



## Meeting Report: Institute for Social Security and Services for State Workers (ISSSTE) on Acute Lymphoblastic Leukaemia, México City, México, 3rd to 4th October 2016

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### ABSTRACT

From October 3 to 4, 2016, the fourth meeting of haematologists who belonged to the institute for social security and services for state workers (ISSSTE) was held, the meeting was held in Mexico City, Mexico. Attending this working meeting, medical fellows of the specialty of Haematology and Paediatric Haematology, as well as attached doctors of both specialties that work in different hospitals in Mexico City and the rest of the country, the purpose of the attendees to this consensus was discuss, update, and homogenize the protocols of diagnostic and therapeutic approach in patients with acute lymphoblastic leukaemia of all ages. All participants appreciated the opportunity to participate in one of the most important cooperation projects of the ISSSTE and to be able to offer updated treatment protocols to this population or, failing that, to send them a Medical Center that can provide hospital care as soon as possible. Physicians took advantage of this meeting for the scientific exchange, the discussion on projects in course and were planned the development of other consensuses being the closest the one of lymphomas. As in the previous consensuses that were published in a National magazine. The coordinator of this project raised to the attendees the possibility of a publication in magazines of greater prestige international since in countries like Mexico the cooperative work is

*not frequent and the group of haematologists belonging to ISSSTE are working towards this goal. This consensus was considered as a very well-organized platform to support the research of young fellows in the specialty to stimulate the team work in protocols of the different haematological pathologies and to inform the world the results achieved in a population of patients attended by the ISSSTE. In agreement with the main objective of this consensus on acute lymphoblastic leukaemia once finished and discussed throughout the haematological group, the coordinator for the consensus of lymphomas was unanimously appointed and thus to stimulate the creation of the national protocol of treatment for this pathology. The coordinator also reported that thanks to teamwork, consensus organization and work with the treatment protocols, patients with multiple myeloma are already being recruited and to be able to announce our results.*

**Keywords:** Acute lymphoblastic leukaemia, treatment child, young adult, adult, chemotherapy, markers and immunophenotype

**Abbreviations:** AIEOP: Associazione Italiana di Ematologia Pediatrica; ALL: Acute Lymphoblastic Leukaemia; BFM: Berlin-Frankfurt-Münster; CoALL: Cooperative ALL (study group); COG: Children's Oncology Group; DCOG: Dutch Children's Oncology Group; DFCI: Dana-Farber Cancer Institute (consortium); EFS: Event-Free Survival; EORTC-CLG: European Organisation for Research and Treatment of Cancer-Children's Leukaemia Group; IC-BFM: Intercontinental BFM; JCCLSG: Japanese Children's Cancer and Leukaemia Study Group; Ma-Spore: Malaysia-Singapore; MRC UKALL: Medical Research Council United Kingdom Acute Lymphoblastic Leukaemia; NA: Not Available; NOPHO: Nordic Society of Paediatric Haematology and Oncology; SJCRH: St Jude Children's Research Hospital; TPOG: Taiwan Paediatric Oncology Group; MRD: Minimal Residual Disease; NADPH: Nicotinamide Adenine Dinucleotide Phosphate.

## INTRODUCTION

Acute lymphoblastic leukaemia (ALL) is a heterogeneous neoplastic disease resulting from somatic mutations [1] in a single lymphoid progenitor cell and characterized by the proliferation of immature lymphoid cells [2]. Lymphoblasts present an altered response to growth and programmed cell death signalling [3], thus establishing competition with normal hematopoietic cells, resulting in bone marrow infiltration and presence of anaemia, thrombocytopenia, and neutropenia, additionally having the ability to infiltrate extramedullary sites [4]. The clonal origin of ALL has been established using cytogenetic analysis [5], restriction fragment analysis in female patients, which are heterozygous for polymorphic genes linked to the X chromosome, and by analysis of T-cell receptor or immunoglobulin gene rearrangements. The clinical manifestations are very variable and insidious. The symptoms generally reflect bone marrow failure characterized by 4 syndromes: Anaemic, haemorrhagic, febrile, and infiltrative. Nearly half of the patients present with some kind of infectious process at diagnosis. Bone infiltration may produce pain and arthralgia. Additionally, close to half the patients have hepatomegaly or splenomegaly [6-8].

The age adjusted incidence rate of ALL in the United States is 1.58 for every 100,000 persons per year. 57.2% of the patients diagnosed are under 20 years of age [9]. 26.8% of patients diagnosed are over 45 years of age, and 11% of patients diagnosed are over 65 years old [10]. ALL comprises 80% of all acute leukaemia in children. In adults, it represents approximately 20% of all leukaemia [2]. In Mexico, 10,400 new cases were registered according to the Compendium of the Histopathological Registry of Malignant Neoplasms in Mexico 2002, corresponding to 9.6% of all diagnosed cancers [11].

The survival rates for patients with ALL have improved in the last decades, especially in children. These improved results are due to advances in the understanding of the molecular genetics and pathogenesis of the disease, the incorporation of therapy adapted to risk, the acknowledgement of the prognostic importance of minimal residual disease (MRD) and its increasing use in therapeutic decision making, and the advent of targeted therapies. Data from the SEER (Surveillance, Epidemiology, and End Results) database have shown a 5-year overall survival (OS) of 86 to 89% for children [12,13], except in children under 1 year of age. The group of adolescent and young adult patients have a 5-year OS of 42% to 63%. Adults have a 5-year OS rate of 24.1% for patients between 40 and 59 years, and 17.7% for patients from 60 to 69 years [14].

The objective of this consensus is to unify criteria for diagnosis and treatment in a group of physicians who work for a single institution on the national level with experience in the act of acute lymphoblastic leukaemia.

Once unified in diagnosis and treatment can get to know our results to the international scientific community and know the effectiveness and safety of the Mexican design protocols.

### Physiopathology

The development of ALL is driven by successive mutations that alter cellular functions promoting [1]:

- Greater ability for self-renewal
- Greater proliferation
- Blockage of differentiation
- Resistance to apoptotic signals

Different hereditary DNA repair disorders can play an important role in the induction of this disease. Furthermore, mutagenic environmental agents which can be: physical (ionizing radiation), chemical (benzene) and biological (HTLV-1), can also be involved. However, in most cases, there are no identifiable etiologic agents.

The precise pathogenic events that lead to the development of ALL are unknown. About 5% of the cases are associated with genetic predisposition syndromes. This is the case for children with Down syndrome, who have a 10-30 times greater risk of leukaemia and present genetic abnormalities such as hyperdiploidy and t (12;21) [ETV6-RUNX1], +X, del (9), and alteration in CEBPD. It has been demonstrated that the fusion P2RY8-CRLF2 and the activation of JAK mutations contribute to 50% of the ALL cases in patients with Down syndrome. Ninety% have a deletion of IKZF12015.

The disorders associated with chromosomal fragility that have been found to predispose to ALL, include ataxia-telangiectasia, Nijmegen syndrome, and Bloom syndrome [15].

Patients with ataxia-telangiectasia have 70 times greater risk of leukaemia and 250 times greater risk of lymphoma, particularly of T-cells. The causal gene, ATM (ataxia-telangiectasia mutated), encodes a protein implicated in DNA repair and regulation of cellular proliferation and apoptosis [16,17].

Complete genome sequencing studies have identified a number of common allelic variants in four genes (IKZF1, ARID5B, CEBPE, y CDKN2A) associated with infant ALL. The allelic variant inherited can affect the response to treatment [18].

In utero exposure to X-rays for diagnostic use can confer a slight increase in risk for ALL, which positively correlates with exposure intensity [19].

Data exist that support a causal role for polymorphisms in genes that encode antioxidant enzymes (for example: glutathione S-transferase, nicotinamide adenine dinucleotide phosphate (NADPH), quinone oxidoreductase), folate metabolic enzymes (serine hydroxy methyltransferase and thymidylate synthase), cytochrome 450, methylenetetrahydrofolate reductase, and cell cycle inhibitors [20].

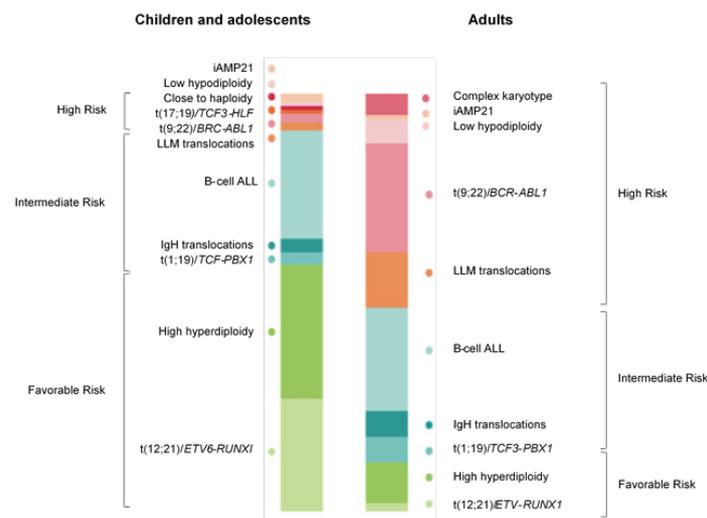
Specific fusion genes have been identified in leukaemia, the most noteworthy being KMT2A/AFF1 (also known as MLL-AF4) and ETV6-RUNX1 or TEL-AML1; additionally, there is hyperploidy and rearrangements of immunoglobulin or T-cell receptor genes [21].

The acquired genetic anomalies are a hallmark, 80% of all cases contain cytogenetic or molecular lesions with abnormalities in chromosome number (ploidy) and structure. The mechanisms involved include aberrant expression of oncoproteins, loss of tumour suppressor genes and chromosomal translocations, which generate fusion genes that encode transcription factors of active kinases [22].

A single genetic rearrangement is not enough to induce leukaemia. Cooperative mutations are necessary for leukemic transformation and include genetic and epigenetic changes in regulatory growth pathways. Candidate genes identified include deletion of the tumour suppressor locus CDKN2A/CDKN2B and NOTCH1 mutations in T cells [23]. The use of SNP microarrays suggests that genomic instability is not characteristic of most cases. There is a great variation in the number of alterations in different subtypes of leukaemia. The infant cases with rearrangements of the *MLL* gene had less than one CNA (copy number alterations) per case, suggesting that few genetic lesions are required.

Conversely, cases with ETV6-RUNX1 [24] and BCR-ABL1 had more than six CNAs, some containing more than 20 lesions, which supports the concept that, despite the initiating events that may occur in early infancy, additional lesions are required for the subsequent development of ALL [25,26].

The lymphoid transcription factor PAX5 encodes a protein involved in evolution and fidelity of the B cell lineage. The second most frequently affected gene was IKZF1 which encodes the protein IKAROS, required for lymphoid differentiation. IKZF1 is absent in most cases with BCR-ABL1. Approximately half of the patients expressing BCR-ABL1 also had deletions in CDKN2A/B and PAX5. This finding suggests that alterations in different signalling pathways are needed to induce leukemia [15]. A special role in this disease is played by the presence of the Philadelphia chromosome t (9;22) which expresses the BCR-ABL fusion gene, and this has diagnostic, prognostic and therapeutic implications [27] (Figure 1).



**Figure 1** Frequency of primary chromosomal anomalies in children and adults with acute lymphoblastic leukaemia of B cell precursors

### Clinical/diagnostic evaluation

The clinical manifestations in both adult and paediatric patients are variable and include constitutional symptoms as well as the different syndromes [17]:

- Anaemic
- Febrile
- Purpuric
- Lymphoproliferative [2,28]

In some cases, the initial manifestation may suggest the leukemic phenotype (lineage), such as leukaemia type T23 which can present with superior vena cava syndrome or involve sanctuary sites (testicle or CNS) [29].

In paediatric population, bone pain may be frequently observed [27]. Thus, the initial evaluation must include a complete clinical history and a detailed physical examination of the lymphoid organs and central nervous system (with a complete neurological exam, as needed) as well as complementary tests to rule out tumour lysis syndrome [30,31] (characterized by hyperuricemia, hyperphosphatemia, hypercalcemia, hypocalcaemia, and renal failure) and CNS infiltration [7,17] (Table 1).

In women of reproductive age, a pregnancy test is recommended (before beginning treatment).

Other studies that must be carried out are:

- Serological profile (HBV, HCV, HIV, TORCH)
- Coagulation tests

- Imaging studies (chest X-ray, USG, CAT scan/MRI)
- Echo and/or MUGA, depending on the clinical evaluation
- Lumbar puncture (ideally at the time of diagnosis, considering as positive a CSF with >5 cells/ $\mu$ L and or unequivocal demonstration of blasts after a centrifugation test.
- Histocompatibility study (HLA determination in high risk adult or paediatric patients before performing a RBC transfusion or 15 days after a non-leukodepleted transfusion) for potential transplant candidates.

**Table 1 General characteristics (modified from Sanz)**

Clinic	Adults (%)	Children (%)
Fever*	50	60
Hemorrhagic diathesis	50	50
Hepatomegaly	40	80
Splenomegaly	60	70
Adenopathy	30	60
Osteoarticular pain	30	25
Mediastinal widening	10	10
CNS infiltration	*8	*3
Testicular infiltration	*0.3	*1
<b>Complete Blood Count</b>		
Normocytic Normochromic non-regenerative Anemia	80-100	85
Leukocytosis	75	50
Hyperleukocytosis (>100 thousand)	10-20	**14
Leucopenia	15-20	-
Thrombocytopenia	70-80	70
<b>Biochemistry</b>		
Hyperuricemia	40-50	-
LDH increase	80-100	-
Hypogammaglobulinemia	25	-

\*75% due to infectious processes and 25% to tumor activity; \*\*Manual of Pediatric Procedures-ISSSTE

### Morphologic diagnosis

The bone marrow aspiration test is fundamental to confirm the presence of lymphoblasts (by morphology and/or cytochemistry with special stains that include a negative MPO in 100% of cells, PAS (+) in 70% to 80% and acid phosphatase (+) in the case of T lymphoblasts). The WHO suggests greater than 20% as DX criteria (if the percentage is lower, one must search for extramedullary disease at the nodal level to differentiate from a diagnosis of lymphoblastic lymphoma) [32].

The bone marrow aspiration is hypercellular 95% to 100% of the time, however, in those cases where the aspirate is “dry” (packed bone marrow), which corresponds to 1% to 2% of the cases, a bone biopsy must be carried out for histopathological confirmation.

Based on morphology, the FAB (French-American-British) classification identifies three types of ALL (Table 2).

**Table 2 FAB classification**

L1	Small cells with homogeneous chromatin and scarce cytoplasm
L2	Large heterogeneous cells with irregular nuclei and variable cytoplasm
L3	Large homogeneous cells with more than 5% mitosis and at least 25% of vacuolated cells. Burkitt.

### Cytogenetic and molecular diagnosis

Although this classification is very useful, currently, more specific types of studies have gained influence, such as multiparametric flow cytometry (immunophenotype to determine cellular lineage) and cytogenetic studies to characterize the disease by risk groups, since they are more reproducible and have been validated (although they are not accessible to many institutions) [33] (Table 3).

Table 3 Markers and immunophenotype

Markers	Immunophenotype
Early cells	CD34, CD117, HLA, TdT
B cells	CD19, CD79a, CD22, CD10, cIgM, mIgM
T cells	CD3, CD5, CD2, CD1a, CD7
Myeloid lineage	CD13 y CD33 (controls)

Another advantage of the immunophenotype, is the possibility of detecting mixed lineage leukaemia (biphenotypic or bilineal) based on the EGIL scale (Table 4) [29].

Table 4 Classification system of the European group for the immunological characterization of leukaemia (EGIL)

EGIL Scale Lineages			
Points	Lymphoid B	Lymphoid T	Myeloid
2	CD79a	CD3	MPO*
	CD22		
	IgM cyt		
1	CD19	CD2	CD13
	CD10	CD5	CD33
0.5	TdT	TdT	CD14, CD15
		CD7	CD11b, CD11c

\*MPO (myeloperoxidase) demonstrated by cytochemical or immunologic methods. Biphenotypic lineage is considered when the score for the myeloid lineage is greater than 2 and 1 for the lymphoid lineage

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In women of reproductive age, a pregnancy test is recommended (before beginning treatment).

Other studies that must be carried out are:

- Serological profile (HBV, HCV, HIV, TORCH)
- Coagulation tests
- Imaging studies (chest X-ray, USG, CAT scan/MRI)
- Echo and/or MUGA, depending on the clinical evaluation

- Lumbar puncture (ideally at the time of diagnosis, considering as positive a CSF with >5 cells/ $\mu$ L and or unequivocal demonstration of blasts after a centrifugation test)
- Histocompatibility study (HLA determination in high risk adult or paediatric patients before performing a RBC transfusion or 15 days after a non-leukodepleted transfusion) for potential transplant candidates.

**Table 5 General characteristics (modified from Sanz)**

Variables	Adults (%)	Children (%)
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Hepatomegaly	40	80
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Adenopathy	30	60
Osteoarticular pain	30	25
Mediastinal widening	10	10
CNS infiltration	8	3
Testicular infiltration	0.3	1
<b>Complete Blood Count</b>		
Normocytic Normochromic non-regenerative Anaemia	80-100	85
Leucocytosis	75	50
Hyperleukocytosis (>100 thousand)	10-20	14 (**)
Leucopenia	15-20	--
Thrombocytopenia	70-80	70
<b>Biochemistry</b>		
Hyperuricemia	40-50	-
LDH increase	80-100	-
Hypogammaglobulinemia	25	-

### Morphologic diagnosis

The bone marrow aspiration test is fundamental to confirm the presence of lymphoblasts (by morphology and/or cytochemistry with special stains that include a negative MPO in 100% of cells, PAS (+) in 70% to 80% and acid phosphatase (+) in the case of T lymphoblasts). The WHO (2008) suggests greater than 20% as DX criteria (if the percentage is lower, one must search for extramedullary disease at the nodal level to differentiate from a diagnosis of lymphoblastic lymphoma [32]).

