T-cell acute lymphoblastic leukemia

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T-cell acute lymphoblastic leukemia (T-ALL) accounts for about 15% and 25% of ALL in pediatric and adult cohorts respectively.[1] Patients usually have high white blood cell counts and may present with organomegaly, particularly mediastinal enlargement and CNS involvement. The biological knowledge of T-ALL has until recently been rather limited. Flow cytometry allowed the stage of differentiation of the leukemic clone to be defined, while cytogenetic analyses were often uninformative. The introduction of novel technologies has allowed an increasing number of alterations to be unraveled. The most relevant results have been obtained by using FISH (Fluorescent In Situ Hybridization), molecular biology and gene expression profiling, which have enabled five subgroups to be recognized, i.e. immature/*LYL1*, *TAL1*, *HOX11*, *HOX11L2* and *HOX*.[2]

The most important biological findings of T-ALL, their role in leukemia initiation and their possible therapeutic implications will be hereby revised.

Role of flow cytometry

The EGIL classification[3] recognizes the differentiation stages of the neoplastic clone: pro-T, pre-T, cortical and mature T-ALL. The cortical stage has been associated with a better outcome. Efforts have been made to identify additional antigens of prognostic significance that may drive therapeutic decisions. In the current issue of Haematologica-the hematology journal, Fischer *et al.*[4] show that CD56, whose expression is correlated with an inferior outcome in acute myeloid leukemia (AML) and aggressive lymphoma, is associated in T-ALL with a lower remission rate and a higher percentage of resistant disease, but not with an inferior overall survival and disease-free survival. Other antigens that have been associated with outcome include CD2[5] CD34 and the coexpression of myeloid markers.

Antigens can also be used for targeted therapies and different monoclonal antibodies for clinical use can be considered for the management of ALL patients, including T-ALL. CD52 is detected in about 60% of T-ALL and its negativity seems to correlate with novel molecular aberrations (A. Guarini *et al.*, personal observation, 2008). The myeloid antigen CD33 is also expressed in a small proportion of T-ALL cases, though at a low level of expression. Careful flow cytometric analysis can define the percent of leukemic cells expressing a given antigen and can also accurately quantify the number of molecules expressed per cell.

Role of genetic aberrations in leukemogenesis

In this paragraph, the most important results will be summarized: due to space limitations, not all genetic aberrations will be considered.

Lesions involving the T-cell receptor (TCR)

Aberrations involving the 14q11 (TCRA/D) and 7q34(TCRB) regions can be detected in 35% of patients.[6] They juxtapose enhancer elements of the TCR genes with transcription factors involved in T-cell differentiation, with deregulation of hemopoiesis (Table 1).

Table 1. Rearrangements involving T-cell receptor genes.

Translocation	Involved gene	Fusion gene function	Frequency
t(7;10)(q34;q24) and t(10;14)(q24;q11)	*TLX1* (*HOX11*)	Transcription factor	7% children 31% adults
t(5;14)(q35;q32)	*TLX3* (*HOX11L2*)	Transcription factor	20% children 13% adults
inv(7)(p15q34), t(7;7)	*HOXA* genes	Transcription factor	5%
t(1;14)(p32;q11) and t(1;7)(p32;q34)	*TAL1*	Transcription factor	3%
t(7;9)(q34;q32)	*TAL2*	Transcription factor	<1%
t(7;19)(q34;p13)	*LYL1*	Transcription factor	<1%
t(14;21)(q11.2;q22)	*BHLHB1*	Transcription factor	<1%
t(11;14)(p15;q11)	*LMO1*	Protein-protein interaction	2%
t(11;14)(p13;q11) and t(7;11)(q35;p13)	*LMO2*	Protein-protein interaction	3%
t(1;7)(p34;q34)	*LCK*	Signal transduction	<1%
t (7;9)(q34;q34.3)	*NOTCH1*	Fate determination, differentiation	<1%
t(7;12)(q34;p13) and t(12;14)(p13;q11)	*CCND2*	Cell cycle activator	<1%

Lesions involving known oncogenes

Notch1. Notch1 mutations are frequently found in T-ALL: they occur in the HD and PEST domains, resulting in the constitutive activation of the Notch pathway or in an increase of its half-life, respectively. Furthermore, the t(7;9)(q34;q34.3) translocation, which occurs in ~1% of T-ALL, leads to the constitutive expression of active Notch1.[7] Notch1 aberrations are found in association with other aberrations, consistent with their occurrence in immature cells, susceptible to additional hits.

HOXA cluster. The HOXA subgroup comprises several lesions: i) MLL rearrangements;[8] ii) inv (7)(p15q34);[9] iii) t(10;11)(p13;q23), which results in the CALM-AF10 rearrangement;[10] iv) a novel cryptic deletion – del(9)(q34.11q34.13) – leading to SET-NUP214.[11] Gene expression profiling analyses have recognized signatures associated with these altered pathways and have highlighted that different genetic lesions converge into the same pathway.

TLX1(HOX11) and TLX3 (HOX11L2). t(10;14)(q24;q11) and t(7;10)(q34;q24) induce high expression levels of TLX1.[12] TLX1 is expressed in 30% of T-ALL, more often in adults. TLX1+ leukemias display an early cortical phenotype and a more favorable outcome.

