [178]. HGSC is composed of epithelial cells displaying papillary, glandular (often slit-like) and solid patterns with high-grade nuclear atypia. Tumor necrosis is frequent, and mitoses are numerous [174]. Nuclear expression of WT1 is considered a useful marker for HGSC and is present in more than 90% of HGSCs [174]. Mutation in the *TP53* gene as well as post-translational induced dysfunction of TP53 is a hallmark for HGSC that is found in close to all cases and is detected immunohistochemically as

aberrant TP53 [179]. Another common feature in this subtype is inactivation (germline or somatic mutation or promoter methylation) of *BRCA1* and *BRCA2* in nearly one-half of HGSCs [173].

LGSC represents 3-5% of all EOC cases [180]. LGSC is an invasive carcinoma that usually appears in distinct patterns showing low-grade malignant cytological atypia [174]. Tumor necrosis is almost never detected, and the mitotic activity is significantly lower than in HGSC. Nuclear expression of WT1 is present in almost 100% of LGSCs [174]. *TP53* mutations are uncommon and have lower levels of chromosomal instability than HGSC. Common mutations are those of *KRAS*, *BRAF* and *ERBB2* oncogenes [177, 181, 182], all upstream of the MAPK, which result in activation of MAPK signaling and proliferation [183].

Endometrioid carcinomas represent the second most common form of EOC, approximately 10-15% of EOCs [174, 180]. Endometrioid carcinoma morphologically resembles endometrioid carcinoma of the uterine corpus. The frequent association of ovarian endometrioid carcinoma with endometriosis and endometrial carcinoma suggests that some ovarian endometrioid carcinomas may share risk factors with endometrial carcinomas [184]. The genetic profile involves mutations in *CTNNB1*, *PTEN*, *ARID1A*, *TP53*, *KRAS* and *BRAF* [174].

Clear cell carcinomas account for 10-12% of all EOC cases [180]. Clear cell carcinoma is a malignant tumor composed of clear eosinophilic and hobnail cells that displays a tubulocystic, papillary and solid pattern. For unknown reasons, this subtype has a higher prevalence in Japan relative to western countries [185]. Similar to endometrioid carcinoma, the majority of clear cell carcinomas originate from endometriotic lesions [174]. Clear cell tumors are normally *TP53* wild-type and have low chromosomal instability. Mutations in *ARID1A* and *PIK3CA* genes have been reported, and low expression of *PTEN* is a common feature [174]. Considering the high risk of relapse and unfavorable OS, clear cell carcinoma, which belongs to Type I tumors behaves as the Type II tumors.

Mucinous carcinomas account for approximately 3% of EOCs [180]. The cell of origin is still unknown, but the tumor is composed of gastrointestinal-type cells containing intra-cytoplasmic mucin. Somatic *KRAS* mutations are the most consistent molecular genetic alterations, and *HER2* amplification is seen in 15-20% of tumors; most such tumors do not harbor mutations in *KRAS* [174, 186].

Undifferentiated carcinomas are uncommon as ovarian tumors. These tumors show no differentiation of any specific Müllerian cell type. They display sheet-like growth, frequently associated with necrosis and high mitotic activity. The tumor cells are often monotonous and non-cohesive [174].

Tumor stage at diagnosis is the most important prognostic factor for predicting survival. Staging of OC is done according to the FIGO guidelines and TNM, as shown in Table 6 [159, 187, 188]. The FIGO stages are based on surgical staging. TNM stages are based on clinical and/or pathological classification. The 5-year disease-specific survival decreases with higher stage, from more than 90% in stage I to less than 20% in stage IV [189]. Unfortunately, the majority of cases are diagnosed at a late stage (stage III or IV), with disseminated disease. WT1 protein expression is used in gynecological pathology as a diagnostic marker of serous differentiation and is frequently coexpressed with estrogen. Since the prognosis is exceptionally poor and therapeutic advances are only made slowly, novel therapy options for this highly aggressive neoplasm are needed. *WT1* has been proposed as a promising target for immunotherapy [6, 110]. Knowledge about *WT1* as a prognostic factor can be useful in the development and evaluation of immunotherapy. One recently published study by Taube *et al.*, [190] demonstrated that WT1 protein expression is an independent favorable prognostic factor for primary HGSC, a finding that could be validated in an independent patient cohort and in silico in a series of publically available gene expression datasets.

Table 6. FIGO stage grouping and TNM classification [159]

FIGO Stages	TNM	Definition
I	T1-N0-M0	Tumor limited to the ovaries (one or both)
IA	T1a-N0-M0	Tumor limited to one ovary (capsule intact). No malignant cells in the ascites or peritoneal washings
IB	T1b-N0-M0	Tumor limited to both ovaries (capsules intact). No malignant cells in the ascites or peritoneal washings
IC	T1c-N0-M0	Tumor limited to one or both ovaries or fallopian tubes, with any of the following: capsule ruptured, tumor on ovarian surface, malignant cells in ascites or peritoneal washings
II	T2-N0-M0	Tumor involves one or both ovaries with pelvic extension
IIA	T2a-N0-M0	Extension and/or implants on uterus and/or tube(s). No malignant cells in the ascites or peritoneal washings
IIB	T2b-N0-M0	Extension to other pelvic tissues. No malignant cells in the ascites or peritoneal washings
IIC	T2c-N0-M0	Pelvic extension (2a or 2b) with malignant cells in the ascites or peritoneal washings
III	T3-N0-M0	Tumor involves one or both ovaries with microscopically confirmed peritoneal metastasis outside the pelvis
IIIA	T3a-N0-M0	Microscopic peritoneal metastasis beyond pelvis
IIIB	T3b-N0-M0	Macroscopic peritoneal metastasis beyond the pelvis up to 2 cm in greatest dimension.
IIIC	T3c-N0/N1-M0	Macroscopic peritoneal metastasis beyond the pelvis more than 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes (includes extension of tumor to capsule of liver and spleen without parenchymal involvement of either organ)
IV	Any T-Any N-M1	Distant metastasis excluding peritoneal metastases

Abbreviations: Primary tumor (T), Regional lymph nodes (N), Distant metastasis (M).

Aims of the Thesis

The main goal of this thesis was to investigate the significance of *WT1* as a biomarker in AL and solid tumors.

Specific aims:

Paper I

- To analyze *WT1* gene expression in relation to clinical characteristics of patients with AML.
- To evaluate the prognostic value of *WT1* gene expression at diagnosis.
- To study *WT1* gene expression as an MRD marker to predict outcome in AML patients.

Paper II

- To study the association between mutation and expression levels of the *WT1* gene in ccRCC.
- To test the clinical relevance of *WT1* mutation in ccRCC.

Paper III

- To investigate the importance of anti-WT1 IgG Ab in blood as a marker of anti-OC immune response and possible relation to disease progression.
- To determine whether anti-WT1 IgG Ab levels in blood are related to WT1 protein expression in cancer tissue specimens.

Paper IV

- To investigate the clinical implications of *WT1* gene variations and mutations in childhood BCP-ALL.
- To identify whether *WT1* variations can be used as biomarkers for predicting clinical outcome in childhood BCP-ALL.

Materials and Methods

Patients and tissue samples (Papers I-IV)

In paper I, 43 adult patients (median age 61 y, range 23 to 85 y) who were diagnosed with AML between 1996 and 2002 were included in the study. These patients were treated at the Department of Hematology, Umeå University Hospital, Sweden, according to standard protocols. Patients with severe comorbidity precluding the initiation of intensive induction chemotherapy were excluded. BM and PB samples were obtained at diagnosis and during treatment. Expression levels of *WT1* mRNA were quantified in BM at diagnosis in 34 patients. Additional follow-up samples from 9 patients were analyzed, yielding a total of 43 patients. *WT1* gene expression levels in PB could be quantified at diagnosis and during follow-up in 14 patients. The total number of samples was 202, and the median number of follow-up samples per patient was 3 (range 1 to 7). The median follow-up time was 22 months (range 1 to 141).

In paper II, the study included 182 adult patients who were diagnosed with ccRCC between 1985 and 2007. These patients were treated at Umeå University Hospital, Sweden, based on guidelines from the European Association of Urology [191]. The median age of the patients was 65.5 years (range 38–87 years), and median survival time was 49.5 months (range 1–300 months). For patients providing corresponding tumor-free specimens, the median age was 67 years (range 38 to 87 years), and median survival time was 55.5 months (range 1 to 115 months). A total of 260 tissue specimens, including 182 ccRCC tumor samples and 78 corresponding tumor-free renal cortical tissue samples, were sequenced for *WT1* exons. Follow-up medical records of the patients were used for survival analysis.

In paper III, the study included a total of 103 ovarian specimens from patients undergoing surgery at the Department of Obstetrics and Gynaecology, Umeå University Hospital, Sweden, between August 1993 and November 2000. Plasma samples were obtained from patients before operation (median 1 day; range 0 to 48 days) and stored at -80°C until use. Medical records of the patients during follow-up and the Swedish Cause of Death Register were retrospectively reviewed and used for identification of progression-free survival (PFS) and overall survival (OS) analysis. Patients in this study did not receive any radiation or chemotherapy before surgery.

In paper IV, 92 patients diagnosed with childhood BCP-ALL between 1987 and 2013 at Umeå University Hospital, Sweden, were included in the study. The study comprised 49 males and 43 females at the median age of 4.5 years

(range 0 to 18 years). Patients were treated according to four different NOPHO-ALL (Nordic Society of Pediatric Hematology and Oncology) protocols, ALL-1986 (n = 9), ALL-1992 (n = 23), ALL-2000 (n = 29) and ALL-2008 (n = 26). Details of the protocols have been described previously [192-194]. Patients below the age of one were treated according to specific protocols for infant ALL (n = 7). BM or PB samples were collected at diagnosis. For six patients, paired diagnostic, remission and relapse samples were available for analyses. Six additional patients had paired diagnostic and relapsed samples available for analyses.

Informed consent was obtained in accordance with the recommendations of the Declaration of Helsinki and institutional regulations. All studies were approved by the Human Ethics Committee of the Medical Faculty, Umeå University, Sweden.

Classification and risk group stratification in AL (Papers I and IV)
The diagnosis and classification of AML were based on criteria according to the FAB classification. The cytogenetic analyses were performed on BM samples obtained at diagnosis, and before treatment at the Department of Medical and Clinical Genetics, Umeå University Hospital, Sweden. At least 20 cells were analyzed. Risk group stratification based on cytogenetic findings was evaluated retrospectively. Cytogenetic risk group stratification on AML patients was performed as described by Grimwade *et al.* [195]. The risk was categorized into favorable, intermediate, or adverse. Molecular data were not available and was therefore not incorporated in the risk stratification. MDS was defined as an antecedent hematological disorder if diagnosed at least 2 months before the diagnosis of leukemia.
For childhood BCP-ALL, the cytogenetic analyses were routinely performed on BM samples taken at diagnosis, and before treatment at the Department of Medical and Clinical Genetics, Umeå University Hospital, Sweden. The risk was categorized as standard, intermediate, or high.

ccRCC grade and stage (Paper II)
All pathology specimens were reviewed by pathologists subspecialized in uropathology. The histological grading of specimens was performed according to the internationally approved Fuhrman grading system [160]. Tumor stages were classified according to the TNM classification 2002 [196].

OC classification, grade and stage (Paper III)
All pathology specimens were reviewed and classified by a subspecialist in gynecologic pathology. The histological grading was determined by pathologists according to the present WHO classification. Tumor stages were classified according to the TNM classification.

Genomic DNA preparation, RNA extraction and cDNA preparation (Papers I, II and IV)

In paper II, genomic DNA was extracted from frozen tissue specimens using MagAttract DNA Mini M48 Kit with Qiagen BioRobot M48 (Qiagen, Hilden, Germany). After extraction and isolation, the DNA was stored at -80°C until use.

In paper IV, DNA was extracted using proteinase K treatment followed by chloroform treatment (Nucleon II, Scotlab, UK) or by using Gentra PURGENE, genomic DNA purification kit (Qiagen, Hilden, Gemany), and both procedures were done according to the manufacturer's instructions.

Total RNA was extracted with the TRIzol method (Invitrogen AB, Stockholm, Sweden). After extraction, the RNA concentration was determined by measuring the optical density at 260 nm, and the RNA samples were stored at -80°C until use. cDNA was synthesized by reverse transcription with the Superscript II Reverse Transcriptase kit according to the manufacturer's protocol (Invitrogen AB, Stockholm, Sweden).

Quantitative assessment of *WT1* transcript expression with real-time quantitative PCR (RQ-PCR) (Papers I and II)

To analyze gene expression, RQ-PCR was performed in paper I and II. RQ-PCR is a very sensitive method with which small amounts of cDNA can be quantified. This method is based on the detection and quantification of a dye-labeled probe (TaqMan probe). The TaqMan probe and primers are designed to hybridize specifically to a complementary sequence. If the probe anneals to its target sequence, which is amplified during PCR, the reporter dye starts to emit fluorescence, which increases in each cycle. Unlike conventional PCR methods detecting the final amount of amplified product, the PCR product is quantified after each round of amplification based on the amount of fluorescence produced. The amplification can be followed in real time during the exponential phase, allowing accurate quantification of gene expression in the starting material. An internal control is used to adjust for variations in RNA isolation and cDNA synthesis. Amplification conditions, primers and probes for the *WT1* gene and the two control genes (CGs)(the *β-actin* gene and the *ABL1* gene) are described in paper I. *WT1* transcription values were normalized against the expression of *β-actin* (papers I and II) and *ABL1* (paper I). Relative expression levels were calculated as the mean of triplicate determinations of the *WT1* gene copy number divided by the mean of duplicate determinations of the copy numbers of the CGs. As an internal experimental control, RNA from K562 cells was reverse-transcribed to produce cDNA for the RQ-PCR assay.

Sequencing analysis of the *WT1* gene (Papers II and IV)

Using intron-exon flanking primer pairs, the polymerase chain reaction (PCR) technique was applied to amplify the whole coding region of 10 exons of the *WT1* gene. Twelve pairs of primers were previously described [144]. Because of the GC-rich sequences of exon 1, Hot Star Plus polymerase (Qiagen, Hilden, Germany) was used for DNA amplification, as previously described [144], and for the remaining exons 2 to 10, AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA, USA) was used. The amplification conditions are described in paper II. Sequence reactions were analyzed on an Applied Biosystems 3730×l DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Nucleotide sequences were aligned using the Sequencher software, v4.7 (Gene Codes Corporation, Ann Arbor, MI, USA). The derived *WT1* gene sequences were identified by comparing them with the corresponding reference genes in GenBank (EMBL) (http://www.ncbi.nlm.nih.gov/genbank/), with the search tools, BLAST (http://blast.ncbi.nlm.nih.gov/) and dbSNP (http://www.ncbi.nlm.nih.gov/snp).

Immunohistochemistry (IHC) (Paper III)

IHC is the localization of antigens or proteins in tissue sections by the use of labelled antibodies as specific reagents through antigen–antibody interactions that are visualized by a marker. In this study, WT1 protein expression using IHC was analyzed on only malignant tumor specimens. Formalin-fixed, paraffin-embedded tissue specimens were cut (4-µm thick sections) and mounted on glass slides. Sections were stained with monoclonal WT1 Ab (clone 6F-H2, Dako, Carpinteria, CA, USA) in a dilution of 1:50 using a fully automated slide preparation system (Ventana Benchmark XT; Ventana Medical Systems, Inc., Tucson, AZ, USA). Monoclonal mouse anti-human WT1 (anti-WT1) recognizes an epitope found in the amino terminal 84 amino acids of WT1. Anti-WT1 reacts with all isoforms of the full-length WT1 and also identifies WT1 lacking exon 2, which is frequently found in subsets of sporadic WTs. In immunoprecipitation assays, anti-WT1 has been shown to recognize full-length and in vitro translated WT1. Anti-WT1 also detects full-length denatured WT1. The intensity of WT1 expression was classified as nonstaining, weak and intensive, as previously described [197]. Tumors with heterogeneous intensity of WT1 were classified according to the highest degree of immunoreactivity if it occupied more than 10% of the tumor. Material from the fallopian tubes was used as positive control tissue.

