T- Acute lymphoblastic leukemia: Does our understanding of molecular pathogenesis will impact future treatment?

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T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignant neoplasm of the bone marrow. It accounts for 20%of all cases of ALL and is somewhat more common in adults than children, although the incidence diminishes with older age. Its clinical presentation can include hyper-leukocytosis with extra-medullary involvement of lymph nodes and other organs, including frequent central nervous system infiltration and the presence of a mediastinal mass, arising from the thymus. The WHO defines lymphoblasts in T-ALL as TdT positive with variable expression of CD1a, CD2, CD3, CD4, CD5, CD7, and CD8. Cytoplasmic CD3 and CD7 are often positive. T-ALL can be subdivided into different stages by intra-thymic differentiation, including pro-T, pre-T, cortical T, and medullary T.

The immunophenotypes of these subtypes are listed in Table 1 ***(Litzow and Ferrando, 2015).***

	cCD3	CD7	CD28	CD1a	CD34	CD4	CD8
Pro-T	+	+	-	-	+/-	-	-
Pre-T	+	+	+	-	+/-	-	-
Cortical T	+	+	+	+	-	+	+
Mature T (medullary)	+	+	+	-	-	+/-	+/-

Table 1: Immunologic classification of T-ALL *(Quoted from Litzow and Ferrando, 2015).*

A subtype of T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) derived from thymic cells at the early T-cell precursor (ETP) differentiation stage has been recognized. ETPs are recent immigrants from the bone marrow to the thymus, derived from hematopoietic stem cells, which retain a certain level of multi-lineage pluripotency. By gene expression profiling, ETP cells share similarities with hematopoietic stem cells and myeloid progenitor cells. The definition of ETP-ALL/LBL (corresponding to pro and pre-T ALL phenotypes) is based on the immunophenotype of the leukemic cells, which are typically CD1a-, CD8-, CD5-(dim), and positive for 1 or more stem cell or myeloid antigens. In the World Health Organization (WHO) classification, ETP-ALL/LBL falls within the early T-ALL/LBL category. ETP ALL has been reported in 11% to 12% of childhood T-ALL/LBL and in 7.4% of adult T-ALL/LBL. ETP-ALL/LBL is also characterized by a distinct molecular profile with a lower incidence of NOTCH1 mutations and frequent presence of FLT3 and DNMT3A mutations ***(Jain et al.,2016).***

ETP ALL were originally described as a high-risk group associated with poor response to therapy and dismal prognosis; however, this group seems to be heterogeneous and not always associated with poor prognosis ***(Litzow and Ferrando, 2015).***

Molecular landscape of T-ALL

In 1991, Ellisen et al. identified a t(7;9)(q34;q34.4) translocation in T-ALL patients, which resulted in fusion of the 3` region of NOTCH1 into the TCR-β locus and consequent overexpression of the active form of Notch1 (ICN1). This translocation appeared to be rare, found in <1% of T-ALL cases. However, 13 years later, Weng et al. (2004) identified activating NOTCH1 mutations in ~56% of T-ALL cases examined, introducing NOTCH1 mutation as the main oncogenic lesion in T-ALL. Two major hotspots of mutations were characterized: mutations in the heterodimerization domain (HD) that induce ligand-independent activation, and mutations in the PEST (proline-glutamate-serine- threonine-rich) carboxy-terminal domain that increase stability of ICN1. Additionally, inactivating mutations were identified in FBXW7, an E3 ubiquitin ligase responsible for ICN1 degradation and subsequent termination of Notch signaling ***(Lobry et al., 2011).***

