A 4-gene expression score associated with high levels of Wilms Tumor-1 (WT1) expression is an adverse prognostic factor in acute myeloid leukaemia

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Summary

Wilms Tumor-1 (WT1) expression level is implicated in the prognosis of acute myeloid leukaemia (AML). We hypothesized that a gene expression profile associated with WT1 expression levels might be a good surrogate marker. We identified high WT1 gene sets by comparing the gene expression profiles in the highest and lowest quartiles of WT1 expression in two large AML studies. Two high WT1 gene sets were found to be highly correlated in terms of the altered genes and expression profiles. We identified a 17-probe set signature of the high WT1 set as the optimal prognostic predictor in the first AML set, and showed that it was able to predict prognosis in the second AML series after adjustment for European LeukaemiaNet genetic groups. The gene signature also proved to be of prognostic value in a third AML series of 163 samples assessed by RNA sequencing, demonstrating its cross-platform consistency. This led us to derive a 4-gene expression score, which faithfully predicted adverse outcome. In conclusion, a short gene signature associated with high WT1 expression levels and the resultant 4-gene expression score were found to be predictive of adverse prognosis in AML. This study provides new clues to the molecular pathways underlying high WT1 states in leukaemia.

Keywords: WT1, gene signature, expression score, AML, prognosis.

Acute myeloid leukaemia (AML) is a heterogeneous disease with variable prognosis depending mainly on the underlying genetic aberrations. AML patients are classified according to different risk-stratification guidelines, including those of the World Health Organization (WHO) (Swerdlow et al, 2008) and the European LeukaemiaNet (ELN) (Dohner et al, 2010). These guidelines are mainly based on the presence or absence of specific cytogenetic aberrations and gene muta-
tions. However, the guidelines have evolved over the years, as more and more new factors are found to affect AML prognosis given our increasing knowledge of the AML biology as well as the emergence of modern powerful high-throughput tools, including gene expression profiling (GEP) and next generation sequencing. Several studies have since attempted to explore the correlation of biologically relevant events to AML-GEP and prognosis using supervised cluster analysis. These studies have led to identification of several prognostic gene signatures related to various biological or clinical characteristics, including gene rearrangements (Camos et al., 2006; Wilson et al., 2006), gene mutations in NPM1 (Verhaak et al., 2005), CEBPA (Wouters et al., 2009), FLT3 (Neben et al., 2005) and WT1 (Becker et al., 2010), gene expression (de Jonge et al., 2010), leukemic stem cells (Gentles et al., 2010) and drug sensitivity (Tagliafico et al., 2006).

High levels of WT1 expression were originally found to be associated with poor prognosis in adult AML patients and used as a marker for the detection of minimal residual disease in AML (Inoue et al., 1994), as well as in acute lymphoblastic (ALL) (Inoue et al., 1994) and chronic myeloid leukaemia (CML) (Inoue et al., 1996). The predictive value of isolated high WT1 expression in AML has been confirmed in several follow-up long-term studies (Bergmann et al., 1997; Trka et al., 2002), and it was in fact extended to therapy subgroups, including haematopoietic stem cell transplantation (Jacobsohn et al., 2009), though it was not a consistent finding in this setting (Ostergaard et al., 2004). However, despite numerous clinical studies providing solid evidence for the role of high WT1 levels in leukaemia, its role is not yet clearly defined in the context of other known risk factors relevant for AML prognosis. Moreover, little is known about the molecular alterations associated to high WT1 levels that can be responsible for its poor prognostic impact. As a transcriptional regulator, WT1 binds to some common DNA binding sites (Rauscher et al., 1990), and it is not surprising that changes in its expression levels are associated with changes in the expression of hundreds of genes (Kim et al., 2009; Vidovic et al., 2013). We hypothesized that high WT1 expression was the sign of a true biological entity associated with a characteristic gene expression profile, and potentially correlated to AML prognosis. We tested this hypothesis by exploring the GEP differences among high- and low-expressing WT1 samples in two large AML series and next attempted to predict AML outcome using a gene signature and a gene expression score determined from high WT1 expression. This can shed some light on the molecular mechanisms underlying the role of high WT1 in AML pathogenesis as well as prognosis.

