# Recent trends in diagnosis and management of Acute lymphoblastic leukemia

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# INTRODUCTION

Acute lymphoblastic leukemia (ALL) also known as acute lymphocytic leukemia is a malignant (clonal) neoplasm of the bone marrow in which lymphocyte precursor cells or lymphoblasts have exaggerated and uncontrolled growth, fail to mount a normal immune response, and cause a drop in production of normal bone marrow cells that leads to a deficiency of circulating red cells (anemia), platelets (thrombocytopenia), and white cells other than lymphocytes (especially neutrophils, or neutropenia). Both T-cell and B-cell precursors can give rise to ALL; B-cell ALL represents about 75% of all cases ***(Pui C. 1996).***

ALL occurs at all ages but displays a bimodal distribution of incidence, with one peak in early childhood and a second in patients older than 50 years. ***(Faderl et al. 2003***). For adult ALL, the yearly incidence is 2 per 100,000, with 75% of the cases being B lineage and the remainder of T cell origin. ***(Rodriguez et al. 2007).*** for pediatric ALL, the yearly incidence is 3 to 4 cases per 100,000 ***(Shu et al. 2002)***.

ALL represents approximately less than 1% of adult cancers, and 25% of all childhood cancers. In the United States of America (USA) among all ages, it represents less than 0.4% of all cancers, 13.6% of all leukemias, and 29.6% of all lymphocytic leukemias. ***(Groves et al. 1995).***

ALL represents less than 1.1% of total US cancer related deaths and 28.9% of all leukemia deaths. In US children, however, ALL represents almost 16% of total cancer mortality and 50% of all leukemia deaths. ***(Ries et al. 2006).***

The clinical presentation of ALL may range from nonspecific symptoms such as progressive malaise, fever, and fatigue to severe life-threatening manifestations, requiring immediate medical intervention such as acute tumor lysis syndrome (ATLS). Symptoms may appear insidiously or acutely. The presenting features generally reflect

the degree of marrow failure and the extent of extramedullary spread. Among the frequently evident findings are pallor, petechiae, and ecchymosis in the skin and mucous membranes and bone tenderness as a result of leukemic infiltration or hemorrhage that stretches the periosteum. Liver, spleen, and lymph nodes are the most common sites of extramedullary involvement, and the degree of organomegaly is more pronounced in children than in adults. Very rarely, ALL produces no signs or symptoms and is detected during routine examination ***(Chessells et al. 1998)***.

The diagnosis of acute lymphoblastic leukemia (ALL) is dependent on the identification and characterization of blast cells in peripheral blood or bone marrow. ***(Weinkauff et al. 1999).***

Diagnosis and classification are generally based on the morphologic, cytochemical, and immunologic features of the blasts. However, cytogenetic and molecular studies are frequently needed to confirm the diagnosis, predict clinical behavior, and stratify patients for therapy ***(Pui et al. 2004).***

The French, American, and British (FAB) classification of ALL, which recognizes three subclasses of ALL (L1, L2, and L3), is based strictly on blast morphology and cytochemistry***, (Bennett et al. 1981)*** whereas the World Health Organization (WHO)classification scheme also incorporates immunophenotyping and cytogenetics. ***(Harris et al. 2000 ).***

Remarkable progress has been made in the treatment and outcome of adult acute lymphoblastic leukemia over the past 3 decades. This progress is the result of an accumulation of a mosaic of knowledge and experience, which have led to a more profound understanding of the biology of the disease, and at the same time the development of new drugs and treatment strategies. The combination of further cytogenetic-molecular dissection of ALL subtypes with the emergence of new and targeted therapies will thus continue to constitute the fundament upon which further progress will hopefully occur in adult ALL ***(Kantarjian H. 2000)***

Most of the initial therapeutic advances in adult ALL have arisen from successful adaptation of ALL treatment strategies in children. ALL therapy incorporates multiple drugs into regimen-specific sequences of dose and time intensity and is divided into several phases: (i) induction; (ii) a sequence of intensified consolidation; (iii) a prolonged maintenance phase; and (iv) CNS prophylaxis. Intensive combination therapy in ALL following this pattern has resulted in complete remission (CR) rates of 80% to 90% and leukemia-free survival rates of between 30% and 40%. ***(G kbuget et al. 2002)***.

