WT1 mutations in T-ALL

Valeria Tosello,1 Marc R. Mansour,2 Kelly Barnes,1 Maddalena Paganin,3 Maria Luisa Sulis,1,4 Sarah Jenkinson,2 Christopher G. Allen,2 Rosemary E. Gale,2 David C. Linch,2 Teresa Palomero,1,5 Pedro Real,1,6 Vundavalli Murty,1 Xiaopan Yao,7 Susan M. Richards,8 Anthony Goldstone,9 Jacob Rowe,10 Giuseppe Basso,3 Peter H. Wiernik,11,12 Elisabeth Paietta,11,12 Rob Pieters,13,14 Martin Horstmann,15,16 Jules P. P. Meijerink,13 and Adolfo A. Ferrando1,4,5

1Institute for Cancer Genetics, Columbia University, New York, NY; 2Cancer Institute, Department of Haematology, University College London, London, United Kingdom; 3Hematology-Oncology Laboratory, Department of Pediatrics, University of Padua, Padua, Italy; Departments of 4Pediatrics and 5Pathology, Columbia University Medical Center, New York, NY; 6Andalusian Stem Cell Bank, Centro de Investigacion Biomedica, Granada, Spain; 7Department of Biostatistics & Computational Biology, Dana-Farber Cancer Institute, Boston, MA; 8Clinical Trial Service Unit, Oxford, United Kingdom; 9University College London Hospitals, London, United Kingdom; 10Rambam Medical Center and Technion, Israel Institute of Technology, Haifa, Israel; 11Montefiore Medical Center North, Bronx, NY; 12New York Medical College, New York, NY; 13Department of Pediatric Oncology/Hematology, Erasmus MC–Sophia Children’s Hospital, Rotterdam, The Netherlands; 14Dutch Childhood Oncology Group, The Hague, The Netherlands; 15Clinic for Pediatric Hematology and Oncology, University Hospital Hamburg-Eppendorf, Hamburg, Germany; and 16German Cooperative Study Group for Childhood Acute Lymphoblastic Leukemia, Hamburg, Germany

The molecular mechanisms involved in disease progression and relapse in T-cell acute lymphoblastic leukemia (T-ALL) are poorly understood. We used single nucleotide polymorphism array analysis to analyze paired diagnostic and relapsed T-ALL samples to identify recurrent genetic alterations in T-ALL. This analysis showed that diagnosis and relapsed cases have common genetic alterations, but also that relapsed samples frequently lose chromosomal markers present at diagnosis, suggesting that relapsed T-ALL emerges from an ancestral clone different from the major leukemic population at diagnosis. In addition, we identified deletions and associated mutations in the WT1 tumor suppressor gene in 2 of 9 samples. Subsequent analysis showed WT1 mutations in 28 of 211 (13.2%) of pediatric and adult T-ALL cases. WT1 mutations present in T-ALL are predominantly heterozygous frameshift mutations resulting in truncation of the C-terminal zinc finger domains of this transcription factor. WT1 mutations are most prominently found in T-ALL cases with aberrant rearrangements of the oncogenic TLX1, TLX3, and HOXA transcription factor oncogenes. Survival analysis demonstrated that WT1 mutations do not confer adverse prognosis in pediatric and adult T-ALL. Overall, these results identify the presence of WT1 mutations as a recurrent genetic alteration in T-ALL. (Blood. 2009;114:1038-1045)

Introduction

T-lineage acute lymphoblastic leukemia (T-ALL) accounts for 10% to 15% of pediatric and 25% of adult ALL cases.1 Once associated with a dismal prognosis, the introduction of intensive combination chemotherapy protocols has led to remarkable improvements in survival for this disease. Thus, more than 70% of children and 50% of adult T-ALL patients achieve long-lasting complete remissions.2 However, in contrast with the favorable response to therapy in patients at diagnosis, the prognosis of T-ALL patients with relapsed leukemia remains poor, underscoring the need to identify molecular mechanisms responsible for disease progression and to develop more effective antileukemic drugs active against relapsed T-ALL.

