T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy of thymocytes characterized by high numbers of bone marrow and circulating blast cells, enlargement of mediastinal lymph nodes, and often central nervous system involvement. T-ALL accounts for approximately 15% of pediatric and 25% of adult ALL cases. Similar to other types of leukemia, T-ALL is caused by genetic alterations in hematopoietic precursor cells leading to a variety of changes, including loss of cell cycle control, unlimited self-renewal capacity, impaired differentiation, hyperproliferation and loss of sensitivity to death signals.

Molecular analysis of T-ALL cases has identified numerous chromosomal aberrations and more subtle genetic defects, most of which are specific to T-ALL. In 1985, a major breakthrough was made in the study of T-ALL, by localizing the TRA@ (T-cell receptor α; old gene symbol: TCRA) locus at 14q11, a region that had been described to be involved in translocations and inversions in T-ALL. At that time, it was hypothesized that these rearrangements could possibly activate proto-oncogenes by bringing them in the proximity of enhancers of the TRA@ locus, and that this could be exploited to clone these loci involved in T-cell malignancies. Now, 20 years later, we possess a detailed list of genes affected by chromosomal rearrangements and other genetic defects, yet a number of questions remain unanswered. The fact that our current insight into the molecular pathogenesis of T-ALL is still incomplete was recently illustrated by the discovery of cryptic ABL1 rearrangements in 6%, and NOTCH1 mutations in more than 50% of T-ALL.

In vitro and in vivo analysis of some of the deregulated genes cloned from T-ALL cases confirmed their oncogenic properties, and provided a better understanding of their specific contribution to the development of T-ALL. The combination of genetic and functional data suggests that a stepwise alteration of at least four specific pathways is required before thymocytes become fully malignant. We associate these pathways with four different classes of mutations: (i) mutations that affect the cell cycle; (ii) mutations that impair differentiation; (iii) mutations that provide a proliferative and survival advantage; (iv) mutations providing self-renewal capacity (Figure 1). This model is deduced from the two-hit hypothesis as proposed for acute myeloid leukemia (AML). It is now well illustrated that class I mutations, providing mainly a proliferative advantage, and class II mutations, impairing differentiation, cooperate with each other and with addi-
tional mutations to cause AML. In this article we extend this model to T-ALL and use it as a strategy to classify the hitherto identified molecular defects in T-ALL.

**Cell cycle defects**

Mitogen-induced progression through the cell cycle is tightly regulated to make sure that cell division is co-ordinated with cell growth, and does not proceed when, for example, DNA damage is detected. Different types of checkpoints ensure the control over this. Since these checkpoints are central to the maintenance of the genomic integrity and basic viability of the cells, defects in these pathways may result in either tumorigenesis or apoptosis, depending on the severity and nature of the defects.

Mitogenic signals induce the formation of active complexes between cyclins and cyclin-dependent kinases (CDK) resulting in phosphorylation of the retinoblastoma protein (RB1), thereby abrogating the ability of RB1 to inhibit cell proliferation (Figure 2). The activity of cyclin-CDK complexes is in turn inhibited by the INK4 proteins (p16, p15, p18 and p19). Another important protein involved in cell cycle control is the p53 tumor suppressor protein (TP53). When DNA damage is detected, TP53 gene expression is upregulated causing transcriptional activation of CDKN1A (p21). The p21 protein is a CDK inhibitor causing arrest of the cell cycle in G1-phase allowing DNA repair or apoptosis in case of irreparable DNA damage. The activity of TP53 is harnessed by HDM2, a protein that binds TP53 and induces its degradation. HDM2 is in turn inhibited by p14 (Figure 2).

RB1 and TP53 are rarely mutated in T-ALL. In contrast, inactivation of CDKN2A and CDKN2B, 2 genes located in close proximity on chromosome region 9p21, is the most common genetic defect in T-ALL (Figure 1). The CDKN2A gene encodes both p16 and p14. The p16 and p14 transcripts have different promoters and first exons (exons 1a and 1b respectively), but share exons 2 and 3. Both gene products are potent regulators of the cell cycle, although they use different mechanisms: p16 inhibits RB1 phosphorylation whereas p14 activates TP53 (Figure 2).