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**Table 6 FAB Classification**

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Based on morphology, the FAB (French-American-British) classification identifies three types of ALL (Table 6).

### Cytogenetic and molecular diagnosis

Although this classification is very useful, currently, more specific types of studies have gained influence, such as multiparametric flow cytometry (immunophenotype to determine cellular lineage) and cytogenetic studies to characterize the disease by risk groups, since they are more reproducible and have been validated (although they are not accessible to many institutions) (Table 7) [33].

**Table 7 Markers and Immunophenotype**

Markers	Immunophenotype
Early cells	CD34, CD117, HLA, TdT
B cells	CD19, CD79a, CD22, CD10, cIgM, mIgM

T cells	CD3, CD5, CD2, CD1a, CD7
Myeloid lineage	CD13 y CD33 (controls)

Another advantage of the immunophenotype, is the possibility of detecting mixed lineage leukaemia (biphenotypic or bilineal) based on the EGIL scale (Table 8) [29].

**Table 8 Classification system of the European group for the immunological characterization of leukaemia (EGIL)**

EGIL Scale Lineages			
Points	Lymphoid B	Lymphoid T	Myeloid
2	CD79a	CD3	MPO*
	CD22		
	IgM cyt		
1	CD19	CD2	CD13
	CD10	CD5	CD33
0.5	TdT	TdT	CD14, CD15
		CD7	CD11b, CD11c

\*MPO (myeloperoxidase) demonstrated by cytochemical or immunologic methods. Biphenotypic lineage is considered when the score for the myeloid lineage is greater than 2 and 1 for the lymphoid lineage

**Note:** A marker is considered to be positive when it is detected on the Surface of >20% of the blasts and in >10% of the cytoplasm.

Cytometry not only defines the cellular origin and degree of maturation but also provides the possibility to evaluate MRD (minimal residual disease), and establishes the basis for the current WHO classification for acute lymphoblastic leukaemia (Tables 9 and 10) [33].

**Table 9 Classification according to B origin**

Subgroups	HLA-DR	TdT	CD19	CD79	CD22	CD10	cIg	mIg	Cytogenetics and molecular biology
Pro-B	++	++	++	++	++	-	-	-	t (4;11) AL1-AF4 MLL
Common Pre-B (CALLA)	+	++	++	++	++	++	-	-	t (9;22) BCR/ABL
Pre-B	+	++	++	++	++	++	++	-	t (12;21) TEL/AML1 (rare in adult) 11q 23
Mature B	+	++	++	++	++	++	++	++	t (8;14) c-myc-IgH

**Table 10 Classification according to T origin**

Subgroups	Ccd3	CD+	CD5	CD2	CD3	CD1	Cytogenetics and molecular biology
Early T	++	++	+	-	-	-	Notch 1 t (10;14) HIX 11-TCR
							t (11;14) LMO/TCR
							SIL-TAL1
							NUP213
							ABL1
							HOX11
Cortical T	-	-	++	++	+	+	-
Mature T	+	-	++	++	++	-	-

The cytogenetic (G banded Karyotype) and molecular (FISH and/or PCR) profiles also define prognostic subtypes that determine therapeutic conduct. The objective is to identify fusion genes (BCR/ABL1 (p190, p210), MLL-AF4, ETV6-RUNX1 and TCF3-PBX1)1, as well as clonal rearrangements in genes that encode immunoglobulin heavy chains (IgH) and/or T-cell receptor genes (TCR) (Table 11) [23].

**Table 11 Risk classification**

Prognosis	Chromosomal alteration	Specifications	Frequency (%)
Good	t (12;21) (p13; q22) */ETV6-RUNX1	2/3 present loss of the normal allele of the ETV6 gene 20-25% trisomy 21 15% to 20% Duplication der (21)	25
	High HYPERDIPLOIDY (HeH) 51 to 65/67 chromosomes	Trisomy X, 4, 6, 10, 17, 18/tetrasomy 14 and 21 DNA index 1.10-1.44	30

Intermediate	t (1;19) (q23; p13)/TCF-PBX1 (E2A-PBX1)		50% unbalanced der (19) t (1;19)	3-5
	t11q23/MLL rearrangements Excluding the t (4;11), which denotes poor prognosis		t (11;19) (q23; p13.3)/MLL-ENL other partners: 6q27 (MLLT4/AF6), 9p21 (MLLT3/AF9), 10p12 (MLLT10/AF10) 1p32(EPS15)	9
	IAMP21**		Amplification of 21q22.11-21q22.12	-
	Normal karyotype		At least 20 metaphases analyzed	-
	Others		Structural alterations, 45 chromosomes, no growth, etc.	-
Poor	Hypodiploidy <45 chromosomes DNA Index <0.8	High Hypodiploidy 40-44 chromosomes	-	0.1
		Low Hypodiploidy 30-39 chr/ almost triploidy 60-78 chr	Monosomies chr 3, 7, 15, 16, 17/Disomies chr. 1, 6, 11, and 18 They tend to double the amount number to almost triploidy	3-5
		Almost HAPLOIDY <30 chromosomes	<30 chromosomes X chrs are retained/Y, 10, 14, 18, 21 are usually doubled until 54 chrs.	1
	t (4;11) q21; q23)/MLL-AFF1 (AF4)		-	2-3
	t (17;19) (q22; p13)/TCF3-HLF (E2A-HLF)		-	0.1

\*There is controversy, but the most recent publications suggest that additional alterations do not modify the prognosis.  
 \*\*iAMP21: some cooperative groups include this alteration in the poor prognosis group

**Risk classification**

Genetic and molecular alterations with prognostic value in ALL.

This series of studies was used by the WHO in 2008 to modify the current classification of cancer from lymphoid precursors and classify them into 4 subtypes.

**Note:** A marker is considered to be positive when it is detected on the Surface of >20% of the blasts and in >10% of the cytoplasm.

Cytometry not only defines the cellular origin and degree of maturation but also provides the possibility to evaluate MRD (minimal residual disease), and establishes the basis for the current WHO classification for acute lymphoblastic leukaemia (Tables 12 and 13) [33].

**Table 12 Classification according to B origin**

Subgroups	HLA-DR	TdT	CD19	CD79	CD22	CD10	cIg	mIg	Cytogenetics and molecular biology
Pro-B	++	++	++	++	++	-	-	-	t (4;11) AL1-AF4 MLL
Common Pre-B (CALLA)	+	++	++	++	++	++	-	-	t (9;22) BCR/ABL
Pre-B	+	++	++	++	++	++	++	-	t (12;21) TEL/AML1 (rare in adult) 11q 23
Mature B	+	++	++	++	++	++	++	++	t (8;14) c-myc-IgH

**Table 13 Classification according to T origin**

Subgroups	Ccd3	CD+	CD5	CD2	CD3	CD1	Cytogenetics and molecular biology
Early T	++	++	+	-	-	-	Notch 1 t (10;14) HIX 11-TCR
							t (11;14) LMO/TCR
							SIL-TAL1
							NUP213
							ABL1
							HOX11
Cortical T	-	-	++	++	+	+	-
Mature T	+	-	++	++	++	-	-

The cytogenetic (G banded Karyotype) and molecular (FISH and/or PCR) profiles also define prognostic subtypes that determine therapeutic conduct. The objective is to identify fusion genes (BCR/ABL1 (p190, p210), MLL-AF4, ETV6-RUNX1 and TCF3-PBX1)1, as well as clonal rearrangements in genes that encode immunoglobulin heavy chains (IgH) and/or T-cell receptor genes (TCR) (Table 14) [23].

Table 14 Risk Classification

Prognosis	Chromosomal Alteration		Specifications	Frequency (%)
Good	t(12;21) (p13;q22)*/ETV6-RUNX1		2/3 present loss of the normal allele of the <i>ETV6</i> gene 20% to 25% trisomy 21 15% to 20% Duplication der (21)	25
	High Hyperdiploidy (HeH) 51 to 65/67 chromosomes		Trisomy X, 4, 6, 10, 17, 18/tetrasomy 14 and 21 DNA index 1.10-1.44	30
Intermediate	t (1;19) (q23;p13)/TCF-PBX1 (E2A-PBX1)		50% unbalanced der (19) t (1;19)	03-05
	t11q23/MLL rearrangements		t (11;19) (q23;p13.3)/MLL-ENL	9
	Excluding the t (4;11), which denotes poor prognosis		Other partners: 6q27 (MLLT4/AF6), 9p21 (MLLT3/AF9), 10p12 (MLLT10/AF10) 1p32(EPS15)	
	IAMP21**		Amplification of 21q22.11-21q22.12	-
	Normal Karyotype		At least 20 metaphases analyzed	-
Others		Structural alterations, 45 chromosomes, no growth, etc.	-	
Poor	HYPODIPLOIDY <45 chromosomes DNA Index <0.8	High Hypodiploidy 40-44 chromosomes	-	0.1
		Low HYPODIPLOIDY 30-39 chr/almost triploidy 60-78 chr	Monosomies chr 3, 7, 15, 16, 17/Disomies chr. 1, 6, 11, and 18. They tend to double the amount number to almost triploidy	03-05
		Almost Haploidy <30 chromosomes	<30 chromosomes X chrs are retained/Y, 10, 14, 18, 21 are usually doubled until 54 chrs.	1
	t (4;11) q21;q23)/MLL-AFF1 (AF4)		-	02-03
	t (17;19) (q22;p13)/TCF3-HLF (E2A-HLF)		-	0.1

\* There is controversy, but the most recent publications suggest that additional alterations do not modify the prognosis; \*\* iAMP21: some cooperative groups include this alteration in the poor prognosis group

**Risk classification**

Genetic and molecular alterations with prognostic value in ALL.

This series of studies was used by the WHO in 2008 to modify the current classification of cancer from lymphoid precursors and classify them into 4 subtypes (Table 15).

Table 15 Precursor lymphoid neoplasm subtypes

B Cell Lymphoblastic Leukemia/Lymphoma, NOS (Not otherwise specified)	-
B Cell Lymphoblastic Leukemia/Lymphoma with recurrent cytogenetic anomalies	B Cell Lymphoblastic Leukemia/Lymphoma with t (9;22) (q34;q11.2); BCR/ABL1
	B Cell Lymphoblastic Leukemia/Lymphoma with t(v;11q23); with MLL rearrangements
	B Cell Lymphoblastic Leukemia/Lymphoma with hyperdiploidy
	B Cell Lymphoblastic Leukemia/Lymphoma with t (5;14) (q31;q32); IL3-IGH
	B Cell Lymphoblastic Leukemia/Lymphoma with t (1;19) (q23;p13.3); E2A-PBX1 (TCF3-PBX1)
T Cell Lymphoblastic Leukemia/Lymphoma	
Acute Leukemias of Ambiguous Lineage	
Undifferentiated Acute Leukemia	
Acute Leukemia with Mixed Phenotype with t (9;22) (q34;q11.2)	
Acute Leukemia with Mixed Phenotype with t (v;11q23); with MLL rearrangements	
Acute Leukemia with Mixed Phenotype B/Myeloid, NOS	
Acute Leukemia with Mixed Phenotype T/Myeloid, NOS	
Lymphoblastic Natural Killer Leukemia/Lymphoma	

Based on these elements, prognostic factors, and group stratification by risk groups, according to age, have been established (Tables 16 and 17).

**Table 16 Risk groups: Adults with ALL**

<b>Standard</b>	<50 years,
	leukocytes <30,000 for the B lineage,
	<100,000 for the T lineage,
	CR (complete remission) in 4-6 weeks after treatment is initiated.
	Cytogenetic indicator of good prognosis: Hyperdiploidy (>50 chromosomes) or hypodiploidy (<40 chromosomes)
	Gene fusion TEL-AML1 and t (1;19)/E2A-PBX1.
	BMA d+14: aplasia and <10% blasts
	BMA d+28: Complete Remission
<b>High</b>	Final induction MRD: <10 <sup>-3</sup> – 10 <sup>-4</sup>
	Standard criteria are not achieved.
	Mature B or pro-B immunophenotype,
<b>Very High</b>	Central nervous system infiltration
	Unfavorable genetics: t (4:11), t (1:19), t (9:22), 11q23 Ph+ ALL or >60 years

**Table 17 Risk groups: Paediatric age**

Standard risk (SR)	GRP (Good Response to Prednisone) at day 8: <1000 blasts/mL
	Age >1 year and/or <6 years
	White blood cell count <20,000/mL
	MRD in BM d15 <0.1%
	BM d15 M1 or M2
Intermediate risk (IR)	BM d 33 M1
	GRP at day 8: <1000 blasts/mL
	Age <1 year and/or ≥ 6 years and/or white blood cell count ≥ 20,000/mL
	MRD in BM d15 <10%
	and BM d15 M1 or M2
High risk (HR)	and BM d 33 M1 or:
	Criteria for SR but: MRD >0.1% and <10% or BM d15 M3
	and BM d33 M1
	IR and BM at d15 M3
	IR with MRD >10%
	BRP (Bad RP) at day 8: >1000 blasts/mL
	BM d 33 M2 or M3
	t (9;22) (BCR/ABL) or t (4;11) (MLL/AF4)
	Hypodiploidy ≤44Cr

The best results are observed in children between 1 and 10 years of age as well as in patients with a marked and rapid decrease of blasts in PB (d+8) and/or BM (d+15) and CR after induction. The T lineage has an adverse prognosis (except cortical T ALL without hyperleukocytosis).

In the patient group between 16 and 39 years of age, known as AYA (Adolescents and young adults), a significant reduction in all-cause mortality has been achieved in patients that received paediatric inspired treatments, compared to conventional adult treatments [34,35].

**Treatment**

Before initiating treatment, having basal studies is highly suggested, including: complete blood count, group and RH, bone marrow aspiration, lumbar puncture, testicular exploration if indicated, fundoscopy, flow cytometry, karyotype, PCR for BCR/ABL and/or FISH, functional status, comorbidity index, left ventricular ejection fraction, renal function, hepatic function, serology, and imaging.

The treatment for patients with acute lymphoblastic leukaemia should be individualized, since the therapeutic conditions and options are different based on the type of leukaemia, risk, age, and comorbidities, among others. The proposal in this document includes: Paediatric patients (will be discussed in the section for paediatric population), young adults (16 to 39 years), adults (40 to 60 years), senior citizens older than 60, and special populations presenting the Philadelphia chromosome.

### Overview of treatment for acute lymphoblastic leukaemia

Special conditions must be identified: Hyperleukocytosis, leukostasis, tumour lysis syndrome and cytokine storm; it is pertinent to individualize the management for any of these circumstances. Historically, the treatment comprises different phases: Induction, early intensification (consolidation), prophylaxis and/or treatment to the central nervous system, late intensification (re-induction), and maintenance.

**Induction:** The goal of induction is to reduce tumoral load and re-establish normal hematopoietic function. The most common treatment includes a combination of 5 drugs, including anthracyclines, Cyclophosphamide, vincristine, a steroid, L-asparaginase and in some protocols, cytarabine. If positive for CD20, and if there are no contraindications, a selective monoclonal antibody should be included. During induction, intrathecal chemotherapy with cytarabine, methotrexate and a steroid should be administered.

After induction, the response levels are defined as:

- 1) **Clinical:** Disappearance of the symptoms attributable to ALL.
- 2) **(Haematological) Morphological:** HB >100 g/L with transfusion independence, granulocytes  $>1 \times 10^9/L$ , platelets  $>100 \times 10^9/L$  (without need for support) and normocellular bone marrow (BM) with less than 5% blasts and without blasts in the CSF.
- 3) **Morphological with incomplete recovery:** Does not comply with the hematologic requirements or unequivocal demonstration of extramedullary leukemic condition.
- 4) **Cytogenetic:** Morphologic CR with normal cytogenetics, if alterations had been previously detected.
- 5) **Immunophenotypic:** <0.1% cells with a leukemic immunophenotype.

**Early intensification:** The goal is to eradicate residual leukemic cells, reduce drug resistance and avoid relapse. The treatment schemes most commonly used include: Methotrexate, Cyclophosphamide, Cytarabine, Vincristine, L-asparaginase, 6-Mercaptopurine, steroids, and in some protocols, Etoposide, Fludarabine, and Clofarabine.

**Prophylaxis and/or treatment to the central nervous system:** This is the direct application of drugs to the central nervous system, with the goal of reaching therapeutic doses that can eradicate the presence of the disease in the sanctuary site. The types of treatment used are: Radiotherapy and intrathecal chemotherapy.

**Prophylaxis:** Apply Cytarabine, Methotrexate and a steroid with the frequency established in the protocol, in combination with systemic treatment with 6-Mercaptopurine and Methotrexate.

The radiotherapy used for prophylaxis is administered at 12 Gy, usually combined with a steroid.

**Treatment:** Apply Cytarabine, Methotrexate and a steroid. In accordance with the risk and regimen used, it must be administered 3 times a week until CSF is negative in 2 determinations. Radiotherapy to the neuraxis with 18 Gy is suggested for the following cases:

- Presence of 5 or more leukocytes/ $\mu L$  in CSF, with the presence of lymphoblasts.
- In the case of patients with blasts in blood and traumatic puncture with leukocytes greater than  $5/\mu L$ . The relationship leukocyte/erythrocyte in blood and CSF must be compared, if this last one is double, it is considered positive.
- In the case of presenting testicular illness, the recommended dose is 24 Gy.

**Late intensification:** Some protocols contemplate a second intensification regimen, also known as reinduction, utilizing the same drugs with the same objective.

**Maintenance:** During the initial treatment phase, which lasts between 2 and 2.5 years, the goal is to avoid relapse. The drugs most frequently used are: 6-Mercaptopurine, Methotrexate, Vincristine, and Prednisone.