Enzyme-linked immunosorbent assay (ELISA) (Paper III)

Anti-WT1 IgG Ab titers were measured by the method described previously [198] with minor modifications. In brief, 96-well ELISA plates were coated with three recombinant glutathione S-transferase tagged, WT1 fragment proteins, WT-Fr1 (1–182 AA), WT-Fr2 (180–324 AA) and WT-Fr3 (318–449 AA) in immobilization buffer overnight. Then, the plates were washed with tris-buffered saline and blocked with blocking solution. Plasma was diluted at 1:100 in blocking solution. Thus, 100 μL of blocking solution was used as the negative control for the assay. Then, 100 μL of the diluted plasma (1:50 dilution) was added to each well for overnight incubation at 4°C. Plates were washed and incubated with ALP-conjugated goat anti-human IgG Ab (Santa Cruz Biotechnology, Dallas, TX, USA) diluted at 1:500. After washing, bound anti-WT1 IgG Ab was visualized for each well using 100 μL of BCIPNBT kit (Nacalai Tesque, Kyoto, Japan). Then, absorbance at 550 nm was measured using a microplate reader MTP-310 (Corona Electric, Ibaraki, Japan). The absorbance for sample plasma was calculated by subtracting the absorbance of the negative control from the measured absorbance of the sample. All samples were examined in duplicate. The titers of anti-WT1 IgG Ab were calculated by interpolation from the corresponding standard line, which was constructed for each assay from the results of simultaneous measurements of serial dilutions of anti-WT1 C19 Ab (8, 40, 200, and 1000 ng/mL), using ALP-conjugated goat anti-rabbit IgG Ab (diluted at 1:500; Santa Cruz Biotechnology) as the second Ab. The level of anti-WT1 IgG Ab in the plasma that produced absorbance at 550 nm, equal to that produced by 0.1 μg/mL of anti-WT1 C19 Ab, were defined as 1.0 WT1-reacting-units (WRUs) in the ELISA system.

Statistical analysis

Statistical analysis was performed with SPSS (version 18 or 21) statistical software (SPSS Inc., Chicago, IL, USA).

The Mann-Whitney U test was used to compare the differences between the two independent variables, and the Kruskal-Wallis one-way analysis of variance was used for group comparisons. Correlations between two variables were tested according to Spearman correlation tests. Fisher's exact test (when the sample size was <5) was used for comparing proportions. The $\chi 2$ test was used to determine the significance of observed differences in proportions (papers II and IV).

The Kaplan-Meier method was used to estimate the distribution of freedom from relapse (FFR), the OS, disease-specific survival (DSS) and PFS, relapse-free survival (RFS) and event-free survival (EFS) (papers I-IV). The log-rank test was used to estimate differences between survival distributions.

OS was defined as being from the date of diagnosis to death of any cause or last follow-up.
For AML patients who achieved complete remission (CR), FFR was measured from the date of diagnosis until the day of disease relapse.
For ccRCC patients, DSS was defined as the time period from diagnosis to death from the disease or to the last follow-up.
For OC patients, PFS was calculated from the date of diagnosis to the date the disease "progressed" or the date on which the patient died of any cause.
For BCP-ALL patients, RFS was calculated from time of CR until date of relapse or last follow-up. EFS were calculated from date of diagnosis to date of induction treatment failure, relapse from CR or death from any cause.

Cox proportional hazard models were used to estimate hazard ratios (HRs) for univariate and multivariate analyses of OS and RFS. *P*-values < 0.05 were considered significant.

Results and Discussion

Paper I

Reduction in *WT1* Gene Expression During Early Treatment Predicts the Outcome in Patients With Acute Myeloid Leukemia

Correlation of WT1 transcript levels normalized against two different CGs

Several genes have been suggested as internal CGs for RQ-PCR to correct for variations in the quality and quantity of RNA and subsequent cDNA synthesis. In this study we compared the usefulness of the *β-actin* gene and *ABL1* gene as CGs. We performed an analysis on the *WT1* expression using these two CGs in a total of 202 samples, including 48 diagnostic (14 PB and 34 BM) and 154 follow-up samples (65 PB and 89 BM). *WT1* transcripts were detected in 133 of 202 samples. We found significant agreement between *WT1* gene expression levels normalized with the *β-actin* gene and the *ABL1* gene ($r = 0.96$, $P<0.001$)

The *β-actin* gene has been frequently used as a CG for normalization at our clinical laboratory and has been used as a CG in previous published MRD studies [112, 199, 200]. More recently, several studies have used the *ABL1* gene as CG for normalizing *WT1* transcripts [131, 201]. The *β-actin* gene has pseudogenes that could cause overestimation of gene expression, but primer combinations are claimed to be mRNA-specific, thus preventing amplification of contaminating genomic DNA (pseudogenes) [202, 203]. Studies have found that repair of gene transcriptional activity induced by chemotherapy with melphalan (alkylating agent) in vivo occurred in the order *β-actin> p53> N-ras> d-globin* [204]. It must be noted that this result cannot automatically be compared with DNA repair following treatment with antracycline-based (anti-tumor antibiotics) and cytarabine-based (antimetabolite) treatment. Levels of *β-actin* mRNAs has also been described as relatively constant posthypoxia in comparison with immediate early gene products [205]. These findings suggest that *β-actin* transcripts are probably less affected by chemical-induced or stress-induced damage to gene activity. An argument against using *β-actin* as a CG in AML patients is that monosomy 7 occurs in 4% of AML patients [195]. Chromosome 7 harbors the *β-actin* gene, and monosomy of this chromosome could result in a lower level of *β-actin* mRNA and cDNA. In our study cohort, however, 3 patients had monosomy 7 in diagnostic BM samples, and their expression of *β-actin* was in the same range as in the other patients.

Tamaki *et al.* [202] demonstrated a strong correlation between *WT1* gene transcription values normalized against *β-actin*, *ABL1*, and *GAPDH* as CGs. A landmark for standardization of PCR methodology for clinical implementation was the Europe Against Cancer program [206], one of whose key achievements was an extensive evaluation of a large amount of potential CGs to identify candidates with stable expression in normal PB and BM, similarly expressed across a range of types of leukemia and with comparable stability to that of the leukemic transcripts. Only *ABL1* was proposed for use as a CG because its mRNA expression was not significantly different in normal and leukemic samples at diagnosis. *β-actin* was excluded after the first round of the study because the presence of pseudogenes was a major criterion for exclusion and further analysis. The *ABL1* gene is ubiquitously expressed at a desirable level and has no pseudogenes, but some primers for *ABL1* can also yield amplification of *ABL1* transcripts from translocations in Philadelphia-positive leukemia [207].

All genes used for normalization can show alterations in one or the other condition because no single RNA has constant expression in all situations during development and experimental treatment [208, 209]. That is why CGs should be validated for each particular condition of interest, and every researcher should first demonstrate a satisfactory function of the CGs used in their specific application. Mathematical combination of CGs may also be considered [210].

Expression levels of WT1 gene mRNA and clinical characteristics at diagnosis and course of the disease

We analyzed a total of 34 BM samples from newly diagnosed adult patients with AML. At diagnosis, the median ratio of *WT1* gene expression was 10.57/10 000 *β-actin* (0.11 to 76.54) and 1268/10 000 *ABL1* (11 to 14151). No significant differences in *WT1* expression were observed regarding age or sex. Regarding FAB classification, the highest expression levels of *WT1* mRNA at diagnosis were observed in the four patients with FAB M3 subtype, which corresponds to acute promyelocytic leukemia, and the lowest levels were seen in the three patients with FAB M5 subtype, which corresponds to acute monoblastic and monocytic leukemia. No significant difference was shown between patients with a history of MDS and patients with de novo AML. The expression of CD34 antigen was reported as negative or positive and was not associated with *WT1* transcription levels ($P = 0.592$ for *WT1/β-actin*, $P = 0.394$ for *WT1/ABL1*). To determine the prognostic relevance of *WT1* expression levels at diagnosis, the patients were divided into two groups according to the median of the initial *WT1* transcript ratio. There was no difference in *WT1* mRNA expression at diagnosis between patients who

relapsed and those who did not relapse. According to cytogenetics, the highest *WT1* expression was observed in the favorable group, which included patients with FAB M3 (P = 0.070 for *WT1/β-actin*, P = 0.042 for *WT1/ABL1*).

A median *WT1* gene expression level of 0.0279/10 000 *β-actin* and 17.38/10 000 *ABL1* was observed in BM samples at first CR in 23 patients with AML. Fourteen patients received hematopoietic stem cell transplantation. In five of these patients, BM samples were available for quantitative assessment of *WT1* expression at diagnosis and during follow-up before and after transplantation. Two induction treatment courses had been given to all but one of the patients. Before transplantation, all patients were in CR and showed significantly lower expression of the *WT1* gene than at diagnosis. After transplantation, a very low level of *WT1* expression was found in two patients, and the *WT1* transcript was undetectable in three patients.

Several studies have reported that expression of the *WT1* gene at diagnosis may be predictive of outcome [87, 136, 137]. These studies are not identically performed but have in common that a low *WT1* expression at diagnosis was associated with better outcome. However, other studies [131, 133, 138], including our study, have not been able to confirm these findings. These results and conclusions are confounded by differences in:

1. The performance of assays (TaqMan or SYBR green dye) and standardized approaches (absolute or RQ-PCR analysis).
2. Patient materials (BM or PB) and differences in age, leukemia subtype, etc.
3. Treatment protocols and length of follow-up.
4. Different *WT1* cutoff levels used for defining the status of MRD.

Quantitative assessment of *WT1* mRNA expression after allogeneic stem cell transplantation has been demonstrated to be a useful tool for monitoring MRD in AML [211, 212]. In the present study, we were unable to prove the usefulness of *WT1* gene expression as a predictor of relapse after BM transplantation because of the restricted patient material. However, all samples after BM transplantation showed reduced *WT1* gene expression compared with the degree of gene expression before transplantation.

Prognostic significance of reduction in WT1 gene expression during treatment

To determine the prognostic relevance of *WT1* gene expression levels in both BM and PB for OS and FFR during follow-up, different intervals were

evaluated as follows: Interval 1: <1 month, samples acquired between 3 and 4 weeks after diagnosis (median 25 d, range 19 to 30 d); and interval 2: samples acquired between 1 and 6 months (median 2.7 mo, range 1.03 to 5.7 mo). We analyzed the effect of a ≥1-log reduction and a ≥2-log reduction in *WT1* mRNA expression. We found that, in interval 1, the achievement of ≥1-log reduction in BM was associated with improved OS and FFR when *β-actin* was used as CG (P = 0.004 for OS and P = 0.010 for FFR). No significant result was achieved regarding a ≥2-log reduction or when *ABL1* was used as CG. These results are probably due to the limited number of patients studied. PB could not be analyzed because very few follow-up samples were available from this interval. In interval 2, the achievement of a ≥2-log reduction in PB was associated with improved OS (P = 0.004) and FFR (P = 0.012) when both *β-actin* and *ABL1* were used as CGs. Similarly, a ≥1-log reduction in PB was also associated with improved OS and FFR.

WT1 mRNA expression has been shown to be a potential candidate for the evaluation of MRD, especially in AML patients in the absence of multiparameter flow cytometric or DNA MRD markers [213-215]. However, widespread implementation of *WT1* MRD measurements has been hampered by the lack of knowledge of how and when to measure MRD levels.

Despite the limited number of patients in this study, our observations were consistent with the results of previous studies, which have shown that early MRD measurements could provide predictive information on patient outcome [131, 216]. *WT1* gene expression analysis in PB samples is likely to be the most informative measure, given the low background level of *WT1* in PB relative to BM samples. Taken together with previous MRD studies [131, 132, 211, 217-219], the data collected in this study suggest that the analysis of *WT1* expression may be a tool for monitoring of MRD in AML, but its usefulness is debatable.

How useful is WT1 expression analysis for defining MRD in AML?

The molecular heterogeneity of AML combined with increasing understanding of the clonal architecture contradicts the notion that one single MRD approach is applicable to all forms of AML [134]. Considering the fact that a significant proportion of adult AML patients lack an informative leukemia-specific marker and in view of the finding that the majority of patients with AML express *WT1*, it has been natural to evaluate *WT1* gene expression as a universal molecular AML-MRD marker. Taking into account the relatively limited sensitivity and lack of specificity of *WT1* assessments, this platform seems unlikely to be widely adopted into routine

clinical practice [134]. A major factor affecting assay sensitivity is that expression of *WT1* is not leukemia specific. In one European LeukemiaNet (ELN) study, Cilloni *et al.* [131] analyzed 204 control samples derived from healthy volunteers using the ELN *WT1* assay. Based on their results, the upper limit of normal *WT1* expression was defined as 250 copies for BM and 50 copies/10,000 *ABL1* copies for PB. The presence of *WT1* in normal samples limits the capacity to distinguish low-level MRD from a normal background. This is in contrast to leukemia-specific markers which generally provide a more sensitive and reliable sample source for MRD assessment.

There is accumulating evidence that multiparameter flow cytometric MRD (MFC-MRD) assessments is a strong candidate for MRD detection in AML when risk stratifying both younger and older patients [220-223]. The clinical value of MFC as an MRD measuring tool is difficult to ignore, particularly with its applicability to the majority of AML patients and the possibility for very exact cell characterization [134]. Recent sequencing-based approaches are also expected to be more informative in terms of applicability and sensitivity in the substantial proportion of AML patients in whom MRD tracking is not suitable using an established RQ-PCR [134]. Newer molecular technology for MRD detection includes next-generation sequencing (NGS) technologies and microfluidics-based systems such as digital PCR. Almost all AMLs harbor genetic mutations, and mutational hotspots are not necessary for NGS MRD measurement; therefore, NGS could potentially be applicable to measure predictive markers in all AMLs [224].

Paper II

Single Nucleotide Polymorphisms in the Wilms' Tumour Gene 1 in Clear Cell Renal Cell Carcinoma

Frequencies and features of WT1 mutations and SNPs in ccRCC patients

We performed sequence analysis on all 10 exons in *WT1* in 182 ccRCC tumor specimens and in 78 corresponding tumor-free renal cortical tissue samples. Previous reported missense, nonsense or frame-shift mutations in the *WT1* gene in leukemia [141] or in WT [225] were not identified in the present study. However, a novel heterozygous missense mutation in exon 1 at nucleotide position 536 C>A was identified in one patient with a stage IV ccRCC at diagnosis. This mutation changes the amino acid from proline to histidine at codon 179. In addition to the 536 C>A mutation, this patient also carried the heterozygous minor allele for the rs2234582 and rs16754.

A total of six different *WT1* gene SNPs were identified in the material. The genotypes of each SNP met Hardy-Weinberg equilibrium. One or two copies of the minor allele were found in exon 1 in 95 tumor specimens, in exon 7 in 52 tumor specimens and in exon 10 in 27 tumor specimens. The minor allele frequencies of these SNPs presented in order with 16.8% of rs2234582 in exon 1, followed by 16% of rs16754 in exon 7, 13.7% of rs1799925 in exon 1, 7.7% of rs5030315 in exon 10, 6.6% of rs2234583 in exon 1 and 0.6% of rs2234581 in exon 1. Similar frequencies of these SNPs were presented in all 78 of the corresponding tumor-free renal cortical tissue samples except for rs2234581. The majority of the minor allele of these SNPs was heterozygous in both tumor and tumor-free tissues. At least one copy of the minor allele could be detected in 111 of the 182 tumor specimens (61%). Furthermore, SNP genotypes were compared in 78 tumor and corresponding tumor-free specimen pairs. A high concordance (95%) was demonstrated.
Our data suggest that *WT1* mutations found in other types of malignancies are not common in ccRCC.

Correlation between WT1 SNP genotypes and WT1 mRNA expression, and clinical and pathological characteristics in ccRCC patients

It has previously been demonstrated that *WT1* mRNA expression is significantly lower in ccRCC compared to tumor-free renal cortical tissue, indicating that *WT1* acts as a tumor suppressor in ccRCC [63]. These findings are supported by the present study. *WT1* mRNA expressions were analyzed by RQ-PCR in a total of 115 tissue samples, including 100 tumor and 15 tumor-free specimens. Significantly lower *WT1* mRNA expression in ccRCC was observed in comparison with tumor-free renal cortical tissue (P = 0.001). *WT1* mRNA expression was compared between different *WT1* SNP genotype groups, and we found no difference in *WT1* mRNA expression levels in tumors (P = 0.726) and tumor-free tissue samples (P = 0.779). No significant differences were found between patients with wild-type and homozygosity or heterozygosity for the minor allele of the *WT1* SNPs with regard to age (P = 0.397), sex (P = 0.542), tumor stage (P = 0.947), tumor size (P = 0.602), tumor grade (P = 0.718), DSS (P = 1) and OS (P = 0.873). One caveat of this study is that cDNA was not synthesized directly after sampling. Since RNA is naturally labile and prone to degradation this may have influenced the estimation of WT1 mRNA expression.