It is clear now that CDKN2A is the primary target of 9p21 deletions in ALL, but the respective importance of p16 and p14 inactivation is still unclear. The 9p deletions vary significantly in size and sometimes cover large genomic regions. Therefore, contiguous genes such as CDKN2B (encoding p15) are co-deleted in a significant fraction of T-ALL. Inactivation of CDKN2A and CDKN2B by homozygous deletion has been described to be present in 65% and 23% of T-ALL samples, respectively. Hemizygous CDKN2A and CDKN2B deletions are found in approximately

---

**Figure 1.** Frequency of the different mutations observed in T-ALL. Molecular analysis of T-ALL shows that four major classes of mutations are involved in the molecular pathogenesis of T-ALL. These four classes are represented by the four diagrams, in which the frequency of each of the different mutations is given. For most genes, no separate mutation frequencies were available for childhood and adult T-ALL. In case these were available, we give the frequency in childhood T-ALL. It is important to note that this scheme is a simplification, and that some indicated genes in fact cannot be unambiguously assigned to only one of these categories. Out of this scheme, it seems reasonable to assume that one or more mutations implicated in survival/proliferation and in self-renewal of T-ALL cells are yet to be identified.

---

**Table:**

<table>
<thead>
<tr>
<th>Class</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell cycle defects</strong></td>
<td></td>
</tr>
<tr>
<td>TP53, RB, p27</td>
<td></td>
</tr>
<tr>
<td>CDKN2A/CDKN2B (96%)</td>
<td></td>
</tr>
<tr>
<td><strong>Differentiation impairment</strong></td>
<td></td>
</tr>
<tr>
<td>PICALM-MLLT10 (5-10%)</td>
<td></td>
</tr>
<tr>
<td>MLL-fusions (4%)</td>
<td></td>
</tr>
<tr>
<td>TAL-2 (&lt;1%)</td>
<td></td>
</tr>
<tr>
<td>TLX1 (7%)</td>
<td></td>
</tr>
<tr>
<td>TLX3 (20%)</td>
<td></td>
</tr>
<tr>
<td><strong>Proliferation and survival</strong></td>
<td></td>
</tr>
<tr>
<td>unknown (&gt;78%)</td>
<td></td>
</tr>
<tr>
<td>PTEN (&lt;1%)</td>
<td></td>
</tr>
<tr>
<td>ETV6-JAK2 (-1%)</td>
<td></td>
</tr>
<tr>
<td>ETV6-PBL2 (&lt;1%)</td>
<td></td>
</tr>
<tr>
<td>FLT3 (1%)</td>
<td></td>
</tr>
<tr>
<td><strong>Self-renewal capacity</strong></td>
<td></td>
</tr>
<tr>
<td>unknown (44%)</td>
<td></td>
</tr>
<tr>
<td>N-RAS (5%)</td>
<td></td>
</tr>
<tr>
<td>LCK (&lt;1%)</td>
<td></td>
</tr>
<tr>
<td><strong>Proliferation and survival</strong></td>
<td></td>
</tr>
<tr>
<td>unknown (1%)</td>
<td></td>
</tr>
<tr>
<td>TP53, RB, p27</td>
<td></td>
</tr>
<tr>
<td>CDKN2A/CDKN2B (96%)</td>
<td></td>
</tr>
<tr>
<td><strong>Differentiation impairment</strong></td>
<td></td>
</tr>
<tr>
<td>PICALM-MLLT10 (5-10%)</td>
<td></td>
</tr>
<tr>
<td>MLL-fusions (4%)</td>
<td></td>
</tr>
<tr>
<td>TAL-2 (&lt;1%)</td>
<td></td>
</tr>
<tr>
<td>TLX1 (7%)</td>
<td></td>
</tr>
<tr>
<td>TLX3 (20%)</td>
<td></td>
</tr>
<tr>
<td><strong>Proliferation and survival</strong></td>
<td></td>
</tr>
<tr>
<td>unknown (1%)</td>
<td></td>
</tr>
<tr>
<td>PTEN (&lt;1%)</td>
<td></td>
</tr>
<tr>
<td>ETV6-JAK2 (-1%)</td>
<td></td>
</tr>
<tr>
<td>ETV6-PBL2 (&lt;1%)</td>
<td></td>
</tr>
<tr>
<td>FLT3 (1%)</td>
<td></td>
</tr>
<tr>
<td><strong>Self-renewal capacity</strong></td>
<td></td>
</tr>
<tr>
<td>unknown (44%)</td>
<td></td>
</tr>
<tr>
<td>N-RAS (5%)</td>
<td></td>
</tr>
<tr>
<td>LCK (&lt;1%)</td>
<td></td>
</tr>
<tr>
<td><strong>Proliferation and survival</strong></td>
<td></td>
</tr>
<tr>
<td>unknown (1%)</td>
<td></td>
</tr>
<tr>
<td>TP53, RB, p27</td>
<td></td>
</tr>
<tr>
<td>CDKN2A/CDKN2B (96%)</td>
<td></td>
</tr>
</tbody>
</table>
10% and 15% of the samples. In addition, inactivation of CDKN2A/B is also caused by mutation and by promoter hypermethylation. A few studies describe CDKN2A inactivation at the transcriptional and post-transcriptional levels, although the mechanisms remain unknown. Overall, the inactivation rates for CDKN2A and CDKN2B in T-ALL at DNA/RNA/protein levels were found to be as high as 95% and 99%, respectively. These findings emphasize the importance of inactivation of CDKN2A/B in the development of T-ALL and identify the RB1 and TP53 pathways as possible targets for therapy.