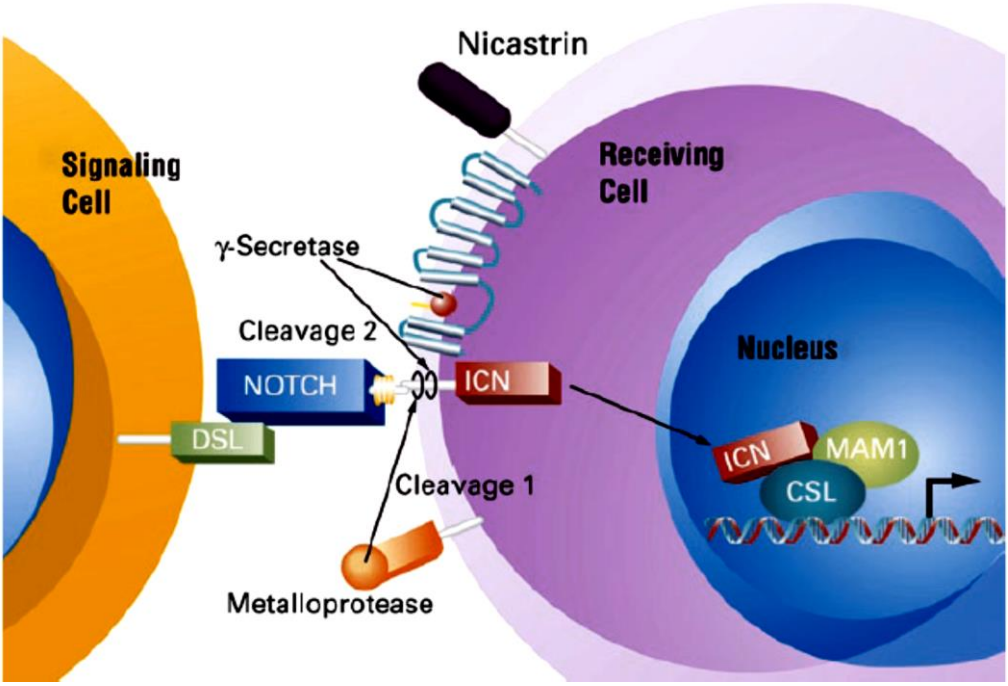


Figure 1: Activation of NOTCH signaling via proteolytic cleavage and nuclear translocation of the intracellular NOTCH domain (ICN). Interaction with delta serrate ligand (DSL) stimulates proteolytic cleavage of NOTCH by metalloproteases and g-segretase. This leads to the release of the intracellular ICN domain, which translocates to the nucleus, displaces corepressors and recruits coactivators (MAM1), thereby converting CSL from a repressor to an activator of gene expression**. Reprinted with permission from Armstrong and Look 2005)**

In addition, T-ALLs characteristically show the translocation and aberrant expression of transcription factor oncogenes. These chromosomal rearrangements place T-ALL transcription factor oncogenes under the control of strong T cell–specific enhancers located in the *TCRB* (7q34) or *TCRA–TCRD* (14q11) loci, resulting in their aberrant expression in T cell progenitors. These oncogenic transcription factors include basic helix-loop-helix (bHLH) family members such as *TAL1*, *TAL2*, *LYL1*, and *BHLHB1*; LIM-only domain (LMO) genes such as *LMO1* and *LMO2*; the *TLX1/HOX11*, *TLX3/HOX11L2*, *NKX2.1*, *NKX2.2*, *NKX2.5* and *HOXA* homeobox(HOX) genes; *MYC* ; *MYB*; and *TAN1*, a truncated and constitutively activated form of the NOTCH1 receptor. In addition, some of these T cell transcription factor oncogenes can be activated as result of alternative genetic rearrangements. Most notably, the *TLX3/HOX11L2* locus is recurrently translocated to T cell regulatory sequences in the proximity of the *BCL11B* locus and only rarely translocated to the TCR loci. In addition, small intra-chromosomal deletions in chromosome 1p32 result in *TAL1* overexpression, and cryptic deletions in chromosome 11p13 can lead to activation of the *LMO2* oncogene ***(Vlierberghe and Ferrando, 2012).***

Figure 2: The landscape of genetic alterations in T-ALL. Schematic representation of the most common genes targeted by chromosomal translocations, deletions, and mutations in T-ALL. Font size is indicative of the relative prevalence of these alterations, with highly prevalent targeted genes shown in in larger font sizes and less frequently altered loci shown in smaller font