Due to the relative large frequency of SNPs in the human genome, synonymous SNPs are often disregarded in many studies based on the assumption that they are silent. The moniker “silent” to describe a synonymous mutation or SNP comes from Anfinsen’s principle that the

amino acid sequence of a protein alone determines the three-dimensional structure of a protein and, hence, its function [226]. It has been proposed that synonymous SNPs may change protein amount, structure or function by altering mRNA structure and stability, kinetics of translation or splicing [226]. However, it is unclear whether RNA expression could be affected by the major or minor allele. One argument could be that *AWT1*, co-expressed with *WT1* in renal and hematopoietic cells [16], may affect detection of *WT1* using primers and probes in exon 1 and 2. In this study, primers and probes for measuring *WT1* RNA level were selected according to a quality-control study involving 11 ELN laboratories spread across eight countries [131]. The mechanism of downregulated *WT1* expression in ccRCC appears unrelated to *WT1* SNP genotypes. Other mechanisms—for example epigenetic modifications such as histone posttranscriptional modifications, DNA methylation or related oncogenic pathways—may be involved in the decreased *WT1* expression in ccRCC.

Homozygous minor allele for WT1 SNPs were associated with favorable clinical outcome

We investigated the prognostic impact of the *WT1* SNPs in our entire cohort of 182 ccRCC patients, who had a median OS of 49.5 months (range 1 to 300 months) and a median DSS of 49 months (range 1 to 293 months). To determine whether outcome was associated with genotype, we also performed separate analyses in patients homozygous for the *WT1* wild-type alleles and those heterozygous and homozygous respectively for the minor allele. In the subgroup of patients with homozygous minor allele (6%), median OS was 91 months (range 9 to 243 months), and median DSS was 96.5 months (range 9 to 243 months). Patients with a heterozygous minor allele (54.9%) had a median OS of 41 months (range 1 to 300 months) and a median DSS of 40 months (range 1 to 276 months). Wild-type patients (39%) had a median OS of 49 months (range 1 to 293 months) and a median DSS of 48 months (range 2 to 293 months). Patients with a homozygous minor allele (6%) had longer OS and DSS than patients with a heterozygous minor allele (P = 0.020 for OS and 0.018 for DSS) and patients who were homozygous for the wild-type allele (P = 0.029 for OS and DSS). No difference in OS or DSS was found between patients with *WT1* wild-type and those heterozygous for the minor allele (P = 0.610 for OS and P = 0.652 for DSS). In addition, we combined and compared patients with different *WT1* genotypes in exon 1. Patients with a homozygous minor allele for *WT1* in exon 1 were observed to have a favorable outcome for both OS and DSS compared to patients with a heterozygous allele (P = 0.026 for OS and P = 0.022 for DSS) or wild-type allele (P = 0.012 for OS and P = 0.010 for DSS).

Patients with wild-type and heterozygous genotypes in exon 1 did not differ significantly in OS or DSS (P = 0.772 for OS and P = 0.809 for DSS).

Because *WT1* SNP rs16754 (*WT1* exon 7) has been proposed as a prognostic factor in AML, a subgroup analysis of the rs16754 SNP in ccRCC was performed. The subgroup analysis of the rs16754 SNP showed that patients with a homozygous minor allele had significantly favorable OS compared to those with a heterozygous genotype (P = 0.036) but longer DSS was only a trend (P = 0.060). In comparison with wild-type genotype, survival time did not differ significantly for patients with a homozygous minor allele (P = 0.107 for OS and P = 0.108 for DSS) from those with a heterozygous genotype (P = 0.123 for OS and P = 0.462 for DSS).

There are conflicting reports concerning the prognostic significance of SNP rs16754 [149, 151-153, 227, 228]. The International HapMap 3 consortium database has collected genetic data from the global population since 2002 (http://hapmap.nbci.nlm.nih.gov/) [229, 230]. The minor allele frequency of rs16754 in the present study was 16%, in agreement with data from the HapMap database for European subjects (15.9%). This suggests that none of the rs16754 genotypes are associated with predisposition for ccRCC.

We were not able to evaluate whether the prognostic impact was independent in a multivariate analysis, since few patients were homozygous for the minor allele. Taken together, these results indicate that the prognostic impact of *WT1* SNPs in ccRCC is weak.

It is worth noting that only exonic variation of *WT1* in ccRCC has been studied. As we have learned from the ENCODE Project Consortium [231], the loci associated with disease by most genome-wide association studies are enriched within non-coding functional elements rather than exonic functional variants. We considered analysis of haplotypes, as these individual SNPs or haplotypes could be associated with another variant with regulatory effect on gene expression. Haplotype analysis may increase the power to map disease genes compared to single marker analysis. However, it is still not clear what type of genetic variants, such as major alleles (common alleles) or minor alleles (rare alleles) cause the disease. Furthermore, the true power of haplotype analysis has yet to be demonstrated in theoretical studies and practice.

Paper III

Prognostic Significance of Specific Anti-WT1 IgG Antibody Level in Plasma in Patients with Ovarian Carcinoma

Anti-WT1 IgG Ab in plasma in patients with ovarian tumors and clinical parameters

No differences in anti-WT1 IgG Ab level were found between patients with malignant, borderline or benign tumors. The association of anti-WT1 IgG Ab with clinical parameters was also examined. Using cut-off at the median of anti-WT1 IgG Ab levels obtained from 52 patients with OC, patients were divided into two groups with high level (≥median) and low level (<median). No significant differences were found between anti-WT1 IgG Ab levels and clinical parameters including age, histological subtype, FIGO stage, grade, disease progression and OS.

There is a great need to develop novel biomarkers for early detection of OC. A previous study of anti-WT1 IgG Ab in non-small cell lung cancer (NSCLC) showed that the level of anti-WT1 IgG Ab was elevated in NSCLC patients compared to healthy controls, indicating a humoral immune response against cancer-derived WT1 protein [198]. In NSCLC and hematopoietic malignancies, WT1-specific immune response has been observed to be biased toward Th1-type cells [198, 232]. Similarly to our results in OCs, the NSCLC study demonstrated no association between anti-WT1 IgG Ab levels and clinical parameters [198]. Unfortunately, no healthy controls were included in our study.

WT1 protein expression in tumor specimens in patients with OCs

When IHC was performed, WT1 was dominantly stained in the nuclei of OC cells. Strong WT1 protein staining was demonstrated in 35 of 50 specimens, and weak WT1 staining was observed in only one tissue sample. Positive WT1 protein staining was found more frequently in the serous histological subtype (60%) than in the non-serous one (12%, $P < 0.001$). Advanced stage of the disease (FIGO stages III and IV) was associated with positive WT1 staining ($P < 0.001$), as well as grade 3 but not grades 1 and 2 ($P = 0.007$). Poor outcome was observed in patients with positive WT1 staining both for disease progression ($P = 0.005$) and OS ($P = 0.016$). No significant differences were observed between patients with positive versus negative WT1 staining with regard to age.

Our data are in line with results from previous studies. Unfortunately, this study was initiated and performed before the revised classification of the serous OCs into HGSC and LGSC. The consequence of this is that WT1 expression as a prognostic marker has not been studied separately in HGSC

and LGSC. However, it should be noted that no negative impact was observed concerning OS and PFS in the serous OCs in this study. WT1 protein expression has been demonstrated as an independent favorable prognostic marker in HGSC [190, 233]. Indeed, there are conflicting data on this topic to date [234-236]. These conflicting results can be attributed to the fact that most studies used cohorts composed of different histological subtypes including LGSCs, which are now regarded as a separate, molecularly distinct subtype. There is reason to reflect on the interesting fact that WT1 protein expression (primarily known as a diagnostic marker for serous differentiation) has been described as a possible favorable prognostic marker in HGSC. *WT1* is generally thought to be an oncogene in adult cancers [237], and this would rather entail a negative prognostic effect. Taube *et al.* [190] speculated that *WT1* could be upregulated in a feedback mechanism and function as a tumor suppressor in HGSC.

Prognostic relevance of anti-WT1 IgG Ab level and WT1 protein expression in patients with OCs

Patients diagnosed with OCs were divided into two groups according to the median of anti-WT1 IgG Ab levels. Survival analysis in patients with OC is shown in Table 7. Anti-WT1 IgG Ab level was not shown to be of overall prognostic significance in OS. However, in women with grade 3 OC, low anti-WT1 IgG Ab levels were related to longer PFS compared with high anti-WT1 IgG Ab levels.

Patients with positive WT1 protein staining in OCs had shorter survival for both OS and PFS. When stratified by subtype, there was no significant difference in OS or PFS with regard to WT1 protein expression in serous OC, whereas in patients with non-serous subtypes there was an association with poor clinical outcome and positive WT1 protein staining for PFS but not OS.
We also investigated the prognostic impact by combining anti-WT1 IgG Ab level in plasma and WT1 protein staining. Patients were divided into subgroups based on positive or negative WT1 IHC staining. No association was found between anti-WT1 IgG Ab and WT1 staining ($P = 0.280$). Survival analysis in the subgroup with high anti-WT1 IgG Ab levels showed that positive WT1 protein staining was related to shorter survival and significantly associated to shorter PFS. In serous OC, patients with both high anti-WT1 IgG Ab level and WT1-positive staining survival did not differ in PFS compared with others, whereas significant poor PFS was found in subgroups with non-serous subtype and tumor grade 3 (Table 7).

Our study has shown that low anti-WT1 IgG Ab levels are of positive prognostic significance in grade 3 OCs, while positive WT1 expression by IHC has a negative impact on survival in the subgroup consisting of non-

serous OCs with high anti-WT1 IgG Ab levels. However, previous studies observed high anti-WT1 IgG Ab levels to be associated with longer survival in both NSCLC and MDS [198, 238]. The reason for this difference is unclear. In accordance with previous reports by Hogdall *et al.* and Hylander *et al.* [235, 236], we showed that WT1 IHC expression was related to advanced stages and histological grade. In contrast, a study by Netinatsunthorn *et al.*, mainly composed of advanced stage III and IV tumors found no association between WT1 IHC expression and tumor stage or tumor grade [234]. An association between higher WT1 IHC expression and the serous OC subtype has been demonstrated in previous studies and is confirmed in this study [103, 233, 239, 240]. Poor survival was associated with positive WT1 expression, which was observed in this study as well as previous studies [233, 235].

Table 7: Schematic summary of survival analysis in patients with OC presented in Paper III

	WT1 Ab titer		**WT1 IHC**		**WT1 Ab + IHC**	
	OS	**PFS**	**OS**	**PFS**	**OS**	**PFS**
All	*P*=0.129	*P*=0.063	*P*=0.046*	*P*=0.006*	*P*=0.123	*P*=0.016*
Serous	*P*=0.273	*P*=0.352	*P*=0.244	*P*=0.728	*P*=0.361	*P*=0.264
Non-serous	*P*=0.288	*P*=0.059	*P*=0.161	*P*=0.024*	*P*=0.502	*P*=0.039*
Stage I + II	*P*=0.124	*P*=0.432	*P*=0.805	*P*=0.288	*P*=0.495	*P*=0.482
Stage III + IV	*P*=0.318	*P*=0.063	*P*=0.801	*P*=0.135	*P*=0.619	*P*=0.104
Grade 1	*P*=0.140	*P*=0.140	*P*=0.140	*P*=0.140	*P*=0.140	*P*=0.140
Grade 2	*P*=0.590	*P*=0.560	*P*=0.144	*P*=0.103	*P*=0.970	*P*=0.496
Grade 3	*P*=0.053	*P*=0.039*	*P*=0.692	*P*=0.185	*P*=0.053	*P*=0.039*

*, $P < 0.05$.

Abbreviations: Antibody (Ab), Immunohistochemistry (IHC), Overall Survival (OS), Progression Free Survival (PFS)

It should be noted that there are limitations with the analyses presented in this study. First, the classification and the grading system of OC (especially the serous subtype) have recently been revised as described in the introduction. This new classification has unfortunately not been possible to be taken into account retrospectively. Second, the ELISA performed by our collaborators in Japan was modified, so the results are not entirely comparable. The ELISA was performed on plasma samples, not on serum samples. Plasma differs from serum in that it contains fibrinogen and clotting factors, and it has not been shown that plasma and serum can be

used interchangeably in detection assays. In addition, we did not include healthy controls or controls with other malignancies. The source of anti-WT1 IgG Ab can be considered unclear and controversial, and studies using ELISA as a detection method have been excluded from the meta-analysis [237]. In summary, our study suggests that anti-WT1 IgG Ab measurements in plasma and WT1 staining in tissue specimens could be useful as biomarkers for patient outcome in the high-risk subtypes of OCs. Combining the ELISA data and IHC added no additional information regarding prognosis. Further data from large multicenter prospective studies are needed to validate the clinical importance of anti-WT1 IgG Ab as a predictive marker in OC.

Paper IV

Analysis of Single Nucleotide Polymorphisms and Mutational Status of the Wilms' Tumor Gene 1 in Childhood B-cell Precursor Acute Lymphoblastic Leukemia

Frequencies and features of WT1 mutations and SNPs in childhood BCP-ALL

We performed sequence analysis on all 10 exons in *WT1* in 92 patients with BCP-ALL. Only one *WT1* mutation was detected in the entire cohort. The mutation was a novel heterozygous missense mutation in exon 1 at nucleotide position 650 (G>A), with the change of the amino acid from glutamic acid to lysine. This *WT1* mutation was not detected in relapse and/or remission samples. The result indicates that mutations in the *WT1* gene are not frequent events in childhood BCP-ALL, unlike in T-ALL or AML [115-118]. Five *WT1* SNPs were identified. The minor allele frequencies of the SNPs are presented in order: 7.1% for rs5030315, 7.6% for rs2234583, 11.9% for rs16754, 15.2% for rs1799925 and 16.8% for rs2234582. The number of patients with heterozygous/homozygous genotype for the minor alleles were 13 (14.1%) for rs5030315 ($WT1^{AG/GG}$), 13 (14.1%) for rs2234583 ($WT1^{GA/AA}$), 21 (22.8%) for rs16754 ($WT1^{AG/GG}$), 26 (28.3%) for rs1799925 ($WT1^{CT/TT}$) and 29 (31.5%) for rs2234582 ($WT1^{GT/TT}$). All genotypes were found to be in Hardy Weinberg equilibrium. Out of the 92 patients, 38 (41.3%) did not have any of the *WT1* SNPs. Fourteen patients (15.2%) carried one SNP minor allele, 34 (37.0%) had the minor allele of two SNPs, four (4.3%) had the minor allele of three SNPs, and two (2.2 %) had the minor allele of four SNPs.

Variations in the *WT1* SNP genotypes were analyzed in 12 paired diagnostic, relapse and/or remission samples. No differences in the SNP genotypes were detected in any of these patients.

The minor allele frequencies of the five SNPs identified in our patient cohort were comparable to that of the European population according to the 1000 Genomes Project [241], suggesting that *WT1* SNPs are not associated with a predisposition to developing childhood BCP-ALL. However, *WT1* SNP rs16754 showed a slightly lower frequency, occurring in 11.9% of children with BCP-ALL, in comparison to 17% in the corresponding European population. Genotype frequencies of rs16754 have been demonstrated to differ greatly between populations [241]. From the genetic perspective, one serious potential confounder is ethnicity. The patients in our study mostly derived from the northern part of Sweden. A study of the internal genetic structure of the Swedish population has recently been performed [242]. The overall structure within Sweden appeared clinal, and the substructure in the southern and middle parts was subtle. In contrast, the northern part of Sweden, Norrland, exhibited pronounced genetic differences both within the area and relative to the rest of the country. These distinctive genetic features of Norrland are probably the result of isolation by distance and genetic drift caused by low population density, which has led to pronounced genetic differences both within the area and relative to the rest of the country [242].

Correlation between WT1 SNP genotypes and patient clinical and molecular characteristics

No significant difference was found between SNP genotypes and clinical and molecular characteristics including sex, age, WBC or cytogenetic aberrations. However, patients heterozygous/homozygous for the minor allele of rs1799925 had a significantly worse survival rate than patients without the minor allele ($P = 0.018$).

Negative prognostic impact of rs1799925 on OS

The prognostic impact of the five *WT1* SNPs identified was evaluated for survival analyses in 66 children with BCP-ALL. Patients treated according to the latest NOPHO ALL-2008 protocol were not included in the survival analysis. The results show that patients with the heterozygous/homozygous combination for the minor allele of rs1799925 have shorter OS compared to patients with the wild type allele ($P = 0.021$). In addition, we investigated the prognostic significance of the minor allele of rs1799925 in specific risk groups and found that in the patient group without high risk factors, the presence of the minor allele of rs1799925 maintained a negative association with worse OS ($P = 0.037$). We could not detect any prognostic significance of rs16754, rs2234582, rs2234583 or rs5030315.

We also performed univariate and multivariate Cox regression to assess whether rs1799925 and other prognostic factors could predict OS and RFS in our patient cohort. In the univariate analysis, patients with the minor allele

of rs1799925 had a lower OS, with an HR of 3.35 (95% CI = 1.12-9.99; *P* = 0.030). In the multivariate analysis, the presence of the rs1799925 minor allele showed a trend toward prognostic significance on OS (HR = 3.07; 95% CI = 0.94-9.94; *P* = 0.062). Age below one year at diagnosis was the only prognostic factor that retained significance on OS in the multivariate analysis (HR = 11.19; 95% CI = 1.63-77.0; *P* = 0.014).

