Genetic insights in the pathogenesis of T-cell acute lymphoblastic leukemia

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Over the past 20 years, a large number of genes involved in the pathogenesis of T-cell acute lymphoblastic leukemia (T-ALL) has been identified by molecular characterization of recurrent chromosomal aberrations and more subtle genetic defects. When reviewing the current list of oncogenes and tumor suppressor genes, it becomes clear that these can be grouped into four classes of mutations, which are involved in: (i) cell cycle deregulation; (ii) impaired differentiation; (iii) proliferation and survival advantage and (iv) unlimited self-renewal capacity. Based on recent studies of T-ALL, we can speculate that at least these four different mutations are required for the development of T-ALL. In this review we summarize our current insights into the molecular pathogenesis of T-ALL, and we discuss how these molecular findings provide new directions for future research and novel therapeutic strategies in T-ALL.

Key words: transcription factor, oncogene, tumor suppressor gene, self-renewal, stem cell, differentiation, proliferation, survival, tyrosine kinase.

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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.[1,2] 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.[1] Now, 20 years later, we possess a detailed list of genes affected by chromosomal rearrangements and other genetic defects,[3-5] and 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.[6,7]

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.[6,7] 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).[8,9] 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-

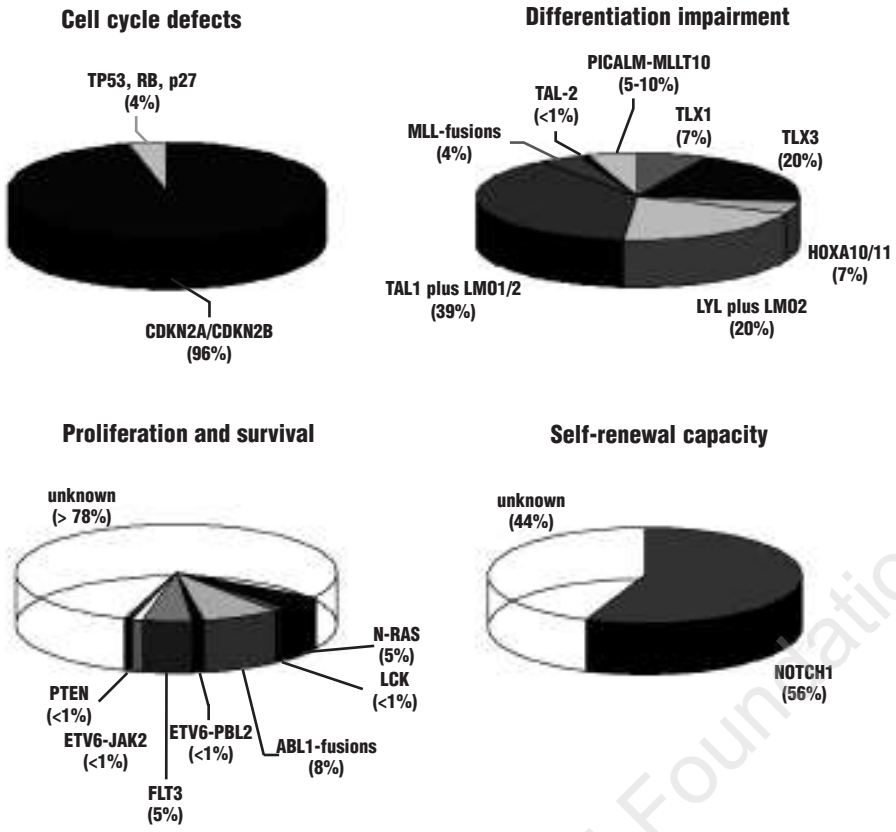

Figure 1. Frequency of the different mutations observed in T-ALL. Molecular analysis of T-ALL shows that four major classes of mutations are involved in the molecular pathogenesis of T-ALL. These four classes are represented by the four diagrams, in which the frequency of each of the different mutations 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.

tional mutations to cause AML.[8,9] 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).[10] 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).[10] *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).[11,12] The CDKN2A gene encodes both p16 and p14. The p16 and p14 transcripts have different promoters and first exons (exons 1α and 1β 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).[13,14]