**Treatment for acute lymphoblastic leukaemia in patients under 40 years of age (LAL-6, ISSSTE):** With the purpose of unifying treatment schemes, the following protocol is recommended for patients younger than 40 years of age:

#### Inclusion criteria

- With de novo ALL, according to the criteria set forth by WHO, immunophenotype and cytogenetic exams

- Between 15 and 40 years of age
- Without evidence of cardiomyopathy
- With left ventricular ejection fraction (LVEF) >50%
- Karnofsky score >70%
- Acceptance through an Informed Consent Form (signed by parents or tutors when required).

**Exclusion criteria**

- History of myelodysplastic syndrome. Previous chemotherapy or radiotherapy
- HIV positive
- Sepsis
- Diabetes Mellitus which precludes the use of steroids
- Serum creatinine >2.0 mg/dL
- Pregnancy (immunologic test, if necessary).

**Elimination criteria**

- Patient refusal to continue treatment
- Cardiotoxicity. Persistent toxicity >WHO Grade 3 in any vital organ
- Patients receiving doses lower than those indicated in the protocol.

**Initial instructions**

- 1) Fill out the questionnaires: “ACUTE LEUKEMIA. INITIAL DATA” and “ACUTE LEUKEMIA. FOLLOW UP”.
- 2) Consult Table 18, in order to adhere to the calendar of exams included therein.
- 3) Consider and treat for Febrile Neutropenia, if it is not likely that the fever is due to activity.
- 4) Request the insertion of a central catheter before initiating induction chemotherapy.
- 5) During each intrathecal chemotherapy treatment, a sample will be taken for CSF cytology.
- 6) The dose of Vincristine will not be greater than 2 mg (total).
- 7) Maintain a haematocrit between 25% and 30% with erythrocyte concentrates.
- 8) Use platelets (from cytopheresis) only if there is haemorrhage or fever.
- 9) Infection prophylaxis with Ciprofloxacin 250 mg PO every 12 h, Acyclovir 200 mg IV, and Fluconazole 100 mg IV, every 12 h.
- 10) Strict isolation measures
- 11) Mouthwash with Nystatin and bicarbonate every 4 h.
- 12) Request Karyotype and/or PCR results during the first 8 days.

**Table 18 Calendar for laboratory and clinical tests**

Study	Induction	Intensification	Follow Up
CBC	2/week	2/week	Before each cycle
PTT, PT, TT	Basal, as indicated	As indicated	As indicated
Fibrinogen	2/week	As indicated	As indicated
Blood Chemistry	Weekly	Weekly	Before each cycle
Amylase	2/week	As indicated	As indicated
Electrolytes	Weekly	Weekly	As indicated

Urinalysis	Basal, as indicated	As indicated	As indicated
Ferritin	NO	YES	Every 6 months
CSF	Basal, as indicated	As indicated	During IT CHT, or as indicated
Immunophenotype	Basal	YES	Maintenance 3, 6, 9 and elective discontinuation
Cytochemistry	Basal	As indicated	As indicated
Karyotype	Basal	As indicated	As indicated
FISH or PCR	Basal	As indicated	As indicated
Bone Marrow	Days 14, 28	As indicated	Every 3 months, as indicated
Chest X-Ray	Basal, as indicated	As indicated	As indicated
Abdominal X-Ray	Basal, as indicated	As indicated	As indicated
LVEF	Basal	As indicated	Month 30 or as indicated

## Therapeutic Management

### Induction (1.0)

**Day 4:** Dexamethasone, 10 mg/m<sup>2</sup>/day, IV, bolus, for 4 days.

**Day 0:** Methotrexate 12.5 mg, IT + Cytarabine 100 mg + Dexamethasone 5 mg Daunorubicin 120 mg/m<sup>2</sup> BSA IV with continuous drip for 48 hours, diluted in an appropriate amount of isotonic saline solution. If daunorubicin is unavailable, use Epirubicin 120 mg/m<sup>2</sup> BSA, continuous IV infusion for 48 hours.

**Day 2:** Cyclophosphamide 1,200 mg/m<sup>2</sup> BSA, IV bolus.

- Vincristine 1.5 mg/m<sup>2</sup> BSA, IV bolus. Repeat on days 9, 16 and 23.
- Prednisone 60 mg/m<sup>2</sup> BSA/day PO, until day 23. Afterward, reduce progressively during the course of 9 days.

**Day 4:** Asparaginase. 4,000 u/m<sup>2</sup> BSA/day IM. Continue Mon-Wed-Fri until finishing consolidation.

**Day 8:** If there is CNS infiltration:

- Methotrexate 12.5 mg IT + Cytarabine 100 mg + Dexamethasone 5 mg. Repeat on days 15 and 22.
- If there is no CNS infiltration: Methotrexate, Cytarabine and Dexamethasone with the same dose and route of administration, only on Day 8.
- Take bone marrow on day 14. If no blasts are found, continue with the program, and evaluate complete remission by aspiration on day 28; if there is CR, go to 2.0.
- If blasts are present, go to Day 28.

**Day 9:** G-CSF 5 µg/kg/day SC. Continue until neutrophil levels are  $> 0.5 \times 10^9/L$ , in two consecutive counts

**Day 28:** Dexamethasone, 25 mg/m<sup>2</sup> BSA, IV on days 28, 30, 32, 34, 36 and 38.

- Daunorubicin, 60 mg/m<sup>2</sup> BSA, IV continuous drip for 24 hours (if unavailable, Epirubicin 60 mg/m<sup>2</sup> BSA).
- Vincristine 1.5 mg/m<sup>2</sup> BSA, IV. Repeat on Day 35.
- Ph+ patients will receive: Dasatinib 100 mg, from Day 4 or Nilotinib 600 mg, permanently.
- No Asparaginase will be given in any of the phases.

In all cases the following must be measured: Minimal residual disease using flow cytometry at the end of induction, intensification, and maintenance (3, 6, 9 months) phases and at elective discontinuation. The remission criteria will be applied on the sample from day 28. If there is no remission, the patient will leave this protocol; if there is remission, go on to 2.0.

\*When Daunorubicin is unavailable, use Epirubicin.

### Intensification (2.0)

**Day 1:** Begin with neutrophils  $>0.5 \times 10^9/L$  and platelets  $>100 \times 10^9/L$ . Place reservoir.

- Cytarabine. 1.5 g/m<sup>2</sup> BSA, IV, diluted in a convenient amount of saline solution, pass in three hours, every twelve hours (8 doses).

- G-CSF 300 µg daily beginning on day 7 post-chemotherapy, until neutrophils are above  $0.5 \times 10^9/L$  in two consecutive counts.

- Ophthalmic Prednisolone every 8 h, suspend 24 h after finishing Cytarabine.

\*At the end of intensification, high-risk patients should be considered for allogeneic transplant. Request RT-PCR for BCR-ABL.

### Consolidation (3.0) (3.1)

**Day 18:** (with neutrophils  $>0.5 \times 10^9/L$  and without infection).

- Vincristine, 1.5 mg/m<sup>2</sup> BSA, IV, one day before giving Methotrexate, without surpassing 2 mg.
- Infuse 0.5 mEq/kg of Na bicarbonate in 15 to 30 minutes, immediately before giving Methotrexate.
- Methotrexate, 1.0 g/m<sup>2</sup> BSA diluted in saline solution (at least 1.0 g of Methotrexate in 1,000 ml of dextrose solution) as a continuous drip in 4 to 6 hours.
- Folinic acid, 75 mg/m<sup>2</sup> BSA, IV every 6 hours, beginning 24 hours after having finished Methotrexate (6 doses).

### Day 25

- Vincristine 1.5 mg/m<sup>2</sup> BSA IV.
- Prednisone 180 mg/m<sup>2</sup> BSA/day PO for 7 days, with progressive reduction until suspension.
- Take bone marrow. If the patient remains in remission continue to *Maintenance*. After this time, bone marrow will be taken every three months. If Ph+, RT-PCR will be repeated every three months.

### Early maintenance (4.0)

Begin with neutrophils  $>1.0 \times 10^9/L$  and platelets  $>50 \times 10^9/L$ ). Use G-CSF when pancytopenia prevents fulfilment of the program. If neutrophil count is lower than  $1.0 \times 10^9/L$  and platelets lower than  $50 \times 10^9/L$ , without evidence of relapse or infection, apply a dose of 50% to 75%, in order to comply with the calendar of treatments.

### Day 1

- Methotrexate 12.5 mg + Cytarabine 100 mg + Dexamethasone 5 mg, IT. Repeat on days 8, 15 and 22.
- Cranial radiotherapy, 1.8 Gy/day, 10 days, only for patients with: leukocytes  $>5 \times 10^9/L$ , CNS infiltration, initial lymphomatous component or T immunophenotype.
- Prednisone 15 mg/m<sup>2</sup> BSA/day PO, only if receiving radiotherapy.
- Drug 6-Mercaptopurine 300 mg/m<sup>2</sup> BSA/day PO, during four consecutive days. If unavailable, use Fludarabine 20 mg/m<sup>2</sup> BSA/day PO, for 3 days.

### Day 5

- Cyclophosphamide 600 mg/m<sup>2</sup> BSA, IV bolus.
- L-Asparaginase 4,000 u/m<sup>2</sup> BSA IM. Continue with one application per week until completing nine doses.

### Day 12

- Rituximab 375 mg/m<sup>2</sup> BSA, IV (if more than 30% CD20).
- Vincristine 1.5 mg/m<sup>2</sup> BSA, IV. Repeat on days 19 and 26 (74 and 81).

### Day 19

- Prednisone 180 mg/m<sup>2</sup> BSA/day, PO. Administer for seven days.

### Day 26

- Methotrexate, 650 mg/m<sup>2</sup> BSA, intravenously diluted in 500 mL of isotonic saline solution with sodium bicarbonate 80 mEq/m<sup>2</sup> BSA, 4-hour infusion (Day 1).

• Folinic acid, 75 mg/m<sup>2</sup> BSA, PO or IM, every 6 hours (6 doses). The first dose will be 24 hours after the treatment with Methotrexate is finished.

**Day 40**

Daunorubicin 40 mg/m<sup>2</sup> BSA (or Epirubicin at 60 mg/m<sup>2</sup> BSA), IV, 4-hour infusion diluted in an adequate amount of saline solution.

**Day 41**

- Ara-C 100 mg/m<sup>2</sup> BSA/day, IM, every 12 hours for three consecutive days.
- Mercaptopurine 50 mg/m<sup>2</sup> BSA, every 12 hours, PO, during three consecutive days (six doses). If unavailable, use Fludarabine 20 mg/m<sup>2</sup> BSA/day, PO, once a day.
- Pegfilgrastim, one vial SC, 24 h after finalizing Ara-C and another one week later.

**Subsequent maintenance (5.0)**

Begin with neutrophils >1.0 × 10<sup>9</sup>/L and platelets >50 × 10<sup>9</sup>/L. Use G-CSF when pancytopenia prevents fulfilment of the program. If neutrophils are lower than 1.0 × 10<sup>9</sup>/L and platelets are lower than 50 × 10<sup>9</sup>/L, without evidence of relapse, infection, or haemorrhage, apply a 50% to 75% dose to comply with the calendar of treatments.

**Day 0:** Methotrexate, same as induction, IT. One dose.

**Day 0, 1, 2 y 3:** Mercaptopurine 300 mg/m<sup>2</sup> BSA/day. PO. If unavailable, use Fludarabine 20 mg/m<sup>2</sup> BSA/day, PO.

**Day 4**

- Cyclophosphamide 1,200 mg/m<sup>2</sup> BSA, IV bolus.
- Pegfilgrastim one vial SC at day 5, and another one week later.

**Day 11**

- Rituximab 375 mg/m<sup>2</sup> BSA, IV (if more than 30% CD20). Use only during maintenance days 2, 4, 6, 8 and 10.
- Vincristine 1.5 mg/m<sup>2</sup> BSA, IV. Repeat at days 18 and 25.

**Day 18:** Prednisone 180 mg/m<sup>2</sup> BSA/day, PO, administered for seven days.

**Day 25**

- Methotrexate, 650 mg/m<sup>2</sup> BSA, IV diluted in 500 mL isotonic saline solution with added 80 mEq/m<sup>2</sup> BSA sodium bicarbonate and infused for 4 hours (Day 1).
- Folinic acid, 75 mg/m<sup>2</sup> BSA, PO or IM, every 6 hours (six doses). The first dose will be 24 hours after finishing the infusion with methotrexate.

**Day 40**

• Daunorubicin 40 mg/m<sup>2</sup> BSA (or Epirubicin 60 mg/m<sup>2</sup> BSA), IV, 4-hour infusion diluted in an adequate amount of saline solution, until completing an accumulated dose of 550 mg/m<sup>2</sup> BSA of Daunorubicin or 750 mg/m<sup>2</sup> BSA Epirubicin. When this occurs, use Cyclophosphamide, as in 4.3.

**Day 41**

- Ara-C 100 mg/m<sup>2</sup> BSA/day, IM, every 12 hours for three consecutive days.
- Mercaptopurine 50 mg/m<sup>2</sup> BSA, PO, every 12 hours, six doses. If unavailable, use Fludarabine 20 mg/m<sup>2</sup> BSA/day, PO.
- Pegfilgrastim, one vial SC 24 h after finishing Ara-C and another one week later.
- Use Deferasirox if more than 10 globular packages have been transfused and/or ferritin is greater than 1,500. If Daunorubicin is unavailable, use Epirubicin.
- Dexrazoxane 600 mg/m<sup>2</sup>/day before Epirubicin administration. Must be used after the 8th subsequent maintenance.

If Mercaptopurine is unavailable, use Fludarabine 20 mg/m<sup>2</sup> BSA/day, independent of the cycle.

Rituximab will be used if there is 30% CD20 as determined by flow cytometry, a total of 6 doses will be administered (initial maintenance, maintenance 2, 4, 6, 8, and 10).

Use Pegfilgrastim, one vial SC, weekly, if necessary for initial and subsequent maintenance.

Repeat the subsequent maintenance as many times as necessary until completing two years in complete remission, from the end of the consolidation. Twelve cycles (early maintenance + subsequent maintenance).

Calendar for the Bone Marrow and CSF after elective discontinuation: Monthly, during the first six months. Bimonthly, for the following six months. Every three months in the second year, with a CSF every six months. Every four months during the remaining time (CSF, as indicated).

The patients will be referred to their original hospital after completing three years of continuous complete remission after elective discontinuation.

### Response criteria

Failure: More than 5% blasts in the bone marrow after finishing induction.

### Complete remission

- **Morphologic:** Disappearance of clinical manifestations attributed to ALL, Hb >100 g/L with transfusion independence, granulocytes >1 × 10<sup>9</sup>/L, platelets >100 × 10<sup>9</sup>/L (without support needed) and normocellular bone marrow (B.M.) with less than 5% blasts and without blasts in CSF.

- **Morphologic with incomplete recovery:** The previous criteria but with neutropenia (5% blasts in BM in a patient that had reached CR or unequivocal demonstration of extramedullary leukemic illness.

- **Cytogenetic:** Morphologic CR with normal cytogenetics in cases where alterations had been detected.

- **Immunophenotypic:** <0.1% of cell having leukemic immunophenotype.

**Partial remission:** Neutrophils in peripheral blood equal or greater than 1,000/mL. Blasts in bone marrow greater than 5% but less than 25% with absolute reduction in one or more series.

**CNS infiltration:** Blasts in CSF as detected by cytology. CNS relapse. Blasts in CSF after reaching disease remission.

**Extramedullary relapse:** Infiltration to organs different from the central nervous system, as demonstrated by histopathology.

**Cytogenetic response:** Disappearance of initial genetic abnormalities.

**Myeloid relapse:** More than 5% blasts in bone marrow with alterations in the proportion or normal cells, after having reached complete remission.

**Absolute drug resistance (must comply with BOTH criteria):** Normal or hypercellular marrow and presence of blasts in bone marrow of 50% or greater than basal levels.

**Death during induction:** Demise after having initiated Induction chemotherapy and before evaluation of possible remission.

**Death during remission:** Demise after having reached remission.

**Negative minimal residual disease:** No detection of malignant clones by flow cytometry <0.001.

**Incipient progression:** Detection of RD >0.1% in two consecutive determinations in patients with morphologic RC that had presented with RD <0.1% at some previous moment.

**Overall survival:** Timespan between diagnosis and the date of all-cause mortality or the date of the last control.

Progression-free survival/leukaemia-free survival: Timespan between the date of CR until relapse, all-cause mortality, or last control.

**Length of CR:** Timespan between the date of CR and relapse or the last control.

**Event-free survival:** Timespan between diagnosis and treatment failure, relapse, all-cause mortality, or last control of the patient.

For those patients that require a second-line therapy, a treatment regimen of chemotherapy should be selected based on the following table. The consensus established that the alternative should be Hyper-CVAD, if it was not received as first-line regimen, and if it is, the LAL-6 (ISSSTE) regimen should be considered as second-line (Table 19).

**Table 19 Alternative regimens for second-line therapy**

PHI Negative	PHI Positive TKI
GRAALL-2003	COG ALL 0031(16 to 60)
PETHEMA-ALL-96	*HYPER-CVAD (16-60)
*HYPER-CVAD	MULTIAGENT (16-60)
BFM	STEROID (OLDER THAN 60)
COG AALL0434	VINCRIStINE
CALGB 10403	STEROID (MAY 60)
COG AALL0232	
USC ALL(CCG-1961)	

\*The treatment elected by consensus for ISSSTE patients

## Transplant

HPC transplant at first remission for young adults treated with paediatric protocols and standard risk are usually not carried out, although it should be considered for high-risk patients (Tables 20-22).