These results suggest that the *WT1* SNP rs1799925 may be useful as a new biomarker for clinical outcome in childhood BCP-ALL without any of the known prognostic markers. However, further studies in larger patient cohorts treated under homogenous conditions are necessary to confirm the clinical use of the rs1799925 as a new biomarker in childhood BCP-ALL.

Conclusions

Paper I

We have found (1) that there is a correlation between *WT1* expression levels normalized against *β-actin* and against *ABL1*; (2) that expression levels of *WT1* mRNA at diagnosis are of no prognostic relevance in AML; (3) that reduction in *WT1* gene expression in BM (≥1-log) <1 month after diagnosis can predict the outcome (regarding OS and FFR) when *β-actin* is used as CG; and (4) that reduction in *WT1* expression in PB (≥2-log) between 1 and 6 months of treatment can predict outcome (regarding OS and FFR) irrespective of the CG used. Taken together with previous MRD studies, the data collected suggest that the analysis of *WT1* expression may be a potential tool for monitoring MRD in AML. However, the usefulness is debatable, and newer molecular technologies for MRD detection are under construction and evaluation.

Paper II

None of the previously reported *WT1* mutations in leukemia were found in ccRCC. Our study showed the occurrence of one or two copies of the *WT1* SNP minor allele in 61% of ccRCCs. Six different SNPs were identified in the *WT1* gene. In order of descending minor allele frequency, SNP rs2234582 was most frequent, followed by rs16754, rs1799925, rs5030315, rs2234583 and rs2234581. No association was found between *WT1* SNP genotypes and clinical and pathological characteristics. However, we found favorable outcomes associated with homozygosity in the SNP that represented the minor allele of *WT1*. Considering its low frequency, larger studies would be necessary to confirm this finding.

Paper III

Our study has shown that low anti-WT1 IgG Ab levels are of positive prognostic significance in grade 3 OCs, while positive WT1 expression by IHC has a negative impact on survival in the subgroup consisting of non-serous OCs. These results suggest that anti-WT1 IgG Ab levels may be used as a prognostic marker in addition to WT1 staining in stratification/classification, particularly in the subgroups of patients with non-serous OC or grade 3 OC. However, further studies, with expanded patient materials applying the most up-to-date classification, grading systems and genetic knowledge, are needed to elucidate the impact of anti-WT1 IgG Abs and WT1 in tumor tissue.

Paper IV

None of the previously reported *WT1* mutations in leukemia were found in childhood BCP-ALL patients. Five *WT1* SNPs were identified with minor allele frequencies: 7.1% for rs5030315, 7.6% for rs2234583, 11.9% for rs16754, 15.2% for rs1799925 and 16.8% for rs2234582. Patients who were heterozygous or homozygous for the minor allele of rs1799925 were found to have worse OS compared to patients with the wild-type allele. Furthermore, the presence of the minor allele of rs1799925 was associated with a decreased OS in a subgroup of patients without known high risk factors. However, further studies in larger patient cohorts treated under homogenous conditions are necessary to confirm the clinical use of the rs1799925 as a new biomarker in childhood BCP-ALL.

Acknowledgments

Many people have supported me throughout this emotionally and scientifically challenging PhD journey. I would like to thank all the people who made this thesis possible. I wish to express my warm appreciation and sincere gratitude to:

Aihong Li (in memory), my supervisor, my colleague, my dear friend and PCR Queen. Your brilliant scientific mind and enthusiasm for life and science are a source of never-ending inspiration. Thank you for introducing me to the research field of *WT1* and for giving me the great opportunity to work in your group. Thank you also for encouraging independency and for giving me a platform to develop as a scientist and gardener. Your journey came to an end too soon. Thank you for everything!

Eva Lundin, my co-supervisor and current supervisor. Your humanity is outstanding! I deeply appreciate your knowledge in many fields. Thank you for unconditionally sharing of life experience, valuable suggestions and discussions!

Xingru Li and Sihan Wang, my closest collaborators in Aihong's group. Thank you for reminding me of the essence of life: "We are here for each other!"

Göran Roos, my clinical mentor and dear senior colleague. Thank you for being the Swedish father to my Chinese mother. Thank you for accepting me even though I am rough, hard and heavy and carry a burden that sometimes spills over. I respect and appreciate you very much!

Anna Rosén, I appreciate your friendship and your ability to focus. Your knowledge of genetics and research has been very valuable during my thesis preparation. I look forward to continued cooperation!

Kjell Grankvist, my examinator. Thank you for your pragmatism and valuable advice, especially during this last year when everything changed so dramatically.

My co-authors: Fryderyk Lorenz, Irina Golovleva, Anders Wahlin, Tumkur Sitaram Raviprakash, Börje Ljungberg, Yusuke Oji, Nina Ohlson, Ulrika Ottander, Haruo Sugiyama, Sofia Ottosson and Sofie Nilsson. Thank you for fruitful collaboration, for friendship and nice companionship.

I wish to acknowledge all my hard-working colleagues at the Department of Clinical Pathology, especially Inga Hägerstrand (in memory), Egle Tolockiene, Karin Sixtensdotter Graffmo, Janos Vasko, Thomas Höckenström and Bertil Lundskog for introducing me to the interesting field of pathology and for taking care of me during the first year as a resident.

Thank you to Lena-Maria Engström and Magnus Hultdin for sharing my deep interest in hematopathology. Your support has been essential for me to complete this project! Magnus, thank you for being an excellent clinical supervisor!

Thank you to Maria Näslund, Semira Gouissem, Linda Rännar and Carina Ulander for sharing my ambition to bring order and clarity to the Department for Clinical Pathology.

I also wish to acknowledge all my colleagues at the Department of Medical Biosciences, especially Karin Nylander, Maréne Landström, Roger Stenling, Anders Bergh, Richard Palmqvist, Thomas Brännström and Alkwin Wanders for all your support.

I am grateful to all the administrative staff: Terry Persson, Åsa Lundsten, Carina Ahlgren, Karin Boden and Clas Wikström. Thank you for friendliness and your practical support and help over the years.

I wish to acknowledge my mother and father, my aunts, my uncles, my cousins and my 101-year-old grandmother and my big family in northern Sweden. Thank you for your love, understanding and support. Thank you for never-ending access to native forest, valuable minerals and generations of wisdom, business mentality and critical and analytical thinking.

Thank you to my brother, Nils, and his fiancée, Fanny, for sharing my interests in healthcare, science and digital distance solutions. Thank you for close contact and the opportunity for our children to grow up together.

I wish to acknowledge my wonderful extended family in Sundsvall, Ånge, Stockholm and Örnsköldsvik. Eva and Ryde Jonsson, you are the best in-laws I could ever imagine! Thanks for letting me become a part of your life and share good food, wine and memorable moments.

I wish to acknowledge all my friends and especially all attractive and deeply intellectual female friends who for many years have shared my interest in hunting and fishing.

Finally, my beloved soul mate, Andreas Jonsson, and our children, Wilford and Katarina. You are wonderful human beings with your feet on the ground. I am infinitely grateful to walk the path of life with you!

This work was supported by grants from the Lion's Cancer Research Foundation, Umeå, Sweden, Kempe foundation, the Children's Cancer Foundation in Sweden, and the County Council of Västerbotten, Umeå, Sweden.

References

1. Zantinga, A.R. and M.J. Coppes, *Max Wilms (1867-1918): the man behind the eponym*. Med Pediatr Oncol, 1992. **20**(6): p. 515-8.
2. Haber, D.A., A.J. Buckler, T. Glaser, K.M. Call, J. Pelletier, R.L. Sohn, E.C. Douglass, and D.E. Housman, *An internal deletion within an 11p13 zinc finger gene contributes to the development of Wilms' tumor*. Cell, 1990. **61**(7): p. 1257-69.
3. Gessler, M., A. Poustka, W. Cavenee, R.L. Neve, S.H. Orkin, and G.A. Bruns, *Homozygous deletion in Wilms tumours of a zinc-finger gene identified by chromosome jumping*. Nature, 1990. **343**(6260): p. 774-8.
4. Call, K.M., T. Glaser, C.Y. Ito, A.J. Buckler, J. Pelletier, D.A. Haber, E.A. Rose, A. Kral, H. Yeger, W.H. Lewis, and et al., *Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus*. Cell, 1990. **60**(3): p. 509-20.
5. Hohenstein, P. and N.D. Hastie, *The many facets of the Wilms' tumour gene, WT1*. Hum Mol Genet, 2006. **15 Spec No 2**: p. R196-201.
6. Oka, Y., A. Tsuboi, Y. Oji, I. Kawase, and H. Sugiyama, *WT1 peptide vaccine for the treatment of cancer*. Curr Opin Immunol, 2008. **20**(2): p. 211-20.
7. Scharnhorst, V., A.J. van der Eb, and A.G. Jochemsen, *WT1 proteins: functions in growth and differentiation*. Gene, 2001. **273**(2): p. 141-61.
8. Yang, L., Y. Han, F. Suarez Saiz, and M.D. Minden, *A tumor suppressor and oncogene: the WT1 story*. Leukemia, 2007. **21**(5): p. 868-76.
9. Rauscher, F.J., 3rd, *The WT1 Wilms tumor gene product: a developmentally regulated transcription factor in the kidney that functions as a tumor suppressor*. FASEB J, 1993. **7**(10): p. 896-903.
10. Menke, A.L., A.J. van der Eb, and A.G. Jochemsen, *The Wilms' tumor 1 gene: oncogene or tumor suppressor gene?* Int Rev Cytol, 1998. **181**: p. 151-212.
11. Gessler, M., A. Konig, and G.A. Bruns, *The genomic organization and expression of the WT1 gene*. Genomics, 1992. **12**(4): p. 807-13.
12. Haber, D.A., R.L. Sohn, A.J. Buckler, J. Pelletier, K.M. Call, and D.E. Housman, *Alternative splicing and genomic structure of the Wilms tumor gene WT1*. Proc Natl Acad Sci U S A, 1991. **88**(21): p. 9618-22.
13. Reddy, J.C. and J.D. Licht, *The WT1 Wilms' tumor suppressor gene: how much do we really know?* Biochim Biophys Acta, 1996. **1287**(1): p. 1-28.
14. Hastie, N.D., *Life, sex, and WT1 isoforms--three amino acids can make all the difference*. Cell, 2001. **106**(4): p. 391-4.
15. Roberts, S.G., *Transcriptional regulation by WT1 in development*. Curr Opin Genet Dev, 2005. **15**(5): p. 542-7.

16. Dallosso, A.R., A.L. Hancock, K.W. Brown, A.C. Williams, S. Jackson, and K. Malik, *Genomic imprinting at the WT1 gene involves a novel coding transcript (AWT1) that shows deregulation in Wilms' tumours.* Hum Mol Genet, 2004. **13**(4): p. 405-15.

17. Sharma, P.M., M. Bowman, S.L. Madden, F.J. Rauscher, 3rd, and S. Sukumar, *RNA editing in the Wilms' tumor susceptibility gene, WT1.* Genes Dev, 1994. **8**(6): p. 720-31.

18. Bruening, W. and J. Pelletier, *A non-AUG translational initiation event generates novel WT1 isoforms.* J Biol Chem, 1996. **271**(15): p. 8646-54.

19. Scharnhorst, V., P. Dekker, A.J. van der Eb, and A.G. Jochemsen, *Internal translation initiation generates novel WT1 protein isoforms with distinct biological properties.* J Biol Chem, 1999. **274**(33): p. 23456-62.

20. Ye, Y., B. Raychaudhuri, A. Gurney, C.E. Campbell, and B.R. Williams, *Regulation of WT1 by phosphorylation: inhibition of DNA binding, alteration of transcriptional activity and cellular translocation.* EMBO J, 1996. **15**(20): p. 5606-15.

21. Smolen, G.A., M.T. Vassileva, J. Wells, M.J. Matunis, and D.A. Haber, *SUMO-1 modification of the Wilms' tumor suppressor WT1.* Cancer Res, 2004. **64**(21): p. 7846-51.

22. Makki, M.S., T. Heinzel, and C. Englert, *TSA downregulates Wilms tumor gene 1 (Wt1) expression at multiple levels.* Nucleic Acids Res, 2008. **36**(12): p. 4067-78.

23. Toska, E. and S.G. Roberts, *Mechanisms of transcriptional regulation by WT1 (Wilms' tumour 1).* Biochem J, 2014. **461**(1): p. 15-32.

24. Reddy, J.C., S. Hosono, and J.D. Licht, *The transcriptional effect of WT1 is modulated by choice of expression vector.* J Biol Chem, 1995. **270**(50): p. 29976-82.

25. Nichols, K.E., G.G. Re, Y.X. Yan, A.J. Garvin, and D.A. Haber, *WT1 induces expression of insulin-like growth factor 2 in Wilms' tumor cells.* Cancer Res, 1995. **55**(20): p. 4540-3.

26. Lee, S.B., K. Huang, R. Palmer, V.B. Truong, D. Herzlinger, K.A. Kolquist, J. Wong, C. Paulding, S.K. Yoon, W. Gerald, J.D. Oliner, and D.A. Haber, *The Wilms tumor suppressor WT1 encodes a transcriptional activator of amphiregulin.* Cell, 1999. **98**(5): p. 663-73.

27. Dame, C., K.M. Kirschner, K.V. Bartz, T. Wallach, C.S. Hussels, and H. Scholz, *Wilms tumor suppressor, Wt1, is a transcriptional activator of the erythropoietin gene.* Blood, 2006. **107**(11): p. 4282-90.

28. Kirschner, K.M., P. Hagen, C.S. Hussels, M. Ballmaier, H. Scholz, and C. Dame, *The Wilms' tumor suppressor Wt1 activates transcription of the erythropoietin receptor in hematopoietic progenitor cells.* FASEB J, 2008. **22**(8): p. 2690-701.

29. Kim, J., D. Prawitt, N. Bardeesy, E. Torban, C. Vicaner, P. Goodyer, B. Zabel, and J. Pelletier, *The Wilms' tumor suppressor gene (wt1) product regulates Dax-1 gene expression during gonadal differentiation.* Mol Cell Biol, 1999. **19**(3): p. 2289-99.

30. Hossain, A. and G.F. Saunders, *The human sex-determining gene SRY is a direct target of WT1*. J Biol Chem, 2001. **276**(20): p. 16817-23.

31. Klattig, J., R. Sierig, D. Kruspe, B. Besenbeck, and C. Englert, *Wilms' tumor protein Wt1 is an activator of the anti-Mullerian hormone receptor gene Amhr2*. Mol Cell Biol, 2007. **27**(12): p. 4355-64.

32. Gross, I., D.J. Morrison, D.P. Hyink, K. Georgas, M.A. English, M. Mericskay, S. Hosono, D. Sassoon, P.D. Wilson, M. Little, and J.D. Licht, *The receptor tyrosine kinase regulator Sprouty1 is a target of the tumor suppressor WT1 and important for kidney development*. J Biol Chem, 2003. **278**(42): p. 41420-30.

33. Wagner, N., K.D. Wagner, H. Scholz, K.M. Kirschner, and A. Schedl, *Intermediate filament protein nestin is expressed in developing kidney and heart and might be regulated by the Wilms' tumor suppressor Wt1*. Am J Physiol Regul Integr Comp Physiol, 2006. **291**(3): p. R779-87.

34. Wagner, K.D., N. Wagner, G. Schley, H. Theres, and H. Scholz, *The Wilms' tumor suppressor Wt1 encodes a transcriptional activator of the class IV POU-domain factor Pou4f2 (Brn-3b)*. Gene, 2003. **305**(2): p. 217-23.

35. Han, X. and R.W. Chesney, *Regulation of taurine transporter gene (TauT) by WT1*. FEBS Lett, 2003. **540**(1-3): p. 71-6.

36. Kim, M.S., S.K. Yoon, F. Bollig, J. Kitagaki, W. Hur, N.J. Whye, Y.P. Wu, M.N. Rivera, J.Y. Park, H.S. Kim, K. Malik, D.W. Bell, C. Englert, A.O. Perantoni, and S.B. Lee, *A novel Wilms tumor 1 (WT1) target gene negatively regulates the WNT signaling pathway*. J Biol Chem, 2010. **285**(19): p. 14585-93.

37. Wagner, N., K.D. Wagner, H. Theres, C. Englert, A. Schedl, and H. Scholz, *Coronary vessel development requires activation of the TrkB neurotrophin receptor by the Wilms' tumor transcription factor Wt1*. Genes Dev, 2005. **19**(21): p. 2631-42.

38. Cook, D.M., M.T. Hinkes, M. Bernfield, and F.J. Rauscher, 3rd, *Transcriptional activation of the syndecan-1 promoter by the Wilms' tumor protein WT1*. Oncogene, 1996. **13**(8): p. 1789-99.

