Molecular Biology: Open Access
ISSN: 2168-9547

Berbis et al., Mol Biol 2015, 4:4
DOI: 10.4172/2168-9547.1000142

Review Article Open Access

Implications of basic research in clinical practice: toward a personalized medicine in T-cell Acute Lymphoblastic Leukemia (T-ALL)

Guillem Berbis[1], Jordi Ribera[1], Josep Maria Ribera[1,2] and Eulàlia Genescà[1]*

[1]*ALL Research Group, Josep Carreras Leukaemia Research Institute (IJC), Badalona, Barcelona, Spain*
[2]*Clinical Hematology Department, Catalan Institute of Oncology (ICO)-Germans Trias i Pujol Hospital (HGTP), Universidad Autónoma de Barcelona (UAB), Badalona, Barcelona, Spain*

Abstract

Acute Lymphoblastic Leukemia (ALL) is the most common cancer in children and one of the main causes of death among childhood blood disorders. There are two subtypes according to the affected lymphoid progenitor: B-ALL and T-ALL. The T-ALL is the less common and historically was associated with poor prognosis in both adults and children, although at present, treatment outcomes do not differ significantly between the two types of ALL. The T-ALL subtype is the most complex and heterogeneous at the genetic level and currently the one with less new therapeutic alternatives available. This trend is changing thanks to the remarkable progress that is being made in understanding the biology involved. Advances in genomic research during last decade have largely contributed to this progress. Moreover many efforts are being made to identify which of this new basic data is relevant for clinical practice. This will allow us to better define the risk and take decisions on the best treatment to apply to each patient. Therefore we are moving towards a personalized patient management that ultimately will result in an increase in survival and progress to T-ALL cure. This review summarizes the most relevant and applicable biological findings in T-ALL made in recent years and their therapeutic implications that will influence the clinical practice in the future.

Keywords: Acute T-cell lymphoblastic leukemia, Molecular markers, Biology, Treatment, Personalized medicine.

Introduction

Acute lymphoblastic leukemia (ALL) is characterized by an oncogenic multistep process leading to the blockage of maturation and malignant transformation of lymphoid progenitors [1]. The incidence of ALL is not homogeneous throughout life; it has an early peak at 4 or 5 years (incidence of 4-5 cases per 100,000 people/ year), a decrease in incidence in young adults and a slight increase after 50 years (incidence rate of up to 2 cases per 100,000 person/ year). The cure rate is lower in adults than in children, with a long term disease-free survival (DFS) of approximately 80% in children and only 35-45% in adults (www.seer.cancer.gov/statistics). Specifically, T-ALL corresponds to 15% of pediatric acute leukemias and 25% of the adult cases and survival does not differ from B-precursor ALL. This subtype is characterized by a high level of genetic heterogeneity and complexity making it attractive for basic research, despite being the rare ALL subtype.

The aim of this review is to summarize the latest scientific advances in T-ALL, both in basic and clinic research highlighting the data that will have an impact in clinical practice. We will try to emphasize where the research in this subtype of ALL is moving forward in order to identify which are the key issues to be solved in the medium to long term to improve the treatment and survival of patients with this disease.

High through-put techniques for detailed genetic information on T-ALL patients

Copy Number Alterations (CNAs) in T-ALL

In recent years the work based on the use of the large scale and high resolution genomic techniques has been of vital importance for the understanding of ALL and especially revealing in T-ALL. Since Irving et al. were successfully able to identify copy neutral losses of heterozygosity (CN-LOH, also known as LOH) in the leukemic cells in 2005, using single nucleotide polymorphism (SNP) arrays [2], many other studies have been published involving new genes in the development of the T-ALL [3]. Among others, focal copy number alterations involving deregulation of *TAL1*, *LMO2*, *PTEN*, *FBXW7* and *MYB*, have been identified [4]. Tables 1 and 2 summarize findings in CNAs in T-ALL.

Expression Arrays

The use of expression arrays has also contributed to an increase in understanding of leukemia development, progression and relapse. When comparing the expression pattern of human T-ALL samples and normal T cells at different stages of differentiation, major clusters of T-ALL are obtained that significantly correlate with the immunologic and oncogenetic expression annotation. This has allowed the redefinition of a limited number of T-ALL subgroups: a) Immature Subtype (that includes Early T-cell Precursor, ETP leukemias), characterized by the absence of CD1a, CD4 and CD8 immunomarkers and the presence of stem cells or myeloid markers such as CD117, CD34, HLA-DR, CD13, CD33, CD11b, or CD65 [5-6], and the expression of high levels of *LYL1* gene [6]; b) Cortical Subtype, characterized by aberrant expression of the transcription factor family members with homeobox domain such as *TLX1*, *TLX3*, and *HOXA* genes [6], and the expression of CD1, CD4 and CD8 immunomarkers [6] and the presence of Pre-T1 or T2/ Pre-T3 (E) (7); c) Mature Subtype, characterized by the expression of the *TAL* oncogene [6], CD4, CD8 and CD3 immunomarkers [6] and Tαβ cell receptor [7] Table 1 summarizes the main chromosome abnormalities that characterize these three molecular subtypes. However, some exceptions in these correlations must be noted, highlighting the genetic diversity of T-ALL and the involvement of the affected genes in different steps of T-cell differentiation. This is the case for *TLX3* gene expression, which is also expressed in leukemias that cluster within the immature subgroup in addition to the cortical subgroup [7]; and the LIM-only domain gene (*LMO2),* whose expression can be found either in mature or immature leukemias [6].