Methods and materials

Patients and samples

The first series of AML patients, hereafter called ‘Netherlands series’, comprised 524 younger adult (≤60 years) cases who have been treated according to sequential the Dutch-Belgian Hae-

mato-Oncology Cooperative Group and the Swiss Group for Clinical Cancer Research (HOVON/SAKK) AML-04, -04A, -29, -32, -42, and -43 protocols (Valk et al., 2004; de Jonge et al., 2010). The second series, hereafter called ‘Germany series’, consisted of 562 adult AML patients who were enrolled in the German AMLCG-99, AMLCG-M3, AMiLSG AML HD98A or HD98B trial protocols (Herold et al., 2014). The third series, hereafter called The Cancer Genome Atlas series (‘TCGA series’), consisted of 163 adult AML patients enrolled in Cancer and Leukemia Group B (CALGB) treatment protocols 8525, 8923, 9621, 9720, 10201, and 19808 including those with survival and immunophenotyping data (The Cancer Genome Atlas Research Network, 2013). The Netherlands (GSE14468) and Germany (GSE37642) gene expression sets were retrieved from NCBI Gene Expression Omnibus (GEO), and the normalized RSEM (RNS sequencing [RNA-Seq] by Expectation-Maximization) and clinical AML data set (LAML) were obtained from the TCGA Data Portal (https://tcga-data.nci.nih.gov/tcga/tcgaDownload.jsp).

Gene expression profiling

The Netherlands Study employed HG-U133 Plus 2.0 arrays (Affymetrix, Santa Clara, CA, USA) for gene expression profiling (Verhaak et al., 2009), while the Germany Study used either HG-U133 Plus 2.0 or HG-U133A and U133B arrays (Affymetrix) (Li et al., 2013). For practical reasons, only common probe-sets among two Germany subgroups were used in this study. The normalized RSEM data obtained from RNA-seq was used as an estimate of TCGA gene expression profiles.

High WT1 gene set

Each data series was separately sorted based on WT1 expression (using probe-set 206067_s_at), resulting in four quartiles. The fourth quartile (Q4) of the samples with highest mean WT1 expression was compared with the first quartile (Q1) with lowest mean WT1 expression. Those probe-sets with significant differential expression in Q4 as compared to Q1 were considered as the high WT1 gene set for that AML series.

Statistical analysis

The two-tailed student’s t-test (IBM SPSS, v.20, IBM, Armonk, NY, USA) was used to compare the means among Q4 versus Q1 samples, with acceptable Benjamini false discovery rate (FDR) of less than 0.05. Linear regression analysis (IBM SPSS, v.20) was performed to study the correlation of the differences among various gene sets. Overall survival (OS) was measured from the date of patient enrolment to the date of death. Event-free survival (EFS) was measured from the date of patient enrolment to the date of failure to achieve complete remission, relapse from complete remission or death. Relapse-free survival (RFS) was measured from the date of complete remission to the date of relapse or death. Cox regression test (IBM SPSS, v.20) was used for univariate and multivariate analysis of sur-
vival in the classifier and validation AML series. Univariate Cox regression analysis was performed using each of the potentially implicated variables. Multivariate Cox regression analysis was performed using all variables with \( P < 0.1 \) in univariate analyses. Kaplan–Meier analysis was performed using Mantel-Cox statistic (IBM SPSS, v.20) in order to test the equality of survival distributions for the different levels of the classifier in both the classifier and validation AML series.

**Outcome prediction**

The online KNNXValidation tool (version 6) from GenePattern suite (version 3.8.1; Broad Institute, Cambridge, MA, USA) (Reich et al, 2006) was employed to predict the prognostic gene signature, with EFS as the favourable event, and all others (dead, no remission, progressive disease/relapse) as adverse events. The Student’s \( t \)-test (median) was used to select the probe-sets. The resultant predicting list was trimmed so that only those probe-sets that repeated in at least 90% of the permutations were kept. GENE-E (http://www.broadinstitute.org/cancer/software/GENE-E/) was used for supervised clustering of each AML series using trimmed probe-set list or candidate gene signature.