**Impaired differentiation caused by transcription factor defects**

Transcriptional deregulation is a common theme in acute leukemias. However, unlike AML and B-ALL, in which predominantly chimeric transcription factor proteins are generated, T-ALL is mainly associated with the deregulated expression of normal transcription factor proteins (Figure 1). This is often the result of chromosomal rearrangements juxtaposing promoter and enhancer elements of T-cell receptor genes TRA@ (14q11), TRB@ (T-cell receptor β, 7q34-35), TRG@ (T-cell receptor γ, 7p15) and TRD@ (T-cell receptor δ, 14q11) to a small number of developmentally important transcription factor genes (Table 1). Deregulated transcription factors may exert their oncogenic potential by altering the gene expression programs that regulate hematopoietic differentiation of a multipotent progenitor.

---

**Table 1. T-cell receptor genes and their involvement in chromosomal aberrations in T-ALL.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene symbol</th>
<th>Chromosome location</th>
<th>Partner gene</th>
<th>Chromosome location</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-cell receptor α</td>
<td>TRA@</td>
<td>14q11</td>
<td>TLX1</td>
<td>10q24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LAL1</td>
<td>1p32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LMO1</td>
<td>11p15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LMO2</td>
<td>11p13</td>
</tr>
<tr>
<td>T-cell receptor β</td>
<td>TRB@</td>
<td>7q34-35</td>
<td>TAL1</td>
<td>7p15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LAL1</td>
<td>19p13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LAL2</td>
<td>9q32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LCK</td>
<td>1p34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NOTCH1</td>
<td>9q34</td>
</tr>
<tr>
<td>T-cell receptor γ</td>
<td>TRG@</td>
<td>7p15</td>
<td>no known chromosomal aberrations</td>
<td></td>
</tr>
<tr>
<td>T-cell receptor δ</td>
<td>TRD@</td>
<td>14q11</td>
<td>TLX1</td>
<td>10q24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LAL1</td>
<td>1p32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LMO1</td>
<td>11p15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LMO2</td>
<td>11p13</td>
</tr>
</tbody>
</table>

Note that TLX3 is not present in this table, as it is not involved in translocations involving T-cell receptor genes.

**Homeobox genes**

The homeobox (HOX) family of transcription factors is divided into two classes. Class I HOX genes are organized in four distinct clusters (HOXA@, HOXB@, HOXC@ and HOXD@), whereas class II HOX genes are dispersed throughout the genome. Class I HOX genes encode a complex network of transcription regulatory proteins whose precise targets remain poorly understood. These HOX genes were shown to play a role not only in anteroposterior patterning and cell differentiation during embryonic development, but also in organizing and regulating hematopoiesis and leukemogenesis. From this class of HOX genes, only the HOXA@ cluster is known to be involved in T-ALL. Among the class II HOX genes, TLX1 (HOX11) and TLX3 (HOX14L2) have been extensively studied in the context of T-ALL.

**TLX1 (HOX11)**

The TLX1 protein is required for spleen development, but is normally not expressed in adult tissues. About 7% of childhood T-ALL samples show ectopic expression of TLX1 in thymocytes. This overexpression has been associated with t(10;14)(q24;q11) and its variant t(7;10)(q35;q24), bringing the TLX1 coding sequence under the transcriptional control of regulatory sequences of the TRA@ or TRB@ genes, respectively. Nevertheless, overexpression of TLX1 in thymocytes has also been demonstrated in the absence of a 10q24 rearrangement, suggesting that other, trans-acting mechanisms can lead to this aberrant gene expression, probably
by disrupting gene silencing mechanisms that operate during normal T-cell development.\textsuperscript{28,33-35} Microarray analysis revealed that TLX1 expressing lymphoblasts show a gene expression pattern resembling that of early cortical thymocytes (Figure 3) and that the highly favorable clinical outcome of these patients may be explained by the lack of expression of anti-apoptotic genes at this stage of thymocyte development.\textsuperscript{29,36}