**Table 20 HPCT in adult ALL**

Question	Recommendation
CR1: Allo-HPCT or CHT?	Allo-HPCT is probably superior for patients with HR <sup>1</sup> Allo-HPCT not recommended for patients with SR <sup>2</sup>
CR2: Allo-HPCT or CHT?	Allo-HPCT is superior
Auto-HPCT or CHT?	Comparable results or slightly inferior for auto-HPCT <sup>3</sup>
Related donor or MUD	Comparable results
Best conditioning guide?	Insufficient data Advantage for guides based on TBI
Allo-HPCT or Auto-HPCT?	Advantage for Allo-HPCT

HR=high risk, SR=standard risk  
<sup>1</sup>Recent studies question the need for Allo-HPCT in patients with negative residual disease after induction and at the end of consolidation.  
<sup>2</sup>Except in patients that do not adequately clear SR. <sup>3</sup>The results of auto-HPCT in patients without previous residual disease are promising.

**Table 21 IDA-FLAG**

Drug	Dose	Days
FLUDARABINE	30 mg/m <sup>2</sup> /day, 30-minute infusion	1, 2, 3 and 4
ARA-C	2000 mg/m <sup>2</sup> /day, 4-hour infusion	1, 2, 3 and 4 after finishing Fludarabine
FILGRAStIM	400 mg/day	Day 0 (24 hours before initiating CHT) until recovery of polymorphonuclears
IDARUBICIN	12 mg/m <sup>2</sup> /day (post Ara-C)	2, 3 and 4

**Table 22 FLANG**

Drug	Dose	Days
FLUDARABINE	30 mg/m <sup>2</sup> /day, 30-minute infusion	1, 2, 3 and 4
ARA-C	2000 mg/m <sup>2</sup> /day, 4-hour infusion	1, 2, 3 and 4 after finishing Fludarabine
FILGRAStIM	400 mg/day	Day 0 (24 hours before starting CHT) until recovery of polymorphonuclears
MITOXANTRONE	10 mg/m <sup>2</sup> /day (post- Ara-C)	2,3 and 4

High-risk is considered as:

- Age >35 years
- Hyperleukocytosis: Leukocytes >30 × 10<sup>9</sup>/L if ALL B lineage precursors; >100 × 10<sup>9</sup>/L if T lineage precursors

- Adverse cytogenetics: t (9;22), t (4;11) or other rearrangements 11q23, t (1;19), complex karyotype (>5 cytogenetic alterations), low hypodiploidy/near triploidy
- Slow responders (no CR at 4-8 weeks, >10% at day 14)
- MRD+ [36,37]
- >2nd CR or active disease.

#### Adults with mature B ALL

The adverse prognosis determined by CD20 expression in adults with de novo acute lymphoblastic leukaemia (ALL) of B lineage precursor cells drove the incorporation of treatment using monoclonal antibodies, like Rituximab, with the intensive chemotherapy regime hyper-CVAD [38,39] (fractionated Cyclophosphamide, Vincristine, Doxorubicin, and Dexamethasone). Other modifications (independent of the expression of CD20) are intensification of anthracyclines, alterations in number of treatments of intrathecal chemotherapy adapted for risk for CNS prophylaxis, additional early intensification, and six-month extension of the maintenance phase. When adding Rituximab to CD20+ ALL patients, the rates for CR and OS were superior with these hyper-CVAD modifications and Rituximab regimen [38] compared to hyper-CVAD (70% vs. 38%, P=0.001 and 75% vs. 47%, P=0.003).

#### Adults with positive Philadelphia chromosome

**Adult Ph+ patient candidates for HPCT:** Chemotherapy associated with Imatinib in similar regimens as for Ph negative patients [40]: 400 mg/day to 600 mg/day (CR 90% to 95%-RMol ± 70%) and HPCT in 1st CR (allogeneic with related or unrelated donor: ALLOT R/UR) [41].

With respect to the role of maintenance post ALLOT (Allogeneic transplant), it is considered that its use in 1<sup>st</sup>-line, after detection of MDR, but independent of it, can reduce the risk of relapse. It must not be administered before 6-8 weeks post ALLOT to avoid toxicity. The length of maintenance has not been clearly established [42].

**Adult Ph+ patients, not candidates for HPCT:** In senior adult patients, TKI treatment associated with corticotherapy [43] has allowed a 90% to 100% CR.

**CNS prophylaxis:** All regimens include CNS prophylaxis.

#### Adults without Philadelphia chromosome with refractory disease or relapse

The most common strategy consists of administering reinduction treatment (IDA-FLAG or related and similar to 1st line for late relapses) and proceed to transplant, if a donor is available.

The probability of obtaining a 2nd CR does not exceed 60%, usually it is short lived (median 3 months) and with a probability of OS of 3 years; without transplant, the probability is only 10%. Seeking the benefit of the graft effect against leukaemia with myeloablative conditioning or reduced intensity (Tables 21 and 22).

The probability of reaching a CR in refractory ALL or in early relapse seems to be above 40% with a short length of response (less than 6 months).

Thus, it is desirable that patients find themselves in a second CR and, if possible, without detectable residual disease (RD).

The main prognostic factors for patient survival with relapsed ALL are:

- Age (better prognosis if under 45-50 years of age).
- Interval between CR and relapse (better prognosis if interval >2 years).

If relapse is suspected, pre-phase treatment with Dexamethasone can be initiated. Once relapse is confirmed, induction treatment is administered. Re-evaluation will be carried out using bone marrow aspiration at day 28-35 or when hematopoietic recovery is documented.

In terms of donor availability and the degree of response, a consolidation treatment or reinduction is administered or proceed directly to Allo-HPCT.

- Patients that present with a cytologic CR after induction, a RD <0.1% and have the option to receive an allogeneic transplant in <30 days will not receive consolidation treatment and will proceed directly to Allo-HPCT.

- Patients that show cytologic CR but with RD >0.1% or those with RD <0.1% for whom it is not deemed possible to receive an Allo-HPCT in <30 days will receive consolidation treatment. The goal of this treatment is to maintain a stable disease (or reduce RD, if present) with minimal toxicity, before HPCT.
- Patients with chemosensitive disease (<10% blasts) after induction, must receive reinduction.
- Incipient progression: For those patients who are positive for RD during first-line treatment, a reconfirmation test will be done after 2 to 3 weeks. If positivity is confirmed (>0.1%) and the patient remains in cytologic remission (incipient progression) a consolidation treatment will be carried out while searching for a donor.
- Late relapse (>2 years from diagnosis): Repeat induction treatment and search for a donor.
- Relapse or progression in patients who have received IDA-FLAG during first-line treatment: It is recommended to directly administer reinduction treatment.

### Support therapy and management of complications

Faced with the complexity and intensity of the treatment, support therapy must be concomitant in order to achieve maximum benefit, compliance and reduction of morbimortality. This must include prevention and treatment of tumour lysis, nutritional support, transfusional support, use of stimulating factors, prevention and treatment of infectious diseases, toxicities, and thrombosis. As well as nursing and palliative care.

Most patients require hospitalization to receive said support therapy, with daily requirements for the following laboratory tests: complete blood count, blood chemistry, serum electrolytes, calcium, phosphorous, uric acid levels, and hepatic function tests.

Among the most feared complications that can develop are: tumour lysis syndrome, thrombosis, haemorrhage, infection, renal insufficiency, and anaphylaxis. Other acute secondary effects include mucositis, pancreatitis, acute hepatic insufficiency, hypertriglyceridemia and hyperglycaemia.

**Tumour lysis syndrome:** Considering an oncometabolic emergency from rapid cell death, it must be monitored in an intensive care unit. The risk factors include a large tumoral load and a high cellular proliferation index. The deposition of uric acid and/or calcium phosphate crystals in renal tubes can result in acute renal lesions [44].

Preventive measures include adequate optimization of perfusion and renal function with:

- **Optimization of renal perfusion:** Hydration, before beginning chemotherapy, using saline solution 0.9%, 2500 ml/m<sup>2</sup>/day to 3000 ml/m<sup>2</sup>/day. Restriction of potassium input.
- **Maintain urinary output:** Use of furosemide, once the intravascular volume has been re-established, ensuring diuresis in 4 ml/kg/h.
- **Use of uricosuric medications:** Rasburicase is the drug of choice. If unavailable, prevention must be carried out with allopurinol at a dose of 200 mg/m<sup>2</sup>/day to 300 mg/m<sup>2</sup>/day (maximum 800 mg/day)
- This must be maintained till 7 days after initiating chemotherapy
- Requires adjustment of renal function, if under 30 ml/min/m<sup>2</sup> it must be reduced to 50%; if <15 ml/min/m<sup>2</sup> its counter indicated
- Urinary alkalization is not recommended.

### Treatment

- Multidisciplinary management with haematologists, nephrologists, and intensivists.
- Hydroelectrolytic imbalance.
- Asymptomatic hypocalcaemia must not be treated. The symptoms must be treated with short infusions with calcium gluconate.
- The use of salbutamol and insulin with glucose for hyperkalaemia may be required, however, most cases require haemodialysis.

### Altered coagulation

Altered coagulation conditions such as haemorrhage, thrombosis and prolonged coagulation times are expected. Chemotherapy directly damages the endothelium, promoting a pro-coagulable state in response to the intense release of endothelial cytokines.

Particularly, L-asparagine reduces the levels of coagulation factors VIII, XI, fibrinogen and K dependent factors (II, VII, IX and X), with increase in risk for haemorrhages [45]. Conversely, it reduces levels of anticoagulants (C, S and antithrombin) and plasminogen, which favours thrombosis. The use of low molecular weight heparin has been demonstrated to reduce the risk of thrombosis. Enoxaparin 40 mg/day has been recommended for patients weighing <80 kg and 60 mg/day for those >80 kg. The dose must be reduced to 50% if platelet count falls below 50,000, and temporarily suspended when under 20,000. For patients with renal failure or at high risk for haemorrhage, unfractionated heparin is preferred.

### Thrombosis treatment

For patients treated with L-asparaginase that develop thrombosis, it is recommended to suspend treatment and begin treatment with heparin, monitoring antithrombin levels and anti-Xa activity. Once adequate anticoagulation is achieved, L-asparaginase may be reinitiated concomitantly with the anticoagulant until treatment is completed. The use of new anticoagulants is still under study. In catheter-associated thrombosis, anticoagulation is recommended for at least 3 months or during the entire time the catheter is in place. The removal of the catheter is not recommended as long as it is functional and there are no signs of infection.

### Antimicrobial prophylaxis

**Antimicrobial prophylaxis:** Begin in those patients where neutropenia is expected <1000 for >7 days [46].

- Ciprofloxacin, 500 mg every 12 h.
- Levofloxacin, 500 mg every 24 h.

**Antiviral prophylaxis:** Recommended for patients seropositive for Herpes virus who are receiving induction or consolidation chemotherapy, during neutropenia and after allogeneic transplant.

- Acyclovir 400 mg every 6 h to 8 h.
- Valacyclovir 500 mg every 8 h to 12 h.

**Antifungal prophylaxis:** Considered for all patients undergoing chemotherapy. Avoid posaconazole, itraconazole and voriconazole for patients treated with vinca alkaloids.

- Fluconazole, 100 mg every 12 h.
- Prevention against *Pneumocystis jirovecii* should be considered for all patients.

### Febrile neutropenia

• In patients with severe neutropenia (<500 cells/mm<sup>3</sup>) and presence of fever (axillary temperature >38.0 in two determinations or >38.2, sustained), empirical treatment for febrile neutropenia should be started with wide spectrum antibiotics that cover known nosocomial microbial flora and clinical and laboratory tests must be requested, in order to identify potential sources of infection.

• The use of glycopeptides is recommended if the patient remains with fever for 4 days after beginning treatment or when there is infection of bland tissues. If febrile events persist for 7 days, an antimycotic agent is indicated.

### Stimulating factors

• The risk of hematologic toxicity during chemotherapy is high. It is estimated that up to 80% of patients will present an event of febrile neutropenia during the duration of treatment.

• The colony stimulating factor is used to prevent the number, severity, and duration of episodes of febrile neutropenia. The recommended doses are:

- Filgrastim 5 mg/kg daily, till recovery.
- Pegfilgrastim 6 mg per cycle.

• Concomitant use during treatments with chemotherapy or radiotherapy are not recommended. The preferred route of administration is subcutaneous.

### Transfusion therapy

The requirement for hemocomponents in the patient with ALL is frequent due to the hematologic toxicity of the treatment. The use of erythrocyte concentrates is not dependent on the amount of haemoglobin and haematocrit; only in the case of anaemic syndrome will erythrocyte concentrates be used to correct symptoms. In patients undergoing chemotherapy or radiotherapy, a haematocrit <25% is indicative of a transfusion.

In candidates for allogeneic transplant, the recommendation is to use leukodepleted, irradiated erythrocyte concentrates from an unrelated donor.

Platelet transfusion has two functions, prophylactic and therapeutic. This depends on the condition of the patient, risk of haemorrhage, number of platelets and their functionality. Prophylactic use is indicated when there is a count <10,000 in patients that are in good general condition and <20,000 in those with fever, infection or have other abnormalities in coagulation. In invasive processes, such as lumbar puncture, a count <50,000 indicates transfusion. This amount is not necessary for bone marrow aspiration. In all procedures where transfusion is used, it will be given just before the procedure [47].

In patients that are actively haemorrhaging, the goal is to stop the haemorrhage as quickly as possible and the maintenance of the platelets to avoid its recurrence. Some authors recommend restrictive transfusions when neutrophils are >500 cells/mm<sup>3</sup>, since this increases the risk of platelet refractoriness.

In order to achieve an increase to >20,000, the indicated platelet transfusion dose in the adult is  $3 \times 10^{11}$  and the amount that is to be transfused depends on the source.

**Platelet concentrates:** The content of each unit is  $0.5 \times 10^{11}$  to  $0.6 \times 10^{11}$ . In order to reach the goal, 5 to 7 units are needed, thus the dosage indication is usually 1 unit per 10 kg of weight of the patient.

**Platelet apheresis:** The amount is fulfilled with one unit.

**Fresh frozen plasma:** Consider if haemorrhage and alteration in coagulation tests coexist.

**Cryoprecipitates:** Contribution of fibrinogen

In ALL patients, this is observed in DIC or secondary to treatment with L-asparaginase. The indicated treatment is fibrinogen 80 mg/L to 100 mg/L. Administration every 72 h.

**Fibrinogen concentrates:** Acquired coagulopathies are limited, with reduction of fibrinogen <100 mg/dL and in the presence of haemorrhage or high risk of presenting it.

### Nutrition

- Establish a basal nutritional state
- In patients with neutropenia, avoid food with high microbial load, like raw or lightly cooked foods
- In patients with mucositis, vomit and hyporexia, give small portions of food with high caloric load, as desired and tolerated
- The type of antiemetic must be chosen depending on the emetogenic potential of the chemotherapeutic regimen used.

### Palliative care

The main objective of care for patients with ALL is to achieve disease-free survival and palliative care must be considered an integral part for the patient, the family, and the medical team.

The traditional focus is that palliation is a synonym for end of life or that we do this when there is “nothing more” available. Many current paradigms of medical attention falsely show that there are two mutually exclusive goals:

to cure leukaemia and prolong life or only provide comfort. "To cure sometimes, to heal often, to comfort always" is a reminder that physicians must provide comfort and believe that suffering can be alleviated or that sickness must be cured.

Some physicians consider that failure to cure and prolong life represents a change in the goals of treatment, which results in transferring the patient to palliative care.

The realization that curative treatment has failed often occurs when the patient still shows many adverse effects from the treatment. This can make it difficult to integrate care with other teams or to recognize the advent of a palliative care phase.

Numerous studies suggest that patients with hematologic cancers have a lower global rate of access to palliative care and a greater rate of hospitalization. Consequently, the decision to focus on the reduction of suffering is typically made after the treatment to prolong life has become inefficient or too onerous and death is imminent.

The introduction of palliative care services at a relatively early stage of the trajectory of the patient with ALL can have significant benefits for the patients and family members, and improve the recognition of the value of palliative care, from the moment of diagnosis of a serious cancer.

The care for patients with advanced disease is a common task within the limits of haematology, since the malignant blood diseases have a high mortality, particularly in the scenario of acute leukaemia.

Studies have demonstrated that early introduction of palliative care, cannot only increase quality of life of the patients with advanced cancer, but also their survival. Furthermore, patients that receive early palliative care have decreased probabilities of receiving chemotherapy within the last 60 days of life, probably because these patients have a better understanding of their prognosis.

In the HABILITAR III study, which examined the results of early versus late initiation of palliative care in patients with advanced cancer, the patients in the group of early palliative care had significantly higher one-year survival rates than the treatment group that began palliative care treatment late (63% vs 48%,  $p=0.038$ ). The guidelines for palliative care must: Provide enough information for patients to be able to make informed decisions with respect to their treatment. Encourage palliative care centred on the symptoms when the therapies directed to the disease fail. Offer the opportunity to participate in clinical trials that may improve their results or that of future patients, and provide the opportunity to die with dignity and serenity.

Part of these care protocols include the management of post-traumatic stress, since ALL is associated with high morbidity and mortality, owed as much to the disease itself as to the aggressiveness of the treatments used. The symptoms of post-traumatic stress (PTS) represent an intense and unstable emotional state that occurs as a response to the immediate experience of the trauma.

This state is characterized by oscillating symptoms of detachment or emotional blockage, hyperexcitation, unreasoned thoughts and attempts not to remember previous trauma. The most severe symptoms of PTS, that occur within one month after exposure to a traumatic event, can fulfil the criteria of the DSM-IV-TR for diagnosis of an acute stress disorder (ASD). The symptoms of PTS are clinically relevant because they are associated with the deterioration of quality of life and the emotional welfare and, when they are sufficiently serious, with an increase of 10 times the risk of suicide.

What must be evaluated by the medical team?

- 1) The benefits of pharmacological treatment
- 2) The potential to respond to an additional treatment
- 3) The potential toxicities related to the treatment
- 4) The patient's understanding about the prognosis of the sickness
- 5) The goals and meaning of the cancer therapy for the patient and the family
- 6) Deterioration of vital organs
- 7) Functional state
- 8) Comorbidities

- 9) Aims, values and personal expectations
- 10) Aims, values and expectations of anticipated planning of care
- 11) Family aims, values and expectations
- 12) The priorities for palliative care
- 13) Objectives and meaning of cancer therapy
- 14) Quality of life.