***Corresponding author:** Dra. Eulàlia Genescà ALL Research Group-IJC Carretera de Canyet s/n, Camí de les Escoles. Edifici IMPPC.08916, Badalona (Barcelona), Spain, Tel: +(93) 557 28 08, Fax: + (93) 465 14 72, E-mail : egenesca@carrerasresearch.org

Received October 07, 2015; **Accepted** October 26, 2015; **Published** November 02, 2015

Citation: Berbis G, Ribera J, Ribera JM, Genescà E (2015) Implications of basic research in clinical practice: toward a personalized medicine in T-cell Acute Lymphoblastic Leukemia (T-ALL). Mol Biol 4: 142. doi:10.4172/2168-9547.1000142

Molecular subtype	Gene target	Chromosomic rearrangement	Frequency in % (ref.)
	LYL1	t(7;19)(q34;p13)	1[55]
IMMATURE	*OLIG2(BHLHB1)*	t(14;21)(q11.2;q22)	1[55]
	HOXA-RCTß	Inv(7)(p15 ;q34); t(7;7)(p15;q34)	3[7]
	HOXA(SET-NUP214)	del9q34; inv(14)(q11.2q13)	3[55]
	HOXA(MLL-ENL)	t(11;19)(q23;p13)	1[56]
CORTICAL	*HOXA(CALM-AF10)*	t(10;11)(p13;q14)	10[57]
	TLX1(HOX11)	t(10;14)(q24;q21); t(7;10)(q34;q24)	8[58]
	NKX2.1	Inv(14)(q13 ;q32.33); t(7;14)(q34;q13)	5[6]
	NKX2.2	t(14;20)(q11;p11)	1[6]
	TAL1- RCTα/δ	t(1;14)(p32;q11); t(1;7)(p32;q34)	3[55]
	SIL-TAL1	del1p32	16-26[59]
MATURE	*TAL2*	t(7;9)(q34;q32)	1[60]
	LMO1	t(11;14)(p15;q11); t(7;11)(q34;p15)	1[55]
	LMO3	t(7;12)(q34;p12)	<1[6]
	TLX3(HOX11L2)	t(5;14)(q35;q32)	24[58]
INCLASSIFIABLE	*LMO2*	t(11;14)(p13;q11); t(7;11)(q34;p13); del11p13	6;3(del)[61]
	c-MYB	t(6;7)(q23;q34)	3[62]

Table 1: Main recurrent chromosomal alterations in T-ALL defining molecular subgroups.

Gene target	Genetic rearrangement	Frequency in % (ref)
NOTCH1	t(7;9)(q34;q13); activating mutation	<1; >60[55]
FBXW7	Inactivating mutation	8-30[55]
CDKN2A/2B	del(9q21); methylation	>70[33]
CCND2	t(7;12)(q34;p12); t(12;14)(p13;q11)	1[55]
RB1	del(13q14)	4[63]
Unknown	del(6q)	12(c)[64]/15(a)[65]
CDKN1B	del(12p13)	2[66]
MYC	t(8;14)(q24;q11)	1[55]
WT1	Inactivating mutation ; deletion	13(c)/12(a)[67]
LEF1	Inactivating mutation ; deletion	10-15[68]
ETV6	Inactivating mutation ; deletion	17(c)/12(a)[69]
BCL11B	Inactivating mutation ; deletion	9[70]
RUNX1	Inactivating mutation ; deletion	4,4(c)[71]/18(a)[72]
GATA3	Inactivating mutation ; deletion	5[8]
PTEN	Point mutation; del(10q22)	10[73]
NUP214-ABL1	Episomal 9q34 amplification	6[74]
EML1-ABL1	t(9;14)(q34;q32)	<1[55]
ETV6-ABL1	t(9;12)(q34;p13)	<1[55]
BCR-ABL1	t(9;22)(q34;q11)	<1[75]
NRAS	Activating mutation	5-10[55]
NF1	Inactivating mutation ; deletion	3[55]
JAK1	Activating mutation	18(a)[76]
ETV6-JAK2	t(9;12)(p24;p13)	<1[55]
JAK3	Activating mutation	5[8]
FLT3	Activating mutation	2-4[77]
IL7R	Activating mutation	10[55]
EZH2	Inactivating mutation ; deletion	10-15[8]
SUZ12	Inactivating mutation ; deletion	10[8]
EED	Inactivating mutation ; deletion	10[8]
PHF6	Inactivating mutation ; deletion	16(c)/38(a)[78]

Table 2: Other recurrent genes altered in T-ALL.

Next Generation Sequencing (NGS)

Another breakthrough in the study of new genes involved in the development of the T-ALL has occurred with the appearance of the next generation sequencing (NGS) platforms. This technique allows the detection of single nucleotide variants (SNVs) and *indels* (insertions or deletions from 1-20 nucleotide) with high sensitivity and high resolution. Table 2 shows recurrent major point mutations affecting genes involved in T-ALL. In early 2012 the group of Mullighan at St. Jude Children's Research Hospital, published the first whole genome sequencing (WGS) study in ALL performed on 12 samples of pediatric immature type (ETP) T-ALL (8). They showed that this subtype of T-ALL presents cytokine activating somatic mutations and mutations in genes involved in the *RAS* signaling pathway such as *NRAS*, *KRAS*, *FLT3*, *IL7R*, *JAK3*, *JAK1*, *SH2B3* and *BRAF*, as well as genetic alterations that inactivate genes involved in hematopoietic development such as *GATA3*, *ETV6*, *RUNX1*,