39. Ryan, G., V. Steele-Perkins, J.F. Morris, F.J. Rauscher, 3rd, and G.R. Dressler, *Repression of Pax-2 by WT1 during normal kidney development*. Development, 1995. **121**(3): p. 867-75.

40. Stanhope-Baker, P. and B.R. Williams, *Identification of connective tissue growth factor as a target of WT1 transcriptional regulation*. J Biol Chem, 2000. **275**(49): p. 38139-50.

41. Wang, Z.Y., S.L. Madden, T.F. Deuel, and F.J. Rauscher, 3rd, *The Wilms' tumor gene product, WT1, represses transcription of the platelet-derived growth factor A-chain gene*. J Biol Chem, 1992. **267**(31): p. 21999-2002.

42. Harrington, M.A., B. Konicek, A. Song, X.L. Xia, W.J. Fredericks, and F.J. Rauscher, 3rd, *Inhibition of colony-stimulating factor-1 promoter activity by the product of the Wilms' tumor locus*. J Biol Chem, 1993. **268**(28): p. 21271-5.

43. Dey, B.R., V.P. Sukhatme, A.B. Roberts, M.B. Sporn, F.J. Rauscher, 3rd, and S.J. Kim, *Repression of the transforming growth factor-beta 1 gene by the Wilms' tumor suppressor WT1 gene product*. Mol Endocrinol, 1994. **8**(5): p. 595-602.

44. Webster, N.J., Y. Kong, P. Sharma, M. Haas, S. Sukumar, and B.L. Seely, *Differential effects of Wilms tumor WT1 splice variants on the insulin receptor promoter*. Biochem Mol Med, 1997. **62**(2): p. 139-50.

45. Werner, H., F.J. Rauscher, 3rd, V.P. Sukhatme, I.A. Drummond, C.T. Roberts, Jr., and D. LeRoith, *Transcriptional repression of the insulin-like growth factor I receptor (IGF-I-R) gene by the tumor suppressor WT1 involves binding to sequences both upstream and downstream of the IGF-I-R gene transcription start site*. J Biol Chem, 1994. **269**(17): p. 12577-82.

46. Zaia, A., G.C. Fraizer, L. Piantanelli, and G.F. Saunders, *Transcriptional regulation of the androgen signaling pathway by the Wilms' tumor suppressor gene WT1*. Anticancer Res, 2001. **21**(1A): p. 1-10.

47. Han, Y., L. Yang, F. Suarez-Saiz, S. San-Marina, J. Cui, and M.D. Minden, *Wilms' tumor 1 suppressor gene mediates antiestrogen resistance via down-regulation of estrogen receptor-alpha expression in breast cancer cells*. Mol Cancer Res, 2008. **6**(8): p. 1347-55.

48. Englert, C., X. Hou, S. Maheswaran, P. Bennett, C. Ngwu, G.G. Re, A.J. Garvin, M.R. Rosner, and D.A. Haber, *WT1 suppresses synthesis of the epidermal growth factor receptor and induces apoptosis*. EMBO J, 1995. **14**(19): p. 4662-75.

49. Englert, C., S. Maheswaran, A.J. Garvin, J. Kreidberg, and D.A. Haber, *Induction of p21 by the Wilms' tumor suppressor gene WT1*. Cancer Res, 1997. **57**(8): p. 1429-34.

50. Guan, L.S., M. Rauchman, and Z.Y. Wang, *Induction of Rb-associated protein (RbAp46) by Wilms' tumor suppressor WT1 mediates growth inhibition*. J Biol Chem, 1998. **273**(42): p. 27047-50.

51. Loeb, D.M., D. Korz, M. Katsnelson, E.A. Burwell, A.D. Friedman, and S. Sukumar, *Cyclin E is a target of WT1 transcriptional repression*. J Biol Chem, 2002. **277**(22): p. 19627-32.

52. Li, R.S., G.L. Law, R.A. Seifert, P.J. Romaniuk, and D.R. Morris, *Ornithine decarboxylase is a transcriptional target of tumor suppressor WT1*. Exp Cell Res, 1999. **247**(1): p. 257-66.

53. Hartwig, S., J. Ho, P. Pandey, K. Macisaac, M. Taglienti, M. Xiang, G. Alterovitz, M. Ramoni, E. Fraenkel, and J.A. Kreidberg, *Genomic characterization of Wilms' tumor suppressor 1 targets in nephron progenitor cells during kidney development*. Development, 2010. **137**(7): p. 1189-203.

54. Kim, M.K., T.J. McGarry, O.B. P, J.M. Flatow, A.A. Golden, and J.D. Licht, *An integrated genome screen identifies the Wnt signaling pathway as a major target of WT1*. Proc Natl Acad Sci U S A, 2009. **106**(27): p. 11154-9.

55. Mayo, M.W., C.Y. Wang, S.S. Drouin, L.V. Madrid, A.F. Marshall, J.C. Reed, B.E. Weissman, and A.S. Baldwin, *WT1 modulates apoptosis by transcriptionally upregulating the bcl-2 proto-oncogene*. EMBO J, 1999. **18**(14): p. 3990-4003.

56. Simpson, L.A., E.A. Burwell, K.A. Thompson, S. Shahnaz, A.R. Chen, and D.M. Loeb, *The antiapoptotic gene A1/BFL1 is a WT1 target gene that mediates granulocytic differentiation and resistance to chemotherapy*. Blood, 2006. **107**(12): p. 4695-702.

57. Morrison, D.J., M.A. English, and J.D. Licht, *WT1 induces apoptosis through transcriptional regulation of the proapoptotic Bcl-2 family member Bak*. Cancer Res, 2005. **65**(18): p. 8174-82.

58. Kim, H.S., M.S. Kim, A.L. Hancock, J.C. Harper, J.Y. Park, G. Poy, A.O. Perantoni, M. Cam, K. Malik, and S.B. Lee, *Identification of novel Wilms' tumor suppressor gene target genes implicated in kidney development*. J Biol Chem, 2007. **282**(22): p. 16278-87.

59. Kreidberg, J.A., H. Sariola, J.M. Loring, M. Maeda, J. Pelletier, D. Housman, and R. Jaenisch, *WT-1 is required for early kidney development*. Cell, 1993. **74**(4): p. 679-91.

60. Szemes, M., A.R. Dallosso, Z. Melegh, T. Curry, Y. Li, C. Rivers, J. Uney, A.S. Magdefrau, K. Schwiderski, J.H. Park, K.W. Brown, J. Shandilya, S.G. Roberts, and K. Malik, *Control of epigenetic states by WT1 via regulation of de novo DNA methyltransferase 3A*. Hum Mol Genet, 2013. **22**(1): p. 74-83.

61. Lee, T.H. and J. Pelletier, *Functional characterization of WT1 binding sites within the human vitamin D receptor gene promoter*. Physiol Genomics, 2001. **7**(2): p. 187-200.

62. Hosono, S., I. Gross, M.A. English, K.M. Hajra, E.R. Fearon, and J.D. Licht, *E-cadherin is a WT1 target gene*. J Biol Chem, 2000. **275**(15): p. 10943-53.

63. Sitaram, R.T., S. Degerman, B. Ljungberg, E. Andersson, Y. Oji, H. Sugiyama, G. Roos, and A. Li, *Wilms' tumour 1 can suppress hTERT gene expression and telomerase activity in clear cell renal cell carcinoma via multiple pathways*. Br J Cancer, 2010. **103**(8): p. 1255-62.

64. Saxen, L. and H. Sariola, *Early organogenesis of the kidney*. Pediatr Nephrol, 1987. **1**(3): p. 385-92.

65. Scholz, H. and K.M. Kirschner, *A role for the Wilms' tumor protein WT1 in organ development*. Physiology (Bethesda), 2005. **20**: p. 54-9.

66. Hosono, S., X. Luo, D.P. Hyink, L.M. Schnapp, P.D. Wilson, C.R. Burrow, J.C. Reddy, G.F. Atweh, and J.D. Licht, *WT1 expression induces features of renal epithelial differentiation in mesenchymal fibroblasts*. Oncogene, 1999. **18**(2): p. 417-27.

67. Armstrong, J.F., K. Pritchard-Jones, W.A. Bickmore, N.D. Hastie, and J.B. Bard, *The expression of the Wilms' tumour gene, WT1, in the developing mammalian embryo*. Mech Dev, 1993. **40**(1-2): p. 85-97.

68. Pelletier, J., M. Schalling, A.J. Buckler, A. Rogers, D.A. Haber, and D. Housman, *Expression of the Wilms' tumor gene WT1 in the murine urogenital system*. Genes Dev, 1991. **5**(8): p. 1345-56.

69. Rackley, R.R., A.M. Flenniken, N.P. Kuriyan, P.M. Kessler, M.H. Stoler, and B.R. Williams, *Expression of the Wilms' tumor suppressor gene WT1 during mouse embryogenesis*. Cell Growth Differ, 1993. **4**(12): p. 1023-31.

70. Moore, A.W., L. McInnes, J. Kreidberg, N.D. Hastie, and A. Schedl, *YAC complementation shows a requirement for Wt1 in the development of epicardium, adrenal gland and throughout nephrogenesis*. Development, 1999. **126**(9): p. 1845-57.

71. Herzer, U., A. Crocoll, D. Barton, N. Howells, and C. Englert, *The Wilms tumor suppressor gene wt1 is required for development of the spleen*. Curr Biol, 1999. **9**(15): p. 837-40.

72. Wagner, K.D., N. Wagner, V.P. Vidal, G. Schley, D. Wilhelm, A. Schedl, C. Englert, and H. Scholz, *The Wilms' tumor gene Wt1 is required for normal development of the retina*. EMBO J, 2002. **21**(6): p. 1398-405.

73. Wagner, N., K.D. Wagner, A. Hammes, K.M. Kirschner, V.P. Vidal, A. Schedl, and H. Scholz, *A splice variant of the Wilms' tumour suppressor Wt1 is required for normal development of the olfactory system*. Development, 2005. **132**(6): p. 1327-36.

74. Gao, Y., E. Toska, D. Denmon, S.G. Roberts, and K.F. Medler, *WT1 regulates the development of the posterior taste field*. Development, 2014. **141**(11): p. 2271-8.

75. Miller, R.W., J.F. Fraumeni, Jr., and M.D. Manning, *Association of Wilms's Tumor with Aniridia, Hemihypertrophy and Other Congenital Malformations*. N Engl J Med, 1964. **270**: p. 922-7.

76. Bickmore, W.A. and N.D. Hastie, *Aniridia, Wilms' tumor and human chromosome 11*. Ophthalmic Paediatr Genet, 1989. **10**(4): p. 229-48.

77. Pelletier, J., W. Bruening, C.E. Kashtan, S.M. Mauer, J.C. Manivel, J.E. Striegel, D.C. Houghton, C. Junien, R. Habib, L. Fouser, and et al., *Germline mutations in the Wilms' tumor suppressor gene are associated with abnormal urogenital development in Denys-Drash syndrome*. Cell, 1991. **67**(2): p. 437-47.

78. Little, M., G. Holmes, W. Bickmore, V. van Heyningen, N. Hastie, and B. Wainwright, *DNA binding capacity of the WT1 protein is abolished by Denys-Drash syndrome WT1 point mutations*. Hum Mol Genet, 1995. **4**(3): p. 351-8.

79. Barbaux, S., P. Niaudet, M.C. Gubler, J.P. Grunfeld, F. Jaubert, F. Kuttenn, C.N. Fekete, N. Souleyreau-Therville, E. Thibaud, M. Fellous, and K. McElreavey, *Donor splice-site mutations in WT1 are responsible for Frasier syndrome*. Nat Genet, 1997. **17**(4): p. 467-70.

80. Klamt, B., A. Koziell, F. Poulat, P. Wieacker, P. Scambler, P. Berta, and M. Gessler, *Frasier syndrome is caused by defective alternative splicing of WT1 leading to an altered ratio of WT1 +/-KTS splice isoforms*. Hum Mol Genet, 1998. **7**(4): p. 709-14.

81. Zhu, J. and S.G. Emerson, *Hematopoietic cytokines, transcription factors and lineage commitment*. Oncogene, 2002. **21**(21): p. 3295-313.

82. Baird, P.N. and P.J. Simmons, *Expression of the Wilms' tumor gene (WT1) in normal hemopoiesis*. Exp Hematol, 1997. **25**(4): p. 312-20.

83. Hosen, N., Y. Sonoda, Y. Oji, T. Kimura, H. Minamiguchi, H. Tamaki, M. Kawakami, M. Asada, K. Kanato, M. Motomura, M. Murakami, T. Fujioka, T. Masuda, E.H. Kim, A. Tsuboi, Y. Oka, T. Soma, H. Ogawa, and H. Sugiyama, *Very low frequencies of human normal CD34+ haematopoietic progenitor cells express the Wilms' tumour gene WT1 at levels similar to those in leukaemia cells*. Br J Haematol, 2002. **116**(2): p. 409-20.

84. Fraizer, G.C., P. Patmasiriwat, X. Zhang, and G.F. Saunders, *Expression of the tumor suppressor gene WT1 in both human and mouse bone marrow*. Blood, 1995. **86**(12): p. 4704-6.

85. Maurer, U., J. Brieger, E. Weidmann, P.S. Mitrou, D. Hoelzer, and L. Bergmann, *The Wilms' tumor gene is expressed in a subset of CD34+ progenitors and downregulated early in the course of differentiation in vitro*. Exp Hematol, 1997. **25**(9): p. 945-50.

86. Bergmann, L., U. Maurer, and E. Weidmann, *Wilms tumor gene expression in acute myeloid leukemias*. Leuk Lymphoma, 1997. **25**(5-6): p. 435-43.

87. Inoue, K., H. Sugiyama, H. Ogawa, M. Nakagawa, T. Yamagami, H. Miwa, K. Kita, A. Hiraoka, T. Masaoka, K. Nasu, and et al., *WT1 as a new prognostic factor and a new marker for the detection of minimal residual disease in acute leukemia*. Blood, 1994. **84**(9): p. 3071-9.

88. Menssen, H.D., H.J. Renkl, U. Rodeck, J. Maurer, M. Notter, S. Schwartz, R. Reinhardt, and E. Thiel, *Presence of Wilms' tumor gene (wt1) transcripts and the WT1 nuclear protein in the majority of human acute leukemias*. Leukemia, 1995. **9**(6): p. 1060-7.

89. Miwa, H., M. Beran, and G.F. Saunders, *Expression of the Wilms' tumor gene (WT1) in human leukemias*. Leukemia, 1992. **6**(5): p. 405-9.

90. Huff, V., *Wilms tumor genetics*. Am J Med Genet, 1998. **79**(4): p. 260-7.

91. Ruteshouser, E.C., S.M. Robinson, and V. Huff, *Wilms tumor genetics: mutations in WT1, WTX, and CTNNB1 account for only about one-third of tumors*. Genes Chromosomes Cancer, 2008. **47**(6): p. 461-70.

92. Smith, S.I., M. Down, A.W. Boyd, and C.L. Li, *Expression of the Wilms' tumor suppressor gene, WT1, reduces the tumorigenicity of the leukemic cell line M1 in C.B-17 scid/scid mice*. Cancer Res, 2000. **60**(4): p. 808-14.

93. Fraizer, G., R. Leahy, S. Priyadarshini, K. Graham, J. Delacerda, and M. Diaz, *Suppression of prostate tumor cell growth in vivo by WT1, the Wilms' tumor suppressor gene*. Int J Oncol, 2004. **24**(3): p. 461-71.

94. Luo, X.N., J.C. Reddy, P.L. Yeyati, A.H. Idris, S. Hosono, D.A. Haber, J.D. Licht, and G.F. Atweh, *The tumor suppressor gene WT1 inhibits ras-mediated transformation*. Oncogene, 1995. **11**(4): p. 743-50.

95. Zamora-Avila, D.E., M.A. Franco-Molina, L.M. Trejo-Avila, C. Rodriguez-Padilla, D. Resendez-Perez, and P. Zapata-Benavides, *RNAi silencing of the WT1 gene inhibits cell proliferation and induces apoptosis in the B16F10 murine melanoma cell line*. Melanoma Res, 2007. **17**(6): p. 341-8.

96. Menke, A.L., A. Shvarts, N. Riteco, R.C. van Ham, A.J. van der Eb, and A.G. Jochemsen, *Wilms' tumor 1-KTS isoforms induce p53-independent apoptosis that can be partially rescued by expression of the epidermal growth factor receptor or the insulin receptor*. Cancer Res, 1997. **57**(7): p. 1353-63.