**Gene expression score**

Receiver operating characteristic (ROC) curve analysis (IBM SPSS, v.20) was performed for individual probe-sets of the gene signature in order to assess their correlations to the EFS in the Netherlands series. Expression scores were developed for each sample using addition of the expression levels for the top two significant probe-sets considering their positive or negative correlation to the EFS. This was repeated after inclusion of the progressively less significant probe-sets, giving rise to expression scores for the lists of 2–17 probe-sets. The median expression scores were used to classify the samples into two groups, and their association to the EFS were assessed using ROC curve analysis.

**Pathway analysis**

Pathway analysis was performed using GeneGo MetaCore (https://portal.genego.com). In brief, the gene set was uploaded to MetaCore and Functional Ontology Analysis was performed to identify the enriched pathway maps. Build Networks Analysis was conducted to identify the Shortest Paths among input genes.

**Results**

**A high degree of correlation was found between the high WT1 gene sets from two AML series**

Normalized gene expression data were used to obtain differentially expressed genes in high WT1 expressing samples. Comparison of the high WT1 (Q4) and low WT1 (Q1) samples in the Netherlands series (Fig 1) identified 19 318 probe-sets as differentially expressed (Table S1), including WT1 expression level showing 10.3 fold difference. Likewise, 23 705 probe-sets were found to be differentially expressed in high WT1 samples in the Germany series (Table SII), including WT1 expression level, which showed 16.4-fold difference. These probe-sets represented genes that were either positively or negatively correlated with WT1 expression levels. About 62% of the probe-sets in the Netherlands high WT1 gene set were also found in the Germany high WT1 set, with 96.7% of them differing in the same direction. Linear regression analysis showed a high degree of correlation of the fold differences between the two high WT1 sets (Fig S1). At the gene level, 57% similarity was observed between the top 100 genes from the two high WT1 gene sets (Tables SI and SII).

A 17-probe set signature was found to be the optimal predictor of prognosis in the Netherlands AML series

The workflow that was used for prediction training and validation is illustrated in Fig 1. Briefly, The Netherlands GEP limited to the high WT1 set was used as a classifier by means of KNNXValidation and EFS as the favourable event. GEP of the AML samples were clustered using lists of 10 to 50 probe-sets as predicted by KNNXValidation. Classification of the Netherlands AML series using each predicted list identified a cluster of patients with distinct GEP that was associated with high WT1 expression levels (Q4). This high WT1 cluster was found to be associated with adverse prognosis, with a list of 17 probe-sets (herein called S17 signature) as the optimal predictor of the long-term prognosis in terms of both significance level and hazard ratio (HR) (Fig S2). The S17 signature (Table SIII) classified a distinct cluster of patients (Fig 2A) associated with high WT1 status [Odds ratio (OR) = 3.0, \( P = 1.6 \times 10^{-4} \)] as well as poor prognosis, with 5-year OS of 9.6% vs. 44.1% [HR = 2.72 [95% confidence interval (CI): 2.15–3.44]], and 5-year EFS of 6.1% vs. 37.7% [HR = 2.69 [95% CI: 2.10–3.44]] (Fig 2B–C, Table SIV). The median OS and EFS for this cluster of patients were 8.3 (95% CI: 6.7–9.9) and 4.9 (95% CI: 3.2–6.5) months respectively, compared to 32.3 (95% CI: 16.81–47.8) and 14.5 (95% CI: 9.4–19.5) months for others. Although the high WT1 cluster was also found to be correlated to other known risk factors including del(5q)(7q), FLT3-internal tandem duplication (ITD), WT1 status (positively), and inv(16), t(15;17), t(8;21), FLT3-tyrosine kinase domain (TKD) and CEBPA (double mutation) status (negatively) (Fig 2A), its prognostic impact remained highly significant after adjustment for baseline characteristics and known prognostic factors (Table 1).