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

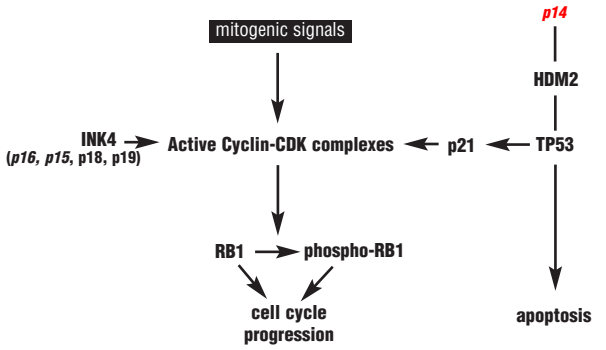

Figure 2. The cell cycle and its defects in T-ALL. Mitogenic signals result in the generation of active cyclin-CDK complexes that phosphorylate RB1, resulting in cell cycle progression (see text for details). The activity of cyclin-CDK complexes is controlled by two alternative pathways: (a) INK4 proteins (p16, p15, p18 and p19) and (b) CDKN1A (p21), which both inhibit cyclin-CDK complexes, resulting in cell cycle arrest. Nomenclature: CDKN2A encodes p14 and p16, CDKN2B encodes p15 and CDKN1A encodes p21. Cell cycle regulators that frequently show defects in T-ALL are indicated in italics.

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

Gene	*T-cell receptor genes*		*Partner gene*	
	Gene symbol	*Chromosome location*	*Gene symbol*	*Chromosome location*
T-cell receptor α	TRA@	14q11	*TLX1*	10q24
			TAL1	1p32
			LMO1	11p15
			LMO2	11p13
T-cell receptor β	TRB@	7q34-35	*TLX1*	10q24
			HOXA@ cluster	7p15
			LYL1	19p13
			TAL2	9q32
			LCK	1p34
			NOTCH1	9q34
T-cell receptor γ	TRG@	7p15	no known chromosomal aberrations	
T-cell receptor δ	TRD@	14q11	*TLX1*	10q24
			TAL1	1p32
			LMO1	11p15
			LMO2	11p13

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

10% and 15% of the samples.[11,12] In addition, inactivation of *CDKN2A/B* is also caused by mutation and by promoter hypermethylation.[15-17] A few studies describe *CDKN2A* inactivation at the transcriptional and post-transcriptional levels, although the mechanisms remain unknown.[15,18] Overall, the inactivation rates for *CDKN2A* and *CDKN2B* in T-ALL at DNA/RNA/protein levels were found to be as high as 93% and 99%, respectively.[19] 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.[19]

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.

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.[20] 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.[21-24] 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* (*HOX11L2*) 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.[25,26] About 7% of childhood T-ALL samples show ectopic expression of *TLX1* in thymocytes.[27,28] 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.[29-32] 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

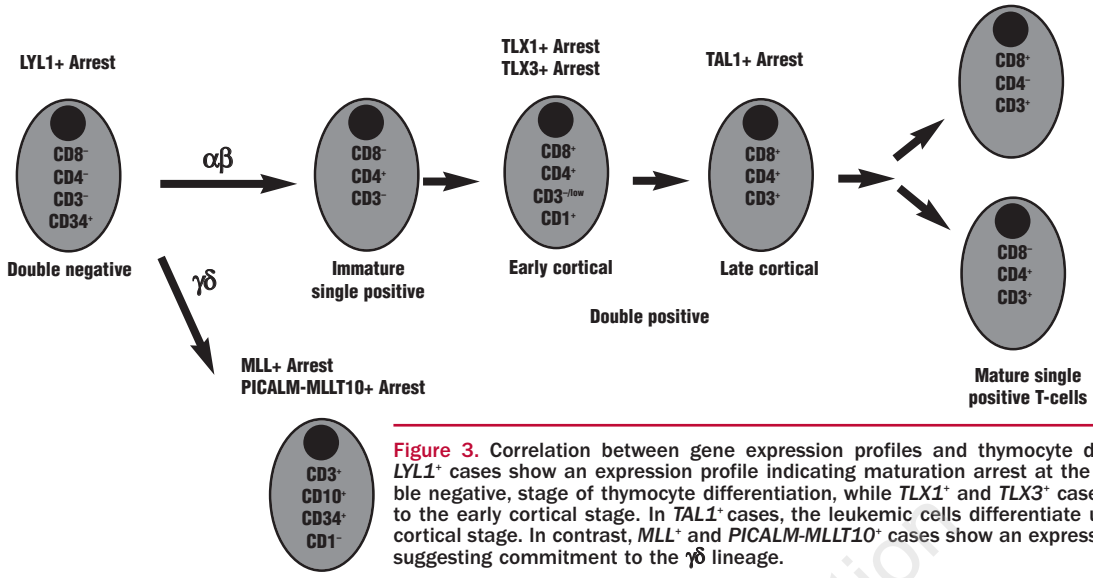

Figure 3. Correlation between gene expression profiles and thymocyte differentiation. *LYL1*⁺ cases show an expression profile indicating maturation arrest at the earliest, double negative, stage of thymocyte differentiation, while *TLX1*⁺ and *TLX3*⁺ cases develop up to the early cortical stage. In *TAL1*⁺ cases, the leukemic cells differentiate up to the late cortical stage. In contrast, *MLL*⁺ and *PICALM-MLLT10*⁺ cases show an expression signature suggesting commitment to the γδ lineage.