IKZF1 and *EP300*. They also found mutations in the histone modifier genes (*EZH2*, *EED*, *SUZ12*, *SETD2* and *EP300*). It is of note that the mutational spectrum identified in the ETP leukemia was similar to that in acute myeloid leukemia (AML) with poor prognosis, with affected pluripotent genes that define the character of the myeloid cell [8]. Recently, a study of whole exome sequencing in adult ETP leukemias has identified mutations in the *DNMT3A* methyltransferase gene in 16% of patients (11/68), with a similar frequency to that detected in AML. Other mutations were found in *FAT1* (25%, 17/68) and *FAT2* (20%, 14/68) cadherins, involving important genes for cell adhesion and interaction between leukemic cells and microenvironment in T-ALL development and/or progression [9]. Using the same approach, De Keersmaecker et al. identified mutations in the *CNOT3* gene (3.8%, 8/211). This mutation causes decreased expression of the gene, suggesting that *CNOT3* could act as a tumor suppressor in T-ALL. In addition, mutations in *RPL10* (5.2%, 11/211) and *RPL5* (1.9%, 4/211) genes were identified. Both genes encode ribosomal proteins that are part of the 60S ribosomal complex. The most frequently observed mutation in *RPL10* was an amino acid change (Arg98Ser) that caused increased proliferation and a defect in ribosome biogenesis in lymphoid cells [10] The authors suggest that these mutations could help leukemic cells to decrease their hyperactive translational activity, which could be beneficial for the fitness of the blasts. Identification of mutations affecting the translational ability of leukemic cells is a new and a very exciting field of research to explore.

A new actor in the T-ALL scenario: the MicroRNAs

Advances in technology have not only contributed to identifying tumor suppressor genes and oncogenes but have also revealed that non-coding genes such as MicroRNAs (miRNA) are deregulated in almost all types of human cancer [11]. MiRNAs are one of the most abundant regulatory molecules in multicellular organisms and influence the expression of many genes. Therefore it is not surprising that they also play a key role in T-cell differentiation [12] and T-cell leukemia development [13]. The miR-17-92 cluster and specifically the miR-19b, was the first miRNA described to be overexpressed in human T-ALL samples [14]. Later, the existence of a translocation in the *TCR/D* locus affecting *NOTCH1* on one side (through the t(9;14)(q34;q11)) and the miR-17-92 cluster (through t(13;14)(q32;q11)) was described by Mavrakis et al. [15]. They demonstrated that the coexpression of the active form of *NOTCH1* and the miR-19 in mouse hematopoietic progenitors accelerated *NOTCH1* induced leukemia in mice. It was also observed that *BIM*, *PTEN*, *PRKAA1*, *DOCK5* and *PPP2R5E* genes were regulated by the miR-19 [15]. The first screening of miRNA in human T-ALL samples was published in 2011. Five miRNA (miR-19b, -20a, -26a, -92 and -223) that accelerate leukemia in a mouse model of T-ALL were identified. These miRNAs act cooperatively inhibiting tumor suppressor genes such as *PTEN*, *BIM*, and *FBXW7* [16] After this, the Baldus group observed overexpression of the miR-221 and the miR-222 and underexpression of the 151-3p, -19a, -20b, -342-3p, -363 and 576-3p miRNAs in adult ETP ALL samples when analyzed by microarrays [17] They showed that overexpression of miR-222 decreases the messenger RNA levels of the *ETS1* gene, a regulator of macrophage differentiation. This group also noted that high expression of miRNAs were positively associated with the expression of genes with prognostic impact on the ETP leukemia [17]. Recently, it has been reported that the expression of the miR-150, one of the miRNAs widely studied in B cells, is increased in $CD4^+$ or $CD8^+$T-cells and decreased in $CD4^+$-$CD8^+$ T cells (DP T-cells). The miR-150 also regulates *NOTCH3* expression in both DP and CD4+ or CD8+ T cells. The authors have suggested a possible role of this miRNA in T-ALL, since it regulates proliferation and apoptosis in T-ALL cell lines [18]. In the case of the mature T-ALL subtype, it has been observed that the expression of the *TAL1* oncogene leads to overexpression of miR-223 and the consequent deregulation of this miRNA's targets such as *FBXW7*, a key tumor suppressor in T-cell leukemias [19]. Finally miR-142-3p, which is overexpressed in T-ALL (16), has also been involved in a possible mechanism of glucocorticoid resistance, since this miRNA is able to regulate the translation of the α glucocorticoid receptor isoform α (GRα) [20]. In table 3 are summarized the miRNAs involved in T-ALL.

More recently, long non coding RNAs (lncRNAs), RNAs defined as transcripts with a length of at least 200 nucleotides that lack protein-coding potential and have evolutionary conservation [21], have also been shown to be involved in T-ALL development [22]. Again, high throughput techniques have introduced a new player in the T-ALL field and add an additional level of complexity to the disease, which needs to be further explored.