**Minimum Residual Disease**

**Overview**

The minimum residual disease (MRD) is the quantification of sickness below the threshold of detection by conventional methods such as light microscopy and cytochemical stains (<5% of blasts). The definition of complete remission is critical for the evaluation and design of the different therapeutic alternatives used in patients with ALL. In the last two decades, the emergence of techniques based on cytogenetics, flow cytometry and molecular biology tools have allowed a more adequate evaluation of remission. The determination of MRD has become one of the main prognostic factors in patients with ALL.

The determination of MRD is useful after induction to establish the need for subsequent intensification, follow up and detection of relapse, with the objective of achieving a directed and standardized therapy to avoid overtreatment in those who demonstrate an adequate response.

Currently, the techniques used are: flow cytometry, cytogenetics (FISH) and molecular biology techniques (PCR). PCR is used to find rearrangements in T-cell receptor and immunoglobulin genes (in T-cell and B-cell leukaemia, respectively); as well as in the search for fusion genes. Flow cytometry detects aberrant immunophenotypes. The sensitivity of PCR is 10<sup>-4</sup> to 10<sup>-5</sup> (one leukemic cell per every 10,000 to 100,00 cells) while for flow cytometry, the sensitivity is 10<sup>-3</sup> to 10<sup>-4</sup> (one leukemic cell for every 1000 to 10,000 cells). Although the advantage of PCR is its sensitivity and speed, in our environment it is not currently available, and additionally, most patients are not evaluated for molecular alterations at the time of diagnosis. Conversely, flow cytometry has adequate sensitivity and is available in most of the country, which makes it the most adequate technique for our institution. However, it does have the disadvantage of requiring specialized personnel for its execution and interpretation.

A determination of basal markers by flow cytometry is indispensable for the initial determination of the leukemic phenotype. The determination of MRD by flow cytometry is recommended at the end of induction (Day 14), intensification (Day 28) and during maintenance (at 3, 6, and 9 months). At minimum, it is recommended after induction, intensification and after termination of treatment. In all cases, it is recommended to carry out this determination in bone marrow (in T-lineage leukaemia it is possible to use peripheral blood) [48]. This can only be carried out if the patient has attained morphologic remission and has enough haematological recovery to reach the minimum recommended number of cells for analysis, 1 × 10<sup>6</sup> to 2 × 10<sup>6</sup>.

Euroflow is a method that aims to standardize the interpretation of MRD, considering <0.1% of leukemic cells after induction as a negative result, and <0.01% during maintenance. The basal panel recommended includes the basic lineage markers (Figure 2A). Depending on the results obtained by the basal determination, the corresponding lineage determinations will be carried out (See Figure 2B and 2C).

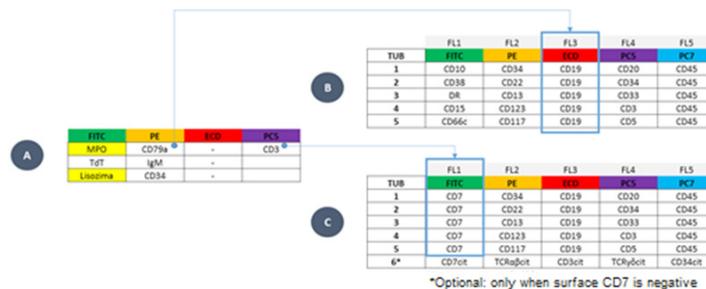


Figure 2 Study panels

Of special use for monitoring MRD are aberrant markers, such as:

- Over or under expression of marker.
- Asynchronism of a marker
- Infidelity of line.

### Paediatrics

**Overview:** The global tendency for applying protocols for ALL treatment in paediatric population is aimed at improving survival, attaining a reduction in medium and long-term toxicity as well as a decrease in relapse to central nervous system [49]. Paediatric population at the ISSSTE is considered up to 18 years of age.

It is estimated that in the United States, 6,000 cases of acute lymphoblastic leukaemia are diagnosed every year, half of the cases have been observed in children and adolescents, making it the most common cause of death in people under 20 years of age [50,51].

From its initial description in 1948, great progress has been made on the use of stratification by risk and the management with chemotherapeutic regimens with multiple agents that allow for greater efficacy based on the clinical findings of the patient, the biology of the leukemic cells, and early response to the treatment. All of these factors simultaneously predict the risk of relapse, and have aided in achieving an increase in survival in some countries >90% [50]. The incidence of ALL in children varies substantially between geographic regions, because of race and ethnic origin [52]. Additionally, it varies from 1 to 40 per million in low income countries, while this range is from 40-50 cases per million in medium and high-income countries [53].

In Mexico, since 2010, cancer is the second cause of mortality in children between 4 and 15 years of age [6]. The incidence of children's cancer at the national level in a period of 6 years (2007-2012) has increased, registering an incidence of 156.9/1,000,000 in 2012. In 2012, the incidence of leukaemia was of 78.1/1,000,000/year with a prevalence of 49.8%; acute lymphoblastic leukaemia was predominant in comparison with other types of leukaemia in paediatric population [54]. From January 2007 to 2012, 14,178 patients with cancer were registered. The most common cancer was leukaemia (49.8%) [55], representing 26% of all cancers and 78% of leukaemia in this age group [52]. The incidence of leukaemia has increased rapidly in children under 5 years of age while it decreased for children between 5-19 years of age [56].

Before initiating treatment, it is important to take into consideration any previous treatments, the functional status of the patient, the cardiopulmonary function (left ventricular ejection fraction), as well as renal and hepatic function, and if suspected, carry out a pregnancy test.

The classification of leukaemia is carried out based on the following aspects:

**Morphologic:** The classification is based on the criteria indicated by the FAB.

**Immunologic:** the acute lymphoblastic leukaemia will be classified from this point of view into:

- B-cell precursors (early pre-B, pre-B, and transitional pre-B)
- Mature B
- T-cell, based on the positivity of the monoclonal antibodies.

**Cytogenetic:** can be carried out by FISH or RT-PCR

**Numeric alterations:** The leukaemia have been classified into seven ploidy groups, considering low-risk as less than or equal to 50 chromosomes, with presence of trisomy of chromosome 4, 10 or 17, as well as a DNA index greater than 1.16; high-risk is considered as less than 45 chromosomes or a DNA index lower than 0.81 [50].

**Structural alterations:** Include translocations (which are the most frequent), deletions, inversions, etc. There are two types of functional translocations:

1) **Affecting regulatory oncogenes:** C-MYC, rearrangements of CRLF2 and in the receptor of erythropoietin (EPOR) in B-cell lineage ALL, and alterations in transcription factors TLX1 and TLX3, and in T-cell receptors (TCR) in T-cell lineage ALL [50].

2) **Affecting chimeric proteins:** ETV6-RUNX1, which is observed in a quarter of the children with ALL; another example is the TCF3-PBX1, t (9;22) (q34; q11.2). Rearrangement in chromosome 11q23 in the gene for mixed lineage leukaemia (MLL) is particularly common in children younger than 1 year of age, having a frequency of close to 75% [50].

ALL involves many entities with different groups of somatic alterations. These alterations include aneuploidy (change in the number of chromosomes), chromosomal rearrangements which deregulate gene expression or result in the expression of a chimeric fusion protein, deletions or gain of DNA, and mutations in DNA sequence [50].

In children with B lineage ALL, 25% to 30% of leukemic cells have high hyperploidy (>50 chromosomes), and 2% to 3% have hypoploidy (<44 chromosomes) [50].

A low hypoploidy (30-39 chromosomes) is associated with the presence of mutations in TP53 and frequently manifest Li-Fraumeni Syndrome [50].

Therefore, it is of vital importance to determine the risk of the patient. This is based on the criteria established by the Oncologic Paediatric Group and the Paediatric Cancer Group in an international meeting, where they determined the guidelines for low-risk and high-risk (Table 23).

**Table 23 The guidelines for low-risk and high-risk**

Characteristic	Requirement
Age	>1 year and < 10 years
Leukocytes	<50,000/ $\mu$ l
Immunophenotype	B precursor
t (12;21) (TEL-AML1)	Positive
BMA: days 19 and 26	M1 (<5% blasts)
MRD day 46	<0.01%
<b>Without any of the following</b>	
CNS infiltration	CNS 3
	Cranial nerve paralysis
Testicular infiltration	Positive echography
Immunophenotype	T
Adverse genetics	t (9;22) BCR-ABL fusion
	t (1;19) E2A-PBX fusion
	t (4;11) MLL rearranged
	Hypodiploidy (<45 chromosomes)

High-risk: All cases of T-cell ALL and those precursor B cells that do not meet the criteria of Low-Risk, and with one or more of the following characteristics (Table 24).

**Table 24 Characteristics of High-risk**

Characteristic	Requirement
Phi Chromosome	t (9;22) BCR-ABL fusion
BMA: day 46	M2
MRD day 46	>1%
MRD before reinduction I	>0.1%

The assessment of treatment results is carried out by morphology, Minimal Residual Disease, and cytologic studies in cerebrospinal fluid (Table 25).

**Table 25 The assessment of treatment results**

Blasts by morphology	CNS infiltration
M1 $\leq$ 6%	CNS1:
	<10 erythrocytes/ $\mu$ L, without Blasts
	>10 erythrocytes/ $\mu$ L (traumatic) without Blasts
M2=6 to 25%	CNS2:
	<10 erythrocytes/ $\mu$ L, <5 leukocytes/ $\mu$ L, Blasts+ >10 erythrocytes/ $\mu$ L (traumatic) with Blasts

M3 ≥ 25%	CNS3:
	<10 erythrocytes/μL, >5 leukocytes/μL, Blasts+
	cranial nerve involved (by physical exam or imaging)

Table 26 Treatment regimens

Age (months)	MTX (mg)	Hydrocortisone (mg)	Ara-C (mg)	Volume (ml)
12 to 23	8	16	24	8
24 to 35	10	20	30	10
>36	12	24	36	12

### Treatment Regimens

Based on the original regimen: “Total therapy study XV for newly diagnosed patients with acute lymphoblastic leukaemia” [49]. The most recognized international groups show results for 5-year disease-free survival, greater than 75% (BFM90: 78%, Children’s Cancer Group: CCG1800 75%, German Cooperative Study Group for ALL: COALL92 77%, Dana Farber Cancer Institute DFCI91 83%, Nordic Society of Paediatric Haematology and Oncologic: NOPHOIII 78%, St. Jude Children’s Hospital: 81%) [57].

#### 1. Induction

**Steroid window:** Dexamethasone, 10 mg/m<sup>2</sup>, day, IV, in 30 ml Dextrose solution, 6-hour infusion, every 24 h. Days 0 to 4.

**Initial IT CHT, Day 5:** Must be delivered in the operating room under anaesthesia in order to avoid traumatic puncture, with triple drugs:

- Prednisone 40 mg/m<sup>2</sup>, daily, PO, every/8 h. Days 5 to 32. Can be substituted by Methylprednisolone 20 mg/m<sup>2</sup>/day IV every/8 h. For patients that cannot tolerate Prednisone or cannot be treated orally.
- Vincristine 1.5 mg/m<sup>2</sup> per day without surpassing 2 mg, before beginning treatment with Daunorubicin. Days 5, 12, 19 and 26 (four doses).
- Daunorubicin: 25 mg/m<sup>2</sup>/day, diluted to 1 mg per 10 ml of 0.9% saline sol. infused as a continuous drip during 4 h. A previous infusion of Dexrazoxane 250 mg/m<sup>2</sup>, diluted in 150 ml Hartman Sol. administered as an infusion for 60 minutes, one hour before Daunorubicin administration. Days 5 and 12 (two doses).

**L-Asparaginase:** 10,000 UI/m<sup>2</sup> IM, three times a week, on days 6, 8, 10, 12, 14, and 16 (six doses).

If the patient is high-risk or the BM shows >de 5% blasts on Day 19 (or MRD positive), three additional doses will be administered. Days 19, 21 and 23.

Bone marrow on Day 26 in patients with positive minimum residual disease or > than 5% blasts in BM on Day 19.

Patients with >5% blasts in BM on Day 19 or residual leukaemia identified on day 29 must receive Cyclophosphamide, 6-mercaptopurine and Cytarabine as programmed, if their clinical condition allows it (without fever and neutropenia or grade IV mucositis). Conversely, the treatment can be delayed for 3 to 7 days to allow for hematopoietic recovery, when neutrophils are <300/μl.

**Cyclophosphamide:** 1,000 mg/m<sup>2</sup> + MESNA 1,000 mg/m<sup>2</sup>, in 100 ml 0.9% saline sol., one hour IV infusion. Day 26.

**6-Mercaptopurine:** 60 mg/m<sup>2</sup>, PO. Days 26 to 39.

**Ara-C:** 75 mg/m<sup>2</sup>, IV, diluted in 100 ml 0.9% Saline sol. One hour infusion. Days 27, 28, 29, 30 and 34, 35, 36, 37.

Treatment may be resumed if fever disappears and neutrophils >300/μl, mucositis has been resolved and C-reactive protein is normal.

If these conditions are found after Day 34, treatment is suspended if half of the dose has been received.

Follow up IT CHT: Triple drug, all patients receive on Day 19.

Patients with the following characteristics, will also receive on Days 8 and 26:

- CNS 2 or 3 or paralysis of the cranial nerve.
- Traumatic initial puncture: >10 g/L of CSF.
- T ALL.
- Leukocytes >50,000/ $\mu$ L at diagnosis.
- Philadelphia chromosome, MLL rearrangement, or Hypodiploidy (<45).

If there is CNS infiltration, apply twice a week until obtaining two infiltration negative cerebrospinal fluid samples.

If infiltration persists after three doses of IT CHT, apply cranial radiotherapy in accordance with the guideline on CNS radiotherapy.

**Folinic Acid:** 5 mg/m<sup>2</sup>/dose, PO, at 24 and 30 h. After each IT CHT, during Induction and Consolidation (Not if it coincides with rescue with MTX IV).

#### End of induction

The bone marrow aspiration will be carried out between days 43 and 46 of the Induction to remission, when neutrophils >300/ $\mu$ l and platelets >50,000/ $\mu$ l.

**Remission:** <5% Blasts in BM.

- The MRD is defined as a bad or positive response if >0.01% (one or more lymphoblasts in 104 mononuclear cells in bone marrow).
- If there is success or the sample is inadequate, MRD is catalogued as negative.
- If MRD is positive, if the patient is provisionally considered to be Low-Risk, then he will be considered as Standard-Risk.
- If MRD>0.01% but less than 1% or High-Risk (MRD>1%) patient must consequently receive the last three Consolidation doses after MTX, at 5 g/m<sup>2</sup>. These cases will receive the first Consolidation therapy MTX dose at 2.5 g/m<sup>2</sup>.
- Days 43 to 46: If in remission (<5% Blasts in BM) and with neutrophils  $\geq$  300/ $\mu$ l, leukocytes  $\geq$  1,000/ $\mu$ l and platelets  $\geq$  50,000/ $\mu$ l, go on to Consolidation.

#### Consolidation

Apply Intrathecal CHT following the corresponding table, by age, on the day of MTX infusion. Days 1, 15, 29, and 43 (total: 4 doses during the complete consolidation phase).

Pre-hydration: Two hours before beginning MTX

5% Dextrose sol., 400 ml/m<sup>2</sup> + 40 mEq of NaHCO<sub>3</sub>/L, 2-hour infusion.

Hydration: 5% dextrose solution at 3,000 ml/m<sup>2</sup>/24 h + 40 mEq of NaHCO<sub>3</sub>/L.

Methotrexate: Loading dose of 10% is administered for 1 hour and the rest in a span of 23 hours, 4 doses are indicated.

Preparation: Dilute in 5% dextrose solution at 50 mg/ml. Days 1, 15, 29 and 43.

Urinary pH every 6 h.

If urinary pH is between 7 and 6, administer 12 mEq/m<sup>2</sup> NaHCO<sub>3</sub>, IV

If urinary pH is <6, administer 25 mEq/m<sup>2</sup> NaHCO<sub>3</sub>, IV

If systemic alkalosis: Acetazolamide 500 mg/m<sup>2</sup>, PO, e/6 h.

#### MTX dose

**Low-Risk:** 2.5 g/m<sup>2</sup> (1 to 3 g/m<sup>2</sup>), with higher doses in patients with creatinine clearance >102 ml/min/m<sup>2</sup> and lower doses in those with creatinine clearance <88 ml/min/m<sup>2</sup>.

**High-risk:** 5 g/m<sup>2</sup> (3 to 6 g/m<sup>2</sup>), with higher doses for patients with creatinine clearance >102 ml/min/m<sup>2</sup> and lower doses for those with creatinine clearance <88 ml/min/m<sup>2</sup>.

**IF:** Total bilirubin: >2 mg or direct: >1.4 mg, ALT: >500 U/L or mucositis, administer one day before a dose of MTX, NaHCO<sub>3</sub> at 1 g/m<sup>2</sup>, PO, e/6 h., or intravenously.

**Folinic acid:** 50 mg/m<sup>2</sup>, IV. Begin at 42 h if Methotrexate levels are measured, if not, 36 h. after beginning MTX infusion. Afterwards use 20 mg/m<sup>2</sup>, IV every 6 h. for 12 more doses. Days 2, 16, 30 and 44.