The S17 signature was an independent prognostic factor in the Germany AML series

In order to validate the prognostic value of the S17 signature, it was tested against the Germany series. Supervised
clustering of the Germany series using the gene signature predicted a cluster of patients with a distinct GEP similar to the Netherlands series (Fig 3A) and predicting both adverse OS and RFS (Fig 3B–C, Table SV). The median OS was 7.6 (95% CI: 5.9–9.3) months for the cluster of patients with high WT1 as compared to 13.9 (95% CI: 11.0–16.8) months for other cases. Similarly, the median RFS was 5.3 (95% CI: 4.2–6.4) months for high WT1 cases as compared to 17.3 (95% CI: 13.4–21.2) months for the remaining patients. This prognostic impact of the S17 signature remained statistically significant after adjustment for baseline characteristics and ELN genetic risk groups (Table II).

The S17 signature predicted adverse survival in the TCGA AML series

We further tested the prognostic value of the high WT1 signature in the TCGA series, which showed a cluster of patients with a similar distinct GEP (Fig 4A), predicting adverse OS (Fig 4B). The median OS was 12.0 (95% CI: 10.1–13.9) months for the cluster of patients with high WT1 as compared to 19.1 (95% CI: 11.2–27.0) months for others. No correlation was found between the high WT1 cluster and gene mutations of prognostic significance, including FLT3-ITD, NPM1, or IDH mutations. Immunophenotyping analysis of the AML cells identified a positive correlation of the high WT1 cluster with CD34+ (OR = 4.5, P = 0.001) and CD7+ (OR = 14.4, P = 0.03) cells.

An expression score based on top four genes predicted adverse outcome in three AML series

ROC curve analysis ranked 17 probe-sets based on their correlation to the EFS in the Netherlands series (Table SVI). CD109 (226545_at) was the most significant gene and the HAVCR2 (1555628_a_at) the least significant. A gene expression score derived from the cumulative expression levels of the top four genes [CD109, KIAA0125 (also termed FAM30A), NGFRAP1 and ZC3H12C], herein called W4, demonstrated very high correlation to the EFS in the Netherlands series (Table SVII). The W4 genes were all overexpressed in the poor risk group. As anticipated, the W4 score...
predicted adverse outcome in the Netherlands series tested by Cox regression analysis (Fig 5A), with a median OS of 10.9 and 85.5 months \((P = 2 \times 10^{-14})\) in the high- and low-W4 cases, respectively. Similarly, the median EFS was 8.0 and 26.7 months \((P = 4 \times 10^{-13})\) in these groups (Fig 5B), and remained statistically significant after adjustment for baseline characteristics and known prognostic factors (Table SVIII). The W4 expression score was also able to...

Table I. Multivariate analysis of the OS and EFS in the Netherlands acute myeloid leukaemia series using Cox Regression analysis of those variables which were significant at the level of \(P < 0.1\) in univariate Cox Regression.

| Variable               | OS     |         |         |         | EFS     |         |         |         |
|------------------------|--------|---------|---------|---------|---------|---------|---------|---------|
|                        | \(P\)-value | HR | Lower | Upper | \(P\)-value | HR | Lower | Upper |
| S17 signature          | <0.001 | 1.852  | 1.387  | 2.473  | <0.001 | 1.934  | 1.465  | 2.555  |
| Age                    | 0.026  | 1.121  | 1.013  | 1.239  | NS      | -      | -      | -      |
| Complex karyotype      | 0.001  | 2.198  | 1.397  | 3.458  | 0.001  | 2.182  | 1.354  | 3.518  |
| del(5q)/del(7q)        | 0.017  | 1.76   | 1.109  | 2.795  | 0.001  | 2.027  | 1.322  | 3.109  |
| inv(16)                | 0.027  | 0.537  | 0.31   | 0.931  | 0.036  | 0.569  | 0.336  | 0.964  |
| t(8;21)                | 0.010  | 0.465  | 0.259  | 0.833  | 0.004  | 0.444  | 0.254  | 0.775  |
| FLT3-ITD               | 0.004  | 1.526  | 1.142  | 2.037  | 0.003  | 1.548  | 1.161  | 2.062  |
| NPM1-mutated           | 0.001  | 0.615  | 0.456  | 0.83   | 0.001  | 0.603  | 0.452  | 0.804  |
| CEBPA-double-mutated   | 0.008  | 0.397  | 0.201  | 0.785  | 0.008  | 0.4    | 0.203  | 0.789  |