Adult T-ALL and pediatric T-ALL

As the number of genes known to be involved in T-ALL increases, and the number of samples tested and analyzed also increases, adult and pediatric T-ALL are emerging as two distinct molecular entities. In the extensive sequencing study done by the group of Cools, 39 matched diagnosis and remission samples (19 pediatric cases and 20 adult cases) were analyzed, it was observed that the number of mutations in adults was 2.5 times higher than in children (21.0 versus 8.2) with a clear correlation between the age and the number of mutations. However,

miRNA	Alteration	Afected genes	Prognosis
miR-19	overexpression[15]	*Bim/Pten/Prkaa1/Dock5/Ppp2r5e*[15]	NA
miR-20a	overexpression[16]	*Pten/Phf6/Bim*[16]	NA
miR-26a	overexpression[16]	*Pten/Phf6*[16]	NA
miR-92	overexpression[16]	*Ikzf1/Fbxw7*[16]	NA
miR-223	overexpression[16,19]	*Fbxw7*[16,19]	NA
miR-221	overexpression[17]	*NA*	unfavorable[17]
miR-222	overexpression[17]	*ETS1*[17]	unfavorable[17]
miR-151-3p	underexpression[17]	*NA*	NA
miR-19a	underexpression[17]	*NA*	NA
miR-20b	underexpression[17]	*NA*	NA
miR-342-3p	underexpression[17]	*NA*	NA
miR-363	underexpression[17]	*NA*	NA
miR-576-3p	underexpression[17]	*NA*	NA
miR-142-3p	overexpression[16,20]	*cAMP*[20]	unfavorable[20]

NA: not analyzed

Table 3. Micro RNA involved in T-cell leukemia.

this fact did not imply any prognostic significance. Likewise, it was observed that adults mostly had mutations in regulatory genes affecting the methylation pattern such as *KDM6A* (4.5%, 3/67) and *MAGEC3* (3.0%, 2/67), transcription regulators like *PHF6* (17.9%, 12/67) and tumor suppressor genes as *CNOT3* and *FBXW7* (11.9%, 8/67) [10]. Furthermore, mutations in proteins that are part of the 60S ribosomal complex were identified to be child specific [10]. All these data suggest that the pathogenesis of T-ALL in adults and children could arise from a different origin with different key signaling pathways affected. While in the adult alterations in epigenetic factors would have a decisive role in the acquisition of a multi-complex cancer genotype, in children a unique genetic alteration such as the *MLL* fusion alone may be sufficient to trigger an aggressive leukemia in infants [23]. This is a new area of research to explore in T-ALL, which will be developed during the coming years with the associated therapeutic consequences.

Clonal heterogeneity and relapse

Genome-wide approaches have also helped to define the clonal heterogeneity of T-ALL at diagnosis. The importance of this subclonal heterogeneity was recently revealed through the analysis of CNAs in paired diagnosis-relapse pediatric T-ALL samples [24]. Clappier et al. noted that while leukemic cells at diagnosis and at relapse share a large number of genetic lesions, additional genetic alterations that are underrepresented in the initial diagnosis sample are also detected at relapse but in a higher frequency. Thus, at the time of diagnosis leukemia could be composed of several subclones that share the same common pre leukemic ancestor, but only one or a few of them would be able to acquire a selective advantage in a particular microenvironment (niche) and proliferate to develop leukemia. Relapse would be mainly composed of clones underrepresented at diagnosis and selected during treatment. The mutational screening of paired samples (diagnosis-relapse) of pediatric ALL by NGS identified the *NT5C2* gene to be specifically mutated at relapse [25]. Sequencing of the gene in a large number of patients confirmed that mutations in *NT5C2* were found in 19% of relapsed T-ALL patients. Mutations in this gene create resistance to 6-mercaptopurine and 6-thioguanine, two nucleoside analogues used in T-ALL treatment. That was the first demonstration that leukemia developed at relapse arises from a clone refractory/resistant to the treatment [25]. A similar study published by Meyer et al., showed mutations in *NT5C2* in 2/7 of the samples tested at diagnosis, at a frequency of mutated alleles of 0.01% and 0.02% respectively, indicating that, in some cases, resistant mutations already exist before treatment at a very low frequency [26]. Collectively, these data clearly suggest that treatment at diagnosis and at relapse should be different, as is currently being carried out in clinics, but it is not informative regarding how to predict or anticipate the existence of a resistant clones before starting treatment. In addition, it is difficult to explain the origin of clone resistance in cases where no genetic alterations are detected at diagnosis. Probably the generation of resistant subclones is much more dynamic and clearly influenced by the selective pressures of the microenvironment, such as the treatment process itself. Therefore new approaches to better identify and define the resistant clones should be developed.

Clinical implication: prognostic value of biomarkers

To assess whether the information obtained in basic research is relevant to the clinics, basic data need to be correlated with clinical information of patients included in treatment protocols, in order to establish the prognostic value of the different molecular markers. Table 4 summarizes molecular markers with prognostic value identified so far. When revising the literature, it is surprising that as the number of

	Biomarker	Prognosis	
		Childhood	**Adult**
T-ALL subtype	ETP	unfavorable [4]	unfavorable [33]
	Cortical	NA	NA
	Mature	NA	unfavorable (79)
Chromosomic rearrangements (translocations)	*CALM-AFA10*	unfavorable [57]	unfavorable [80]
	TAL1	Favorable [81]/ no impact[82]	NA
	TLX1	favorable (83)	favorable [84]
	TLX3	unfavorable [85]/ no impact[81]	unfavorable [86]
Chromosomic rearrangements(deletions)	*CDKN2A/2B*	favorable [87]	unfavorable [88]/ favorable (homozygous deletions) [33]
	BCL11B	NA	favorable (homozygous deletions) [33]
	TP53	NA	unfavorable (homozygous deletions) [33]
Chromosomic rearrangements(amplifications)	*NUP214-ABL1*	Unfavorable associated to the expression of *TLX3*[82]	NA
Chromosomic rearrangements(point mutations)	*NOTCH1/FBWX7*	favorable [26],[27]/ favorable in early response[29]/	favorable [30],[31],[33]/ no impact [32]
		no impact[28]	
	DNMT3A	NA	unfavorable [33]
	IDH1/2	NA	unfavorable [33]
	WT1	no impact[67]	unfavorable in a risk standard of thymic origin subgroup [67]
	RUNX1	NA	unfavorable [89]
TCR	Lack of biallelic deletion in TCRG	unfavorable [90]	unfavorable [33]
Immunological markers	CD13+ (myeloid marker)	NA	unfavorable [33]
	CD8+	NA	favorable [33]
	CD62L+	NA	favorable [33]
Minimal residual disease (MRD)		Prognostic factor independent of treatment response[35],[91]	Prognostic factor independent of treatment response[36],[92]