TLX1 has been reported to immortalize murine hematopoietic progenitors \textit{in vitro} and to induce T-ALL-like malignancies in mice engrafted with MSCV-TLX1-transduced bone marrow cells after long latency, suggesting that progression to a fully malignant state indeed requires supplemental mutations.\textsuperscript{37,38} These studies provide convincing evidence supporting the oncogenic potential of TLX1, and efforts are now being made to elucidate the exact molecular mechanism of TLX1-induced leukemogenesis.\textsuperscript{29}

\textbf{TLX3 (HOX11L2)}

Approximately 20% of childhood and 15% of adult T-ALL cases are characterized by ectopic TLX3 expression.\textsuperscript{27,40,41} In most cases, TLX3 expression is caused by the cryptic t(5;14)(q35;q32) juxtaposing TLX3 to the distal region of \textit{BCL11B}, a gene highly expressed during T-cell differentiation.\textsuperscript{32,43} Interestingly, a variant t(5;14)(q35;q32) has been identified in a pediatric T-ALL cell line, in which a different homeobox gene, \textit{NKX2-5}, is ectopically activated instead of TLX3.\textsuperscript{44} \textit{NKX2-5} expression was not reported in other T-ALL cases. Other variant chromosomal aberrations, each targeting TLX3, have been observed as well, including a t(5;7)(q35;q21), in which the \textit{CDK6} gene is involved on 7q21.\textsuperscript{45}

Although TLX1 and TLX3 themselves and the gene expression profiles of TLX1 and TLX3-expressing T-ALL samples are very similar, some studies indicate that TLX3 confers a worse response to treatment, whereas other studies are not in accordance with this unfavorable prognosis.\textsuperscript{27,28,40} It is possible that the prognostic meaning of TLX3 expression is modulated by the presence of specific oncogenes (such as \textit{NUP214-ABL1} or \textit{NOTCH1} mutations, see further), but this has not been looked at so far. Larger follow up studies will be required to determine the exact prognostic meaning of TLX3 expression alone or in combination with other markers.

\textbf{HOXA@ cluster}

Only very recently, a cytogenetically invisible chromosomal inversion inv(7)(p15q34) has been observed in approximately 5% of T-ALL cases. This inversion juxtaposes part of the TRB@ locus (7q34-35) to part of the HOXA@ cluster (7p15), resulting in deregulated expression of HOXA10 and HOXA11.\textsuperscript{46} In addition, 2% of the cases showed elevated HOXA10 and HOXA11 expression in the absence of inv(7), suggesting that other activating mechanisms may exist. In contrast to TLX1 and TLX3, which are normally not expressed in the hematopoietic system, HOXA10 and HOXA11 are expressed in developing thymocytes. While HOXA11 is expressed at different stages of T-cell differentiation, HOXA10 expression is only detected at the earliest stages of differentiation, suggesting that its downregulation is required for full maturation of T cells to the CD4 and CD8 single positive stages.\textsuperscript{47,48} This is supported by \textit{in vitro} and \textit{in vivo} mouse models in which the effect of \textit{HOXA10} overexpression on differentiation of both myeloid and lymphoid cells was confirmed.\textsuperscript{49,50}
MLL fusions

Although MLL itself is not a homeobox gene, it is closely linked to the family of HOX genes. MLL fusion proteins possess enhanced transcriptional activity resulting in increased expression of HOXA9, HOXA10, HOXC6, and also of the MEIS1 HOX co-regulator. T-ALL cells with MLL fusions are a distinct subtype, characterized by differentiation arrest at an early stage of thymocyte differentiation, after commitment to the γδ lineage (Figure 3).]

Basic helix-loop-helix family members

The basic helix-loop-helix (bHLH) family of transcription factors all share a bHLH motif (about 60 amino acids), which allows them to dimerize through the HLH domain and to bind to DNA through the basic regions of the dimerized proteins. bHLH proteins are classified in two main groups. Class A proteins, such as E2A (TCF3), are widely expressed and form homodimers as well as heterodimers with other bHLH proteins. In contrast, class B proteins are expressed in a tissue-specific manner and do not homodimerize, but form heterodimeric complexes with class A proteins.

LYL1

The LYL1 gene was initially identified upon the molecular characterization of the translocation t(7;19)(q34;q11) associated with T-ALL. As a consequence of this chromosomal rearrangement, LYL1 coding sequences are juxtaposed to the TRB@ locus, and are constitutively expressed, whereas LYL1 expression is absent in normal T cells. Although ectopic LYL1 expression is found in some human T-cell leukemias, suggesting that it may participate in T-cell leukemogenesis, its oncogenic potential has thus far not been demonstrated in a transgenic mouse model.