97. Oji, Y., S. Miyoshi, H. Maeda, S. Hayashi, H. Tamaki, S. Nakatsuka, M. Yao, E. Takahashi, Y. Nakano, H. Hirabayashi, Y. Shintani, Y. Oka, A. Tsuboi, N. Hosen, M. Asada, T. Fujioka, M. Murakami, K. Kanato, M. Motomura, E.H. Kim, M. Kawakami, K. Ikegame, H. Ogawa, K. Aozasa, I. Kawase, and H. Sugiyama, *Overexpression of the Wilms' tumor gene WT1 in de novo lung cancers*. Int J Cancer, 2002. **100**(3): p. 297-303.

98. Mundlos, S., J. Pelletier, A. Darveau, M. Bachmann, A. Winterpacht, and B. Zabel, *Nuclear localization of the protein encoded by the Wilms' tumor gene WT1 in embryonic and adult tissues*. Development, 1993. **119**(4): p. 1329-41.

99. Loeb, D.M., E. Evron, C.B. Patel, P.M. Sharma, B. Niranjan, L. Buluwela, S.A. Weitzman, D. Korz, and S. Sukumar, *Wilms' tumor suppressor gene (WT1) is expressed in primary breast tumors despite tumor-specific promoter methylation*. Cancer Res, 2001. **61**(3): p. 921-5.

100. Koesters, R., M. Linnebacher, J.F. Coy, A. Germann, Y. Schwitalle, P. Findeisen, and M. von Knebel Doeberitz, *WT1 is a tumor-associated antigen in colon cancer that can be recognized by in vitro stimulated cytotoxic T cells*. Int J Cancer, 2004. **109**(3): p. 385-92.

101. Oji, Y., S. Nakamori, M. Fujikawa, S. Nakatsuka, A. Yokota, N. Tatsumi, S. Abeno, A. Ikeba, S. Takashima, M. Tsujie, H. Yamamoto, M. Sakon, R. Nezu, K. Kawano, S. Nishida, K. Ikegame, M. Kawakami, A. Tsuboi, Y. Oka, K. Yoshikawa, K. Aozasa, M. Monden, and H. Sugiyama, *Overexpression of the Wilms' tumor gene WT1 in pancreatic ductal adenocarcinoma*. Cancer Sci, 2004. **95**(7): p. 583-7.

102. Andersson, C., Y. Oji, N. Ohlson, S. Wang, X. Li, U. Ottander, E. Lundin, H. Sugiyama, and A. Li, *Prognostic significance of specific anti-WT1 IgG antibody level in plasma in patients with ovarian carcinoma*. Cancer Med, 2014. **3**(4): p. 909-18.

103. Shimizu, M., T. Toki, Y. Takagi, I. Konishi, and S. Fujii, *Immunohistochemical detection of the Wilms' tumor gene (WT1) in epithelial ovarian tumors*. Int J Gynecol Pathol, 2000. **19**(2): p. 158-63.

104. Oji, Y., T. Suzuki, Y. Nakano, M. Maruno, S. Nakatsuka, T. Jomgeow, S. Abeno, N. Tatsumi, A. Yokota, S. Aoyagi, T. Nakazawa, K. Ito, K. Kanato, T. Shirakata, S.

Nishida, N. Hosen, M. Kawakami, A. Tsuboi, Y. Oka, K. Aozasa, T. Yoshimine, and H. Sugiyama, *Overexpression of the Wilms' tumor gene W T1 in primary astrocytic tumors*. Cancer Sci, 2004. **95**(10): p. 822-7.

105. Athale, U.H., S.A. Shurtleff, J.J. Jenkins, C.A. Poquette, M. Tan, J.R. Downing, and A.S. Pappo, *Use of reverse transcriptase polymerase chain reaction for diagnosis and staging of alveolar rhabdomyosarcoma, Ewing sarcoma family of tumors, and desmoplastic small round cell tumor*. J Pediatr Hematol Oncol, 2001. **23**(2): p. 99-104.

106. Rodeck, U., A. Bossler, C. Kari, C.W. Humphreys, T. Gyorfi, J. Maurer, E. Thiel, and H.D. Menssen, *Expression of the wt1 Wilms' tumor gene by normal and malignant human melanocytes*. Int J Cancer, 1994. **59**(1): p. 78-82.

107. Amin, K.M., L.A. Litzky, W.R. Smythe, A.M. Mooney, J.M. Morris, D.J. Mews, H.I. Pass, C. Kari, U. Rodeck, F.J. Rauscher, 3rd, and et al., *Wilms' tumor 1 susceptibility (WT1) gene products are selectively expressed in malignant mesothelioma*. Am J Pathol, 1995. **146**(2): p. 344-56.

108. Tuna, M., A. Chavez-Reyes, and A.M. Tari, *HER2/neu increases the expression of Wilms' Tumor 1 (WT1) protein to stimulate S-phase proliferation and inhibit apoptosis in breast cancer cells*. Oncogene, 2005. **24**(9): p. 1648-52.

109. Wagner, N., J.F. Michiels, A. Schedl, and K.D. Wagner, *The Wilms' tumour suppressor WT1 is involved in endothelial cell proliferation and migration: expression in tumour vessels in vivo*. Oncogene, 2008. **27**(26): p. 3662-72.

110. Oka, Y., A. Tsuboi, T. Taguchi, T. Osaki, T. Kyo, H. Nakajima, O.A. Elisseeva, Y. Oji, M. Kawakami, K. Ikegame, N. Hosen, S. Yoshihara, F. Wu, F. Fujiki, M. Murakami, T. Masuda, S. Nishida, T. Shirakata, S. Nakatsuka, A. Sasaki, K. Udaka, H. Dohy, K. Aozasa, S. Noguchi, I. Kawase, and H. Sugiyama, *Induction of WT1 (Wilms' tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resultant cancer regression*. Proc Natl Acad Sci U S A, 2004. **101**(38): p. 13885-90.

111. Hartkamp, J., B. Carpenter, and S.G. Roberts, *The Wilms' tumor suppressor protein WT1 is processed by the serine protease HtrA2/Omi*. Mol Cell, 2010. **37**(2): p. 159-71.

112. Inoue, K., H. Ogawa, Y. Sonoda, T. Kimura, H. Sakabe, Y. Oka, S. Miyake, H. Tamaki, Y. Oji, T. Yamagami, T. Tatekawa, T. Soma, T. Kishimoto, and H. Sugiyama, *Aberrant overexpression of the Wilms tumor gene (WT1) in human leukemia*. Blood, 1997. **89**(4): p. 1405-12.

113. Ariyaratana, S. and D.M. Loeb, *The role of the Wilms tumour gene (WT1) in normal and malignant haematopoiesis*. Expert Rev Mol Med, 2007. **9**(14): p. 1-17.

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

115. Summers, K., J. Stevens, I. Kakkas, M. Smith, L.L. Smith, F. Macdougall, J. Cavenagh, D. Bonnet, B.D. Young, T.A. Lister, and J. Fitzgibbon, *Wilms' tumour 1 mutations are associated with FLT3-ITD and failure of standard induction chemotherapy in*

patients with normal karyotype AML. Leukemia, 2007. **21**(3): p. 550-1; author reply 552.

116. Paschka, P., G. Marcucci, A.S. Ruppert, S.P. Whitman, K. Mrozek, K. Maharry, C. Langer, C.D. Baldus, W. Zhao, B.L. Powell, M.R. Baer, A.J. Carroll, M.A. Caligiuri, J.E. Kolitz, R.A. Larson, and C.D. Bloomfield, *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, 2008. **26**(28): p. 4595-602.

117. Virappane, P., R. Gale, R. Hills, I. Kakkas, K. Summers, J. Stevens, C. Allen, C. Green, H. Quentmeier, H. Drexler, A. Burnett, D. Linch, D. Bonnet, T.A. Lister, and J. Fitzgibbon, *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, 2008. **26**(33): p. 5429-35.

118. Tosello, V., M.R. Mansour, K. Barnes, M. Paganin, M.L. Sulis, S. Jenkinson, C.G. Allen, R.E. Gale, D.C. Linch, T. Palomero, P. Real, V. Murty, X. Yao, S.M. Richards, A. Goldstone, J. Rowe, G. Basso, P.H. Wiernik, E. Paietta, R. Pieters, M. Horstmann, J.P. Meijerink, and A.A. Ferrando, *WT1 mutations in T-ALL*. Blood, 2009. **114**(5): p. 1038-45.

119. Huff, V., *Wilms' tumours: about tumour suppressor genes, an oncogene and a chameleon gene*. Nat Rev Cancer, 2011. **11**(2): p. 111-21.

120. Jaffe, E.S., N.L. Harris, H. Stein, and J.W. Vardiman, eds. *Pathology and genetics of tumours of hematopoietic and lymphoid tissues*. World Health Organization Classification of tumours. 2001, IARC press: Lyon.

121. Sandler, D.P. and J.A. Ross, *Epidemiology of acute leukemia in children and adults*. Semin Oncol, 1997. **24**(1): p. 3-16.

122. Bennett, J.M., D. Catovsky, M.T. Daniel, G. Flandrin, D.A. Galton, H.R. Gralnick, and C. Sultan, *Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group*. Br J Haematol, 1976. **33**(4): p. 451-8.

123. Bennett, J.M., D. Catovsky, M.T. Daniel, G. Flandrin, D.A. Galton, H.R. Gralnick, and C. Sultan, *Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group*. Ann Intern Med, 1985. **103**(4): p. 620-5.

124. Swerdlow, S.H., E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein, J. Thiele, and J.W. Vardiman, eds. *WHO classification of tumours of hematopoietic tissues*. 2008, IARC: Lyon.

125. Dohner, H., E.H. Estey, S. Amadori, F.R. Appelbaum, T. Buchner, A.K. Burnett, H. Dombret, P. Fenaux, D. Grimwade, R.A. Larson, F. Lo-Coco, T. Naoe, D. Niederwieser, G.J. Ossenkoppele, M.A. Sanz, J. Sierra, M.S. Tallman, B. Lowenberg, and C.D. Bloomfield, *Diagnosis and management of acute myeloid leukemia in*

adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood, 2010. **115**(3): p. 453-74.

126. Pui, C.H., *Recent research advances in childhood acute lymphoblastic leukemia*. J Formos Med Assoc, 2010. **109**(11): p. 777-87.

127. Pieters, R., M. Schrappe, P. De Lorenzo, I. Hann, G. De Rossi, M. Felice, L. Hovi, T. LeBlanc, T. Szczepanski, A. Ferster, G. Janka, J. Rubnitz, L. Silverman, J. Stary, M. Campbell, C.K. Li, G. Mann, R. Suppiah, A. Biondi, A. Vora, and M.G. Valsecchi, *A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): an observational study and a multicentre randomised trial*. Lancet, 2007. **370**(9583): p. 240-50.

128. Meeker, N.D., J.J. Yang, and J.D. Schiffman, *Pharmacogenomics of pediatric acute lymphoblastic leukemia*. Expert Opin Pharmacother, 2010. **11**(10): p. 1621-32.

129. Vrooman, L.M. and L.B. Silverman, *Childhood acute lymphoblastic leukemia: update on prognostic factors*. Curr Opin Pediatr, 2009. **21**(1): p. 1-8.

130. Holmfeldt, L., L. Wei, E. Diaz-Flores, M. Walsh, J. Zhang, L. Ding, D. Payne-Turner, M. Churchman, A. Andersson, S.C. Chen, K. McCastlain, J. Becksfort, J. Ma, G. Wu, S.N. Patel, S.L. Heatley, L.A. Phillips, G. Song, J. Easton, M. Parker, X. Chen, M. Rusch, K. Boggs, B. Vadodaria, E. Hedlund, C. Drenberg, S. Baker, D. Pei, C. Cheng, R. Huether, C. Lu, R.S. Fulton, L.L. Fulton, Y. Tabib, D.J. Dooling, K. Ochoa, M. Minden, I.D. Lewis, L.B. To, P. Marlton, A.W. Roberts, G. Raca, W. Stock, G. Neale, H.G. Drexler, R.A. Dickins, D.W. Ellison, S.A. Shurtleff, C.H. Pui, R.C. Ribeiro, M. Devidas, A.J. Carroll, N.A. Heerema, B. Wood, M.J. Borowitz, J.M. Gastier-Foster, S.C. Raimondi, E.R. Mardis, R.K. Wilson, J.R. Downing, S.P. Hunger, M.L. Loh, and C.G. Mullighan, *The genomic landscape of hypodiploid acute lymphoblastic leukemia*. Nat Genet, 2013. **45**(3): p. 242-52.

131. Cilloni, D., A. Renneville, F. Hermitte, R.K. Hills, S. Daly, J.V. Jovanovic, E. Gottardi, M. Fava, S. Schnittger, T. Weiss, B. Izzo, J. Nomdedeu, A. van der Heijden, B.A. van der Reijden, J.H. Jansen, V.H. van der Velden, H. Ommen, C. Preudhomme, G. Saglio, and D. Grimwade, *Real-time quantitative polymerase chain reaction detection of minimal residual disease by standardized WT1 assay to enhance risk stratification in acute myeloid leukemia: a European LeukemiaNet study*. J Clin Oncol, 2009. **27**(31): p. 5195-201.

132. Kern, W., D. Voskova, C. Schoch, W. Hiddemann, S. Schnittger, and T. Haferlach, *Determination of relapse risk based on assessment of minimal residual disease during complete remission by multiparameter flow cytometry in unselected patients with acute myeloid leukemia*. Blood, 2004. **104**(10): p. 3078-85.

133. Weisser, M., W. Kern, S. Rauhut, C. Schoch, W. Hiddemann, T. Haferlach, and S. Schnittger, *Prognostic impact of RT-PCR-based quantification of WT1 gene expression during MRD monitoring of acute myeloid leukemia*. Leukemia, 2005. **19**(8): p. 1416-23.

134. Grimwade, D. and S.D. Freeman, *Defining minimal residual disease in acute myeloid leukemia: which platforms are ready for "prime time"?* Blood, 2014. **124**(23): p. 3345-55.

135. Owen, C., J. Fitzgibbon, and P. Paschka, *The clinical relevance of Wilms Tumour 1 (WT1) gene mutations in acute leukaemia.* Hematol Oncol, 2010. **28**(1): p. 13-9.

136. Trka, J., M. Kalinova, O. Hrusak, J. Zuna, O. Krejci, J. Madzo, P. Sedlacek, V. Vavra, K. Michalova, M. Jarosova, and J. Stary, *Real-time quantitative PCR detection of WT1 gene expression in children with AML: prognostic significance, correlation with disease status and residual disease detection by flow cytometry*. Leukemia, 2002. **16**(7): p. 1381-9.

137. Bergmann, L., C. Miething, U. Maurer, J. Brieger, T. Karakas, E. Weidmann, and D. Hoelzer, *High levels of Wilms' tumor gene (wt1) mRNA in acute myeloid leukemias are associated with a worse long-term outcome.* Blood, 1997. **90**(3): p. 1217-25.

138. Schmid, D., G. Heinze, B. Linnerth, K. Tisljar, R. Kusec, K. Geissler, C. Sillaber, K. Laczika, M. Mitterbauer, S. Zochbauer, C. Mannhalter, O.A. Haas, K. Lechner, U. Jager, and A. Gaiger, *Prognostic significance of WT1 gene expression at diagnosis in adult de novo acute myeloid leukemia.* Leukemia, 1997. **11**(5): p. 639-43.

139. Siehl, J.M., M. Reinwald, K. Heufelder, H.D. Menssen, U. Keilholz, and E. Thiel, *Expression of Wilms' tumor gene 1 at different stages of acute myeloid leukemia and analysis of its major splice variants.* Ann Hematol, 2004. **83**(12): p. 745-50.

140. Andersson, C., X. Li, F. Lorenz, I. Golovleva, A. Wahlin, and A. Li, *Reduction in WT1 gene expression during early treatment predicts the outcome in patients with acute myeloid leukemia.* Diagn Mol Pathol, 2012. **21**(4): p. 225-33.

141. Hollink, I.H., M.M. van den Heuvel-Eibrink, M. Zimmermann, B.V. Balgobind, S.T. Arentsen-Peters, M. Alders, A. Willasch, G.J. Kaspers, J. Trka, A. Baruchel, S.S. de Graaf, U. Creutzig, R. Pieters, D. Reinhardt, and C.M. Zwaan, *Clinical relevance of Wilms tumor 1 gene mutations in childhood acute myeloid leukemia*. Blood, 2009. **113**(23): p. 5951-60.

142. Fitzgibbon, J., L.L. Smith, M. Raghavan, M.L. Smith, S. Debernardi, S. Skoulakis, D. Lillington, T.A. Lister, and B.D. Young, *Association between acquired uniparental disomy and homozygous gene mutation in acute myeloid leukemias*. Cancer Res, 2005. **65**(20): p. 9152-4.

143. Algar, E.M., T. Khromykh, S.I. Smith, D.M. Blackburn, G.J. Bryson, and P.J. Smith, *A WT1 antisense oligonucleotide inhibits proliferation and induces apoptosis in myeloid leukaemia cell lines.* Oncogene, 1996. **12**(5): p. 1005-14.