by disrupting gene silencing mechanisms that operate during normal T-cell development.[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.[28,36]

TLX1 has been reported to immortalize murine hematopoietic progenitors *in vitro* and to induce T-ALL-like malignancies in mice engrafted with MSCV-*TLX1*-transduced bone marrow cells after long latency, indicating that progression to a fully malignant state indeed requires supplemental mutations.[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.[39]

TLX3 (HOX11L2)

Approximately 20% of childhood and 13% of adult T-ALL cases are characterized by ectopic *TLX3* expression.[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 *BCL11B*, a gene highly expressed during T-cell differentiation.[42,43] Interestingly, a variant t(5;14)(q35;q32) has been identified in a pediatric T-ALL cell line, in which a different homeobox gene, *NKX2-5*, is ectopically activated instead of *TLX3*.[44] *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 *CDK6* gene is involved on 7q21.[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.[27,28,40] It is possible that the prognostic meaning of *TLX3* expression is modulated by the presence of specific oncogenes (such as *NUP214-ABL1* or *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.

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*.[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.[47,48] This is supported by *in vitro* and *in vivo* mouse models in which the effect of *HOXA10* overexpression on differentiation of both myeloid and lymphoid cells was confirmed.[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.[51] 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).[51]

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.[52] LYL1, TAL1 and TAL2 are three class B bHLH proteins that are ectopically expressed in T-ALL. They all dimerize with the E2A proteins and bind the same E box element, reflecting their highly similar bHLH motif.[53,54,54-56] For these reasons, *LYL1*, *TAL1* and *TAL2* have been proposed to constitute the subgroup TAL in the large bHLH family.[57]

LYL1

The *LYL1* gene was initially identified upon the molecular characterization of the translocation t(7;19)(q35;p13) associated with T-ALL.[58] 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.[58,59] 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.[60-64] 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 transcipts by fusing 5' *TAL1* to the promoter region of the *SIL* gene.[27,65] In addition, T-ALL cell lines and patients' samples without detectable *TAL1* rearrangements often express high levels of *TAL1* mRNA.[28,66]

Two theoretical models for *TAL1*-induced leukemogenesis 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.[67] In agreement with this model, it has also been shown that *TAL1* tumors undergo apoptosis after administration of histone deacetylase (HDAC) inhibitors.[68] 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*.

Ectopic *TAL1* and *LYL1* expression in thymocytes is associated with maturation arrest of thymocytes in the late cortical and double negative stage, respectively (Figure 3).[28] In children, these T-ALL subgroups are associated with an unfavorable clinical outcome, probably due to upregulation of anti-apoptotic molecules.[28]

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.[69] The properties of TAL2 broadly resemble those described previously for TAL1, supporting the idea that both proteins promote T-ALL by a common mechanism.[56]

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 t(11;14)(p15;q11) and t(11;14)(p13;q11) juxtaposing *LMO1* or *LMO2* to the *TRA@* or *TRD@* loci.[70-72]

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.[28,28,73] 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 *LMO1* or *LMO2* is strictly required.[67,74] This explains the high frequency of overlap between *TAL1* and *LMO* expression. As a consequence, ectopic expression of *LMO1* and *LMO2* cannot be shown independently on the graphs in Figure 1, but is shown overlapping with *TAL1* and *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.[75-78] This translocation is often not detected by routine cytogenetics and the corresponding fusion gene, *PICALM-MLLT10* can be detected in up to 10% of T-ALL cases, of which only half show the translocation in their karyotype.[79,80] The exact function of the fusion protein is not known, but interestingly, both *PICALM* and *MLLT10* are known fusion partners of *MLL*.[81,82]