TAL1 (SCL, TCL5)

Alteration of the TAL1 locus (1p32) is the most common transcription factor defect in (childhood) T-ALL. In 3% of childhood T-ALL, TAL1 disruption is caused by t(1;14)(p32;q11), providing another example of ectopic expression in T cells caused by juxtaposition to regulatory TCR gene elements. However, in a much larger fraction (17%) of T-ALL, TAL1 is overexpressed as a consequence of a cryptic interstitial deletion, generating SIL-TAL1 fusion transcripts by fusing TAL1 to the promoter region of the SIL gene. In addition, T-ALL cell lines and patients’ samples without detectable TAL1 rearrangements often express high levels of TAL1 mRNA.

Two theoretical models for TAL1-induced leukaemogenesis exist. In the first model, the oncogenic potential of TAL1 is explained by inappropriate activation of TAL1 target genes. The second model proposes a dominant-negative mechanism in which ectopically expressed TAL1 binds to, and inactivates its normal interacting proteins, such as the E47 and E12 variants of E2A transcription factors. Experimental data are in favor of the second model as it has been shown that mice transgenic for the SIL-TAL1 fusion lacking the TAL1 transactivation domain still develop aggressive T-cell malignancies. In agreement with this model, it has also been shown that TAL1 tumors undergo apoptosis after administration of histone deacetylase (HDAC) inhibitors. These results indicate that overexpression of TAL1 causes gene silencing and that reactivation of silenced genes by administering HDAC inhibitors may prove efficacious in T-ALL patients expressing TAL1.

TAL2

As a consequence of t(7;9)(q34;q32), a recurring translocation associated with T-ALL, the TAL2 gene is juxtaposed to the TRB@ locus. The properties of TAL2 broadly resemble those described previously for TAL1, supporting the idea that both proteins promote T-ALL by a common mechanism.

LIM-domain only genes LMO1 and LMO2

The genes encoding the LIM-domain only proteins LMO1 (RBTN1 or TTG1, 11p15) and LMO2 (RBTN2 or TTG2, 11p13) are frequently rearranged in T-ALL. Most common are the (11;14)(p15;q11) and t(11;14)(p15;q11) juxtaposing LMO1 or LMO2 to the TAL1 locus.

In agreement with the model proposed in Figure 1, ectopic expression of TLX1, TLX3, TAL1 and LYL1 is mutually exclusive, although rare exceptions to this rule have been described. In contrast, expression of LMO1 and LMO2 is found in T-ALL cases that already have deregulated TAL1 or LYL1 expression. This seems to be in conflict with the proposed model. Studies in transgenic mice, however, have shown that TAL1 expression in itself is not sufficient to induce T-cell malignancies, and that co-expression...
of \textit{LMO1} or \textit{LMO2} is strictly required.\textsuperscript{6,7,4} This explains the high frequency of overlap between \textit{TAL\textsubscript{1}} and \textit{LMO} expression. As a consequence, ectopic expression of \textit{LMO1} and \textit{LMO2} cannot be shown independently on the graphs in Figure 1, but is shown overlapping with \textit{TAL\textsubscript{1}} and \textit{LYL1} expression.

**PICALM-MLLT10 (CALM-AF10)**

The t(10;11)(p13;q14) is a recurring translocation in T-ALL, but it has also been observed in other leukemias, including AML, and it is thus not exclusively associated with T-cell defects.\textsuperscript{7,6,7} This translocation is often not detected by routine cytogenetics and the corresponding fusion gene, \textit{PICALM-MLLT10} can be detected in up to 10\% of T-ALL cases, of which only half show the translocation in their karyotype.\textsuperscript{7,5,8} The exact function of the fusion protein is not known, but interestingly, both \textit{PICALM} and \textit{MLLT10} are known fusion partners of \textit{MLL}.\textsuperscript{8,12}