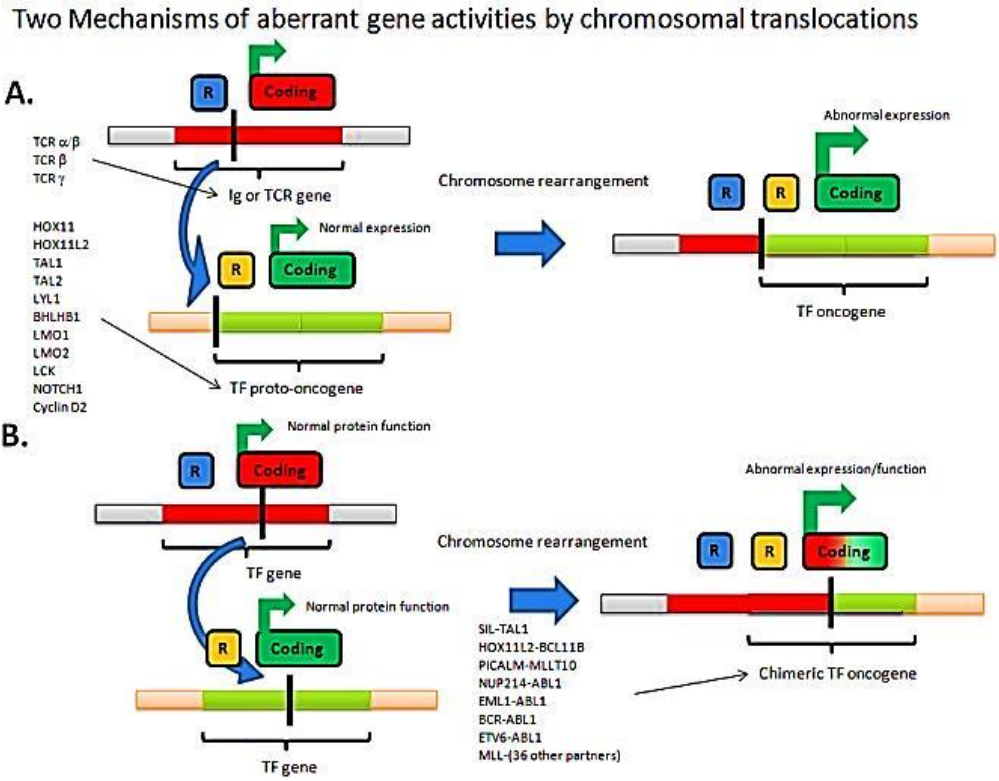
size ***(Quoted from Litzow and Ferrando, 2015).***

Other type of rearrangement in T-ALL, mostly translocations, results in formation of 'fusion genes' that are associated with specific subgroups of T-ALL

(CALM-AF10, NUP98-t, MLL-t and ABL1-fusions). In these translocations, parts of both genes located at the chromosomal breakpoints are fused

'in frame' and encode a new chimeric protein with oncogenic properties ***(Gorello et al., 2010).***

Figure 3: Two mechanisms of aberrant activities caused by chromosomal translocations. A. A strong promoter or enhancer is rearranged next to a proto-oncogene resulting in abnormal expression of the proto-oncogene. The TCR loci elements and recurring gene targets involved in T-cell leukemogenesis are indicated to the left. B. Chromosomal rearrangement between two transcription



factors result in a chimeric transcription factor with oncogenic activity. Recurring gene fusions in T-cell leukemogenesis are indicated in the center below the arrow ***(Litt et al., 2013).***

The introduction of microarray gene expression profiling analyses showed a marked relationship between the expression of T-ALL oncogenes activated by chromosomal translocations, gene expression signatures related to differential arrest at different stages of T-cell development, immunophenotype and clinical outcome. Supervised analysis of microarray data from pediatric T-ALLs based on the expression of T-ALL transcription factor oncogenes activated by chromosomal translocations identified a distinct group of T-ALLs with the highest

levels of expression of the LYL1 oncogene and coexpression of LMO2. Notably, LYL1 tumors not only showed a gene expression signature distinct from leukemias driven by TLX1 and TAL1 oncogenes, but also showed a gene expression signature reflecting an early arrest in T-cell differentiation. In contrast TLX1 positive tumors were more related to early cortical thymocytes and expressed high levels of CD1 genes, while TAL1 positive leukemias showed transcriptional upregulation of CD3 and TCR genes corresponding with a late cortical thymocyte stage of development arrest ***(Haydu and Ferrando, 2013).***

Role of CDKN2A:

Inactivation of the tumor suppressor gene, CDKN2A, can occur by deletion, methylation, or mutation. It has been reported that CDKN2A and CDKN2B are frequently inactivated in various hematologic malignancies. Loss of heterozygosity of chromosome arm 9p, including the CDKN2A locus, is one of the most frequent genetic events in childhood acute lymphoblastic leukemia (ALL), suggesting inactivation of the second allele or, possibly, haploinsufficiency. Haploinsufficiency of a tumor suppressor gene, eg, CDKN2A, has been shown to be adequate to promote tumor progression. Homozygous deletion of CDKN2A has been suggested as the dominant mechanism of its inactivation in leukemogenesis. However, the reported frequencies of both heterozygous and homozygous deletions in childhood ALL vary, 9% to 27% and 6% to 33% in B-cell precursor ALL and 7% to 18% and 30% to 83% in T-ALL, respectively ***(Sulong et al., 2009).***

Management

Application of pediatric inspired protocols in adolescents and young adults (AYA)

In the last two decades great improvements have been made in the treatment of childhood acute lymphoblastic leukemia, with 5-year overall survival rates currently approaching almost 90%. In comparison, results reported in adolescents and young adults (AYAs) are relatively poor. In adults, results have improved, but are still lagging behind those obtained in children. Possible reasons for this different pattern of results include an increased incidence of unfavorable and a decreased incidence of favorable cytogenetic abnormalities in AYAs

compared with children. Furthermore, in AYAs less intensive treatments (especially lower cumulative doses of drugs such as asparaginase, corticosteroids and methotrexate) and longer gaps between courses of chemotherapy are planned compared to those in children. There is considerable evidence from retrospective analyses that treating AYAs with a pediatric protocol may improve clinical outcomes compared with treatment adopted in adult protocols ***(Rizzari et al., 2014).***

Treatment of older adults with T-ALL

The hyper-CVAD (cyclophosphamide, vincristine, Adriamycin, and dexamethasone alternating with high dose methotrexate and cytarabine) regimen is considered intensive and in spite of this is reasonably well tolerated in fit older individuals. However, in a small series of T-ALL patients, although a high CR rate was seen with the hyper-CVAD regimen, and it was safely administered, there was a high risk of relapse after achievement of remission. In 2009, the German ALL Group (GMALL) reported their experience with 744 T-ALL patients between the ages of 15 and 55 years. The CR rate was 86%, with OS at 10 years of 47%. OS at 5 years improved from 44%to 56% with the addition of pegaspargase in induction and high-dose methotrexate with pegaspargase in consolidation in latter trials ***(Litzow and Ferrando, 2015).***

Nelarabine

Nelarabine is of interest as a targeted T cell–directed drug. Nelarabine is a water-soluble pro-drug of 9 β D-arabinofuranoyslguanine (ara-G), a deoxyguanosine analog. In contrast to deoxyguanosine, ara-G is resistant to degradation by purine nucleoside phosphorylase. The mechanism of action is based on the phosphorylation of ara-G by deoxycytidine kinase and deoxyguanosine kinase to its triphosphate (ara-GTP) that is required for cytotoxic action. It has been demonstrated that T-lymphatic cells compared with B cells have a decreased catabolism of ara-GTP. A higher initial ara-GTP accumulation in T cells compared with B cells has also been reported. The accumulation of ara-GTP leads to inhibition of ribonucleotide reductase, inhibition of DNA synthesis, and subsequent cell death ***(Gökbuget et al., 2011).***

Patients with relapsed/refractory T-cell acute lymphocytic leukemia (T ALL) and T-cell lymphoblastic lymphoma (T-LBL) have a dismal prognosis. Prior

to the development of novel purine analogs, salvage chemotherapy was of limited efficacy. Nelarabine, followed by, clofarabine and forodesine have demonstrated significant anti-tumor activity in relapsed/refractory T-ALL and T-LBL. As a single agent, nelarabine induces response rates in between 33% to 50% of adult or pediatric patients with T-ALL/T-LBL, respectively. On the other hand, limited activity was observed in mature T-cell neoplasms. Significant neurotoxicity has been the major obstacle for the further clinical development of nelarabine **(Hernandez-Ilizaliturri and Czuczman, 2009).**

Forcade et al. (2013) had conducted a retrospective analysis on behalf of the Group of Research in Adult ALL (GRAALL), of patients with T-ALL receiving Nelarabine following relapse after transplantation. Eleven patients received Nelarabine as salvage therapy in this situation, either alone as single agent or combined with other drugs. The overall hematological response rate was 81%. Event free survival and overall survival at 1 year were 70 and 90% respectively ***(Forcade et al., 2013).***