WT1 was originally identified as a tumor suppressor gene in patients with the WAGR (Wilms tumor, aniridia, genitourinary abnormalities, mental retardation) tumor predisposition syndrome, caused by inherited germline deletions in the chromosomal band 11p13.3 Germline mutations in WT1 are also present in patients affected with the Denys-Drash syndrome (DDS), a related tumor predisposition syndrome characterized by pseudohermaphroditism, nephropathy, genital abnormalities, and an increased risk of Wilms tumors.4 Moreover, biallelic somatic mutations in WT1 account for 15% of sporadic Wilms tumors.5 In addition to its prominent role in the pathogenesis of Wilms tumors, WT1 mutations have been reported in 10% of cases of acute myeloid leukemia (AML), 20% of biphenotypic leukemias, and sporadic cases of T-ALL.6-9

In this study, we aimed to characterize the mechanisms of disease progression and relapse in T-ALL via single nucleotide polymorphism (SNP) array analysis of paired diagnostic and relapsed T-ALL samples. Our results show a remarkable lack of genomic instability in most high-risk T-ALL cases during progression from diagnosis to relapse. In addition, we identified the presence of recurrent chromosomal deletions involving the WT1 locus in T-ALL. Subsequent mutation analysis revealed the presence of WT1 mutations in approximately 10% of pediatric and adult T-ALL samples at diagnosis. WT1 mutations in T-ALL primarily consist of heterozygous frameshift insertions and deletions encoding truncated proteins devoid of the C-terminal zinc finger domains of WT1. Notably, WT1 mutations were characteristically associated with aberrant expression of the
*TLX1*, *TLX3*, and *HOXA* transcription factor oncogenes. These results identify *WT1* mutations as a recurrent genetic abnormality associated with aberrant HOX gene expression in T-ALL.

**Methods**

**Patient samples and cell lines**

Leukemic DNA and cryopreserved lymphoblast samples were provided by collaborating institutions in the United States (Eastern Cooperative Oncology Group [ECOG], Pediatric Oncology Group), United Kingdom (Medical Research Council [MRC]), The Netherlands (Dutch Childhood Oncology Group [DCOG]), Germany (German Cooperative Study Group for Childhood Acute Lymphoblastic Leukemia [COALL]), and Italy (Hematology Laboratory, Department of Pediatrics, University of Padua, Padua, Italy). All samples were collected in clinical trials with informed consent and under the supervision of the Institutional Review Board committees of all participating institutions. Consent was obtained from all patients at trial entry according to the Declaration of Helsinki. T-cell phenotype was confirmed by flow cytometry.

**SNP array analysis**

DNA samples from pediatric T-ALL patients (n = 9) obtained at diagnosis, remission, and relapse were analyzed with the GeneChip Human Mapping 250K Sty Array (Affymetrix), which contains 23,800 SNPs. Copy number and loss of heterozygosity (LOH) analysis was performed with dCHIP. Probe intensity data for each array were normalized to a baseline array with median signal intensity using the “invariant set” model, as previously described.11 We used matched remission samples as a reference for copy number estimation and LOH analysis. LOH was analyzed using a Hidden Markov Model in dChip and an inferred LOH call threshold of 0.5. SNP array data were deposited in the Gene Expression Omnibus (GEO; National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/geo/) under accession no. GSE15931.

**Karyotype and fluorescence in situ hybridization analysis**

Interphase preparations from T-ALL cells obtained at diagnosis were subjected to fluorescence in situ hybridization (FISH) analysis using PAC clones RP11-299P16, RP1-259N9, and RP1-74J1. DNA from BACS RP11-299P16 and RP1-259N9 located telomeric and centromeric to the *WT1* locus, respectively, were labeled using spectrum orange dUTP fluorochrome (Vysis). BAC RP1-74J1, expanding the *WT1* gene, was labeled with spectrum green-dUTP fluorochrome (Vysis). FISH was performed by standard methods as previously described.10 Hybridization signals were scored on at least 500 interphase nuclei on 4,6-diamidino-2-phenylindole-stained slides using the Cytovision Imaging system attached to a Nikon Eclipse 600 microscope (Applied Imaging).

**Molecular characterization of T-ALL samples**

*WT1* mutations were analyzed by polymerase chain reaction (PCR) amplification of *WT1* exons 1 to 10 followed by direct bidirectional DNA sequencing. DNA samples from T-ALL patients enrolled in the UKALLXII/COEG2993 (n = 85) protocols according to overall survival and event-free survival. Kaplan-Meier curves were used to assess survival, and differences between groups compared by the log-rank test.