### Mutations providing a proliferative and survival advantage

**Components of the T-cell receptor signaling pathway**

Signals generated upon engagement of the T-cell antigen receptor (TCR) are critical in the regulation of T-cell immune responses, in particular for T-cell survival and proliferation. As the TCR is devoid of intrinsic kinase activity, proximal signaling is mediated by recruitment and activation of multiple tyrosine kinases, which act in concert to activate a diverse set of signaling molecules (Figure 4). The SRC family protein tyrosine kinases, LCK and FYN, play a central role in the initiation of this signaling cascade. Activated LCK and FYN cause phosphorylation of the immunoreceptor tyrosine based activation motifs (ITAM) of the TCR-CD3 complex leading to recruitment of the ZAP70 tyrosine kinase and phosphatidylinositol 3-kinase (PI3K).\textsuperscript{6,11} Recent studies indicate a role for the ABL1 kinase downstream of LCK and upstream of ZAP70.\textsuperscript{6} Activated ZAP70 then in turn phosphorylates the adaptor molecules SLP-76 (LCP2) and LAT. Association of PLC\textgamma\textsubscript{1} with LAT allows it to be activated and to be positioned in the proximity of its substrate PIP\textsubscript{2}, which is then hydrolysed into DAG and IP\textsubscript{3}, second messengers causing, respectively, activation of PKC and release of intracellular Ca\textsuperscript{2+}. The increased Ca\textsuperscript{2+} levels cause initiation of calcineurin phosphatase activity. PKC and GRB2 initiate activation of the RAS-MAP kinase pathway.\textsuperscript{6,8,9,7} PI3K is another important player which becomes active after TCR engagement. PI3K catalyzes phosphorylation of mainly PIP\textsubscript{2} to PIP\textsubscript{3}, causing activation of AKT followed by inactivation of GSK3B kinase. Generation of PIP\textsubscript{3} also contributes to activation of RAS, PLC\textgamma\textsubscript{1} and other mediators of TCR-signaling. PTEN catalyzes dephosphorylation of PIP\textsubscript{3} to PIP\textsubscript{2}, opposing PI3K activity.\textsuperscript{64} Activated ERK and calcineurin, and inactivated GSK3, act in concert to initiate activation of multiple transcription factors resulting in \textit{IL2} gene expression. The resulting secreted interleukin-2 stimulates T cells to proliferate. In the following section of this text, we will give an overview of the molecules involved in the TCR signaling pathway that have been shown to play a role in the pathogenesis of T-ALL.

**ABL1-fusions**

ABL1 is a ubiquitously expressed tyrosine kinase that has recently been shown to play a role in the TCR pathway (Figure 4).\textsuperscript{6,6} ABL1 is typically found to be fused to the \textit{BCR} gene in chronic myeloid leukemia and precursor B-cell acute lymphoblastic leukemia as a result of the Philadelphia translocation t(9;22) (q34;q11).\textsuperscript{6,8,9} Although the \textit{BCR-ABL1} fusion is exceptionally rare in T-ALL, recent findings clearly indicate that ABL1-fusions do play a role in the pathogenesis of T-ALL.\textsuperscript{6,9} An example of this is the identification of the \textit{NUP214-ABL1} fusion in approximately 6\% of T-ALL patients.\textsuperscript{6} Interestingly, this fusion was found to be present on small, cytogenetically invisible, extrachromosomal elements (episomes), and is associated with \textit{TLX1} or \textit{TLX3} expression and deletion of \textit{CDKN2A}.\textsuperscript{6} Apart from the \textit{NUP214-ABL1} fusion, variant ABL1-fusions, such as the \textit{ETV6-ABL1} and the \textit{EML1-ABL1} fusions, have also been reported with a lower incidence in T-ALL.\textsuperscript{6,10,11} These ABL1 fusion kinases are constitutively phosphorylated, resulting in excessive activation of survival and proliferation pathways, which can be inhibited upon addition of imatinib, a selective inhibitor of ABL1 kinase activity.\textsuperscript{6,8,13} These findings suggest that imatinib could improve outcome for T-ALL patients with ABL1-fusions. It will be interesting to investigate whether these oncogenic ABL1-fusions interact with the SRC kinases and other signaling molecules in the same way as ABL1 does in T-cells (see Figure 4).

**LCK**

LCK, a member of the SRC family of tyrosine kinases, is highly expressed in T-cells and plays a critical role in proximal TCR signaling events (Figure 4).\textsuperscript{94} In the past, rare cases have been described with activation of \textit{LCK} by t(1;7)(p34;q34), joining \textit{LCK} and \textit{TRB@loci}.\textsuperscript{9,5,6} Interestingly, recent findings indicate that ABL1 is located downstream of LCK in the TCR signaling pathway (Figure 4).\textsuperscript{95} Based on these results, SRC kinase inhibitors and the dual SRC/ABL kinase inhibitors could be used for treating T-ALL patients with hyperactive LCK.
**RAS**

RAS proteins are anchored at the cytosolic side of the plasma membrane and are required to transmit proliferation stimulating signals from several types of receptors, including tyrosine kinase receptors, non-tyrosine kinase receptors (including the TCR) and G protein-coupled receptors (Figure 4). Activating mutations of the NRAS isoform have been identified in 4-10% of T-ALL cases. Nevertheless, there are results indicating that RAS is highly activated in half of T-ALL patients, suggesting a major contribution of RAS activation in the pathogenesis of T-ALL, either by RAS mutations or by activation of molecules upstream of RAS. As RAS is constitutively activated by mutations in about one-third of all human malignancies, RAS and its downstream effectors have been the subject of intensive research by the pharmaceutical industry, resulting in the availability of a variety of farnesyltransferase inhibitors. These inhibitors are currently being tested for the treatment of AML, and they could also turn out to be relevant as targeted therapy of T-ALL.