OS, overall survival; EFS, event-free survival; HR, Hazard ratio.
predict adverse outcome in the Germany series. The median OS was 8.5 and 24.5 (P = 2 × 10⁻¹²) among the high- and low-W4 cases, respectively, and the median RFS was 7.6 and 33.7 months (P = 3 × 10⁻¹¹) in these groups (Fig 5C, D). This prognostic impact remained statistically significant after adjustment for baseline characteristics and ELN genetic groups (Table SIX). The positive predictive value (PPV) and negative predictive value (NPV) of the marker for prediction of adverse OS were 85.8% and 39.3%, respectively. They were found to be 84.6% and 43.8%, respectively, for prediction of adverse RFS. Finally, the W4 score was found to be predictive of adverse OS in the TCGA series. The median OS was found to be 11.0 and 26.0 months (P = 7 × 10⁻⁸) among high- and low-W4 cases, respectively (Fig 5E). The PPV and NPV of the marker for prediction of adverse OS were 75.3% and 46.3%, respectively.

**Pathway analysis**

MetaCore pathway analysis of the Netherlands high WT1 set of >2-fold difference identified Antigen Presentation by major histocompatibility complex (MHC) Class II as the top

Table II. Multivariate analysis of the OS and RFS in the Germany AML series using Cox Regression analysis of the ELN genetic groups and those variables which were significant at the level of P < 0.1 in univariate Cox Regression.

| Variable      | OS P-value | HR  | 95% CI for HR | RFS P-value | HR  | 95% CI for HR |
|---------------|------------|-----|---------------|-------------|-----|---------------|
|                |            |     |               |             |     |               |
| S17 signature | <0.001     | 1.485 | 1.186 - 1.860 |             |     |               |
| Age           | <0.001     | 1.291 | 1.197 - 1.391 |             |     |               |
| ELN2*         | <0.001     | 2.395 | 1.734 - 3.291 |             |     |               |
| ELN3*         | <0.001     | 2.395 | 1.732 - 3.312 |             |     |               |
| ELN4*         | <0.001     | 3.306 | 2.376 - 5.099 |             |     |               |
|               |            |     |               | <0.001      | 1.834 | 1.326 - 2.536 |
|               |            |     |               | <0.001      | 1.198 | 1.081 - 1.327 |
|               |            |     |               | <0.001      | 3.147 | 2.124 - 4.663 |
|               |            |     |               | <0.001      | 2.153 | 1.405 - 3.299 |
|               |            |     |               | <0.001      | 6.401 | 4.027 - 10.175 |

OS, overall survival; RFS, relapse-free survival; HR, Hazard ratio.

*The ELN2, ELN3, and ELN4 indicate the corresponding genetic ELN groups compared to the ELN1 group, respectively.

Fig 3. (A) Supervised clustering of the Germany acute myeloid leukaemia series using the S17 signature. The marked cluster of the high WT1 patients, comprising 31% of the cases, showed distinct gene expression profiling as compared to the remaining clusters. (B, C) Kaplan–Meier analysis of the survival in the Germany validation series clustered by S17 signature. Log-Rank (Mantel–Cox) P-values included 2.2 × 10⁻¹³ and 5.4 × 10⁻¹² for OS (B) and RFS (C), respectively. OS, overall survival; RFS, relapse-free survival;
relevant pathway (Fig S3). Most of the pathway genes were found to show low expression, indicating the downregulation of the whole pathway. Likewise, Antigen Presentation by MHC-II was found as the most biologically relevant pathway in the Germany high WT1 set of >2-fold difference (FDR = 8.5 × 10^{-3}). MetaCore analysis of the S17 plus WT1 identified a single dense network, indicating their frequent interactions (Fig S4). Three canonical pathways were identified, including those terminating on NFKB1, CREB1, and MEF2 transcription factors. The upregulated HDAC4 seemed to be in a hub position for the network.