**Results**

**Genetic alterations in diagnostic and relapsed T-ALL**

Recent studies have illustrated the power of SNP array-based copy number analysis to identify important cancer-related genes associated with the pathogenesis of pediatric and adult acute lymphoblastic leukemia.18-20 To analyze genomic alterations involved in the pathogenesis of high-risk T-ALL and chromosomal changes associated with disease progression at relapse, we analyzed matched diagnostic, remission, and relapsed DNA samples from 9 pediatric T-ALL patients using Affymetrix 250K Sty SNP arrays. This analysis demonstrated the presence of known recurrent chromosomal alterations associated with the pathogenesis of T-ALL in each of the cases at diagnosis (Table 1). Most prominently, 6 of 9 cases (samples 1Id.r, 3Id.r, Vd.r, VlId.r, VHId.r, and VIIIId.r) showed homozygous deletions in the short arm of chromosome 9 centered on band 9p21, which result in loss of the *CDKN2A/p16INK4A* and *p14ARF* tumor suppressor genes. Similarly, 2 patients (samples IIId and IVd) showed discrete deletions at chromosome 4q31.2-31.3, with a common deleted region centered on...
Table 1. Chromosomal abnormalities identified in T-ALL by SNP array analysis

<table>
<thead>
<tr>
<th>Cases</th>
<th>Gain/loss</th>
<th>Chromosome</th>
<th>Cytogenetic location</th>
<th>Size, Mb</th>
<th>No. of genes</th>
<th>Candidate genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIIID,r</td>
<td>Loss</td>
<td>1</td>
<td>p21.1-31.1</td>
<td>28</td>
<td>&gt;50</td>
<td>LMO4</td>
</tr>
<tr>
<td>VIIID,r</td>
<td>Loss</td>
<td>1</td>
<td>p13.1-2</td>
<td>2.8</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>VIIID,r</td>
<td>Loss</td>
<td>1</td>
<td>q32.3-44</td>
<td>35</td>
<td>&gt;50</td>
<td>WNT9A</td>
</tr>
<tr>
<td>IVd</td>
<td>Loss</td>
<td>2</td>
<td>q21.1-31.2</td>
<td>20</td>
<td>&gt;50</td>
<td>TANK</td>
</tr>
<tr>
<td>IVd</td>
<td>Loss</td>
<td>3</td>
<td>q12.1-21.3</td>
<td>34</td>
<td>&gt;50</td>
<td></td>
</tr>
<tr>
<td>VIlr</td>
<td>Loss</td>
<td>3</td>
<td>q26.3-27.1</td>
<td>8.6</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>IXd</td>
<td>Loss</td>
<td>4</td>
<td>q21.1</td>
<td>0.06</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>IXd, IVd, IXr</td>
<td>Loss</td>
<td>4</td>
<td>q31.3-32.1*</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>IXd, IXr</td>
<td>Loss</td>
<td>5</td>
<td>q23.3-32*</td>
<td>16</td>
<td>&gt;50</td>
<td></td>
</tr>
<tr>
<td>Id, Ixr, VIIId,r</td>
<td>Loss</td>
<td>6</td>
<td>q13-16.3*</td>
<td>27</td>
<td>&gt;50</td>
<td></td>
</tr>