The dosage of Folinic Acid will be increased for patients with high plasma concentrations of MTX (>1.0 µm in 42 hours) and will continue until the concentration of MTX is <0.10 µm. Additional measures, such as hydration or plasmatic turnover can be considered for patients with levels of MTX >10 µm at 42 hours. Patients with a history of grade 3 or 4 gastrointestinal toxicity previous to MTX administration, or history of typhlitis with any type of chemotherapy, must receive Folinic acid at 36 hours after MTX initiation. If toxicity is repeated, the baseline dose of Folinic Acid must be increased. Conversely, patients with a minimal toxicity (Grade 1 or less for gastrointestinal toxicity and there are no delays in therapy) after complete rescue with Folinic Acid, must only receive 6 doses in the following MTX treatment round, as long as the plasma levels are not >110% of those in the previous course of MTX.

6-Mercaptopurine (6-MP): 50 mg/m<sup>2</sup>, PO. Days 1 to 56.

- Must be taken on an empty stomach (that is >2 hours after any meal), when going to bed. Milk or other dairy products must not be taken with 6-MP nor taken less than 2 hours before.
- In patients, whose MTX treatment was delayed, doses can continue until 14 days after the last MTX course, even though this goes beyond the 56 days from initiation of 6-MP. However, 6-MP can be continued in the presence of neutrophils <300/µl, platelet count <50,000/µl, or grade 3 or 4 mucositis.
- For patients who have continued to show neutropenia after MTX and 6-MP, the doses of 6-MP may be lowered in the following treatments, 25 mg/m<sup>2</sup>/day.

The patient should rest for a week and continue to the initial *Maintenance* phase if Neutrophils >1,000/µL, platelets >100,000/µL, and without active infections.

#### Initial Maintenance (Indicated in chronological order by weeks)

Follow the provisions found in the “General Guidelines for Treatment”.

- Ophthalmic Prednisolone: Apply 2 drops in each eye, three times a day, until one day after the treatment with Cytarabine.
- Paracetamol: 15 mg/kg, PO, every 6 h, together with Cytarabine administration.
- Apply intrathecal chemotherapy in accordance with the corresponding table, by age group. One dose is given on day 1 of weeks 4, 8, 12, and 17 (\*) (Table 27).

**Table 27 Chemotherapy general guidelines for treatment**

Week	Chemotherapy	
	Low-risk	High-risk
1	6-MP + DEX + VCR	DEXA, DOXO, VCR, 6-MP, L-ASP
2	6-MP + MTX	6-MP, L-ASP
3	6-MP + MTX	6-MP, L-ASP
4*	6-MP + DEX + VCR	DEXA, DOXO, VCR, 6-MP, L-ASP
5	6-MP + MTX	6-MP, L-ASP
6	6-MP + MTX	6-MP, L-ASP
7	Reinduction I (DEX + VCR + Doxo + L-Asp x 3)	REINDUCTION I
8*	Reinduction I (VCR + L-ASP x 3)	REINDUCTION I
9	Reinduction I (DEX + VCR + ASP x 3)	REINDUCTION I
10	6-MP + MTX	6-MP, L-ASP
11	6-MP + MTX	DOXO, VCR, 6-MP, L-ASP
12*	6-MP + MTX	6 MP, L-ASP
13	6-MP + MTX	6 MP, L-ASP
14	6-MP + DEX + VCR (5)	DEXA, DOXO, VCR, 6-MP, L-ASP
15	6-MP + MTX	6-MP, L-ASP

16	6-MP + MTX	6-MP, L-ASP
17*	Reinduction II (DEX + VCR + DOXO + L-ASP x 3)	REINDUCTION II
18	Reinduction II (VCR + ASP x 3)	REINDUCTION II
19	Reinduction II (DEX + VCR + L-ASP x 3)	REINDUCTION II
20	6-MP + MTX	NO CHEMOTHERAPY

#### Doses and days corresponding to weeks 1 to 6 and 10 to 16

- Dexamethasone: 12 mg/m<sup>2</sup>, Oral administration, Days 1 to 5.
- Doxorubicin: 30 mg/m<sup>2</sup> in 4 h infusion. Dexrazoxane 500 mg/m<sup>2</sup>, diluted in 150 ml of Hartman Sol. for 30 min. infusion, one hour before administration of Doxorubicin. Day 1.
- Vincristine: 2 mg/m<sup>2</sup>, without surpassing 2 mg, IV bolus. Day 1.
- 6-Mercaptopurine: 50 mg/m<sup>2</sup>, Oral administration. Days 1 to 7.
- L-Asparaginase: 20,000 UI/m<sup>2</sup> IM. Day 1. Follow diagnostic and therapeutic program for L- Asparaginase.

#### Doses and days corresponding to reinduction I

- Dexamethasone: 8 mg/m<sup>2</sup>, PO. Days 1 to 8 and 15 to 21.
- Doxorubicin: 30 mg/m<sup>2</sup> in 4 h infusion. Dexrazoxane 500 mg/m<sup>2</sup> diluted in 150 ml of Hartman Sol. for a 30-minute infusion, one hour before administration of Doxorubicin. Days 1 and 8.
- Vincristine: 1.5 mg/m<sup>2</sup> IV bolus. Days 1, 8 and 15.
- L-Asparaginase: 10,000 UI/m<sup>2</sup> IM. Days 1, 8 and 15. Follow diagnostic and therapeutic program for L- Asparaginase.
- Apply intrathecal CHT in accordance with the corresponding table, by age group. Give one dose on day 1 of Reinduction I.

#### Doses and days corresponding to reinduction II

- Dexamethasone: 8 mg/m<sup>2</sup>, Oral administration, Days 1 to 8 and 15 to 21.
- Vincristine: 1.5 mg/m<sup>2</sup> IV bolus, Days 1, 8 and 15.
- L-Asparaginase: 10,000 UI/m<sup>2</sup>, IM. Days 1, 8, and 15. Follow diagnostic and therapeutic program for L- Asparaginase.
- Ara-C: 2,000 mg/m<sup>2</sup>, in 250 ml of 0.9% Saline sol., 3-hour infusion, every 12 hours. Days 15 and 16 of Reinduction II (total: 4 doses).
- Apply intrathecal CHT in accordance with the corresponding table, by age group. Give one dose on day 1 of Reinduction II.

#### Subsequent maintenance

- Follow the provisions found in the “General Guidelines for Treatment”.
- Ophthalmic Prednisolone: Apply 2 drops in each eye, three times a day, until one day after the treatment with Cytarabine.
- Paracetamol: 15 mg/kg, PO, every 6 h, together with Cytarabine administration.
- Apply intrathecal CHT in accordance with the corresponding table, by age group. Treatment will be programmed on day 1 of the corresponding week.
- Low-risk, weeks:
- High-risk, weeks: 24, 28, 32, 36, 40, 44, 48, 56, 64, 72 and 80

**Subsequent maintenance 1** (continue in chronological order indicated by weeks of treatment, weeks 21 to 84). (Table 27).

Table 28 Subsequent maintenance 1 of chemotherapy

Week	Chemotherapy	
	Low-risk	High-risk
1	6-MP, MTX	6-MP, MTX
2	6-MP, MTX	6-MP, MTX
3	6-MP, MTX	CP, ARA-C
4	6-MP, DEXA, VCR	DEXA, VCR
5	6-MP, MTX	6-MP, MTX
6	6-MP, MTX	6-MP, MTX
7	6-MP, MTX	CP, ARA-C
8	6-MP, DEXA, VCR	DEXA, VCR

Doses and days corresponding to weeks 1 to 8:

- Dexamethasone: 12 mg/m<sup>2</sup>, PO. Days 1 to 5.
- Vincristine: 2 mg/m<sup>2</sup> IV bolus. Day 1.
- Ara-C: 300 mg/m<sup>2</sup> in 150 ml. In 0.9% saline sol., 1 hour infusion. Day 1.
- Cyclophosphamide: 300 mg/m<sup>2</sup> diluted at 1 ml per 1 ml 0.9% Saline Sol., 2-hour infusion. Day 1.
- 6-Mercaptopurine: 75 mg/m<sup>2</sup> Oral administration. Days 1 to 7.
- Methotrexate: 40 mg/m<sup>2</sup> Oral administration, Day 1.

The treatments for weeks 1 to 8 will be repeated 8 more times.

**Subsequent maintenance 2** (continue in chronological order indicated by weeks of treatment, corresponding to 8 cycles, weeks 85 to 124) (Table 29).

Table 29 Subsequent maintenance 2 of chemotherapy

Week	Chemotherapy	
	Low-risk	High-risk
1	6-MP + MTX	6-MP, MTX
2	6-MP + MTX	6-MP, MTX
3	6-MP + MTX	6-MP, MTX
4	6-MP + DEX + VCR	6-MP, MTX
5	6-MP + MTX	DEXA, VCR

Doses and days corresponding to weeks 1 to 5:

- 6-Mercaptopurine: 75 mg/m<sup>2</sup> Oral administration. Days 1 to 7.
- Methotrexate: 40 mg/m<sup>2</sup> Oral administration, Day 1.
- Dexamethasone: 12 mg/m<sup>2</sup>, Oral administration, Days 1 to 5.
- Vincristine: 2 mg/m<sup>2</sup> IV bolus, Day 1.
- Intrathecal CHT triple-drug.
  - Low-risk
  - High-risk: weeks 88, 96, 106, 116, 126 and 130.

From this time on, 6-MP will be administered daily and MTX weekly with DEXA pulses and VCR every 4 weeks until week 124 for women and 140 for men.

### CNS Radiotherapy

#### Indications are:

- 1) Patients will ALL with refractory CNS infiltration at diagnosis (persistence of infiltration after 3 intrathecal chemotherapy procedures).

• Treatment: Cranial radiotherapy at 24 Gy with 5 intrathecal therapies (with folinic acid rescue) at one year of remission (weeks 48 to 50).

2) Patients with ALL with evidence of blasts on 2 occasions during remission (independent of cell count).

• Treatment: Induction to remission with 4 intrathecal chemotherapies followed by radiotherapy, in the following manner:

➤ If blasts present in CSF in the first 18 months of remission:

<5 Leukocytes in CSF: Cranial radiotherapy at 24 Gy in 16 fractions

>5 Leukocytes in CSF: Craniospinal radiotherapy (24 Gy in 16 fractions to the cranium plus radiation in 15 Gy in 10 fractions at the spinal level).

➤ If blasts present in CSF after 18 months of remission

Any number of leukocytes in CSF: Cranial radiotherapy of 18 Gy in 12 fractions.

During radiation, the patient must only receive 4 to 5 intrathecal therapies and 6-MP and MTX will be suspended for at least one week before and during radiation. The radiation treatment will include dexamethasone and vincristine, with or without L-asparaginase.

After concluding 120 weeks of subsequent maintenance for women and 140 for men, continue with observation until 60 months in complete continuous remission are completed. Check-ups will be monthly (first 6 months), bimonthly (second semester), trimonthly (second year) and biannual (fourth and fifth years), with the following tests:

- Complete blood count and LDH at each appointment.
- Bone marrow and minimum residual disease at the time of elective discontinuation of treatment. Afterward as indicated.
- Testicular or ovarian ultrasound at elective discontinuation.
- Cerebral spinal fluid cytology and cytochemistry every 2 months during the first year after elective discontinuation of chemotherapy.
- LVEF: at the moment of discharge

The patients will be referred to their original hospital after completing 60 months of continuous complete remission, after elective discontinuation of chemotherapy.

### **Relapse in acute lymphoblastic anaemia**

Notwithstanding the high cure rates (70% to 80%) of children's ALL, it still represents a cause of death in children with cancer, related to resistant forms of this disease, and thus poses formidable challenges for the future [49].

The most common cause for failure in ALL treatment is relapse, which occurs in 15% to 20% of the patients. The survival after relapse may be predicted based of the site of relapse, the amount of time in complete remission and the immunophenotype of the relapse. Being defined as:

- Early relapse: <30 months from diagnosis
- Late relapse: >30 months from diagnosis

Relapse represents a population of clonal cells that were not eliminated completely during treatment. Sophisticated studies on cytogenetic rearrangements or clones of genes typical of ALL at the time of diagnosis may help to clear up the origin of the recurrence of leukaemia. The risk factors, such as the time and location of the recurrence, the immunophenotype of the disease, and the response to treatment, allow the determination of a group of patients with an acceptable DFS only with chemotherapeutic treatment (patients with standard risk) and another group that require intensification with HPCT after having attained remission (high-risk patients). An early relapse, as well as T-lineage are associated with worse prognosis compared to extramedullary or late relapse. Most relapses occur during treatment or within the first 2 years after completing the treatment, although there are reports that they may occur up to 10

years after diagnosis. The most recent protocols for the treatment of relapse include patients that have been divided according to their clinical characteristics at diagnosis and relapse, thus providing different therapeutic options as well as different indications for HPCT [58].

Chemotherapy will be initiated as soon as diagnosis is confirmed by cytomorphology and immunophenotype, and it has been shown that the cytogenetic and molecular studies have been initiated satisfactorily. Before beginning chemotherapy, the proper support treatments based on the state of the patient (prophylaxis and, if necessary treatment for infection, correction of metabolic imbalances, prevention of tumour lysis syndrome, etc.) are prepared in order to minimize the immediate toxic effects of the treatments (see support therapies).

### **Acute lymphoblastic Leukaemia in Early Relapse (BFM 90) [59]**

#### **Inclusion criteria**

- Patients younger than 18 years of age with diagnosis of acute lymphoblastic leukaemia, in first relapse, according to the following characteristics: Combined relapse with more than 25% blasts in bone marrow and presence of extramedullary disease; relapse in bone marrow with more than 25% blasts without evidence of extramedullary disease
- Life expectancy greater than one month
- Without evidence of cardiopathy
- With left ventricular ejection fraction (LVEF) >60%
- With ALT <2.5 times normal
- Bilirubin <1.5 times normal
- Without kidney failure (not generated by tumoral lysis)
- In women of fertile age, negative pregnancy test
- Signed informed consent.

#### **Exclusion criteria**

- Patients with down syndrome
- HIV positive
- Active infection

#### **Elimination criteria**

- Cardiotoxicity (LVEF <50%)
- Refusal by the patient's parents or tutors to continue with the protocol.

#### **1. Induction**

Day 4: Steroid window: Prednisone: 100 mg/m<sup>2</sup>/day, PO, for 5 days.

#### **Course 1**

- Dexamethasone: 20 mg/m<sup>2</sup>/day IV. Days 1 to 5.
- 6-Mercaptopurine: 100 mg/m<sup>2</sup>/day PO. Days 1 to 5.
- Vincristine: 1.5 mg/m<sup>2</sup>, IV for 2 doses. Days 1 and 7.
- Methotrexate: 200 mg/m<sup>2</sup> IV bolus, followed by 800 mg/m<sup>2</sup> diluted in 600 ml/m<sup>2</sup> 5% dextrose solution + 40 mEq of sodium bicarbonate, IV in continuous infusion for 36 hours. Day 1.
- Folinic Acid: 15 mg/m<sup>2</sup> IV every 6 hours. Starting 24 h after starting methotrexate, for 6 doses.
- Cytarabine: 2,000 mg/m<sup>2</sup> IV, dissolved in 250 ml of 0.9% Saline Sol., for 3 h infusion every 12 h. Day 5.
- L-Asparaginase: 6,000 UI/m<sup>2</sup>, IM. Day 6 and continue for three doses, every third day.
- IT CHT with triple drug. Days 1, 8 and 14 if there is no infiltration. If there is infiltration, apply every third day until having two negative cerebrospinal fluid counts.

Rest for two weeks and continue with Course 2, if remission is established (<6% Blasts in BM).

If there is testicular infiltration, request radiotherapy and administer only:

- Vincristine: 1.5 mg/m<sup>2</sup>, IV for 2 doses. Days 1 and 7.
- Methotrexate: 200 mg/m<sup>2</sup> IV bolus, followed by 800 mg/m<sup>2</sup> diluted in 600 ml/m<sup>2</sup> of 5% Dextrose solution + 40 mEq sodium bicarbonate, IV in 36-hour continuous infusion. Day 1.
- Folinic Acid: 15 mg/m<sup>2</sup>, IV every six hours. Beginning 48 h after having started Methotrexate for 6 doses
- After finishing, continue with Course 2.

### **Course 2**

- IT CHT: Day 1.
- Dexamethasone: 20 mg/m<sup>2</sup>/day IV. Days 1 to 5.
- 6-Mercaptopurine: 100 mg/m<sup>2</sup>/day PO. Days 1 to 5.
- Vincristine: 1.5 mg/m<sup>2</sup>, IV for 2 doses. Days 1 and 7.
- Methotrexate: 200 mg/m<sup>2</sup> IV bolus, followed by 800 mg/m<sup>2</sup> diluted in 600 ml/m<sup>2</sup> 5% Dextrose solution + 40 mEq sodium bicarbonate, IV in continuous infusion for 36 hours. Day 1.
- Folinic Acid: 15 mg/m<sup>2</sup> IV every six hours. Starting 48 h after beginning Methotrexate. For 6 doses.
- Daunorubicin: 50 mg/m<sup>2</sup> IV. Dilution 1 mg for 10 ml of 0.9% Saline Sol. for continuous infusion for 24 h. Day 5.
- Ifosfamide: 400 mg/m<sup>2</sup> IV. Days 1 to 5. Apply MESNA: 600 mg/m<sup>2</sup>.
- L-Asparaginase: 6,000 UI/m<sup>2</sup>, IM, Day 6 and continue for three doses every third day.

Rest for two weeks and continue if remission is maintained.

### **Course 3**

- IT CHT Day 1.
- Dexamethasone: 20 mg/m<sup>2</sup>/day IV. Days 1 to 5.
- Cytarabine: 2,000 mg/m<sup>2</sup> IV, dissolved in 250 ml 0.9% Saline Sol. for 3-hour infusion, every 12 h. Days 1 and 2.
- Etoposide: 150 mg/m<sup>2</sup> IV. Dilution 1 mg in 4 ml 0.9% Saline Sol. for one hour infusion. Days 3 to 5.
- L-Asparaginase: 6,000 UI/m<sup>2</sup>, IM day 6 and continue for three doses every third day.