**PTEN**

PTEN is another key component of the TCR signaling pathway whose activity is opposed by PTEN, a known tumor suppressor protein (Figure 4). Consequently, inactivation of PTEN abrogates the balance between PI3K and PTEN activity, causing uncontrolled proliferation of T cells. Pharmacological inhibition of PI3K has been shown to result in apoptosis of PTEN null T-ALL cell lines, indicating that the PI3K/AKT pathway plays a major role in the growth and survival of PTEN-null T-ALL cells, thereby identifying this cascade as a promising target for therapeutic intervention in T-ALL.
Other tyrosine kinase mutations

**FLT3**

The FLT3 gene encodes a receptor tyrosine kinase (RTK) playing an important role in the development of hematopoietic stem cells. Although activating mutations of FLT3 are the most common genetic abnormalities in AML, these mutations are quite rare in T-ALL and seem to be restricted to CD117/KIT+ T-ALL lymphoblasts with high expression levels of LYL1 and LMO2. Similar to what has been described in AML, the mutations in T-ALL are internal tandem duplications (ITD) in the juxtamembrane domain of the receptor and point mutations in the activation loop of the kinase domain, leading to constitutive RTK activity in the absence of ligand. Despite the low number of T-ALL patients with FLT3 mutations, the FLT3 inhibitors that are currently being tested in clinical trials in the context of AML could be valuable for treating these T-ALL patients.

**ETV6-JAK2**

The Janus family of tyrosine kinases (JAK) plays an essential role in development and in coupling cytokine receptors to downstream intracellular signaling events. A t(9;12)(p24;p13) in a childhood T-ALL patient was shown to result in an ETV6-JAK2 fusion protein with constitutive tyrosine kinase activity. In addition, the role of ETV6-JAK2 in causing T-ALL was confirmed by the observation that mice transgenic for ETV6-JAK2 in their lymphoid cells developed a fatal leukemia with preferential expansion of CD8-positive T-cells.

Mutations providing self-renewal properties

Unlimited self-renewal potential is one of the hallmarks of all cancers. Several studies suggest that only some specific oncogenes can provide leukemic cells with self-renewal capacity. Recently, NOTCH signaling has been identified as an important regulator of stem cell maintenance, and NOTCH1 was also implicated in the pathogenesis of T-ALL, suggesting that NOTCH1 defects in T-ALL may predominantly serve to provide the leukemic cells with self-renewal capacity.

NOTCH1 was discovered as a fusion partner of the T-cell receptor β gene in t(7;9)(q34;q34.3) occurring in <1% of T-ALL cases. It has been shown to be an important player in T-cell lineage commitment of pluripotent progenitors and the subsequent assembly of pre-T-cell receptors in immature thymocytes.

The mature heterodimeric NOTCH1 transmembrane receptor consists of an extracellular (NEC) and a transmembrane (NTM) subunit, which are non-covalently kept together by the heterodimerization domain. Binding of Delta-Serrate-Lag2 (DSL) family ligands to the NEC results in activation of the NOTCH1 receptor by removal of the NEC and by initiating a cascade of proteolytic cleavages of the NTM. The final cleavage is catalyzed by the γ-secretase complex of proteases and generates intracellular NOTCH1 (ICN), which translocates to the nucleus where it associates with other proteins to form a transcription activator complex. ICN normally has a short half-life, being subject to ubiquitination and degradation via mechanisms involving the C-terminal PEST domain.

Recent findings suggest a central role for aberrant NOTCH1 signaling in the pathogenesis of T-ALL as 56% of analyzed primary T-ALL samples display NOTCH1 activating mutations. The heterodimerization domain was found to be mutated in 44% of the samples, destabilizing NEC and NTM intersubunit association and consequently resulting in increased ICN production rates without ligand stimulation. In addition, 50% of the tumors displayed PEST domain mutations, causing extended half-life of the ICN-containing transcriptional activation complex. Combined heterodimerization domain and PEST domain mutations were shown to cause synergistic activation of NOTCH1 signaling pathways and were observed in 17% of analyzed tumors. The NOTCH1 mutations were identified in T-ALL cases with expression of LYL1, TLX1, TLX3, TAL, MLL-MLLT1 or PICALM-MLLT10, which together define all major molecular subtypes of T-ALL. This observation is consistent with the notion that NOTCH1 mutations occur in immature T-lineage cells or uncommitted pluripotent marrow progenitors. In the future, it will be interesting to investigate whether some of the ectopically expressed transcription factors identified in T-ALL can also provide self-renewal capacity to the leukemic cells. If this is the case, as has been shown recently in AML, the exact contribution of these transcription factors and of NOTCH1 mutations with respect to self-renewal capacity will need to be addressed in detail.