The backbone of induction therapy consists of vincristine, steroids, and anthracyclines to which various other drugs such as L-asparaginase, cyclophosphamide, or cytarabine have been added. Dexamethasone has replaced prednisone for better antileukemia activity and achievement of higher levels in the CSF. ***(Bostrom et al. 2003).***

Consolidation strategies include repetition of a modified induction regimen, use of rotational consolidation programs frequently involving high doses of cytarabine, methotrexate, cyclophosphamide, or L-asparaginase, and stem cell transplant. There is evidence that some of these components may contribute to subset specific improvements in outcome. For example, high dose methotrexate may be especially effective in low risk B-lineage ALL and T-ALL, whereas cyclophosphamide and L-asparaginase have led to improved outcome in T-lineage ALL patients. ***(Thomas et al. 1999).***

Early use of stem cell transplantation (SCT) remains disputed. Although recommended for patients with poor-prognosis ALL (Philadelphia-chromosome-positive, 11q23 translocations), the benefit of SCT for standard-risk patients in first CR is not established. The Eastern Cooperative Oncology Group (ECOG) together with the Medical Research Council of the United Kingdom (MRC UK) is investigating early matched-related allogeneic SCT for those CR patients less than 50 years who have a

histocompatible donor, whereas all other patients are randomized between autologous SCT and consolidation therapy followed by maintenance for 2.5 years**.** In the standard risk group, the 5-year EFS rates were 66% with allogeneic SCT and 45% for the randomized group whereas the rates were 44% and 26% for high-risk patients**.** ***(Song K. et al. 2007)***

The backbone of maintenance therapy has remained fairly constant throughout the various ALL regimens and consists of vincristine, prednisone, 6-mercaptopurine and methotrexate for the duration of 2–3 years. Although maintenance therapy is proven to be beneficial in ALL, there is so far no evidence that intensification of maintenance provides any additional benefit. ***(Mandelli et al. 1996).***

Several novel agents are being investigated in ALL The two groups of agents that have the potential to make the biggest impact currently are the tyrosine kinase inhibitors (imatinib) and the monoclonal antibodies (rituximab, alemtuzumab) CD20 is expressed in around 35% of ALL patients with higher expression found in Philadelphia chromosome-positive ALL and mature B-cell ALL. In addition, it was found that presence of CD20 on ALL blasts is associated with worse outcome ***(Thomas et al. 2000).*** Thus, including rituximab as part of the induction/consolidation part of therapy may improve the prognosis of ALL patients further. ALL blasts also show high expression of the CD52 antigen. Unlike CD20, CD52 is also expressed on cells of T lineage and use of the anti-CD 52 monoclonal antibody alemtuzumab may therefore provide additional benefit to patients with particularly T-cell leukemias ***(Thomas et al. 2001).***

A number of other novel agents are investigated in relapsed in refractory disease states and include new nucleoside analogs (clofarabine, nelarabine), liposomal agents (liposomal vincristine), or hypomethylating agents (decitabine). ***(Deangelo et al. 2007).***

# Epidemiology and Etiology

## Epidemiology

### (1) Incidence

ALL accounts for approximately 1% of all adult cancers but nearly 25% of all childhood cancers. There is a slight male to female predominance of 1.3:1.0 (male to female ratio). In the USA, in 2008, there were approximately 5,400 new cases of ALL ***(Reis L. et al. 2008).*** Among all ages, this represents 13% of all leukemias and 28% of all lymphocytic leukemias ***(Reis L. et al. 2008).*** The overall age-adjusted incidence of ALL in the USA is 1.6 per 100,000 according to the most recent National Cancer Institute report based on cases between 2001 and 2005 ***(Reis L. et al. 2008).***

### (2) Survival

Long-term survival rates for adult ALL cases have not significantly improved over the past two decades. Five-year survival is still only 30–40% for patients 20–60 years old, worsening to less than 15% for those older than 60 years and less than 5% for those older than 70 years ***(Carpenter P. et al. 2007).*** By contrast, survival for childhood ALL has improved markedly over the past five decades ***(Reis L. et al. 2008).***

### (3) Mortality

The age-adjusted death rate for all ALL cases in the USA was 0.5 per 100,000 people for the period 2001–2005 ***(Reis L. et al. 2008).***