Discussion

Several studies have attempted to risk-stratify AML patients using gene signatures obtained from GEP, though most of these signatures were correlated with known risk factors and hence of no further clinical benefit, including those associated with mutations in NPM1 (Verhaak et al, 2005), double mutant CEBPA (Wouters et al, 2009) and FLT3-ITD (Neben et al, 2005). Metzeler et al (2008) used an unbiased approach to identify all genes whose expression levels were correlated to the OS, which led to a prognostic score based on a 66-gene signature. More recently, Li et al (2013) conducted a similar global study with more extensive training and validation steps and identified a 24-gene signature that improved ELN risk classification of AML. On the other hand, Becker et al (2010) described a signature of 131 genes that was associated with the WT1 mutation, though without any clinical impact.

We showed here that differentially expressed genes in high WT1 samples were highly correlated in two AML series in terms of altered genes, expression profiles and implicated pathways, collectively corroborating the biological relevance of the high WT1 gene signature. Hence, we inferred a 16-gene signature using differentially expressed genes in high WT1 samples that was able to predict AML outcome in two independent AML series. Although the S17 signature was also associated with other known risk factors including some cytogenetic aberrations, FLT3-ITD and NPM1 status, as anticipated from a marker which is overexpressed in the vast majority of AML samples (Ostergaard et al, 2004), it was also predictive of clinical outcome independently of the currently accepted ELN genetic groups (Dohner et al, 2010). This might reflect the prognostic value of WT1 expression level in the vast majority of AML patients regardless of their underlying genetic risk factors (Bergmann et al, 1997; Trka et al, 2002).

WT1 mutation has been controversially reported to be associated with adverse prognosis in AML (Virappane et al, 2008; Gaidzik et al, 2009). We did not observe any prognostic impact of WT1 mutation in the Netherlands series. However, given the association of the WT1 mutation with the S17 signature, one cannot rule out the possibility of its indirect impact through WT1 expression level. Given the growing list of gene mutations of prognostic implication in AML, including...
recently identified mutations in DNMT3A, TET2, and ASXL1, a good surrogate marker can be a more global one, such as the S17 signature, which is correlated to several markers, in addition to its independent impact. We also identified a CD34⁺ CD7⁺ phenotype for high WT1 cluster of AML cells, which has already been found to be associated with poor risk in AML (Del Poeta et al., 1995) and possibly involved in clonal evolution of CML (Kosugi et al., 2005). However, the implication of this finding needs further investigation.

Antigen Presentation by MHC Class II was found to be the most relevant biological pathway in our study, which is in line with findings of Wilson et al. (2006), who demonstrated that a cluster of AML patients with high WT1 expression also showed low expression of MHC-II genes. The fact that targetable HDAC4 has been found to be a master regulator of the S17 network might be of potential therapeutic relevance [reviewed in (Tan et al., 2010)].

Finally, the S17 signature consistently predicted long term outcome in different clinical settings, including age groups, karyotype status and a wide variety of treatment regimens. The S17 signature also demonstrated a similar prognostic value in the much smaller series of TCGA-AML samples assessed using a different GEP platform, i.e. RNA-seq, demonstrating robust cross-platform predictive value. This was particularly promising, given that the RNA-seq gene expression data show broad dynamic range (Zhao et al., 2014) and a very high correlation to qRT-PCR results (Rapaport et al., 2013).