<tr>
<td>VIIid</td>
<td>Gain</td>
<td>6</td>
<td>q15-27</td>
<td>84</td>
<td>&gt;50</td>
<td></td>
</tr>
<tr>
<td>VIlr</td>
<td>Gain</td>
<td>7</td>
<td>p11.2-12.3</td>
<td>7.5</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>VIlr</td>
<td>Gain</td>
<td>7</td>
<td>p21.3</td>
<td>1.6</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>VIlr</td>
<td>Gain</td>
<td>7</td>
<td>q31.3</td>
<td>0.8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>VIIId</td>
<td>Gain</td>
<td>8</td>
<td>p11.2-23</td>
<td>43</td>
<td>&gt;50</td>
<td></td>
</tr>
<tr>
<td>IId, IIId,r, VId,r, VIId,r, VIIId,r</td>
<td>Loss</td>
<td>9</td>
<td>p21.3*</td>
<td>0.16</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>VId,r</td>
<td>Gain</td>
<td>9</td>
<td>q34.12</td>
<td>0.6</td>
<td>2</td>
<td>NUP214-ABL1</td>
</tr>
<tr>
<td>VId,r</td>
<td>Loss</td>
<td>9</td>
<td>q34.13</td>
<td>0.5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>VIIId,r</td>
<td>Loss</td>
<td>9</td>
<td>q22.2-34.3</td>
<td>39</td>
<td>&gt;50</td>
<td></td>
</tr>
<tr>
<td>IVd</td>
<td>Loss</td>
<td>10</td>
<td>q26.2</td>
<td>1.5</td>
<td>1</td>
<td>MGMT</td>
</tr>
<tr>
<td>VDr</td>
<td>Loss</td>
<td>11</td>
<td>p13-14.1</td>
<td>2.8</td>
<td>18</td>
<td>WT1, PAX6</td>
</tr>
<tr>
<td>Id, IVd, IXd</td>
<td>Loss</td>
<td>12</td>
<td>p12.3-13.3*</td>
<td>10</td>
<td>&gt;50</td>
<td>ETV6, CDKN1B, BCL2L14</td>
</tr>
<tr>
<td>Id</td>
<td>Gain</td>
<td>14</td>
<td>q13.1-21.3</td>
<td>25</td>
<td>&gt;50</td>
<td></td>
</tr>
<tr>
<td>Id, VIIId,r</td>
<td>Loss</td>
<td>14</td>
<td>q23.1*</td>
<td>1.5</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Id, VIIId,r</td>
<td>Loss</td>
<td>14</td>
<td>q23.2*</td>
<td>1.8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Id, VIIId,r</td>
<td>Loss</td>
<td>14</td>
<td>q24.4-24.3*</td>
<td>6.3</td>
<td>&gt;50</td>
<td></td>
</tr>
<tr>
<td>Id, VDr, VIIId,r</td>
<td>Loss</td>
<td>14</td>
<td>q32.1*</td>
<td>2.2</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>VDr</td>
<td>Loss</td>
<td>14</td>
<td>q32.2</td>
<td>1.1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>IXd,r</td>
<td>Loss</td>
<td>14</td>
<td>q31.1-1.2</td>
<td>1</td>
<td>3</td>
<td>BCL11B</td>
</tr>
<tr>
<td>IXd,r</td>
<td>Gain</td>
<td>14</td>
<td>q32.1-3</td>
<td>17</td>
<td>&gt;50</td>
<td>TRAF3, DICER1</td>
</tr>
<tr>
<td>IXd,r</td>
<td>Loss</td>
<td>22</td>
<td>q12.3-13.3</td>
<td>48.5</td>
<td>&gt;50</td>
<td>WNT7B, CERK</td>
</tr>
<tr>
<td>VId,r</td>
<td>Loss</td>
<td>22</td>
<td>q11.2</td>
<td>0.2</td>
<td>1</td>
<td>VPREB1</td>
</tr>
</tbody>
</table>