ETP : Early T-cell Precursor; TCR: T-cell cellular receptor; NA: not analyzed

Table 4: Relevant prognosis factors in T-ALL.

these markers increases, the number of studies with different results, and sometimes with discordant results, also increases. The clearest example is the predictive value of mutations in *NOTCH1/FBWX7* genes. In children, two different groups with two different treatment protocols (the English MRC UKALL 2003 [27], and the German ALL-BFM 2000 [28]) confirmed *NOTCH1* mutations as markers of favorable prognosis in this subtype of ALL. However, the American Pediatric Oncology Group (POG) found no predictive value in a study on 47 patients analyzing 5 different treatment protocols [29]. Likewise, in the multicenter French study, which included children treated with two different protocols (EORTC 5881/58951), *NOTCH1* mutations only had favorable prognostic value on early response to treatment [30]. In the case of adults, the prognosis of *NOTCH1/FBWX7* value was initially even more difficult to analyze because there were fewer and discordant results, probably, due to the use of different treatment protocols (GRAALL03 LALA-94/05, by the French group [31] and GMALL 05/93 and 06/99 24 by the German group [32] (Table 4)). Recently, some light has been shed on the role of *NOTCH1/FBWX7* and its prognostic value. A study of the Eastern Cooperative Oncology Group (E2993ECOG) has confirmed the favorable prognostic value of mutations in *NOTCH1/FBWX7* in a series of 53 adult patients [33]; and last year the GRAALL group identified a subgroup within the high risk treatment protocol with a clear favorable outcome (5-year OS approximately of 90%) defined by negative MRD ($<10^{-4}$), the presence of *NOTCH1/FBWX7* mutations and the absence of *N/K RAS* and *PTEN* mutations [34]. In summary, the different impact depending on the use of different treatment protocols by the cooperative ALL groups makes necessary to perform additional studies with large series of patients, with detailed biological and clinical data. This is only possible if cooperative international initiatives are developed.

Minimal Residual Diseases (MRD)

Analysis of minimal residual disease (MRD) by flow cytometry (for aberrant immunophenotypes), or by detecting fusion genes by qPCR (for *TCR* translocated leukemias or others), or TCR rearrangements, is currently the most powerful prognostic factor for treatment response and survival in T-ALL, in both children and adults [35]. MRD allows us to track the response to treatment in each patient and in each phase of treatment, and the early identification of those patients with a high risk of relapse. This allows us to tailor treatment in these patients and even anticipate the rescue therapy if subclinical relapse appears. Consequently, all therapeutic ALL protocols currently incorporate the analysis of MRD to monitor patients at key moments to decide on their treatment.

Current techniques of massive high resolution genomic analysis are being also applied to monitor MRD in ALL. Three different research groups have shown that it is possible to apply NGS in ALL for MRD detection [36]. With the use of NGS, MRD moves from tracking a single clone to clone screening (including the annotation of normal lymphocyte rearrangements). This us to increase the sensitivity of the test and detect minor clones that are important for later relapse, avoiding false negative results.

Treatment

Most of the drugs used in the treatment of ALL were developed before 1970. Since then, the treatment of ALL has continued to improve and reached a survival rate in pediatric ALL of about 90% and between 30-40% in adults. This is thanks to the development of numerous treatment protocols by different cooperative ALL working groups, who optimized them by testing different combinations of drugs, dosage and administration sequences. An important improvement has been the inclusion of MRD detection, measured in centralized specialized platforms to give robustness to the MRD data. Along with this, the identification of specific risk groups according to the molecular characteristics of the diseases, such as the Philadelphia chromosome subgroup (Ph^+) has allowed the administration of specific and effective treatment with ABL tyrosine kinase inhibitors. However, using similar treatment strategies in children and adults, the cure rate in adults continues to be very low. This could be explained by a higher presence of genetic variants of poor prognosis in adults and, in many cases, the adult does not tolerate the intensity and density of the treatment that is administered in a child.

Standard treatment

In Spain, patients diagnosed with an ALL follow treatment protocols from the PETHEMA (Programa Español de Tratamientos en Hematología) cooperative group. T-ALL patients are included in high-risk protocols except those with cortical subtype and less than $100x10^9$ leucocytes/L at the time of diagnosis. The key elements of these protocols are the use of high-dose methotrexate and asparaginase and the assessment of MRD to take the decision of post-consolidation therapy (delayed intensification and maintenance or allogeneic stem cell transplantation). It is striking that they are the same as the ones used for B-precursor high risk Ph-negative ALL. There are, however, differences in treatment protocols between adults and children. Pediatric treatment protocols use more intensive regimens of essential cytotoxic drugs. In young adults physicians are using treatment protocols inspired by pediatric treatment regimens, which has led to an improvement in overall survival [37]. However, the application of these regimens is only feasible in patients up to 45-55 years as over that age the increase in mortality counterbalances the positive effect of the treatment [37]. In general, adult patients with T-ALL and high-risk criteria are treated with chemotherapy followed by allogeneic stem cell transplantation.