TLX3 expression is generally caused by the cryptic t(5;14)(q35;q32) translocation[13] and is detected in 20% of children and 13% of adults with T-ALL. Gene expression profiling has shown highly similar patterns between TLX1$^+$ and TLX3$^+$ cases.[2]

TAL1 and LYL1. *TAL1* (SCL, TCLS) involvement can be secondary to t(1;14) (p32;q11)[14] or to a submicroscopic interstitial deletion, resulting in the SIL-TAL1 fusion gene. In addition, high expression levels of TAL1 are observed in about 40% of T-ALL. Aberrant TAL1 expression may interfere with differentiation and proliferation through the inhibition of the transcriptional activity of E47/HEB.

LYL1 is involved in t(7;19)(q34;p13)[15] and can also be constitutively overexpressed; it is usually expressed in cases with an immature phenotype, expressing CD34 and sometimes myeloid markers.[2]

Lesions involving tyrosine kinases

ABL1. The *ABL1* gene, involved in t(9;22) (9q34;q11.2), hallmark of chronic myeloid leukemia (CML), results in the constitutive activation of BCR-ABL1. BCR-ABL1 can be also detected in B-lineage ALL and in AML. Recent progress has highlighted its involvement in T-ALL: 6% of cases harbor the *NUP214-ABL1* fusion gene.[16] This anomaly can also be detected by gene expression profiling, since it is characterized by high levels of ABL1 expression.[17] Another mechanism is t(9;14)(q34;q32),[18] which leads to the *EML1-ABL1* fusion gene. *NUP214-ABL1* and *EML1-ABL1* are associated with other aberrations, indicating a multigene contribution to T-cell leukemogenesis.

Lesions involving JAK2 and JAK1. *JAK2* can be constitutively activated as a consequence of t(9;12)(p24;p13), encoding the *ETV6-JAK2* fusion gene.[19]

JAK1 is mutated in 18% of adult patients with T-ALL; clinically, these patients may represent an unfavorable prognostic subgroup[20] (Table 2).

Novels findings: overlapping areas between acute myeloid leukemia and T-ALL

Wouters *et al.*[21] recently described a subset of AML cases whose genomic profile is similar to that of CEBPA mutated patients, characterized by the expression of a set of T-lineage genes, *Notch1* mutations and hypermethylation of the CEBPA promoter, suggesting that CEBPA hypermethylation together with *Notch1* mutations may lead to a mixed T-lineage/myeloid lineage scenario. Our group (S. Chiaretti *et al.*, personal observation, 2008) has recently identified a subgroup of adult cases with T-ALL, accounting for 10% of the whole cohort analyzed, which reveal a gene expression signature that resembles that of AML patients (*myeloid-like cluster*). Intriguingly, these cases have high levels of CEBPA expression; accordingly, several myeloid genes are expressed at high levels.

Overall, these results indicate that CEBPA is involved in lineage orientation and that its deregulation may be responsible for mixed genotypes; moreover, they also indicate the presence of overlapping areas between distinct diseases.

Targeted therapies: where do we stand?

Identification of genetic aberrations is important in an attempt to design novel forms of targeted therapies: examples include *Notch1*, *ABL1* and *JAK* kinases.

1) Notch1 activation is sustained by the presenilin γ-secretase complex. γ-secretase inhibitors (GSI), already investigated in Alzheimer's disease, have *in vitro* activity in Notch1$^+$ T-ALL cell lines. However, the use of these agents is only partly effective, because of the complexity of the Notch1 pathway.

Table 2. Lesions involving known oncogenes.

Translocation	Involved gene	Fusion gene function	Frequency
1p32 deletion	*SIL-TAL1*	Transcription factor	9-30%
t(10;11)(p13;q14)	*CALM-AF10*	Transcription factor	10%
t(9;9)(q34;q34)	*NUP214-ABL1*	Signal transduction	~5%
t(9;14)(q34;q32)	*EML1-ABL1*	Signal transduction	<1%
t(9;22)(q34;q11)	*BCR-ABL1*	Signal transduction	<1%
t(9;12)(p24;p13)	*ETV6-JAK2*	Signal transduction	<1%
Mutations			
Notch1	*Notch1*	Fate determination, differentiation	50%
JAK1	*JAK1*	Signal transduction	18%

Table 3. Genetic lesions and potential therapeutic drugs.

Gene involved	Therapeutic compound	Rationale
TAL1 overexpression	HDAC inhibitors	Inhibition of gene silencing
Notch1	γ-secretase inhibitors	Inhibition of extracellular domain cleavage and inhibition of Notch1 signaling activation
ABL1	ABL1 kinase inhibitors	Inhibition of ABL1 activity
JAK2	JAK2 inhibitors	Inhibition of JAK2 activity
JAK1	Tyrosine kinase inhibitors?	Inhibition of JAK1 activity
	Jak2 inhibitors?	

2) ABL1 inhibitors have been tested in T-ALL: dasatinib inhibits NUP214-ABL1$^+$ cell proliferation, while EML1-ABL1$^+$ cells are sensitive to imatinib.

3) JAK2 inhibitors, tested in myeloproliferative disorders, may play a role also in T-ALL treatment. The evidence that JAK1 mutations are common in T-ALL will prompt the identification of kinase inhibitors potentially active in such cases (Table 3).

Concluding remarks and future perspectives

Extensive research has allowed a molecular profile of T-ALL to be defined. Some lesions have been well characterized, while others require further research. As shown by Fischer *et al.* in the present issue of the journal, flow cytometry can be utilized for prognostication, while molecular approaches can define: i) the presence of different subgroups, ii) novel lesions and iii) deregulated pathways. The identification of molecular lesions is leading to the generation of specific inhibitors, the clinical use of which may lead to a revision of the management of T-ALL patients. Finally, other areas of research are rapidly developing, such as microRNA analysis. The analysis of small non-coding RNAs is likely to identify novel mechanisms of transformation.

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