d indicates diagnostic samples; and r, relapse samples.

*Common minimally deleted regions are indicated.

FBXW7, a tumor suppressor gene encoding an F-box protein responsible for the proteasomal degradation of several oncoproteins involved in the pathogenesis of T-ALL, including NOTCH1, c-MYC, and mTOR. Larger deletions in chromosome regions 5q23.3-32 and 6q13-16.3, previously described as commonly deleted in T-ALL, were detected in 2 of 9 (22%); samples IVd and IXd and 3 of 9 (33%); samples IId, Ixr, and VIIId,r patients, respectively. In addition, we observed a focal area of amplification followed by a small deletion in chromosome band 9q34 in one patient (samples Vd,r). This pattern of amplification and deletion corresponds to the rearrangement and amplification of the NUP214 and ABL1 loci to generate the NUP214-ABL1 fusion oncogene present in 5% of T-ALLs. Larger deletions in chromosome regions 5q23.3-32 and 6q13-16.3, previously described as commonly deleted in T-ALL, were detected in 2 of 9 (22%); samples IVd and IXd and 3 of 9 (33%); samples IId, Ixr, and VIIId,r patients, respectively. In addition, we observed a focal area of amplification followed by a small deletion in chromosome band 9q34 in one patient (samples Vd,r). This pattern of amplification and deletion corresponds to the rearrangement and amplification of the NUP214 and ABL1 loci to generate the NUP214-ABL1 fusion oncogene present in 5% of T-ALLs. In addition, we extended our mutation analysis of WT1 to 28 of 211 (13.2%) pediatric and in 10 of 85 (11.7%) adult T-ALLs (supplemental Tables 1-2, available on the Blood website; see the Supplemental Materials link at the top of the online article). The...
WT1 gene encompasses 10 exons encoding an N-terminal proline and glutamine-rich domain (exons 1-6) involved in transcriptional regulation followed by 4 C-terminal Kruppel-like DNA-binding zinc fingers (exons 7-10). WT1 mutations found in T-ALL were primarily frameshift insertions and deletions and occasional nonsense point mutations located in exons 7 (30 of 38) and 8 (1 of 38), which encode prematurely truncated WT1 proteins devoid of the C-terminal zinc finger domains (Figure 2A-C). Splice donor mutations in exons 1 and 7 were found in one T-ALL sample each (Figure 2A,D). Additional frameshift mutations predicted to encode N-terminal truncations in WT1 were detected in exons 1 (4 cases) and 2 (1 case; Figure 2A,E). Two extra cases harbored missense mutations in codon 462 (R462Q and R462P) located in exon 9, which encodes the third zinc finger of the WT1 protein (Figure 2A,F). The functional relevance of these alleles is highlighted by a related WT1 mutation, R462W, which has been described as the most frequent genetic abnormality in patients with DDS. Notably, the R462W mutation has also been identified in a sporadic Wilms tumor sample and is predicted to impair the DNA-binding activity of WT1. Three additional WT1 mutations were identified in the CCRF-CEM, RPMI8402, and ALL-SIL cell lines. CCRF-CEM and RPMI8402 cells showed 2 heterozygous nonsense point mutations in exon 1 of WT1 encoding truncated proteins of 115 and 188 amino acids, respectively, whereas the ALL-SIL cell line harbored a heterozygous frameshift mutation in exon 3, encoding a truncated WT1 protein of 293 amino acids (supplemental Table 3). Analysis of paired leukemic and remission DNA samples in 10 primary T-ALL cases showed loss of the WT1 mutant allele at remission, demonstrating that WT1 mutations are somatically acquired in the leukemic clone (data not shown). In addition to these well-defined WT1 mutations, we also detected additional nonsynonymous nucleotide substitutions located in exon 1 in 4 T-ALL samples for which no remission DNA was available (supplemental Table 4). Each of these alleles, none of which has been described before, represents 0.002% of the chromosomes analyzed in this study and may constitute somatic mutations or as yet unreported private polymorphisms in the WT1 gene.

Although our analysis of WT1 mutations in T-ALL was guided by the identification of WT1 deletions in our SNP array and FISH analysis, 32 of 38 (84%) of the WT1 mutations identified in our series showed retention of the wild-type allele. Consistently, FISH analysis of 5 T-ALL samples harboring frameshift mutations in WT1 demonstrated the absence of deletions or translocations in 11p13 involving the WT1-wild-type allele. Notably, each of the 2 T-ALL cases harboring a R462 WT1 missense mutation showed complete inactivation of WT1 via loss of the WT1-wild-type allele in the case of the R462P mutation or the result of an associated frameshift mutation in exon 7 in the case of the R462Q sample (Figure 2F; supplemental Table 1).

Mutation analysis of 10 paired diagnostic and relapsed T-ALL samples showed that WT1 mutations were present both at diagnosis and relapse in 5 cases (supplemental Table 5). Interestingly, we noted that in one sample the signal of the DNA sequence corresponding to the mutant WT1 allele was weaker than that of the corresponding wild-type allele at diagnosis, with both alleles becoming equally abundant at relapse. In addition, in 1 additional sample, the WT1 mutation was present only in the relapse sample. Notably, in the 4 remaining sample pairs, we observed evidence of WT1 mutations only at diagnosis (supplemental Table 5).
The development of Wilms tumors in patients with WARG syndrome, who harbor germline deletions of the WT1 locus, is invariably associated with mutational loss of the second WT1 allele. However, 32 of 38 (84%) of the WT1 mutations found in our T-ALL series were heterozygous, suggesting that epigenetic mechanisms could account for the inactivation of the second copy of this tumor suppressor gene in T-cell lymphoblasts. To test this hypothesis, we performed DNA methylation analysis of regulatory sequences in the WT1 promoter using a combined bisulfite restriction analysis assay. Notably, this analysis failed to detect any significant methylation in the WT1 locus in WT1-wild-type (n = 110) and mutated (n = 110) T-ALL samples (data not shown). Consistently, direct sequence analysis of reverse-transcribed PCR-amplified WT1 transcripts in 2 primary T-ALL samples harboring truncating mutations in WT1 with available cDNA demonstrated biallelic expression of both the wild-type and mutant alleles of WT1 (data not shown). Overall, only 3 WT1 mutations found in our series were homozygous, with 3 additional cases showing compound heterozygous WT1 mutations.