After this course, continue with a hematopoietic progenitor cell transplant, if not possible, rest for three weeks and reinitiate the sequence. C1-C2-C3 (Intensification), patients will receive nine cycles in total. Continue to CNS radiotherapy and maintenance.

**CNS radiotherapy:** 12 Gy only if there was relapse to bone marrow and 18 Gy if the relapse was also to central nervous system.

### **Maintenance**

- 6-Mercaptopurine: 50 mg/m<sup>2</sup>/day PO.
- Methotrexate: 50 mg/m<sup>2</sup> IV dissolved in 100 ml 5% dextrose sol, for 30-minute infusion. Every two weeks.

Patients will be in maintenance for two years. After completing treatment, continue to observation until completing 60 months in continuous complete remission.

### **Acute Lymphoblastic Leukaemia in First Late Relapse (UKALLR1) [60]**

#### **Overview**

Patients with childhood acute lymphoblastic lymphoma (ALL) in first late relapse (>30 months after Induction to Remission). The probability of attaining disease remission again is 50% and thus they are candidates for allogeneic

bone marrow transplant. However, only a third of them will have a related compatible donor and so there is the option of a transplant using Umbilical Cord cells, although with a lower chance of cure; furthermore, while this procedure is taking place, disease relapse may occur. The substitution of Prednisone for Dexamethasone may increase the probability of initial remission and continuing with constant intensive chemotherapy will increase the permanence of remission. Most patients with ALL present surface antigens for CD20 and are susceptible to being treated with the corresponding monoclonal antibody (Rituximab).

The published results and those observed in our Service, suggest that the initial response to Rituximab is high, thus it will be important to determine the role that this drug will have in the treatment of ALL.

### Induction

- Intrathecal CHT: days 1, 14 and 28 in patients without central nervous system (CNS) infiltration, and twice a week if there is CNS infiltration, until two CSF determinations are negative.
- Steroid window; Prednisone: 60 mg/m<sup>2</sup>, PO, every 24 hours. Days -4 to 0.
- Vincristine: 1.5 mg/m<sup>2</sup>, without surpassing 2 mg, IV, on days 1, 8, 15, 22 and 29.
- pirubicin: 50 mg/m<sup>2</sup>, IV, dilute 1 mg in 10 ml 0.9% Saline Sol, for continuous 24 h infusion, on days 1 and 2.
- L-Asparaginase: 6,000 UI/m<sup>2</sup>, IM, three times a week, for 9 doses.
- Dexamethasone: 10 mg/m<sup>2</sup>, IV, on days 1 to 16. Reduce 2 mg/m<sup>2</sup>, every day until it is suspended on day 21.
- CD20+ Patients: Rituximab: 375 mg/m<sup>2</sup>, IV, diluted in 400 ml 5% Dextrose sol. for 4-hour infusion on days -4, +14 and +28. Premedication: 30 minutes before Diphenhydramine: 1 mg/kg IV, hydrocortisone: 2 mg/kg IV, paracetamol: 15 mg/kg.

### Bone marrow on day 35

- If there are more than 5% Blasts, the patient leaves this program.
- If there are <6% Blasts and normal cells, go on to consolidation on Day 36.
- If the cellularity is subnormal, repeat the study on Day 42 and apply the previous criteria; if the patient is still hypocellular, he leaves this program.

### Consolidation

Intrathecal CHT on days 1, 27 and 34.

- Cytarabine: 300 mg/m<sup>2</sup>, in 250 ml saline solution, 3-hour infusion on days 1, 4, 8 and 12.
- Etoposide: 150 mg/m<sup>2</sup>, in saline solution at 4 mg per ml, IV, one hour infusion on days 1, 4, 8 and 12.
- Epirubicin: 50 mg/m<sup>2</sup>, dilute 1 mg in 10 ml 0.9% Saline Sol. IV infusion for 4 hours, on days 27 and 34.
- Dexrazoxane: 500 mg/m<sup>2</sup>, diluted in 150 ml Hartmann Sol. for 30-minute infusion, one hour before administering Epirubicin.
- Vincristine: 1.5 mg/m<sup>2</sup>, IV on days 27 and 34.
- L-Asparaginase: 6,000 UI/m<sup>2</sup>, IM, on days 27, 29, 31 and 34.
- Dexamethasone: 10 mg/m<sup>2</sup> PO, days 27 to 36, reduce 2 mg/m<sup>2</sup> every day until suspended on day 40.
- Cyclophosphamide: 1 g/m<sup>2</sup>, IV on Day 42.
- Cytarabine: 75 mg/m<sup>2</sup>, IM every 12 hours. Days 42, 43, 49 and 50.
- Mercaptopurine: 60 mg/m<sup>2</sup>, PO, Days 41 to 55.
- CD20 positive patients: Rituximab, as in induction.

### Support therapy

Prophylaxis for infection, from Day 0 and until Neutrophils >1,000/μL. Prevention of emesis during treatments with

anthracyclines, Cytarabine, Etoposide and intrathecal. Filgastrim: 5 µg/kg, day, SC, each week after Day 15, for six doses and after day 56 for 6 doses. Prophylactic platelet transfusion if platelets <20,000/µL, every 48 hours.

#### **After two weeks, begin intensification**

Patients with a compatible donor (brother, umbilical cord, or unrelated), will receive intensification and maintenance until the date of the allogeneic transplant. Patients that are candidates for autologous transplant, will go on to mobilization and harvest of hematopoietic stem cells and will continue to receive intensification and maintenance until the date of the transplant.

Those patients with less than one year receiving central nervous system radiotherapy, continue directly to maintenance.

#### **Intensification**

Hydrate with 5% Dextrose solution at 100 ml/m<sup>2</sup>/h + 40 mEq Sodium bicarbonate until reaching a urinary density <1.015 and a urinary pH >6.5. Days 1 to 3.

- Furosemide: 0.5 mg/kg/dose to 1 mg/kg/dose every 8 to 12 h, based on diuresis and liquid balance.
- Vincristine: 2 mg/m<sup>2</sup> (without surpassing 2 mg) IV. Day 1, 21 and 42.
- Methotrexate: 300 mg/m<sup>2</sup> IV bolus, followed by 2,700 mg/m<sup>2</sup> diluted in 600 ml/m<sup>2</sup> 5% Dextrose solution + 40 mEq sodium bicarbonate, IV in continuous 24 h infusion. Day 1, 21 and 42.
- Folinic Acid: 30 mg/m<sup>2</sup> IV, beginning 12 h after completing infusion. Afterward, 15 mg/m<sup>2</sup> IV, every 6 h for two doses and every 8 h PO or IM for six more doses.
- CD20 positive patients: Rituximab, as in Induction. Day 54.

Intrathecal CHT with triple drugs two hours after the initial bolus of Methotrexate on Day 1.

#### **Support therapy**

Prophylaxis for infection, from Day 0 and until Neutrophils >1,000/µL. Antiemetic on the days of treatment with Methotrexate. Filgastrim: 5 µg/kg, day, SC, at the 7th day of each dose of Methotrexate, for 6 days. Maintain haematocrit above 30%. Prophylactic platelet transfusion if platelets <20,000/µL.

#### **Bone marrow on Day 61**

If there are more than 5% Blasts, the patient leaves this program. If there are <5% de Blasts and normal cellularity, continue to *Maintenance* on day 62. If cells are subnormal, repeat the study on day 67 and apply the previous criteria; if hypocellularity persists, the patient leaves this program.

#### **Maintenance**

Intrathecal chemotherapy with triple drug. Day 1.

- Vincristine: 1.5 mg/m<sup>2</sup>, IV, Day 1.
- rednison: 40 mg/m<sup>2</sup>/day, PO, Day 1 to 7.
- Methotrexate: 20 mg/m<sup>2</sup>/day PO. Once a week for 6 weeks.
- Mercaptopurine: 60 mg/m<sup>2</sup>/day, PO, Day 1 a 42.

Intrathecal chemotherapy with triple drug. Day 49.

- Mercaptopurine: 60 mg/m<sup>2</sup>/day PO, Days 49 to 59.
- Cyclophosphamide: 300 mg/m<sup>2</sup> IV, Days 49 and 56.
- Etoposide: 150 mg/m<sup>2</sup> IV, dilution the same as in consolidation, Days 49 and 56.
- Cytarabine: 50 mg/m<sup>2</sup> IM, Days 49 to 52 and 56 to 59.
- CD20 positive patients: Rituximab, as in induction. Day 48, every 3 cycles.

After two weeks, reinitiate maintenance, until 8 cycles are completed.

After the treatment is fulfilled, the patient passes on to observation until completing 60 months in continuous complete remission.

### **Refractory Acute Lymphoblastic Leukaemia**

#### **Salvage chemotherapy with Bortezomib [61]**

##### **Induction**

- Vincristine: 1.5 mg/m<sup>2</sup>/day, bolus, on days 1, 8, 15 and 22.
- Dexamethasone: 10 mg/m<sup>2</sup> IV bolus on days 1 to 14 and reduction in 7 days.
- Doxorubicin: 60 mg/m<sup>2</sup>, dilute 10 mg/ml in 0.9% saline solution, as 24-hour infusion on day 1.
- Bortezomib: 1.3 mg/m<sup>2</sup> IV rapid bolus on days 1, 4, 8 and 11.
- L-Asparaginase: 4,000 U/m<sup>2</sup> IM, three times a week for six doses.
- G-CSF 5 µg/kg from day +7 of CHT and until neutrophils > 1,500/µL.

Intrathecal CHT with triple drugs. Days 1, 14 and 28. In the case of positive CSF, test twice per week until reaching negativity.

Once in remission, the patient must be immediately passed to transplant or repeat the same cycle until achieving transplant. If this is not possible, the continuation of the treatment will be dependent on the session of the service.

#### **Salvage chemotherapy with IDA-FLAG [62]**

- Fludarabine: 30 mg/m<sup>2</sup>/day, oral administration on Days 1 to 4.
- Cytarabine: 2,000 mg/m<sup>2</sup> IV dissolved in 250 ml of 0.9% saline solution for 4-hour infusion on Days 1 to 4 Administer 4 hours after administration of Fludarabine.
- Idarubicin: 12 mg/m<sup>2</sup>, dilute 1 mg in 10 ml of 0.9% saline solution, for 4-hour infusion on Days 2, 3 and 4 Administer one hour before initiating Cytarabine.
- G-CSF: 10 µg/kg/day from Day 0 and until neutrophils are above 1,500/µL.

Intrathecal CHT with triple drug, days 1, 14 and 28. In the case of positive CSF, test twice per week until reaching negativity.

Once in remission, the patient must be immediately passed to transplant or repeat the same cycle until achieving transplant. If this is not possible, the continuation of the treatment will be dependent on the session of the service.

#### **Salvage chemotherapy with Clofarabine [58]**

Clofarabine has been demonstrated to exhibit a strong significant activity as single agent in children with ALL in relapse or refractoriness, having limited hepatic or neurologic toxicity.

The regimens using Clofarabine were more effective when given after the first relapse, with a CR of 86%, compared to 40% and 20% when administered after the second and third relapse, respectively.

##### **Single agent**

- Clofarabine 52 mg/m<sup>2</sup> BSA/day, on Days 1 to 5.
- Combined therapy with Etoposide or Cyclophosphamide.
- Maximum dose of Clofarabine 40 mg/m<sup>2</sup> BSA/day on Days 1 to 5.

##### **Other options**

There is limited information in paediatric population on the use of Blinatumab for the management of refractory precursor B acute leukaemia or in relapse, with negative Philadelphia chromosome. The publication in the Journal of

Clinical Oncology in 2016 has been the most important [63]. This study included 70 paediatric patients with average age of eight years old (range from seven months to 17 years), and showed complete response in 39% after two cycles of treatment (with a CI 95%: 27% to 51%). In 52% of the patients that responded there was also a complete molecular response. It is of interest that 57% of the patients had already been subjected to a previous bone marrow transplant. The most frequently reported adverse effects were anaemia 36%, thrombocytopenia 21%, hypokalaemia 17%, and cytokine syndrome 4%. In three cases, there were severe convulsive crises causing suspension of the treatment. For this reason, the FDA fast-tracked approval, in August of 2016, to the use of this drug for paediatric population, as long as it continues to show positive results in further clinical trials.

### **Transplant in paediatric patients with acute lymphoblastic anaemia**

Allogeneic transplant of HPCs is more commonly used after relapse, in over 50% of patients, than during primary therapy (5% to 10% of patients). Minimal residual disease can help to determine which patients should undergo transplant after a second remission and which should not [50]. ALL is frequently a polyclonal disease and the mutations in the subclones can be selected for by the chemotherapy and thus promote resistance. This includes mutations in CREBBP, which are associated with glucocorticoid resistance, and mutations in NT5C2 and PRPS1, which are associated with resistance to thiopurines.

The standard indication for children, in accordance with the consensus of the European group, 2009, suggests the following:

Carry out a HPC transplant from an HLA-identical sibling donor in those patients with:

- ALL in CR1 (very high-risk), t (9;22) or BCR/ABL rearrangement
- Age >1 year with t (4;11) or MLL rearrangement
- No complete remission after induction
- Slow response during induction or poor response to corticosteroids associated with leukocytes >100,000  $\mu$ L and/or age >1 year or >10 years and/or T immunophenotype
- CR2 after precocious medullary or extramedullary relapse. In isolated extramedullary relapse or very late relapse, chemotherapy or autologous HPCT, in the absence of an identical familial donor, can be considered
- CR3 or later stages.

Carry out HPC transplant from an unrelated donor with 9 or 10/10 HLA matches in patients with ALL in CR1 (high-risk), CR2, >CR2.

In children, the allogeneic transplant presents a transplant related mortality of 10% to 15% if HPCT is carried out in very high-risk patients in CR1 and 15% to 25% if HPCT is carried out in patients with ALL in CR2. DFS is 60% to 80% if ALL patients are high-risk and in CR1 and of 40% to 50% if they are in CR2. Post-transplant relapse is on the order of 10% to 20% for patients in CR1 and 20% to 40% for those in CR2.

For unrelated donor transplants, transplant-related mortality is 39% to 52%, DFS for patients in CR1 or CR2 is 36% to 62%, and in relapse is 8% to 10%. Post-transplant relapse is 29% to 41%.

Allogeneic HPC transplant is the only curative option for some patients with high-risk leukaemia in CR1 or CR2 after a medullary relapse. Its use is limited by the lack of HLA-identical donors. However, practically all the patients have a haploidentical related donor (parents, children, siblings), which offers an opportunity for treating those patients that do not have an adequate donor. This type of transplant has a 25% to 40% transplant-related mortality rate and DFS of 30% to 50%.

An HPC transplant from umbilical cord cells is another therapeutic option for those patients who do not have a histocompatible donor. Multicentre studies have shown better results using UCB with a compatibility of 6/6 or 5/6 and a high infused cell dose (MNC  $>3 \times 10^7$ /kg). The related mortality is 29% to 46%, being higher if compatibility is 5/6 with low infused cell dose and when UCB with 4/6 compatibility are used. 5-year DFS varies from 33% to 60%, being better with the use of units with 6/6 compatibility and higher cell dose.

Acute lymphoblastic leukaemia is the most common type of cancer in children, it comprises approximately 30% of all childhood cancers. LLA is five times more common than acute myeloid leukaemia (AML) [51]. Every year in the United States, approximately 2500 to 3500 new cases of ALL are diagnosed in children. The incidence is slightly greater in Caucasians (36 cases/million) and Hispanics (41 cases/million) than in African Americans (15 cases/million).

The rates of survival for ALL have increased since the 1980's, with an estimated current 5-year overall survival rate of 85% [50], and over 90% for first world countries [27] (Table 22). Approximately 75% to 80% of recently diagnosed children with ALL participate in clinical trials that aim to improve clinical results and minimize the acute toxic effects and adverse events, which partly explains the high success rate in this population (Table 30).

**Table 30 Patient characteristics and results from treatments utilized in selected clinical trials**

Study Group	Length of the Study	Number of Patients	Age range (years)	T-Cell ALL (%)	5-year Cumulative rate of isolated CNS relapse (% ± SE)	5-year EFS (% ± SE)	5-year Overall Survival (% ± SE)	References
AIEOP-95	1995-2000	1,743	0-18	11	1.2 ± 0.3	75.9 ± 1.0	85.5 ± 0.8	Conter, et al. [1]
BFM-95	1995-1999	2,169	0-18	13	1.8 ± 0.3	79.6 ± 0.9	87.0 ± 0.7	Möricke, et al. [2]
CoALL-97	1997-2003	667	0-18	14	4.0 ± 0.8	76.7 ± 1.7	85.4 ± 1.4	Escherich, et al. [3]
COG	2000-2005	7,153	0-21	7	NA	NA	90.4 ± 0.5	Hunger, et al. [4]
DCOG-9	1997-2004	859	0-18	11	2.6 ± 0.6	80.6 ± 1.4	86.4 ± 1.2	Veerman, et al. [5]
DFCI 00-01	2000-2004	492	0-18	11	NA	80.0 ± 2	91 ± 1	Vrooman, et al. [6]
EORTC-CLG	1998-2008	1,947	0-18	15.2	1.7 ± 0.3	82.7 ± 0.9	89.7 ± 0.7	Domenech, et al. [7]
IC-BFM 2002	2002-2007	5,060	0-18	13.3	1.9 ± 0.1	74 ± 1	82 ± 1	Stary, et al. [8]
JCCLSG ALL 2000	2000-2004	305	0-15	9.8	0.9 ± 0.1	79.7 ± 2.4	89.2 ± 1.8	Yamaji, et al. [9]
Ma-Spore ALL 2003	2002-2011	556	0-18	8.8	1.4	80.6 ± 3.5	89.2 ± 2.7	Yeoh, et al. [10]
MRC UKALL 2003	2003-2011	3,126	0-25	12	1.9 ± 0.6	87.3 ± 1.4	91.6 ± 1.2	Vora, et al. [11]
NOPHO-2000	2002-2007	1,023	0-15	11	2.7 ± 0.6	79.4 ± 1.5	89.1 ± 1.1	Schmiegelow, et al. [12]
SJCRH XV	2000-2007	498	0-18	15	2.7 ± 0.8	87.3 ± 2.9	93.5 ± 1.9	Pui, et al. [13]
TPOG	1999-2010	152	0-18	7.2	1.4 ± 1.0	84.2 ± 3.0	90.2 ± 2.4	Liu, et al. [14]

**Paediatric diagnosis:** The diagnosis and classification of leukaemia is based on specialized studies (Karyotype, FISH profile, flow cytometry, immunohistochemistry, molecular biology) that serve to immunophenotype the lymphoblasts. These studies are carried out on cells obtained from bone marrow aspiration, bone biopsy and/or biopsies from infiltrated tissues. When clinical circumstances counter-indicate a bone marrow exam, the diagnosis can be made based on cells obtained from peripheral blood or pleural effusion. The minimum percentage of blasts in peripheral blood needed to establish a diagnosis has not yet been defined [27].