We hence attempted to derive a gene expression score using the most significant genes amongst those included in the S17 signature. This led to the 4-gene W4 score, which faithfully
predicted the adverse outcome in the TCGA series tested by RNA-seq, as well as the other two AML series examined by microarray analyses. Therefore, the W4 score can be easily used in a short AML series, or in its normalized form, as a surrogate marker for the prognostication of AML patients on a day-to-day basis. Among the W4 genes, CD109, which is also found in the gene signature reported by Metzeler et al (2008), is known to be expressed on a subset of haematopoietic stem and progenitor cells (Lin et al, 2002) as well as activated platelets and T-lymphoblasts (Sutherland et al, 1991), corroborating our immunophenotyping findings. CD109 overexpression has also been observed in many human cancers (Hashimoto et al, 2004). The other overexpressed gene, NGFRAP1, belongs to the neurotrophin signalling pathway, of which many genes, including NTRK1, NTRK2 and NTRK3, are known to be implicated in leukaemogenesis (Eguchi et al, 1999; Meyer et al, 2007; Li et al, 2009). To our knowledge, the W4 risk score is the simplest gene expression score predicting long-term AML prognosis (Gentles et al, 2010; Bou Samra et al, 2012; Marcucci et al, 2014).

In conclusion, we identified a short gene signature associated with high WT1 expression and demonstrated its adverse and independent prognostic impact in adult AML patients. A 4-gene expression score was next derived, which similarly predicted AML prognosis in the three series examined. Our study proposes a novel way to incorporate a candidate prognostic factor, i.e. WT1 expression level, into the current models of AML risk stratification and provides new clues to the molecular mechanisms underlying WT1 regulation. These promising results will have to be validated in further trials.

Author contributions

AN performed the research and wrote the manuscript; AN and DB designed the research study; AN and TH analysed the data; TH, YR, MCS, TB, WH, SKB, PJMV, and DB contributed essential data and reviewed the manuscript.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig S1. Differential gene expression in shared probe sets among Netherlands and Germany high-WT1 gene sets. The differences in expression levels were significantly correlated among two gene sets (r² = 0.81, P < 10⁻¹⁸).

Fig S2. Univariate survival analysis of the supervised clustering by candidate lists of probe sets in order to obtain the optimal survival predictor.

Fig S3. Antigen Presentation by MHC Class II as the most significant MetaCore pathway deregulated in Netherlands 2-fold high-WT1 set (826 probe sets). Those genes with an adjacent color bar showed differed expression in high-WT1 state, with the height of the bar as the relative decrease (blue) or increase (red) in expression level of the gene. Most key genes showed decreased expression, and hence the entire pathway seems to be downregulated in high-WT1.

Fig S4. MetaCore Shortest Network as defined by the network formed among the S17 gene products and WT1, with not more than one connection.

Table S1. Top 100 genes differentially expressed between the highest (Q4) and lowest (Q1) quartiles of WT1 expression level in the Netherlands series, or Netherlands high-WT1 set.

Table SII. Top 100 genes differentially expressed between the highest (Q4) and lowest (Q1) quartiles of WT1 expression level in the Germany series, or Germany high-WT1 set.

Table SIII. The S17 signature (consisting of 16 unique gene transcripts) which optimally classified the high-WT1 cluster associated with poor prognosis in Netherlands high-WT1 state.

Table SIV. Univariate analysis of the OS and EFS in the Netherlands AML series using Cox Regression analysis of the basic covariates and cytogenetic and molecular aberrations.

Table SV. Univariate analysis of the OS and RFS in Germany AML series using Cox Regression analysis of all potentially implicated variables.

Table SVI. Correlation of the individual S17 probe sets to the EFS in the Netherlands series using ROC curve analysis. Those probe-sets with AUCs (area under the curve) significantly above 0.5 correlate positively with the EFS, while those with AUCs less than 0.5 correlate negatively.

Table SVII. Correlation of the gene scores obtained from cumulative gene expression of the n most significant probe-sets of the S17 to the EFS in the Netherlands series using ROC curve analysis. Both the AUC (area under the curve) and the P-value did change little after W4.

Table SVIII. Multivariate analysis of the OS and EFS in the Netherlands AML series using Cox Regression analysis of those variables which were significant at the level of P < 0.1 in univariate Cox Regression.

Table SXI. Multivariate analysis of the OS and RFS in Germany AML series using Cox Regression analysis of the ELN genetic groups and those variables which were significant at the level of P < 0.1 in univariate Cox Regression.

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