Association of WT1 mutations with expression of TLX1, TLX3, and HOXA oncogenes

The malignant transformation of T-cell progenitors requires the cooperative action of different oncogenic pathways. Most prominently, aberrant activation of NOTCH signaling resulting from activating mutations in NOTCH1 or mutational loss of FBXW7 is found in more than 60% of T-ALL cases. In addition, aberrant expression of transcription factor oncogenes, such as TAL1, LMO1, LMO2, HOXA, TLX1, and TLX3, plays a major role in the pathogenesis of T-ALL and defines distinct molecular groups with prognostic significance.

Mutation analysis of NOTCH1 and FBXW7 in 85 adult and 37 pediatric T-ALLs showed a similar incidence of these NOTCH-activating lesions in WT1-wild-type (63 of 102 [61.7%] for NOTCH1 and 19 of 102 [18.6%] for FBXW7) and WT1 mutated (11 of 20 [55%] for NOTCH1 and 5 of 20 [25%] for FBXW7) T-ALL cases (Fisher exact test: P = .62 for NOTCH1 and P = .54 for FBXW7). In contrast, analysis of associated genomic lesions associated with activation of T-ALL transcription factor oncogenes in 127 pediatric T-ALL patients showed that WT1 mutations were associated with genetic rearrangements resulting in oncogenic activation of HOX genes. Thus, 8 of 13 WT1 mutant cases had an associated TLX1/HOX11L2 translocation (Fisher exact test: P < .001). In addition, 1 patient had a TLX1/HOX11 translocation and 2 patients demonstrated aberrant activation of HOXA genes because of the presence of inv(7)(p15q34), which involves the HOXA and TCRB loci (1 patient), or because of the SET-NUP214 fusion associated with the del(9)(q34.1q34.13). Overall, 11 of
13 (85%) WT1 mutant cases showed aberrant expression of oncogenic HOX factor gene. In 1 additional patient, the WT1 mutation was found in association with an LMO2 translocation, whereas for 2 other patients no specific translocations were identified.

**Prognostic impact of WT1 mutations in T-ALL**

Analysis of the prognostic impact of WT1 mutations in AMLs has recently demonstrated that AML cases harboring genetic lesions in WT1 have a poor prognosis. To analyze whether WT1 mutations are also associated with an adverse clinical outcome in T-ALL, we performed survival analysis in pediatric and adult T-ALL cases.

We investigated the presence of WT1 mutations in 2 independent cohorts of pediatric T-ALL patients treated on DCOG protocols (n = 64) or the COALL-97 protocol (n = 63). WT1 mutations were identified in 8 of 64 (12.5%) and 6 of 63 (9.5%) patients treated on DCOG or COALL-97 protocols, respectively. The disease-free survival at 5 years for the DCOG cohort was 62.5% plus or minus 17% versus 70% plus or minus 6% (log-rank: P = .58) for WT1 mutated and wild-type patients, respectively, whereas overall survival at 5 years was 62.5% plus or minus 17% for WT1 mutated samples compared with 66% plus or minus 6% for WT1-wild-type T-ALLs (log-rank: P = .58; Figure 3A). For the COALL cohort, the 5-year disease-free survival for WT1 mutated versus wild-type patients was 60% plus or minus 21% versus 78% plus or minus 6% (log-rank: P = .29) and the 5-year overall survival was 60% plus or minus 21% versus 70% plus or minus 8% (log-rank: P = .54; Figure 3B). Given the higher prevalence of WT1 mutations in T-ALL cases characterized by aberrant expression of TLX1/HOX11, TLX3/HOX11L2, and HOXA homeotic transcription factor oncogenes, we analyzed the specific prognostic significance of WT1 mutations in a group of 23 TLX3/HOX11L2-positive T-ALLs. This analysis showed that the presence of WT1 mutations did not predict outcome in TLX3/HOX11L2 group. The 5-year disease-free survival for TLX3/HOX11L2-positive WT1-mutant cases was 37.5% plus or minus 17% versus 56% plus or minus 15% for TLX3/HOX11L2-negative WT1-wild-type T-ALLs (log-rank: P = .29; Figure 3C).