144. Gaidzik, V.I., R.F. Schlenk, S. Moschny, A. Becker, L. Bullinger, A. Corbacioglu, J. Krauter, B. Schlegelberger, A. Ganser, H. Dohner, and K. Dohner, *Prognostic impact of WT1 mutations in cytogenetically normal acute myeloid leukemia: a study of the German-Austrian AML Study Group*. Blood, 2009. **113**(19): p. 4505-11.

145. Komar, A.A., *Silent SNPs: impact on gene function and phenotype.* Pharmacogenomics, 2007. **8**(8): p. 1075-80.

146. Chamary, J.V., J.L. Parmley, and L.D. Hurst, *Hearing silence: non-neutral evolution at synonymous sites in mammals*. Nat Rev Genet, 2006. **7**(2): p. 98-108.

147. Hunt, R., Z.E. Sauna, S.V. Ambudkar, M.M. Gottesman, and C. Kimchi-Sarfaty, *Silent (synonymous) SNPs: should we care about them?* Methods Mol Biol, 2009. **578**: p. 23-39.

148. Hunt, R.C., V.L. Simhadri, M. Iandoli, Z.E. Sauna, and C. Kimchi-Sarfaty, *Exposing synonymous mutations*. Trends Genet, 2014. **30**(7): p. 308-21.

149. Damm, F., M. Heuser, M. Morgan, H. Yun, A. Grosshennig, G. Gohring, B. Schlegelberger, K. Dohner, O. Ottmann, M. Lubbert, W. Heit, L. Kanz, G. Schlimok, A. Raghavachar, W. Fiedler, H. Kirchner, H. Dohner, G. Heil, A. Ganser, and J. Krauter, *Single nucleotide polymorphism in the mutational hotspot of WT1 predicts a favorable outcome in patients with cytogenetically normal acute myeloid leukemia*. J Clin Oncol, 2010. **28**(4): p. 578-85.

150. Renneville, A., N. Boissel, N. Helevaut, O. Nibourel, C. Terre, C. Pautas, C. Gardin, X. Thomas, P. Turlure, O. Reman, C. Berthon, H. Dombret, S. Castaigne, and C. Preudhomme, *Wilms' tumor 1 single-nucleotide polymorphism rs16754 does not predict clinical outcome in adult acute myeloid leukemia*. Leukemia, 2011. **25**(12): p. 1918-21.

151. Ho, P.A., J. Kuhn, R.B. Gerbing, J.A. Pollard, R. Zeng, K.L. Miller, N.A. Heerema, S.C. Raimondi, B.A. Hirsch, J.L. Franklin, B. Lange, A.S. Gamis, T.A. Alonzo, and S. Meshinchi, *WT1 synonymous single nucleotide polymorphism rs16754 correlates with higher mRNA expression and predicts significantly improved outcome in favorable-risk pediatric acute myeloid leukemia: a report from the children's oncology group*. J Clin Oncol, 2011. **29**(6): p. 704-11.

152. Becker, H., K. Maharry, M.D. Radmacher, K. Mrozek, K.H. Metzeler, S.P. Whitman, S. Schwind, J. Kohlschmidt, Y.Z. Wu, B.L. Powell, T.H. Carter, J.E. Kolitz, M. Wetzler, A.J. Carroll, M.R. Baer, J.O. Moore, M.A. Caligiuri, R.A. Larson, G. Marcucci, and C.D. Bloomfield, *Clinical outcome and gene- and microRNA-expression profiling according to the Wilms tumor 1 (WT1) single nucleotide polymorphism rs16754 in adult de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study*. Haematologica, 2011. **96**(10): p. 1488-95.

153. Choi, Y., J.H. Lee, E.H. Hur, M.J. Kang, S.D. Kim, D.Y. Kim, S.N. Lim, K.S. Bae, H.S. Lim, M. Seol, Y.A. Kang, and K.H. Lee, *Single nucleotide polymorphism of Wilms' tumor 1 gene rs16754 in Korean patients with cytogenetically normal acute myeloid leukemia*. Ann Hematol, 2012. **91**(5): p. 671-7.

154. Siegel, R., D. Naishadham, and A. Jemal, *Cancer statistics, 2012*. CA Cancer J Clin, 2012. **62**(1): p. 10-29.

155. Eble, J.N., G. Sauter, J.I. Epstein, and I.A. Sesterhenn, eds. *Pathology and genetics of tumours of the urinary system and male genital organs*. World Health Organization classification of tumours. 2004, IARC Press: Lyon.

156. Lipworth, L., R.E. Tarone, L. Lund, and J.K. McLaughlin, *Epidemiologic characteristics and risk factors for renal cell cancer*. Clin Epidemiol, 2009. **1**: p. 33-43.

157. Choyke, P.L., G.M. Glenn, M.M. Walther, B. Zbar, and W.M. Linehan, *Hereditary renal cancers*. Radiology, 2003. **226**(1): p. 33-46.

158. Kovacs, G., M. Akhtar, B.J. Beckwith, P. Bugert, C.S. Cooper, B. Delahunt, J.N. Eble, S. Fleming, B. Ljungberg, L.J. Medeiros, H. Moch, V.E. Reuter, E. Ritz, G. Roos, D. Schmidt, J.R. Srigley, S. Storkel, E. van den Berg, and B. Zbar, *The Heidelberg classification of renal cell tumours*. J Pathol, 1997. **183**(2): p. 131-3.

159. Sobin, L.H., M.K. Gospodarowicz, and C. Wittekind, eds. *TNM. Classification of malignant tumours*. Seventh ed. 2009, Wiley-Blackwell: Oxford.

160. Fuhrman, S.A., L.C. Lasky, and C. Limas, *Prognostic significance of morphologic parameters in renal cell carcinoma*. Am J Surg Pathol, 1982. **6**(7): p. 655-63.

161. Iliopoulos, O. and W.G. Kaelin, Jr., *The molecular basis of von Hippel-Lindau disease*. Mol Med, 1997. **3**(5): p. 289-93.

162. Nickerson, M.L., E. Jaeger, Y. Shi, J.A. Durocher, S. Mahurkar, D. Zaridze, V. Matveev, V. Janout, H. Kollarova, V. Bencko, M. Navratilova, N. Szeszenia-Dabrowska, D. Mates, A. Mukeria, I. Holcatova, L.S. Schmidt, J.R. Toro, S. Karami, R. Hung, G.F. Gerard, W.M. Linehan, M. Merino, B. Zbar, P. Boffetta, P. Brennan, N. Rothman, W.H. Chow, F.M. Waldman, and L.E. Moore, *Improved identification of von Hippel-Lindau gene alterations in clear cell renal tumors*. Clin Cancer Res, 2008. **14**(15): p. 4726-34.

163. Gnarra, J.R., K. Tory, Y. Weng, L. Schmidt, M.H. Wei, H. Li, F. Latif, S. Liu, F. Chen, F.M. Duh, and et al., *Mutations of the VHL tumour suppressor gene in renal carcinoma*. Nat Genet, 1994. **7**(1): p. 85-90.

164. Ogawa, O., Y. Kakehi, K. Ogawa, M. Koshiba, T. Sugiyama, and O. Yoshida, *Allelic loss at chromosome 3p characterizes clear cell phenotype of renal cell carcinoma*. Cancer Res, 1991. **51**(3): p. 949-53.

165. Campbell, C.E., N.P. Kuriyan, R.R. Rackley, M.J. Caulfield, R. Tubbs, J. Finke, and B.R. Williams, *Constitutive expression of the Wilms tumor suppressor gene (WT1) in renal cell carcinoma*. Int J Cancer, 1998. **78**(2): p. 182-8.

166. Li, X., S. Wang, R.T. Sitaram, C. Andersson, B. Ljungberg, and A. Li, *Single nucleotide polymorphisms in the Wilms' tumour gene 1 in clear cell renal cell carcinoma*. PLoS One, 2013. **8**(3): p. e58396.

167. Niu, Z., M. Ito, Y. Awakura, T. Takahashi, E. Nakamura, N. Ito, and O. Ogawa, *The expression of NOV and WT1 in renal cell carcinoma: a quantitative reverse transcriptase-polymerase chain reaction analysis*. J Urol, 2005. **174**(4 Pt 1): p. 1460-2.

168. Ferlay, J., H.R. Shin, F. Bray, D. Forman, C. Mathers, and D.M. Parkin, *Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008*. Int J Cancer, 2010. **127**(12): p. 2893-917.

169. Klint, A., G. Engholm, H.H. Storm, L. Tryggvadottir, M. Gislum, T. Hakulinen, and F. Bray, *Trends in survival of patients diagnosed with cancer of the digestive organs in the Nordic countries 1964-2003 followed up to the end of 2006*. Acta Oncol, 2010. **49**(5): p. 578-607.

170. Purdie, D.M., C.J. Bain, V. Siskind, P.M. Webb, and A.C. Green, *Ovulation and risk of epithelial ovarian cancer*. Int J Cancer, 2003. **104**(2): p. 228-32.

171. Hunn, J. and G.C. Rodriguez, *Ovarian cancer: etiology, risk factors, and epidemiology*. Clin Obstet Gynecol, 2012. **55**(1): p. 3-23.

172. Morch, L.S., E. Lokkegaard, A.H. Andreasen, S. Kruger-Kjaer, and O. Lidegaard, *Hormone therapy and ovarian cancer*. JAMA, 2009. **302**(3): p. 298-305.

173. *Integrated genomic analyses of ovarian carcinoma*. Nature, 2011. **474**(7353): p. 609-15.

174. Kurman, R.J., M.L. Carcangiu, C.S. Herrington, and R.H. Young, eds. *WHO classification fo tumours of female reproductive organs*. 2014, IARC: Lyon.

175. Gurung, A., T. Hung, J. Morin, and C.B. Gilks, *Molecular abnormalities in ovarian carcinoma: clinical, morphological and therapeutic correlates*. Histopathology, 2013. **62**(1): p. 59-70.

176. Kurman, R.J. and M. Shih Ie, *Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer--shifting the paradigm*. Hum Pathol, 2011. **42**(7): p. 918-31.

177. Vang, R., M. Shih Ie, and R.J. Kurman, *Ovarian low-grade and high-grade serous carcinoma: pathogenesis, clinicopathologic and molecular biologic features, and diagnostic problems*. Adv Anat Pathol, 2009. **16**(5): p. 267-82.

178. Nik, N.N., R. Vang, M. Shih Ie, and R.J. Kurman, *Origin and pathogenesis of pelvic (ovarian, tubal, and primary peritoneal) serous carcinoma*. Annu Rev Pathol, 2014. **9**: p. 27-45.

179. Kobel, M., S.E. Kalloger, J. Carrick, D. Huntsman, H. Asad, E. Oliva, C.A. Ewanowich, R.A. Soslow, and C.B. Gilks, *A limited panel of immunomarkers can reliably distinguish between clear cell and high-grade serous carcinoma of the ovary*. Am J Surg Pathol, 2009. **33**(1): p. 14-21.

180. Kobel, M., S.E. Kalloger, D.G. Huntsman, J.L. Santos, K.D. Swenerton, J.D. Seidman, and C.B. Gilks, *Differences in tumor type in low-stage versus high-stage ovarian carcinomas*. Int J Gynecol Pathol, 2010. **29**(3): p. 203-11.

181. Singer, G., R. Oldt, 3rd, Y. Cohen, B.G. Wang, D. Sidransky, R.J. Kurman, and M. Shih Ie, *Mutations in BRAF and KRAS characterize the development of low-grade ovarian serous carcinoma*. J Natl Cancer Inst, 2003. **95**(6): p. 484-6.

182. Sieben, N.L., P. Macropoulos, G.M. Roemen, S.M. Kolkman-Uljee, G. Jan Fleuren, R. Houmadi, T. Diss, B. Warren, M. Al Adnani, A.P. De Goeij, T. Krausz, and A.M. Flanagan, *In ovarian neoplasms, BRAF, but not KRAS, mutations are restricted to low-grade serous tumours*. J Pathol, 2004. **202**(3): p. 336-40.

183. Hsu, C.Y., R. Bristow, M.S. Cha, B.G. Wang, C.L. Ho, R.J. Kurman, T.L. Wang, and M. Shih Ie, *Characterization of active mitogen-activated protein kinase in ovarian serous carcinomas*. Clin Cancer Res, 2004. **10**(19): p. 6432-6.

184. Daly, M.B., *The epidemiology of ovarian cancer*. Hematol Oncol Clin North Am, 1992. **6**(4): p. 729-38.

185. Yoshikawa, H., H. Jimbo, S. Okada, K. Matsumoto, T. Onda, T. Yasugi, and Y. Taketani, *Prevalence of endometriosis in ovarian cancer*. Gynecol Obstet Invest, 2000. **50 Suppl 1**: p. 11-7.

186. Anglesio, M.S., S. Kommoss, M.C. Tolcher, B. Clarke, L. Galletta, H. Porter, S. Damaraju, S. Fereday, B.J. Winterhoff, S.E. Kalloger, J. Senz, W. Yang, H. Steed, G. Allo, S. Ferguson, P. Shaw, A. Teoman, J.J. Garcia, J.K. Schoolmeester, J. Bakkum-Gamez, A.V. Tinker, D.D. Bowtell, D.G. Huntsman, C.B. Gilks, and J.N. McAlpine, *Molecular characterization of mucinous ovarian tumours supports a stratified treatment approach with HER2 targeting in 19% of carcinomas*. J Pathol, 2013. **229**(1): p. 111-20.

187. Prat, J., *Staging classification for cancer of the ovary, fallopian tube, and peritoneum*. Int J Gynaecol Obstet, 2014. **124**(1): p. 1-5.

188. Prat, J., *FIGO's staging classification for cancer of the ovary, fallopian tube, and peritoneum: abridged republication*. J Gynecol Oncol, 2015. **26**(2): p. 87-9.

189. Heintz, A.P., F. Odicino, P. Maisonneuve, M.A. Quinn, J.L. Benedet, W.T. Creasman, H.Y. Ngan, S. Pecorelli, and U. Beller, *Carcinoma of the ovary. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer*. Int J Gynaecol Obstet, 2006. **95 Suppl 1**: p. S161-92.

190. Taube, E.T., C. Denkert, J. Sehouli, C.A. Kunze, M. Dietel, I. Braicu, A. Letsch, and S. Darb-Esfahani, *Wilms tumor protein 1 (WT1) - Not only a diagnostic but also a prognostic marker in high-grade serous ovarian carcinoma*. Gynecol Oncol, 2015.

191. Ljungberg, B., D.C. Hanbury, M.A. Kuczyk, A.S. Merseburger, P.F. Mulders, J.J. Patard, and I.C. Sinescu, *Renal cell carcinoma guideline*. Eur Urol, 2007. **51**(6): p. 1502-10.

192. Schmiegelow, K., E. Forestier, M. Hellebostad, M. Heyman, J. Kristinsson, S. Soderhall, and M. Taskinen, *Long-term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia*. Leukemia, 2010. **24**(2): p. 345-54.

193. Gustafsson, G., A. Kreuger, N. Clausen, S. Garwicz, J. Kristinsson, S.O. Lie, P.J. Moe, M. Perkkio, M. Yssing, and U.M. Saarinen-Pihkala, *Intensified treatment of acute childhood lymphoblastic leukaemia has improved prognosis, especially in non-high-risk patients: the Nordic experience of 2648 patients diagnosed between 1981 and*

1996. Nordic Society of Paediatric Haematology and Oncology (NOPHO). Acta Paediatr, 1998. **87**(11): p. 1151-61.

194. Frandsen, T.L., M. Heyman, J. Abrahamsson, K. Vettenranta, A. Asberg, G. Vaitkeviciene, K. Pruunsild, N. Toft, H. Birgens, H. Hallbook, P. Quist-Paulsen, L. Griskevicius, L. Helt, B.V. Hansen, and K. Schmiegelow, *Complying with the European Clinical Trials directive while surviving the administrative pressure - an alternative approach to toxicity registration in a cancer trial.* Eur J Cancer, 2014. **50**(2): p. 251-9.

195. Grimwade, D., H. Walker, F. Oliver, K. Wheatley, C. Harrison, G. Harrison, J. Rees, I. Hann, R. Stevens, A. Burnett, and A. Goldstone, *The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties.* Blood, 1998. **92**(7): p. 2322-33.

196. Greene, F.L. and L.H. Sobin, *The TNM system: our language for cancer care.* J Surg Oncol, 2002. **80**(3): p. 119-20.

197. Yamamoto, S., H. Tsuda, T. Kita, K. Maekawa, K. Fujii, K. Kudoh, K. Furuya, S. Tamai, J. Inazawa, and O. Matsubara, *Clinicopathological significance of WT1 expression in ovarian cancer: a possible accelerator of tumor progression in serous adenocarcinoma.* Virchows Arch, 2007. **451**(1): p. 27-35.