A diagnosis of leukaemia in central nervous system (CNS) requires one of the following:

- Cytological confirmation of the presence of leukemic cells in cerebrospinal fluid (CSF)
- Clinical signs of leukaemia in CNS, such as paralysis of the facial nerve, cerebral and/or ocular damage, or hypothalamic syndrome
- A tumoral mass that implicates the central nervous system, as determined by imaging studies
- These can present clinically as a tumoral lesion or as leukaemia. Although the distinction in some patients is arbitrary, ALL is the preferred term in the USA when the bone marrow contains more than 25% of lymphoblasts, while the

term lymphoblastic lymphoma is preferred when the process is limited to a tumoral mass with minimal or no disease in blood or bone marrow.

The infiltration of blasts in the CNS is commonly classified into three groups:

1. CNS1: no blasts in CSF.
2. CNS2: <5 blasts in CSF with or without RBC.
3. SNC3: >5 blasts in CSF.

Patients with blasts in CSF, independently of whether their number is greater or lower than 5%, have a greater rate of CNS relapse [64,65].

**Differential diagnosis:** A variety of malignant and non-malignant conditions must be considered for differential diagnosis [7]:

- Idiopathic juvenile arthritis.
- Osteomyelitis.
- Epstein-Barr virus.
- Immune thrombocytopenia (ITP).
- Aplastic anaemia.
- Acute infectious lymphocytosis.
- Other malignant tumours that affect the bone marrow (for example, neuroblastoma, retinoblastoma, rhabdomyosarcoma, and Ewing's sarcoma).

### Hyper-eosinophilic syndrome

**Classification/risk stratification:** The current treatment protocols for all children emphasize therapy based on risk, with the goal of reducing toxicity in low-risk patients, simultaneously guaranteeing an appropriate, more aggressive treatment for patients with a high risk of relapse. The stratification into risk groups incorporates information about immunophenotype, cytogenetic findings, the patient's age, white blood count at the moment of diagnosis, and response to the initial treatment, including minimal residual disease (MRD) [66-68].

### Treatment [69]

#### Induction therapy

Induction therapy constitutes the initial phase of treatment. The objective is to achieve complete remission (CR), defined as the eradication of all detectable leukemic cells (less than 5% of blasts) from bone marrow and blood, and to restore normal haematopoiesis. The choice of induction regimen depends on whether the Philadelphia chromosome is present t (9;22) [70,71].

ALL with negative t (9;22)/BCR-ABL1-More than 90% of children and adolescents with ALL enter complete remission (CR). Induction therapy generally implies weekly administration of Vincristine during three to four weeks plus daily corticosteroids (prednisone, prednisolone, or dexamethasone), and Asparaginase [72]. Asparaginase is available as a derivative of *E. coli*, in its native form (L-asparaginase) or in its pegylated form. For patients who are allergic to *E. coli* Asparaginase, it is also available from *Erwinia* (Erwinase) [73]. A fourth agent, generally an anthracycline (for example, doxorubicin or daunorubicin), can be added to the three-drug regimen for high-risk patients [74]. Attaining a complete haematological response and the presence or absence of MRD at the end of the induction therapy are important indicators of the final result. Patients that respond quickly to induction therapy are likely to have a more favourable result, while those who have a slow response or fail induction therapy, have a more conservative prognosis [75,76].

ALL with positive t (9;22)/BCR-ABL1-This is an infrequent mutation in children with ALL, with an incidence rate of <5%. In spite of the fact that t (9;22)/BCR-ABL1 was initially associated with a very poor prognosis, the result has improved significantly with the introduction of tyrosine kinase inhibitors (TKIs) like Imatinib or Dasatinib [77,78].

In a prospective clinical trial carried out by the children's oncology group (COG), 91 children (ages 1 to 21 years) positive for Philadelphia chromosome, were treated with intensive chemotherapy plus Imatinib. Those treated with Imatinib from induction until the end of the therapy, obtained better results, with a 5-year disease-free survival of 70% [79,80].

ALL with Down Syndrome (DS-ALL)-These patients are particularly susceptible to the adverse effects and treatment-related mortality. The intensive chemotherapeutic regimens are frequently complicated by severe mucositis, and the DS-ALL children have greater risk of severe infections. One analysis of 635 children demonstrated that these patients have both a high rate of relapse and an increase in treatment-related mortality rates [81].

### **Post-remission therapy**

Consolidation or intensification therapy constitutes the second phase of treatment and is initiated shortly after attaining complete remission (CR). Continuous treatment is required because a small number of leukemic lymphoblasts remain in the bone marrow even though histological and molecular evidence show CR. In those cases, relapse occurs quickly if therapy is not continued. The purpose of post-remission chemotherapy is to avoid new leukemic growth, reduce the residual tumoral load and prevent the emergence of drug resistance in the remaining leukemic cells [82].

During induction, a great number of leukemic cells are eliminated that originated from a dominant subclone. However, initiating leukemic cells often arise from a heterogeneous population, so at the moment of diagnosis the patients already have multiple leukemic genetic subclones. These subclones have a complex clonal architecture at diagnosis. Consolidation therapy can help to prevent the appearance of drug resistance through elimination of these dominant subclones that were resistant to induction therapy [83].

Consolidation. Consolidation therapy generally lasts between four and eight months. It implies the use of combinations of drugs with mechanisms of action that are different from those used during the induction phase. The regimens often include the following drugs, administered on different schedules in order to maximize synergy and minimize the development of drug resistance [84]:

- Cytarabine.
- Methotrexate.
- Anthracyclines (daunorubicin, doxorubicin).
- Alkylating agents (Cyclophosphamide, Ifosfamide).
- Epipodophyllotoxins (etoposide).

The intensification of the therapeutic regimens is based on the risk group classification of the patient. This has allowed a reduction in intensification therapy for patients with a good prognosis while permitting a more intensive treatment for those that are in the high-risk group. Patients with detectable MRD have a greater risk of relapse after conventional chemotherapy [27,85-87].

Maintenance therapy. The total treatment time for the majority of children with ALL is 30 to 42 months. After a consolidation or intensification therapy phase, patients often receive a less intensive continuation regimen (for example, maintenance therapy) using 6-mercaptopurine (6-MP) [89], weekly Methotrexate with periodic Vincristine, prednisone, and intrathecal therapy. 6-MP can be administered as a tablet or an oral suspension.

Although it is not clear whether all the patients profit from all of the benefits of the maintenance therapy, which includes a combination of pulses of Vincristine and steroids, as well as a daily regimen of 6-MP and weekly Methotrexate, patients with ALL considered to be of standard risk that receive this combination seem to have a more favourable long term result than those treated only with 6-MP and Methotrexate [88,89].

### **Support therapy**

Chemotherapy is highly toxic, especially because of the expected myelosuppression. Most of the patients require hospitalization with support from hemoderivatives. The laboratory tests must be carried out daily and generally include a complete blood count, full blood chemistry, electrolyte panel, calcium, phosphorus, and uric acid levels. Hepatic function tests must be carried out each week. Support therapy is a critical component for the treatment of

a patient with acute leukaemia. Toxicity can be the result of the chemotherapy or of the rapid elimination of the tumoral load (tumoral lysis syndrome). Among the most feared complications are tumour lysis syndrome, thrombosis, haemorrhage, infection, renal failure, and anaphylaxis. Other secondary effects include mucositis, pancreatitis, acute hepatic failure, hypertriglyceridemia and hyperglycaemia.

**Tumour lysis syndrome:** Tumour lysis syndrome is an oncological emergency caused by the massive destruction of tumour cells and the release of large quantities of intracellular components: potassium, phosphate, and uric acid into systemic circulation [91]. Uric acid and/or calcium phosphate crystals can be deposited in the renal tubules and result in an acute renal lesion. There is a significant risk of tumour lysis syndrome in patients treated for ALL.

**Preventive measures include:** hyperhydration with isotonic saline solution, allopurinol or Rasburicase, and the correction of the electrolytic alterations mentioned previously. For patients that present high leucocytosis, a three to seven day “pre-phase” treatment with corticoids and Cyclophosphamide should be considered for cytoreduction in order to reduce the probability that tumour lysis syndrome will be established when induction therapy with intensive polychemotherapy is initiated [90].

**Thrombosis:** Venous thrombosis is a complication of Asparaginase treatment, due to its effect on cytoreduction of anticoagulants, such as antithrombin III, protein C and protein S. Deep venous thrombosis in the legs or the inferior vena cava, and pulmonary embolism can occur during treatment with this drug. Cerebral venous thrombosis (for example, thrombosis of the dural sinus) is less frequent but more serious and can be complicated by secondary haemorrhaging. The symptoms of thrombosis of the dural sinus can include headache, focal neurological symptoms, or encephalopathy. Close vigilance with adequate laboratory tests, like fibrinogen quantification, are necessary and neuroimaging studies should be requested opportunely when there is clinical suspicion of this complication [91,92].

**Anaemia and thrombocytopenia:** Patients with ALL will develop anaemia and thrombocytopenia with requirement for transfusions at some point during their treatment. In general, transfusions of erythrocyte concentrates are given to all patients with anaemic syndrome. Our objective is to maintain haemoglobin levels between 8 g/dl and 9 g/dl, depending on the age, symptoms, and comorbidities of each patient. Platelet transfusions are used prophylactically for patients with platelet counts <10,000/ $\mu$ l or for any patient with manifest signs of haemorrhage, such as wet purpura. For patients who are not bleeding but with platelet count <10,000/ $\mu$ l, the transfusion of multiple units of platelets beyond a single unit, does not offer obvious benefit and could be a risk factor for the fearsome platelet transfusion refractoriness. The blood products must be irradiated or leukodepleted to minimize the risk of adverse immunologic events. Always search for cytomegalovirus (CMV) negative hemoderivatives for those patients who are candidates for hematopoietic cell transplants [47,93].

**Infections:** A prolonged period of neutropenia is expected during polychemotherapeutic treatment in patients with ALL, and this is frequently associated with fever. These patients must be considered as high-risk for infection with bacteria or fungi, and viral reactivation. In order to minimize risk of infection patients must be kept “isolated”, with or without prophylactic antibiotic, antifungal or antiviral treatment, during chemotherapy administration. Patients that develop fever and neutropenia require a quick evaluation and immediate administration of parenteral antibiotics adapted for the predominant organisms and the patterns of resistance in each institution.

**Anaphylaxis:** Some chemotherapeutic or support drugs, such as Asparaginase, Etoposide and Rasburicase, can cause significant allergic reactions, including anaphylaxis. The medications used to treat anaphylaxis must be easily available when these drugs are administered.

**Hepatotoxicity:** Abnormal hepatic function tests in patients with ALL can be the result of leukemic infiltration, medications (for example, Asparaginase, antifungals), infections (for example, reactivation of hepatitis), and other comorbidities (fatty liver, cirrhosis). Asparaginase can be associated with an increase in serum transaminases, alkaline phosphatase, bilirubin, and triglycerides. Patients with fatty liver can be at greater risk for hepatotoxicity caused by Asparaginase.

**Hyperglycaemia and acute pancreatitis:** Acute pancreatitis is observed in 5% of adults receiving asparaginase, while hyperglycaemia related to the use of Asparaginase and steroids is observed in approximately 25%. In most hyperglycaemia cases, it can be regulated with insulin without the need to suspend Asparaginase treatment. Asparaginase is usually continued in asymptomatic pancreatitis, identified only by laboratory or radiological findings.

Conversely, Asparaginase is interrupted in cases of clinical pancreatitis (for example, vomit, severe abdominal pain) [94,95].

### **Prognosis**

The most useful prognostic indicators in acute lymphoblastic leukaemia (ALL) are age, leukocyte count, immunophenotype, detection of minimal residual disease, and karyotype.

Cytogenetic analysis and molecular cytogenetic studies, such as fluorescent in situ hybridization (FISH), reveal recurrent cytogenetic chromosomal abnormalities in approximately 80% of the B immunophenotype acute lymphoblastic leukaemia (ALL), including numeric and structural changes, such as translocations, inversions or deletions. There are substantial differences in frequencies of recurrent abnormalities between children and adults with ALL.

The translocation t (8;14), present in high proportion in Burkitt leukaemia/lymphoma cells (leukemic lymphoblasts with basophilic cytoplasm and prominent vacuoles), occurs in approximately 1% of adults with ALL (ALL-3 of the FAB). It is associated with a high incidence of central nervous system infiltration at diagnosis and a poorer prognosis than any other group of patients classified by chromosomal abnormalities.

Translocation t (4;11) is present in up to 60% of the babies under 12 months of age, but is rarely observed in adult patients. It produces a fusion gene, KMT2A/AFF1, and is associated with a poor prognosis, both in children and in adults. It is commonly characterized by high leukocyte count with L1 morphology of the FAB classification (small cells with scarce cytoplasm, condensed chromatin, and few nucleoli) or L2 (larger cells with moderate amount of cytoplasm, dispersed chromatin, and multiple nucleoli), they frequently have co-expression of myeloid antigens.

Translocation t (9;22) which produces the Philadelphia chromosome, is observed in approximately 2% to 5% of children and 30% of adults. This translocation was classically associated with poor prognosis, however, with the advent of Imatinib and other TKIs, the results have changed.

Translocation t (1;19) is produced in approximately 30% of patients with childhood pre-B ALL and with lower frequency in other B-lineage ALLs in children and adults. In the past, patients with t (1;19) typically showed early treatment failure. However, the adverse prognosis can be surmounted by more intensive chemotherapy. Thus, t (1;19) translocation is currently associated with a favourable prognosis.

Translocation t (12;21) results in a gene fusion, ETV6/RUNX1, detectable by FISH or PCR in approximately 25% of children with B-lineage ALL, and in 3% of adults with ALL. Patients generally have a favourable prognosis.

Patients with hyperdiploidy, with more than 50 chromosomes, often have good prognosis. The individual structural abnormalities do not appear to influence the results of these patients [96]. Approximately 5% to 6% of patients, independently of age, can have clonal loss of different chromosomes, resulting in less than 46 chromosomes. All patients with hypodiploidy have a poor prognosis.

In T-cell ALL, approximately 60% have an abnormal karyotype. These patients have a different pattern of recurrent abnormalities, which implicate both the T-cell receptor (TCR) and not TCR, including mutations in NOTCH1 in over 50%. Both children and adults with T-cell ALL are generally treated with protocols for high-risk individuals, and frequently have favourable results.

### **Relapse**

More than 80% of the adult patients with a recent diagnosis of acute lymphoblastic leukaemia will achieve complete remission (CR) with intensive induction chemotherapy. After consolidation and maintenance chemotherapy, less than half will reach long-term leukaemia-free survival. Additionally, 20% will have resistant disease from the beginning. Thus, most adult ALL patients will have relapse or refractory disease.

**Definitions:** Refractory disease (resistant) is defined as those patients that do not achieve CR with induction therapy, that is, the failure to eradicate all the detectable leukemic cells (less than 5% blasts) in bone marrow and peripheral blood. Disease relapse describes the reappearance of leukemic cells in bone marrow or peripheral blood after having reached a CR.

Once disease relapse has been identified, one must proceed to carry out a new battery of studies, such as: compatibility

of human leukocyte antigen (HLA) in patients that are candidates for hematopoietic cell transplant (HCT). This is extremely important, since HCT is the most probable therapy to achieve a cure for ALL patients in relapse. The complete set of initial laboratory studies must be requested again (CBC, BCP, electrolyte panel, hepatic function tests, hepatitis virus profile, etc.). A chest X-ray, an electrocardiogram (EKG) and a cardiac function study (for example, ejection fraction in the echocardiogram or MUGA) now acquire special relevance, especially in patients with previous exposure to anthracyclines or those with cardiovascular symptoms. Patients with neurologic signs or symptoms must be submitted to imaging studies to evaluate meningeal disease. Lumbar puncture is indicated for all patients in order to examine the cerebrospinal fluid (CSF), which must be sent for cytology and flow cytometry to determine CNS blast infiltration.

**Rescue chemotherapy:** Patients with refractory disease or those in their first relapse may still have complete response rates after rescue chemotherapy as high as 30% to 50%. No standard chemotherapy exists, and these patients should consider joining prospective trials. In general, the election of rescue chemotherapy regime will greatly depend on the condition of the patient and the amount of disease-free survival time the patient had reached, for example: if relapse was detected more than two years after the initial treatment, a second remission can be achieved using a similar induction regimen as was used initially. In turn, patients with resistant disease (failed induction) or with early relapse (relapse while undergoing induction, consolidation, or maintenance therapy), require reinduction with new therapies. After reaching a second CR with rescue chemotherapy, patients must proceed, as soon as possible, to receive an allogeneic transplant, since second complete remissions are generally short lived and HCT offers the best possibility of a cure in this context.

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#### COMPETING INTERESTS

No conflict of interest

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