Next we analyzed the effects of WT1 mutations on the survival of 85 adult T-ALL patients treated in the MRC UKALLXII/ECOG E2993 trial. WT1 mutations were present in 9 of 85 (10.5%) T-ALL cases in this series. Complete remission was achieved in 76 of 85 (90%) of the patients, with no difference in therapy response between WT1-wild-type and WT1-mutant samples in this cohort (P = .65). Similarly, log-rank test analysis of overall survival showed no difference between these 2 groups. Overall survival at 2 years was 60% plus or minus 6% in WT1-wild-type T-ALLs versus 56% plus or minus 17% in WT1 mutated cases (P = .7; Figure 3D). Overall, these results support that, in contrast with AML, WT1 mutations are not associated with poor prognosis in T-ALL.

**Clonal evolution and disease relapse in T-ALL**

Although analysis of clonality was not the primary aim of our study, careful examination of the pattern of genetic alterations observed in our SNP arrays and the pattern of concordance of additional genetic markers such as NOTCH1 and WT1 mutations and TCR rearrangements between diagnosis and relapse shows that in 9 of 9 cases the relapsed clone shared at least one genetic marker present in leukemic cells at diagnosis (Table 1, supplemental Table

---

**Figure 3. Survival analysis of WT1 mutations in pediatric and adult T-ALL.** (A-B) Kaplan-Meier plots of overall survival for pediatric T-ALL patients treated on DCOG (A) and COALL-97 (B) protocols. (C) Kaplan-Meier plot of overall survival for TLX3-positive pediatric T-ALL patients. (D) Overall survival analysis in adult T-ALL patients treated in the MRC UKALLXII/ECOG E2993 trial.
5). However, we also noted that matched diagnostic and relapsed samples were not identical and that most relapsed samples showed the loss of at least one genetic marker present at diagnosis (Table 1, supplemental Table 5). Acquisition of new chromosomal abnormalities and mutations at relapse was more limited, with only 3 of 9 samples showing the acquisition of new genetic lesions at relapse (Table 1, supplemental Table 5). The model that emerges from these data are that relapsed T-ALLs in our series did not derive from the most predominant clone present at diagnosis, but from an ancestral clone, which represents a related (shares some genetic markers) but different (shows loss of genetic markers and in some cases gain of new ones) subpopulation of leukemic cells.

Discussion

Over the last 2 decades, the study of recurrent chromosomal alterations has led to the identification of numerous oncogenes and tumor suppressor genes involved in the pathogenesis of this disease. More recently, the introduction of molecular cytogenetic techniques in the study of T-ALL has identified new chromosomal rearrangements previously unrecognized using standard cytogenetic assays. However, few studies have addressed the mechanisms of disease progression leading to relapse in T-ALL. Early studies on the role of TP53 in the pathogenesis of T-ALL revealed TP53 mutations are rare in T-ALL samples at diagnosis but can be found in approximately 30% of cases at relapse, suggesting TP53 inactivation is selected in the leukemic clone as a mechanism to escape the cytotoxic effects of chemotherapy. Given the safeguard function of TP53 in preserving the integrity of the genome and the mutagenic and genotoxic effects of chemotherapy, one could predict that relapsed leukemias may be characterized by increased genomic instability and harbor an increased number of genetic mutations and chromosomal alterations. However, our SNP array analysis comparing the pattern of chromosomal alterations failed to detect a major increase in chromosomal abnormalities at relapse, suggesting that spontaneous or chemotherapy-induced chromosomal instability may not be a major mutagenic mechanism contributing to therapeutic failure.

After our identification via SNP array and FISH analysis of chromosomal deletions in 11p13 encompassing the WT1 locus, we have performed an extensive mutation analysis of the WT1 gene in T-ALL. Our results demonstrate the presence of WT1 mutations in approximately 10% of pediatric and adult T-ALL cases at diagnosis. Notably, previous reports have shown the absence of WT1 mutations in pre-B-ALL, suggesting that mutational loss of WT1 may contribute to transformation of T-cell and myeloid but not B-cell progenitor cells.