198. Oji, Y., Y. Kitamura, E. Kamino, A. Kitano, N. Sawabata, M. Inoue, M. Mori, S. Nakatsuka, N. Sakaguchi, K. Miyazaki, M. Nakamura, I. Fukuda, J. Nakamura, N. Tatsumi, T. Takakuwa, S. Nishida, T. Shirakata, N. Hosen, A. Tsuboi, R. Nezu, H. Maeda, Y. Oka, I. Kawase, K. Aozasa, M. Okumura, S. Miyoshi, and H. Sugiyama, *WT1 IgG antibody for early detection of nonsmall cell lung cancer and as its prognostic factor.* Int J Cancer, 2009. **125**(2): p. 381-7.

199. Ogawa, H., H. Tamaki, K. Ikegame, T. Soma, M. Kawakami, A. Tsuboi, E.H. Kim, N. Hosen, M. Murakami, T. Fujioka, T. Masuda, Y. Taniguchi, S. Nishida, Y. Oji, Y. Oka, and H. Sugiyama, *The usefulness of monitoring WT1 gene transcripts for the prediction and management of relapse following allogeneic stem cell transplantation in acute type leukemia.* Blood, 2003. **101**(5): p. 1698-704.

200. Kreuzer, K.A., A. Saborowski, J. Lupberger, C. Appelt, I.K. Na, P. le Coutre, and C.A. Schmidt, *Fluorescent 5'-exonuclease assay for the absolute quantification of Wilms' tumour gene (WT1) mRNA: implications for monitoring human leukaemias.* Br J Haematol, 2001. **114**(2): p. 313-8.

201. Willasch, A.M., B. Gruhn, T. Coliva, M. Kalinova, G. Schneider, H. Kreyenberg, D. Steinbach, G. Weber, I.H. Hollink, C.M. Zwaan, A. Biondi, V.H. van der Velden, D. Reinhardt, G. Cazzaniga, P. Bader, and J. Trka, *Standardization of WT1 mRNA quantitation for minimal residual disease monitoring in childhood AML and implications of WT1 gene mutations: a European multicenter study.* Leukemia, 2009. **23**(8): p. 1472-9.

202. Tamaki, H., M. Mishima, M. Kawakami, A. Tsuboi, E.H. Kim, N. Hosen, K. Ikegame, M. Murakami, T. Fujioka, T. Masuda, Y. Taniguchi, S. Nishida, K. Osumi, T. Soma, Y.

Oji, Y. Oka, I. Kawase, H. Sugiyama, and H. Ogawa, *Monitoring minimal residual disease in leukemia using real-time quantitative polymerase chain reaction for Wilms tumor gene (WT1).* Int J Hematol, 2003. **78**(4): p. 349-56.

203. Kreuzer, K.A., U. Lass, O. Landt, A. Nitsche, J. Laser, H. Ellerbrok, G. Pauli, D. Huhn, and C.A. Schmidt, *Highly sensitive and specific fluorescence reverse transcription-PCR assay for the pseudogene-free detection of beta-actin transcripts as quantitative reference.* Clin Chem, 1999. **45**(2): p. 297-300.

204. Souliotis, V.L., M.A. Dimopoulos, H.G. Episkopou, S.A. Kyrtopoulos, and P.P. Sfikakis, *Preferential in vivo DNA repair of melphalan-induced damage in human genes is greatly affected by the local chromatin structure.* DNA Repair (Amst), 2006. **5**(8): p. 972-85.

205. Gubits, R.M., R.E. Burke, G. Casey-McIntosh, A. Bandele, and F. Munell, *Immediate early gene induction after neonatal hypoxia-ischemia.* Brain Res Mol Brain Res, 1993. **18**(3): p. 228-38.

206. Beillard, E., N. Pallisgaard, V.H. van der Velden, W. Bi, R. Dee, E. van der Schoot, E. Delabesse, E. Macintyre, E. Gottardi, G. Saglio, F. Watzinger, T. Lion, J.J. van Dongen, P. Hokland, and J. Gabert, *Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) - a Europe against cancer program.* Leukemia, 2003. **17**(12): p. 2474-86.

207. Cilloni, D., E. Gottardi, D. De Micheli, A. Serra, G. Volpe, F. Messa, G. Rege-Cambrin, A. Guerrasio, M. Divona, F. Lo Coco, and G. Saglio, *Quantitative assessment of WT1 expression by real time quantitative PCR may be a useful tool for monitoring minimal residual disease in acute leukemia patients.* Leukemia, 2002. **16**(10): p. 2115-21.

208. Suzuki, T., P.J. Higgins, and D.R. Crawford, *Control selection for RNA quantitation.* Biotechniques, 2000. **29**(2): p. 332-7.

209. Bustin, S.A., *Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays.* J Mol Endocrinol, 2000. **25**(2): p. 169-93.

210. Vandesompele, J., K. De Preter, F. Pattyn, B. Poppe, N. Van Roy, A. De Paepe, and F. Speleman, *Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes.* Genome Biol, 2002. **3**(7): p. RESEARCH0034.

211. Candoni, A., M. Tiribelli, E. Toffoletti, D. Cilloni, A. Chiarvesio, A. Michelutti, E. Simeone, C. Pipan, G. Saglio, and R. Fanin, *Quantitative assessment of WT1 gene expression after allogeneic stem cell transplantation is a useful tool for monitoring minimal residual disease in acute myeloid leukemia.* Eur J Haematol, 2009. **82**(1): p. 61-8.

212. Jacobsohn, D.A., W.T. Tse, S. Chaleff, A. Rademaker, R. Duerst, M. Olszewski, W. Huang, P.M. Chou, and M. Kletzel, *High WT1 gene expression before haematopoietic*

stem cell transplant in children with acute myeloid leukaemia predicts poor event-free survival. Br J Haematol, 2009. **146**(6): p. 669-74.

213. Grimwade, D., P. Vyas, and S. Freeman, *Assessment of minimal residual disease in acute myeloid leukemia.* Curr Opin Oncol, 2010. **22**(6): p. 656-63.

214. Inoue, K., H. Ogawa, T. Yamagami, T. Soma, Y. Tani, T. Tatekawa, Y. Oji, H. Tamaki, T. Kyo, H. Dohy, A. Hiraoka, T. Masaoka, T. Kishimoto, and H. Sugiyama, *Long-term follow-up of minimal residual disease in leukemia patients by monitoring WT1 (Wilms tumor gene) expression levels.* Blood, 1996. **88**(6): p. 2267-78.

215. Kern, W., C. Haferlach, T. Haferlach, and S. Schnittger, *Monitoring of minimal residual disease in acute myeloid leukemia.* Cancer, 2008. **112**(1): p. 4-16.

216. Rubnitz, J.E., H. Inaba, G. Dahl, R.C. Ribeiro, W.P. Bowman, J. Taub, S. Pounds, B.I. Razzouk, N.J. Lacayo, X. Cao, S. Meshinchi, B. Degar, G. Airewele, S.C. Raimondi, M. Onciu, E. Coustan-Smith, J.R. Downing, W. Leung, C.H. Pui, and D. Campana, *Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML02 multicentre trial.* Lancet Oncol, 2010. **11**(6): p. 543-52.

217. Ostergaard, M., L.H. Olesen, H. Hasle, E. Kjeldsen, and P. Hokland, *WT1 gene expression: an excellent tool for monitoring minimal residual disease in 70% of acute myeloid leukaemia patients - results from a single-centre study.* Br J Haematol, 2004. **125**(5): p. 590-600.

218. Nowakowska-Kopera, A., T. Sacha, I. Florek, M. Zawada, S. Czekalska, and A.B. Skotnicki, *Wilms' tumor gene 1 expression analysis by real-time quantitative polymerase chain reaction for monitoring of minimal residual disease in acute leukemia.* Leuk Lymphoma, 2009. **50**(8): p. 1326-32.

219. Sugiyama, H., *Wilms tumor gene (WT1) as a new marker for the detection of minimal residual disease in leukemia.* Leuk Lymphoma, 1998. **30**(1-2): p. 55-61.

220. Loken, M.R., T.A. Alonzo, L. Pardo, R.B. Gerbing, S.C. Raimondi, B.A. Hirsch, P.A. Ho, J. Franklin, T.M. Cooper, A.S. Gamis, and S. Meshinchi, *Residual disease detected by multidimensional flow cytometry signifies high relapse risk in patients with de novo acute myeloid leukemia: a report from Children's Oncology Group.* Blood, 2012. **120**(8): p. 1581-8.

221. Freeman, S.D., P. Virgo, S. Couzens, D. Grimwade, N. Russell, R.K. Hills, and A.K. Burnett, *Prognostic relevance of treatment response measured by flow cytometric residual disease detection in older patients with acute myeloid leukemia.* J Clin Oncol, 2013. **31**(32): p. 4123-31.

222. Buccisano, F., L. Maurillo, M.I. Del Principe, G. Del Poeta, G. Sconocchia, F. Lo-Coco, W. Arcese, S. Amadori, and A. Venditti, *Prognostic and therapeutic implications of minimal residual disease detection in acute myeloid leukemia.* Blood, 2012. **119**(2): p. 332-41.

223. Kern, W., U. Bacher, C. Haferlach, S. Schnittger, and T. Haferlach, *The role of multiparameter flow cytometry for disease monitoring in AML.* Best Pract Res Clin Haematol, 2010. **23**(3): p. 379-90.

224. Ommen, H.B., *Monitoring minimal residual disease in acute myeloid leukaemia: a review of the current evolving strategies*. Ther Adv Hematol, 2016. **7**(1): p. 3-16.

225. Royer-Pokora, B., M. Beier, M. Henzler, R. Alam, V. Schumacher, A. Weirich, and V. Huff, *Twenty-four new cases of WT1 germline mutations and review of the literature: genotype/phenotype correlations for Wilms tumor development*. Am J Med Genet A, 2004. **127A**(3): p. 249-57.

226. Sauna, Z.E., C. Kimchi-Sarfaty, S.V. Ambudkar, and M.M. Gottesman, *Silent polymorphisms speak: how they affect pharmacogenomics and the treatment of cancer*. Cancer Res, 2007. **67**(20): p. 9609-12.

227. Hollink, I.H., M.M. van den Heuvel-Eibrink, M. Zimmermann, B.V. Balgobind, S.T. Arentsen-Peters, M. Alders, A. Willasch, G.J. Kaspers, J. Trka, A. Baruchel, U. Creutzig, R. Pieters, D. Reinhardt, and C.M. Zwaan, *No prognostic impact of the WT1 gene single nucleotide polymorphism rs16754 in pediatric acute myeloid leukemia*. J Clin Oncol, 2010. **28**(28): p. e523-6; author reply e527-e528.

228. Chen, X., Y. Yang, Y. Huang, J. Tan, Y. Chen, J. Yang, H. Dou, L. Zou, J. Yu, and L. Bao, *WT1 mutations and single nucleotide polymorphism rs16754 analysis of patients with pediatric acute myeloid leukemia in a Chinese population*. Leuk Lymphoma, 2012. **53**(11): p. 2195-204.

229. Altshuler, D.M., R.A. Gibbs, L. Peltonen, E. Dermitzakis, S.F. Schaffner, F. Yu, P.E. Bonnen, P.I. de Bakker, P. Deloukas, S.B. Gabriel, R. Gwilliam, S. Hunt, M. Inouye, X. Jia, A. Palotie, M. Parkin, P. Whittaker, K. Chang, A. Hawes, L.R. Lewis, Y. Ren, D. Wheeler, D.M. Muzny, C. Barnes, K. Darvishi, M. Hurles, J.M. Korn, K. Kristiansson, C. Lee, S.A. McCarrol, J. Nemesh, A. Keinan, S.B. Montgomery, S. Pollack, A.L. Price, N. Soranzo, C. Gonzaga-Jauregui, V. Anttila, W. Brodeur, M.J. Daly, S. Leslie, G. McVean, L. Moutsianas, H. Nguyen, Q. Zhang, M.J. Ghori, R. McGinnis, W. McLaren, F. Takeuchi, S.R. Grossman, I. Shlyakhter, E.B. Hostetter, P.C. Sabeti, C.A. Adebamowo, M.W. Foster, D.R. Gordon, J. Licinio, M.C. Manca, P.A. Marshall, I. Matsuda, D. Ngare, V.O. Wang, D. Reddy, C.N. Rotimi, C.D. Royal, R.R. Sharp, C. Zeng, L.D. Brooks, and J.E. McEwen, *Integrating common and rare genetic variation in diverse human populations*. Nature, 2010. **467**(7311): p. 52-8.

230. *The International HapMap Project*. Nature, 2003. **426**(6968): p. 789-96.

231. *An integrated encyclopedia of DNA elements in the human genome*. Nature, 2012. **489**(7414): p. 57-74.

232. Wu, F., Y. Oka, A. Tsuboi, O.A. Elisseeva, K. Ogata, H. Nakajima, F. Fujiki, T. Masuda, M. Murakami, S. Yoshihara, K. Ikegame, N. Hosen, M. Kawakami, M. Nakagawa, T. Kubota, T. Soma, T. Yamagami, M. Tsukaguchi, H. Ogawa, Y. Oji, T. Hamaoka, I. Kawase, and H. Sugiyama, *Th1-biased humoral immune responses against Wilms tumor gene WT1 product in the patients with hematopoietic malignancies*. Leukemia, 2005. **19**(2): p. 268-74.

233. Kobel, M., S.E. Kalloger, N. Boyd, S. McKinney, E. Mehl, C. Palmer, S. Leung, N.J. Bowen, D.N. Ionescu, A. Rajput, L.M. Prentice, D. Miller, J. Santos, K. Swenerton,

C.B. Gilks, and D. Huntsman, *Ovarian carcinoma subtypes are different diseases: implications for biomarker studies*. PLoS Med, 2008. **5**(12): p. e232.

234. Netinatsunthorn, W., J. Hanprasertpong, C. Dechsukhum, R. Leetanaporn, and A. Geater, *WT1 gene expression as a prognostic marker in advanced serous epithelial ovarian carcinoma: an immunohistochemical study*. BMC Cancer, 2006. **6**: p. 90.

235. Hogdall, E.V., L. Christensen, S.K. Kjaer, J. Blaakaer, I.J. Christensen, S. Gayther, I.J. Jacobs, and C.K. Hogdall, *Expression level of Wilms tumor 1 (WT1) protein has limited prognostic value in epithelial ovarian cancer: from the Danish "MALOVA" ovarian cancer study*. Gynecol Oncol, 2007. **106**(2): p. 318-24.

236. Hylander, B., E. Repasky, P. Shrikant, M. Intengan, A. Beck, D. Driscoll, P. Singhal, S. Lele, and K. Odunsi, *Expression of Wilms tumor gene (WT1) in epithelial ovarian cancer*. Gynecol Oncol, 2006. **101**(1): p. 12-7.

237. Qi, X.W., F. Zhang, H. Wu, J.L. Liu, B.G. Zong, C. Xu, and J. Jiang, *Wilms' tumor 1 (WT1) expression and prognosis in solid cancer patients: a systematic review and meta-analysis*. Sci Rep, 2015. **5**: p. 8924.

238. Tamura, H., K. Dan, N. Yokose, R. Iwakiri, M. Ohta, H. Sakamaki, K. Tohyama, A. Kondo, H. Hyodo, K. Nakamura, T. Yamashita, O.A. Elisseeva, Y. Oka, Y. Oji, H. Sugiyama, and K. Ogata, *Prognostic significance of WT1 mRNA and anti-WT1 antibody levels in peripheral blood in patients with myelodysplastic syndromes*. Leuk Res, 2010. **34**(8): p. 986-90.

239. Hwang, H., L. Quenneville, H. Yaziji, and A.M. Gown, *Wilms tumor gene product: sensitive and contextually specific marker of serous carcinomas of ovarian surface epithelial origin*. Appl Immunohistochem Mol Morphol, 2004. **12**(2): p. 122-6.

240. Waldstrom, M. and A. Grove, *Immunohistochemical expression of wilms tumor gene protein in different histologic subtypes of ovarian carcinomas*. Arch Pathol Lab Med, 2005. **129**(1): p. 85-8.

241. Abecasis, G.R., A. Auton, L.D. Brooks, M.A. DePristo, R.M. Durbin, R.E. Handsaker, H.M. Kang, G.T. Marth, and G.A. McVean, *An integrated map of genetic variation from 1,092 human genomes*. Nature, 2012. **491**(7422): p. 56-65.

242. Salmela, E., T. Lappalainen, J. Liu, P. Sistonen, P.M. Andersen, S. Schreiber, M.L. Savontaus, K. Czene, P. Lahermo, P. Hall, and J. Kere, *Swedish population substructure revealed by genome-wide single nucleotide polymorphism data*. PLoS One, 2011. **6**(2): p. e16747.