New treatments

Targeting the *BCR-ABL1*rearrangement with *ABL1* tyrosine kinas inhibitors (imatinib, dasatinib, nilotinib, and ponatinib, has represented a major advance in the treatment of children and adults with Ph^+ ALL [38]. A minority of patients with T-ALL show genetic alterations involving the *ABL1* gene, as *NUP214-ABL1*, *EML1-ABL1*, *ETV6-ABL1*, for which the efficacy of these inhibitors is under investigation [39].

One of the drugs approved for the treatment of patients with T-ALL who are resistant or in second or subsequent relapse is nelarabine. This deoxyguanosine analog, which accumulates specifically in T cells, has shown promising results in Phase I and II, in either adults or children with T-ALL and T-lymphoblastic lymphoma at relapse [40]. The most important toxicity is neurologic [40]. The Children's Oncology Group conducted a pilot trial with nelarabine combined with intensive chemotherapy treatment in newly diagnosed children with high-risk T-ALL, with encouraging results [41], and has included nelarabine in the current treatment protocol (COGAALL0434) for children with T-ALL.

Immunotherapy

A breakthrough in the treatment of ALL has been the incorporation of specific therapies using monoclonal antibodies, such as anti-CD20 (rituximab), anti-CD19 (blinatumumab, which is also anti-CD3), anti-CD52 (alemtuzumab), anti-CD22 (epratuzumab andinotuzomab ozogamycin) and anti-CD33 (gemtuzumab ozogamycin), among others [42]. Recently, genetically modified T-lymphocytes (CAR T-cells) designed to recognize surface antigens of B-cells such as CD19 and effectively kill leukemic cells have been included in the therapeutic

armamentarium [43]. Most of the monoclonal antibodies and all the CAR T-cells are directed against malignant B-cells, although normal B-cell lymphocytes are also target. All of the immunotherapeutic approaches mentioned before are directed to B-cell precursors ALL. For T-ALL there only are few studies with alemtuzumab. This monoclonal antibody, that recognizes both T-and B-cells, has been investigated in small trials, but its development has been slow because of its modest activity and significant side effects [44].

Future Therapies

New therapies on T-ALL should be based on the improving understanding of the basic molecular data and the specific subtype to be applied. In other words, they must be as personalized as possible. In this regard, the molecular characterization of the poor prognosis ETP-ALL subtype opens the possibility of using drugs tested for the AML treatment such as FLT3 [45] and the JAK/STAT inhibitors [46]. The overwhelming evidence for an inappropriate regulation of epigenetic factors during malignant transformation has created an enormous interest in pharmacological intervention of epigenetic processes. In this sense, new targeted therapies for T-ALL could be based on the use of methyltransferase inhibitors or histone deacetylase inhibitors, such as 5-azacytidine and vorinostat, respectively [47]. Another new possible targeted therapy strategy could be the use of cyclin-CDK complex: D3 CDK4/6inhibitors, which have been shown to be effective in inhibiting proliferation of blasts in samples from patients at diagnosis and at relapse, *in vivo* [48].

One form of targeted treatment is to use a downstream signaling molecule that has regulatory functions in the signaling pathway affected in the leukemic. This could be the case for protein phosphatases. Inhibition of phosphatase activity of calcineurin by using FK506 and cyclosporine drugs blocks proliferation and increases apoptosis in a mouse model of T-ALL [49]. In addition, blasts that do not express calcineurin are not capable of developing leukemia when these cells are re-injected in secondary recipient mice [50]. Another good target could be the PI3K cellular intermediate, since there is a lot of evidence that the PI3K/Akt/mTOR signal pathway contributes to T-ALL development [51].

Conclusions

The improvement in survival and the progress towards healing patients with T-ALL will ultimately come from the relevant molecular information that will allow us to define the risk and the treatment to apply to each patient and increase the therapeutic armamentarium with new alternative therapies. Progress in the molecular knowledge of T-ALL is increasing quickly, since the technological support in which this knowledge is based is continually evolving. The sequencing platforms are continuously improving to detect very rare alterations and to process a big number of samples at lower cost. Also, new techniques such as single cell sequencing will help us to understand and identify the resistant clones. However, the biggest challenge lies in being able to transfer this information to clinical practice. This requires studies with human samples and the development of humanized animal models for T-ALL, such as xenotransplantation. This is not possible without preserving good quality of samples from patients within collections or biobanks, in which samples are collected and stored according to a strict protocol for handling and covering the legal aspects of the collection. In addition, the evaluation of the prognostic significance of new putative biomarkers in patients treated homogeneously should continue, and the establishment of partnerships with other national and international groups to increase the series and compare the data is mandatory. The identification of resistant clones and the development of specific therapies directed against these resistant cells localized in specific niches is highly desirable. These therapies should be able to remove the resistant clone before the patients experience overt relapse (preventive therapy).