Notably, 32 of 38 (84%) of the WT1 mutated T-ALL samples in our series were heterozygous with integrity of the second WT1 allele. In addition, we failed to detect epigenetic inactivation of the wild-type WT1 allele. Consistent with these results, allelic expression analysis in WT1-mutated T-ALLs demonstrated the presence of both wild-type and mutant WT1 transcripts in T-ALL lymphoblasts. Moreover, WT1 mutations found in T-ALL are typically frameshift insertions and deletions predicted to encode C-terminal truncated WT1 proteins devoid of DNA-binding activity. The clustering of WT1 mutations in exon 7 could reflect some dominant-negative effect of the resulting truncated WT1 protein products. In this regard, one of the original papers on the characterization of WT1 had already suggested that disruption of the DNA-binding domain of WT1 could have a dominant-negative effect. Moreover, WT1 truncating mutations have been described as dominant-negative alleles associated with the pathogenesis of the DDS. Nevertheless, the presence of associated deletions in the WT1-wild-type allele in some of our cases suggests that WT1 mutations may only have a partial dominant-negative effect.

Interestingly, WT1 mutations were particularly prevalent in T-ALL cases harboring chromosomal rearrangements associated with the expression of TLX1/HOX11, TLX2/HOX11L2, and HOXA9 transcription factor oncogenes, suggesting that WT1 loss and aberrant expression of oncogenic homeobox factors may be mechanistically linked in the pathogenesis of T-ALL.

Several studies have reported an association of WT1 mutations with resistance to chemotherapy and poor prognosis in AML. However, we failed to detect any significant differences in response to induction therapy and survival between WT1-mutated and WT1-wild-type T-ALLs. This discrepant result suggests that WT1 mutations may confer different biologic properties to AML and T-ALL lymphoblasts.

Finally, examination of the pattern of copy number alterations from our SNP array analysis and additional genetic markers, such as NOTCH1 and WT1 mutations and TCR rearrangements, showed that most T-ALL cases at relapse are not the result of clonal evolution from the predominant leukemic population present at diagnosis. Thus, although all relapsed T-ALLs shared at least one genetic marker with the predominant clone at diagnosis, they also showed frequent loss of copy number alterations and mutations, resulting in a genetically related but distinct disease. These results are in agreement with those reported in an extensive study analyzing the clonality of ALLs at diagnosis and relapse using SNP array analysis. In that study, most ALLs revealed changes in the pattern of alterations during disease progression. Thus, although 88% of ALLs shared genetic markers between the diagnosis and relapse, 52% of the cases showed loss of at least one genetic marker, demonstrating that clonal evolution from an ancestral clone is a frequent mechanism associated with relapse in this disease.

In conclusion, our results identify the presence of WT1 mutations as a recurrent genetic abnormality associated with aberrant HOX gene expression in T-ALL.

Acknowledgments

This work was supported by the National Institutes of Health (R01CA120196, R01CA129382, CA114737, and CA021111), ECOG’s Leukemia Tissue Bank, the WOLF Foundation (A.A.F.), the Leukemia & Lymphoma Society (grants 1287-08 and 6237-08; A.A.F.), the Charlotte Geyer Foundation (A.A.F.), the Cancer Research Institute (A.A.F.), the Swim Across America Foundation (A.A.F.), the Golfers Against Cancer Foundation (A.A.F.), the Leukemia Research Fund (R.E.G., D.C.L.), the Medical Research Council of Great Britain (M.R.M.), and the Fondo de Investigacion Sanitaria (grant CD07/00033; P.R.). A.A.F. is a Leukemia & Lymphoma Society Scholar. T.P. is a recipient of a Young Investigator Award from the Alex’s Lemonade Stand Foundation.

A complete list of the DCOG and the German COALL investigators can be found in the supplemental Appendix.
Authorship


Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Adolfo A. Ferrando, Institute for Cancer Genetics, Columbia University, 1130 St Nicholas Ave, Rm 505A, New York, NY 10032; e-mail: af2196@columbia.edu.

References


WT1 mutations in T-ALL

Valeria Tosello, Marc R. Mansour, Kelly Barnes, Maddalena Paganin, Maria Luisa Sulis, Sarah Jenkinson, Christopher G. Allen, Rosemary E. Gale, David C. Linch, Teresa Palomero, Pedro Real, Vundavalli Murty, Xiaopan Yao, Susan M. Richards, Anthony Goldstone, Jacob Rowe, Giuseppe Basso, Peter H. Wiernik, Elisabeth Paietta, Rob Pieters, Martin Horstmann, Jules P. P. Meijerink and Adolfo A. Ferrando