The identification of NOTCH1 mutations in T-ALL also has therapeutic implications. Most mutant forms of NOTCH1 still require γ-secretase activity to generate critical downstream signals. Because of the involvement of γ-secretase in the production of amyloidogenic peptides in patients with Alzheimer’s disease, efforts have already been made to develop potent and selective γ-secretase inhibitors. Unfortunately, long-term therapy of Alzheimer’s disease seems not appropriate because of the side effects of the current γ-secretase inhibitors, such as disturbances of lymphocyte development and gut epithelial cell differentiation. However, these compounds could provide a rational, molecularly targeted therapy in T-ALL patients with an acceptable level of toxicity when used periodically or for a short time.
Conclusions and future perspectives

Twenty years of molecular studies of T-ALL have provided us with a detailed list of gene defects that are involved in the pathogenesis of these leukemias, and we begin to distinguish certain patterns in the different mutations that are observed, as shown in Figure 1. It is important to realize that different types of mutations need to co-operate to transform a normal thymocyte into a leukemic T-cell. As depicted in Figure 1, we have most likely identified the majority of mutations involved in impaired differentiation and in cell cycle deregulation. In contrast, we still lack significant insight into the different mutations providing self-renewal capacity, and especially those providing a proliferative advantage. Genome-wide approaches for the discovery of oncogenes, such as large-scale sequencing of the kinase genes,\textsuperscript{123} microarray-comparative genomic hybridization (array-CGH),\textsuperscript{4} and gene expression profiling\textsuperscript{28} may lead to the identification of new mutations in T-ALL.

The introduction of microarray technology made it possible to compare global gene expression profiles of T-ALL samples. This approach contributed significantly to the classification of T-ALL into molecular subtypes and led to the discovery of connections between the activation of particular oncogenes and defined stages of normal thymocyte development which are seen in the leukemic thymocytes (Figure 3).\textsuperscript{28,51,73,124} In addition, expression profiles have shed light on the molecular causes of the different response of T-ALL patients to antileukemic therapy.\textsuperscript{28} By using this technique, it may become possible to elucidate signal transduction pathways involved in the development of the distinct T-ALL subgroups, making proteins within these pathways attractive targets for new therapeutic approaches.\textsuperscript{28}

Although current therapies result in relatively good responses in T-ALL patients, there are still specific T-ALL subgroups with bad prognosis and there is the drawback of severe therapy-related toxicity. The next challenge to further improve the outcome of T-ALL patients is the translation of the current genetic insights into new targeted therapies (Table 2). If these targeted therapies are to find their way into the clinic, it will be crucial to assign each T-ALL patient correctly to a molecular subgroup, based on fluorescent in situ hybridization, quantitative RT-PCR and mutational analysis, or micro-array based techniques. We can expect that an ideal targeted therapy will try to correct the different defects that co-operate with each other. Therefore, again, besides the identification of individual oncogenes that may serve as targets for therapy, it will be important to obtain further insights into the different classes of oncogenes and to determine whether targeting several of these different classes at the same time could improve the survival. Continuous genetic and functional studies will be required to validate, correct and extend the model we propose in Figure 1.

Kim De Keersmaecker is an Aspirant, and Jan Cools is a Postdoctoral Researcher of the FWO-Vlaanderen. Our work is supported by grants from the FWO-Vlaanderen, the Belgian Federation against Cancer (BFK), and the European Hematology Association (EHA) (José-Carreras Fellowship grant to J.C.).

We thank the laboratory members for suggestions and critical reading of the manuscript.

Manuscript received March 16, 2005. Accepted June 8, 2005.
References


68. Dreiling MH, Martinez-Climent JA, Billing M, Mao J, Rowe JD, Bohlander SK. The t(10;11)(p15;q24) in the U957 cell line results in the fusion of the AF10 gene and CALM, encoding a new member of the AF-3 clathrin assembly protein family. Proc Natl Acad Sci USA 1996;93:4804-9.
75. Zhang W, Samelson LE. The role of