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).[83,84] Recent studies indicate a role for the ABL1 kinase downstream of LCK and upstream of ZAP70.[85] Activated ZAP70 then in turn phosphorylates the adaptor molecules SLP-76 (LCP2) and LAT. Association of PLCγ1 with LAT allows it to be activated and to be positioned in the proximity of its substrate PIP_2, which is then hydrolysed into DAG and IP_3, second messengers causing, respectively, activation of PKC and release of intracellular Ca^{2+}. The increased Ca^{2+} levels cause initiation of calcineurin phosphatase activity. PKC and GRB2 initiate activation of the RAS-MAP kinase pathway.[86,87] PI3K is another important player which becomes active after TCR engagement. PI3K catalyzes phosphorylation of mainly PIP_2 to PIP_3, causing activation of AKT followed by inactivation of GSK3B kinase. Generation of PIP_3 also contributes to activation of RAS, PLCγ1 and other mediators of TCR-signaling. PTEN catalyzes dephosphorylation of PIP^3 to PIP^2, opposing PI3K activity.[84] Activated ERK and calcineurin, and inactivated GSK3, act in concert to initiate activation of multiple transcription factors resulting in *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).[85] *ABL1* is typically found to be fused to the *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).[88,89] Although the *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.[90] An example of this is the identification of the *NUP214-ABL1* fusion in approximately 6% of T-ALL patients.[6] Interestingly, this fusion was found to be present on small, cytogenetically invisible, extrachromosomal elements (episomes), and is associated with *TLX1* or *TLX3* expression and deletion of *CDKN2A*.[6] Apart from the *NUP214-ABL1* fusion, variant *ABL1*-fusions, such as the *ETV6-ABL1* and the *EML1-ABL1* fusions, have also been reported with a lower incidence in T-ALL.[91,92] 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.[6,91,93] 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).[94] In the past, rare cases have been described with activation of *LCK* by t(1;7)(p34;q34), joining *LCK* and *TRB@* loci.[95,96] Interestingly, recent findings indicate that ABL1 is located downstream of LCK in the TCR signaling pathway (Figure 4).[85] 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.