Gamma secretase inhibitors (GSI)

The high frequency of NOTCH1/FBXW7 mutations in T-ALL suggests the potential for therapeutic targeting. The NOTCH1 receptor is a class I transmembrane protein. Its activation is mediated by a transmembrane proteolytic cleavage catalyzed by the gamma-secretase complex, which is involved in the deposition of amyloid fibrils in the brains of patients with Alzheimer disease and has been the focus of research, resulting in the development of highly active small-molecule γ-secretase inhibitor (GSI) drugs. In preclinical models, inhibition by GSI of NOTCH1 receptor activation resulted inG0/G1 cell-cycle arrest and decreased proliferation. Several GSIs are in clinical development for the treatment of T-ALL ***(Litzow and Ferrando, 2015).***

Tyrosine kinase inhibitors, do they have a role?

The NUP214-ABL1 gene is the second most prevalent fusion gene involving ABL1 in malignant hemopathies, with a frequency of 5% in T-cell ALL ***(De Braekeleer E et al., 2011).***

It results into the episomal amplification and expression of the NUP214-ABL1 fusion oncogene. Interestingly, the NUP214-ABL1 rearrangement is almost exclusively found in TLX1 and TLX3 T-ALLs, which suggests a specific functional

interaction between oncogenic ABL1 signaling and TLX1 expression in the pathogenesis of T-ALL. Related ABL1 rearrangements present in T-ALL include EML1-ABL1 and ETV6-ABL1. Notably, preclinical testing of small-molecule tyrosine kinase inhibitors (developed for the treatment of BCR-ABL1–positive leukemias) in NUP214-ABL1 tumors support the hypothesis that ABL1 inhibition may be used as a targeted therapy in these patients ***(Vlierberghe and Ferrando, 2012).***

Monoclonal antibodies in T-ALL: Do they have a role?

For patients with T-ALL, the development of T cell-directed monoclonal antibody therapies are lagging compared with B-ALL. Alemtuzumab is a humanized monoclonal antibody against CD52. CD52 is expressed in 36% to 66% of leukemia cases, including B- and T-ALL and acute myeloid leukemia. Alemtuzumab has been investigated in small trials, but its development has been slow because of its modest activity and significant side effects. In 1 series, 3 children with relapsed T-ALL received alemtuzumab; none responded ***(Jabbour et al., 2015).***

Role of Hematopoietic stem cell transplantation

Patients with ETP-ALL have a particularly poor response to chemotherapy, very high risk of remission failure and subsequent relapse indicating the need for alternative approaches to treatment. On the basis of this, ETP-ALL is regarded as a high-risk subgroup and allogeneic hematopoietic SCT is recommended in first complete remission (CR) ***(Iqbal et al., 2014).***

The role of autologous SCT in T-ALL is limited. The MRC/ECOG trial showed that 5-year OS of the 99 patients randomized between autologous HCT and chemotherapy was 51% in both arms (P=.09) ***(Litzow and Ferrando, 2015).***

Prognosis

Prognostic factors are less clear in patients with T-ALL than in patients with B-ALL. In the series of T-ALL patients treated in the MRC/ECOG trial, the traditional prognostic factor of a leukocyte count >100 *109/L resulted in a poorer OS at 5 years, compared with patients with a leukocyte count <100 *10^9/L. Patients with a complex cytogenetic karyotype (≥5 chromosomal abnormalities) had a significantly lower OS at 5 years, compared with patients with simple or normal karyotypes (19% vs 51%, P 5 .006), and this impact was not affected by a higher

leukocyte count or age. Patients with activating mutations of NOTCH1 and/or FBXW7 had a higher event-free survival of 51% compared with 27% without these abnormalities, although this difference did not reach statistical significance. By immunophenotype, patients who were CD1a positive in the MRC/ECOG trial had an OS at 5 years of 64% (95% CI, 48%-80%) vs 39%(26%-52%) in CD1a-negative patients (P5.01). This appeared to be caused by a higher risk of relapse in CD1a-negative patients (50%; 36%-65%) at 5 years compared with 23%(8%-38%) in CD1a-positive patients (P 5 .02).1 The GMALL study of 744 patients identified patients with early T-ALL and mature T-ALL as high risk ***(Litzow and Ferrando, 2015).***

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