References

1. Pui CH, Robison LL, Look AT (2008) Acute lymphoblastic leukaemia. Lancet 371: 1030-1043.
2. Irving JA, Bloodworth L, Bown NP, Case MC, Hogarth LA, Hall AG. Loss of heterozygosity in childhood acute lymphoblastic leukemia detected by genome-wide microarray single nucleotide polymorphism analysis. Cancer Res. 2005 Apr 15;65(8):3053-8.
3. Van Vlierberghe P, Ferrando A (2012) The molecular basis of T cell acute lymphoblastic leukemia. J Clin Invest 122: 3398-3406.
4. Mullighan CG, Downing JR (2009) Genome-wide profiling of genetic alterations in acute lymphoblastic leukemia: recent insights and future directions. Leukemia 23: 1209-1218.
5. Coustan-Smith E, Mullighan CG, Onciu M, Behm FG, Raimondi SC, et al. (2009) Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. Lancet Oncol 10: 147-156.
6. Ferrando AA, Neuberg DS, Staunton J, Loh ML, Huard C, et al. (2002) Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. Cancer Cell 1: 75-87.
7. Soulier J, Clappier E, Cayuela JM, Regnault A, García-Peydró M, et al. (2005) HOXA genes are included in genetic and biologic networks defining human acute T-cell leukemia (T-ALL). Blood 106: 274-286.
8. Zhang J, Ding L, Holmfeldt L, Wu G, Heatley SL, et al. (2012) The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature 481: 157-163.
9. Neumann M, Heesch S, Schlee C, Schwartz S, Gökbuget N, et al. (2013) Whole-exome sequencing in adult ETP-ALL reveals a high rate of DNMT3A mutations. Blood 121: 4749-4752.
10. De Keersmaecker K, Atak ZK, Li N, Vicente C, Patchett S, et al. (2013) Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia. Nat Genet 45: 186-190.
11. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, et al. (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 103: 2257-2261.
12. Jeker LT, Bluestone JA (2013) MicroRNA regulation of T-cell differentiation and function. Immunol Rev 253: 65-81.
13. Slavov SN, Gimenes Teixeira HL, Rego EM (2010) The role of micro-ribonucleic acids in normal hematopoiesis and leukemic T-lymphogenesis. Braz J Med Biol Res 43: 619-626.
14. Landais S, Landry S, Legault P, Rassart E (2007) Oncogenic potential of the miR-106-363 cluster and its implication in human T-cell leukemia. Cancer Res 67: 5699-5707.
15. Mavrakis KJ, Wolfe AL, Oricchio E, Palomero T, de Keersmaecker K, et al. (2010) Genome-wide RNA-mediated interference screen identifies miR-19 targets in Notch-induced T-cell acute lymphoblastic leukaemia. Nat Cell Biol 12: 372-379.
16. Mavrakis KJ, Van Der Meulen J, Wolfe AL, Liu X, Mets E, et al. (2011) A cooperative microRNA-tumor suppressor gene network in acute T-cell lymphoblastic leukemia (T-ALL). Nat Genet 43: 673-678.
17. Coskun E, Neumann M, Schlee C, Liebertz F, Heesch S, et al. (2013) MicroRNA profiling reveals aberrant microRNA expression in adult ETP-ALL and functional studies implicate a role for miR-222 in acute leukemia. Leuk Res 37: 647-656.
18. Ghisi M, Corradin A, Basso K, Frasson C, Serafin V, et al. (2011) Modulation of microRNA expression in human T-cell development: targeting of NOTCH3 by miR-150. Blood 117: 7053-7062.
19. Mansour MR, Sanda T, Lawton LN, Li X, Kreslavsky T, et al. (2013) The TAL1 complex targets the FBXW7 tumor suppressor by activating miR-223 in human T cell acute lymphoblastic leukemia. J Exp Med 210: 1545-1557.
20. Lv M, Zhang X, Jia H, Li D, Zhang B, et al. (2012) An oncogenic role of miR-142-3p in human T-cell acute lymphoblastic leukemia (T-ALL) by targeting glucocorticoid receptor-Î± and cAMP/PKA pathways. Leukemia 26: 769-777.

21. Guttman M, Rinn JL (2012) Modular regulatory principles of large non-coding RNAs. Nature 482: 339-346.

22. Durinck K, Wallaert A, Van de Walle I, Van Loocke W, Volders PJ, et al. (2014) The Notch driven long non-coding RNA repertoire in T-cell acute lymphoblastic leukemia. Haematologica 99: 1808-1816.

23. Andersson AK, Ma J, Wang J, Chen X, Gedman AL, et al. (2015) The landscape of somatic mutations in infant MLL-rearranged acute lymphoblastic leukemias. Nat Genet 47: 330-337.

24. Clappier E, Gerby B, Sigaux F, Delord M, Touzri F, et al. (2011) Clonal selection in xenografted human T cell acute lymphoblastic leukemia recapitulates gain of malignancy at relapse. J Exp Med 208: 653-661.

25. Tzoneva G, Perez-Garcia A, Carpenter Z, Khiabanian H, Tosello V, et al. (2013) Activating mutations in the NT5C2 nucleotidase gene drive chemotherapy resistance in relapsed ALL. Nat Med 19: 368-371.

26. Meyer JA, Wang J, Hogan LE, Yang JJ, Dandekar S, et al. (2013) Relapse-specific mutations in NT5C2 in childhood acute lymphoblastic leukemia. Nat Genet 45: 290-294.

27. Jenkinson S, Koo K, Mansour MR, Goulden N, Vora A, et al. (2013) Impact of NOTCH1/FBXW7 mutations on outcome in pediatric T-cell acute lymphoblastic leukemia patients treated on the MRC UKALL 2003 trial. Leukemia 27: 41-47.

28. Kox C, Zimmermann M, Stanulla M, Leible S, Schrappe M, et al. (2010) The favorable effect of activating NOTCH1 receptor mutations on long-term outcome in T-ALL patients treated on the ALL-BFM 2000 protocol can be separated from FBXW7 loss of function. Leukemia 24: 2005-2013.