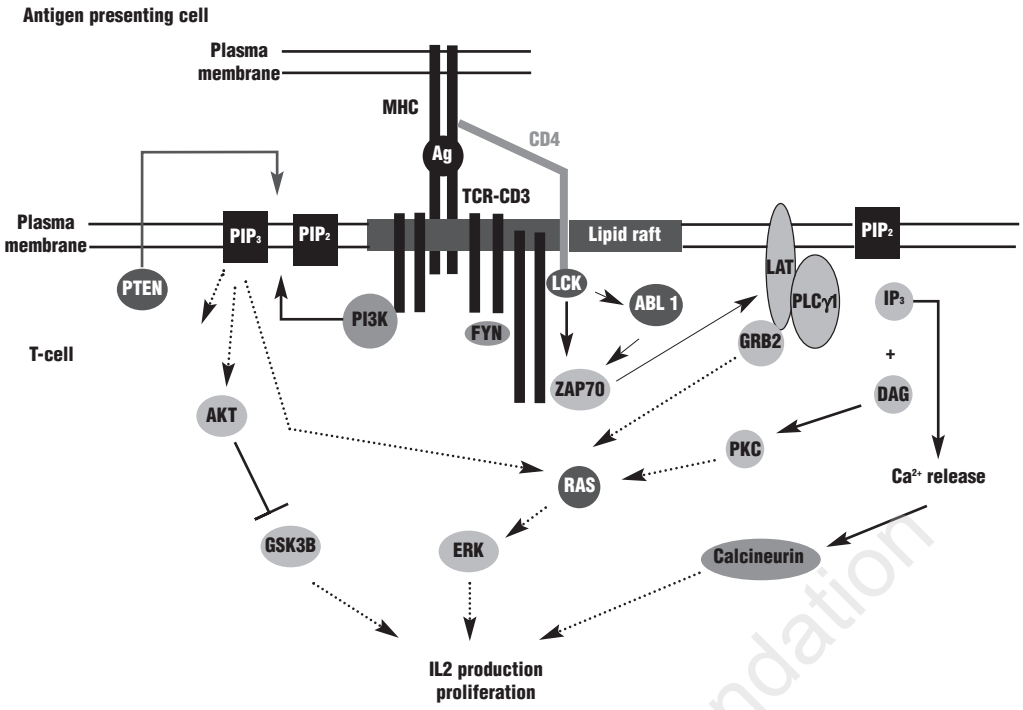

Figure 4. Schematic representation of the T-cell receptor (TCR) signaling pathway and its defects in T-ALL. Stimulation of the TCR-CD3 complex by an MHC bound antigen leads to activation of the SRC family protein tyrosine kinases, LCK and FYN. LCK and FYN in turn catalyze phosphorylation of tyrosine residues within the immunoreceptor tyrosine based activation motifs (ITAM) of the TCR-CD3 complex leading to recruitment of ZAP70 and PI3K. After recruitment, ZAP70 is activated either directly by LCK, or indirectly via ABL1. Activated ZAP70 causes phosphorylation of SLP-76 (not depicted) and LAT. LAT then acts as a linker, binding PLCγ1, GRB2 and several other molecules which are not depicted in the figure for clarity. Association of PLCγ1 with LAT allows it to be activated and to be positioned in the proximity of its substrate PIP_2, which is then hydrolysed into DAG and IP_3, second messengers leading to activation of PKC and to release of intracellular $Ca2^{+}$ respectively. The increased $Ca2^{+}$ levels cause activation of the phosphatase calcineurin. PKC and GRB2 initiate activation of the RAS guanine nucleotide exchange factors SOS and RAS-GRP, resulting in activated RAS. (For graphical reasons, RAS is depicted as cytoplasmic, although it is in fact membrane-anchored.) RAS stimulates the MAP kinase pathway eventually resulting in active ERK. PI3K is another important player which becomes active after TCR engagement. PI3K catalyzes phosphorylation of mainly PIP_2 to PIP_3, causing activation of AKT followed by inactivation of GSK3B kinase. Generation of PIP_3 also contributes to activation of RAS, PLCγ1 and other mediators of TCR-signaling. PTEN catalyzes dephosphorylation of PIP_3 to PIP_2, opposing PI3K activity. Activated ERK and calcineurin, and inactivated GSK3B, initiate activation of multiple transcription factors resulting in *IL2* gene expression. Secreted IL2 eventually stimulates T-cells to proliferate. LCK, ABL1, RAS and PTEN have been shown to be implicated in the pathogenesis of T-ALL.

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).[97] Activating mutations of the *NRAS* isoform have been identified in 4-10% of T-ALL cases.[98-100] 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.[101] As RAS is constitutively activated by mutations in about one-third of all human malignancies,[102] 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.[103] These inhibitors are currently being tested for the treatment of AML,[104] and they could also turn out to be relevant as targeted therapy of T-ALL.[101]

PTEN

PI3K 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.[105,106] 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.[107]

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.[108] 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*.[109] 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.[109] 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.[109]

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.[110] 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.[111]

Mutations providing self-renewal properties

Unlimited self-renewal potential is one of the hallmarks of all cancers.[112] Several studies suggest that only some specific oncogenes can provide leukemic cells with self-renewal capacity.[113,114] Recently, NOTCH signaling has been identified as an important regulator of stem cell maintenance,[115] 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.[116] 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.[117-119]

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.[120,121] 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.[122]

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.[7] 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.[7] In addition, 30% of the tumors displayed PEST domain mutations, causing extended half-life of the ICN-containing transcriptional activation complex.[7] 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.[7] The *NOTCH1* mutations were identified in T-ALL cases with expression of *LYL1*, *TLX1*, *TLX3*, *TAL1*, *MLL-MLLT1* or *PICALM-MLLT10*, which together define all major molecular subtypes of T-ALL.[7,28] 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,[113,114] 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.[7] 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.

Table 2. Overview of gene targeted therapies.

Genetic defect	*Targeted therapy*	*Rationale*
Cell cycle defects		
CDKN2A/B inactivation	CDK inhibitors	CDKN2A inactivation causes activation of cyclin-CDK complexes[125,126]
Transcription factor defects		
TAL1 overexpression	HDAC inhibitors	Gene silencing caused by overexpression of TAL1 is opposed by inhibition of HDAC activity[68]
Proliferative and survival advantage		
ABL1 hyperactivity	ABL kinase inhibitors	Inhibition of ABL1 kinase activity[93]
LCK hyperactivity	SRC/ABL kinase inhibitors	Inhibition of LCK and its downstream target ABL1[127]
RAS hyperactivity	Farnesyltransferase inhibitors	Inhibition of RAS farnesylation, a critical post-translational modification to generate functional RAS[103]
Inactive *PTEN*	PI3K inhibitors	Opposition of PI3K function by PTEN is replaced by PI3K inhibitors[107]
FLT3 hyperactivity	FLT3 inhibitors	Inhibition of FLT3[128]
JAK2 hyperactivity	JAK2 inhibitors	Inhibition of JAK2[129]
Self-renewal capacity		
NOTCH1 hyperactivity	γ-secretase inhibitors	Inhibition of γ–secretase required to generate downstream NOTCH1 signaling[7]

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,[123] microarray-comparative genomic hybridization (array-CGH),[6] and gene expression profiling[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).[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.[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.[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.

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