### (4) Age Differences

There is a clear peak of incidence of ALL between 2 and 4 years old followed by falling rates during later childhood, adolescence, and early childhood ***(Swensen A. et al. 1997).*** There is a bimodal pattern with a smaller secondary peak gradually trending up beyond 60 years old ***(Reis L. et al. 2008).***

## Etiology

### (1) Genetic Syndromes

Inherited, predisposing genetic syndromes are associated with less than 5% of ALL cases. Germline abnormalities associated with ALL include Down syndrome (trisomy 21), ataxia-telangiectasia, Fanconi's anemia, Nijmegen breakage syndrome, Klinefelter's syndrome, Bloom syndrome, and neurofibromatosis ***(Vanasse G. et al. 1999).***

### (2) Acquired Genetic Abnormalities

The major cytogenetic abnormalities are clonal translocations. The most common of these in adults is t(9;22), or the Philadelphia chromosome, resulting in the BCR-ABL fusion gene, occurring in 11–29% of adult ALL. This results in an activated kinase that confers a proliferative advantage ***(Secker-Walker L. et al. 1997).***

### (3) Ionizing Radiation

ionizing radiation has been associated with increased incidence of leukemia including ALL ***(Berrington A. et al. 2001).*** Survivors of the atomic bomb explosions in Nagasaki and Hiroshima were noted to have a ninefold increase in risk of ALL peaking at 5–10 years after exposure ***(Preston D. et al. 1994).***

### (4) Nonionizing Radiation

Exposure to high levels of low-frequency electromagnetic fields has been purported to confer an increased risk of ALL, particularly in children ***(Ahlbom A. et al. 2000).*** Meta-analyses also show conflicting results. There was no significant increase in risk of leukemia in a study by Kheifets and colleagues ***(Kheifets L. et al. 1997).*** However, Wartenberg reported an increased risk of leukemia with high exposure levels ***(Wartenberg D. 2001).***

# Clinical Features and Diagnosis

## Clinical Features of ALL

The clinical presentation of acute lymphocytic leukemia (ALL) may range from insidious nonspecific symptoms to severe acute life threatening manifestations, reflecting the extent of bone marrow involvement and degree of extramedullary spread. In younger patients anemia-induced fatigue may be the only presenting feature. Dyspnea, angina, dizziness, and lethargy may reflect the degree of anemia in older patients presenting with ALL ***(Simone J. et al. 1975).*** Approximately half of all patients may present with fever attributable to the pyrogenic cytokines, such as IL-1, IL-6, TNF, released from the leukemic cells, infection, or both. Arthralgia and bone pain due to the bone marrow expansion by the leukemic cells and occasionally necrosis can be observed, although less commonly in adults compared to children ***(Gur H. et al. 1999).***

Pallor, petechiae, and ecchymosis in the skin and mucous membranes due to thrombocytopenia, DIC, or a combination of the above may be observed. ALL may present with either leukopenia (~20%) or moderate (50%–5–25 × 109/L) and severe leukocytosis (10%–>100 ×109/L). Neutropenia is common. The majority of patients present with platelet counts less than 100 × 109/L (75%), while 15% have platelet counts of less than 10 × 109/L ***(Simone J. et al. 1975).***

Severe metabolic abnormalities may accompany the initial diagnosis of ALL ***(O'Regan S. et al. 1977).*** Patients with high leukemic burden are at risk of developing acute tumor lysis syndrome (ATLS), manifested by hyperuricemia, hyperkalemia, hyperphosphatemia, and secondary hypocalcemia. Such electrolyte abnormalities may lead to the development of oliguric renal failure due to the tubular precipitation of urate and calcium phosphate crystals, fatal cardiac arrhythmias, hypocalcemic tetany, and seizures. Hyperkalemia, defined by a serum potassium concentration of >6 mmol/L, caused by massive cellular degradation, may precipitate significant neuromuscular and potentially life threatening cardiac abnormalities ***(Jeha S. 2001).***

## Diagnosis of ALL

The diagnosis of acute lymphoblastic leukemia (ALL) is dependent on the identification and characterization of blast cells in peripheral blood or bone marrow. ALL can be reliably diagnosed using peripheral blood or bone marrow blasts when blasts are in circulation ***(Weinkauff R. et al. 1999).***