29. Larson Gedman A, Chen Q, Kugel Desmoulin S, Ge Y, LaFiura K, Haska CL, et al. The impact of NOTCH1, FBW7 and PTEN mutations on prognosis and downstream signaling in pediatric T-cell acute lymphoblastic leukemia: a report from the Children's Oncology Group. Leukemia. 2009 Aug;23(8):1417-25.

30. Clappier E, Collette S, Grardel N, Girard S, Suarez L, et al. (2010) NOTCH1 and FBXW7 mutations have a favorable impact on early response to treatment, but not on outcome, in children with T-cell acute lymphoblastic leukemia (T-ALL) treated on EORTC trials 58881 and 58951. Leukemia 24: 2023-2031.

31. Asnafi V, Buzyn A, Le Noir S, Baleydier F, Simon A, Beldjord K, et al. NOTCH1/FBXW7 mutation identifies a large subgroup with favorable outcome in adult T-cell acute lymphoblastic leukemia (T-ALL): a Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) study. Blood. 2009 Apr 23;113(17):3918-24.

32. Baldus CD, Thibaut J, Goekbuget N, Stroux A, Schlee C, et al. (2009) Prognostic implications of NOTCH1 and FBXW7 mutations in adult acute T-lymphoblastic leukemia. Haematologica 94: 1383-1390.

33. Van Vlierberghe P, Ambesi-Impiombato A, De Keersmaecker K, Hadler M, Paietta E, et al. (2013) Prognostic relevance of integrated genetic profiling in adult T-cell acute lymphoblastic leukemia. Blood 122: 74-82.

34. Beldjord K, Chevret S, Asnafi V, Huguet F, Boulland ML, Leguay T, et al. Oncogenetics and minimal residual disease are independent outcome predictors in adult patients with acute lymphoblastic leukemia. Blood. 2014 Jun 12;123(24):3739-49.

35. Borowitz MJ, Devidas M, Hunger SP, Bowman WP, Carroll AJ, Carroll WL, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. Blood. 2008 Jun 15;111(12):5477-85.

36. Faham M, Zheng J, Moorhead M, Carlton VE, Stow P, et al. (2012) Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. Blood 120: 5173-5180.

37. Huguet F, Leguay T, Raffoux E, Thomas X, Beldjord K, et al. (2009) Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 study. J Clin Oncol 27: 911-918.

38. Schultz KR, Bowman WP, Aledo A, Slayton WB, Sather H, et al. (2009) Improved early event-free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: a children's oncology group study. J Clin Oncol 27: 5175-5181.

39. Quintás-Cardama A, Tong W, Manshouri T, Vega F, Lennon PA, et al. (2008) Activity of tyrosine kinase inhibitors against human NUP214-ABL1-positive T cell malignancies. Leukemia 22: 1117-1124.

40. Kurtzberg J, Ernst TJ, Keating MJ, Gandhi V, Hodge JP, et al. (2005) Phase I study of 506U78 administered on a consecutive 5-day schedule in children and adults with refractory hematologic malignancies. J Clin Oncol 23: 3396-3403.

41. Dunsmore KP, Devidas M, Linda SB, Borowitz MJ, Winick N, Hunger SP, et al. Pilot study of nelarabine in combination with intensive chemotherapy in high-risk T-cell acute lymphoblastic leukemia: a report from the Children's Oncology Group. J Clin Oncol. 2012 Aug 1;30(22):2753-9.

42. Hoelzer D, Gökbuget N (2012) Chemoimmunotherapy in acute lymphoblastic leukemia. Blood Rev 26: 25-32.

43. Kochenderfer JN, Dudley ME, Carpenter RO, Kassim SH, Rose JJ, Telford WG, et al. Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. Blood. 2013 Dec 12;122(25):4129-39.

44. Jabbour E, O'Brien S1, Ravandi F1, Kantarjian H1 (2015) Monoclonal antibodies in acute lymphoblastic leukemia. Blood 125: 4010-4016.

45. Lhermitte L, Ben Abdelali R, Villarèse P, Bedjaoui N, Guillemot V, et al. (2013) Receptor kinase profiles identify a rationale for multitarget kinase inhibition in immature T-ALL. Leukemia 27: 305-314.

46. Roberts KG, Morin RD, Zhang J, Hirst M, Zhao Y, Su X, et al. Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. Cancer Cell. 2012 Aug 14;22(2):153-66.

47. Peirs S, Van der Meulen J, Van de Walle I, Taghon T, Speleman F, et al. (2015) Epigenetics in T-cell acute lymphoblastic leukemia. Immunol Rev 263: 50-67.

48. Sawai CM, Freund J, Oh P, Ndiaye-Lobry D, Bretz JC, et al. (2012) Therapeutic targeting of the cyclin D3:CDK4/6 complex in T cell leukemia. Cancer Cell 22: 452-465.

49. Medyouf H, Alcalde H, Berthier C, Guillemin MC, dos Santos NR, et al. (2007) Targeting calcineurin activation as a therapeutic strategy for T-cell acute lymphoblastic leukemia. Nat Med 13: 736-741.

50. Gachet S, Genescà E, Passaro D, Irigoyen M, Alcalde H, et al. (2013) Leukemia-initiating cell activity requires calcineurin in T-cell acute lymphoblastic leukemia. Leukemia 27: 2289-2300.

51. Gutierrez A, Sanda T, Grebliunaite R, Carracedo A, Salmena L, et al. (2009) High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia. Blood 114: 647-650.