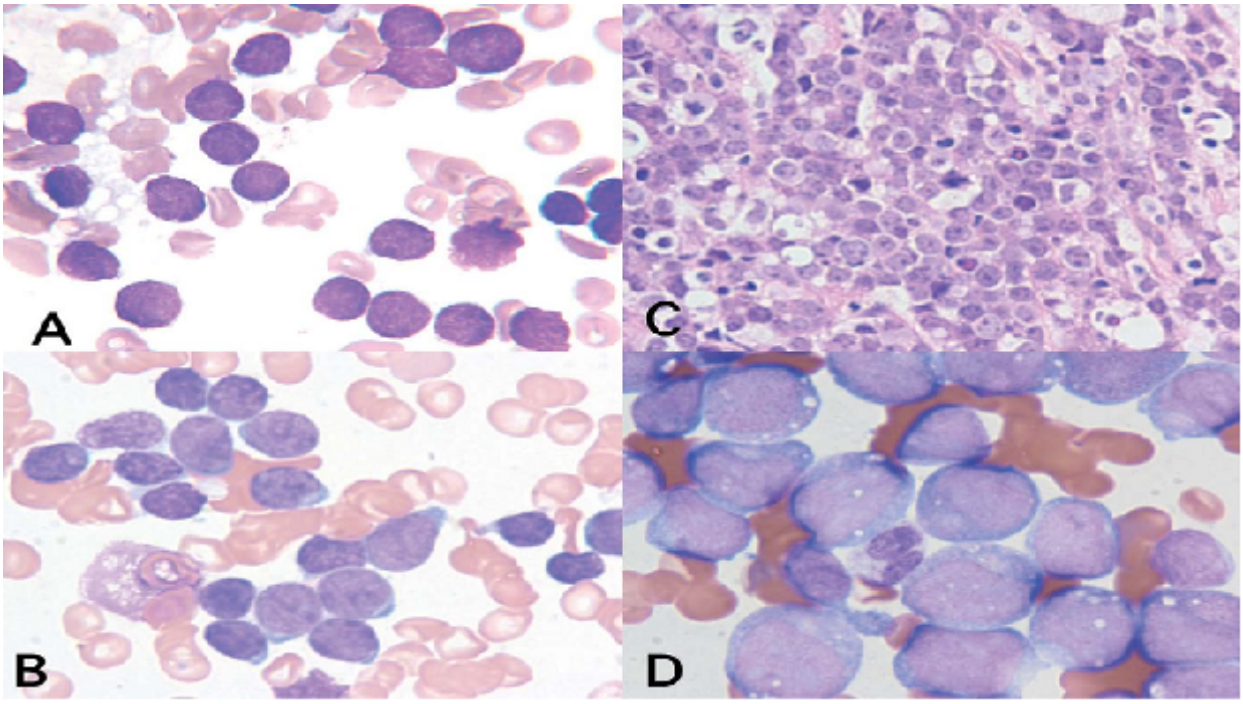
Diagnosis and classification are generally based on the morphologic, cytochemical, and immunologic features of the blasts. However, cytogenetic and molecular studies are frequently needed to confirm the diagnosis, predict clinical behavior, and stratify patients for therapy ***(Pui C. et al. 2004).***

### (1) Morphology

Lymphoblasts in patients with ALL tend to be heterogeneous in size and shape. the FAB classification of ALL emphasizes the presence of subgroups of precursor lymphoblasts: L1, which is more common in children than in adults (85% vs. 30%) and L2, which is more common in adults than in children (60% vs. 15%). The FAB and WHO classifications both recognize the more mature subtypes of B-cells as Burkitt L3 cells ***(Bennett J. et al. 1981).***

L1 precursor lymphoblasts are small with scant cytoplasm, fine chromatin, and indistinct nucleoli (>90% of total blasts). L2 precursor lymphoblasts, on the other hand, are typically medium-to-large cells with high nucleus-to-cytoplasm ratios, prominent nucleoli, and irregular or folded nuclear membrane outlines ***(Potter V. et al. 1984).***

L3 (Burkitt) blasts have distinct morphology, with medium-sized and more uniformly rounded nuclei and finely clumped chromatin. The diagnostic feature of this cell subtype is a deeply basophilic and vacuolated cytoplasm. The vacuoles in L3-type cells contain lipids and stain positively with oil-red O stain. Nucleoli are seen but are not dominant ***(Bennett J. et al. 1981).***




**(Fig. 1):** shows Morphology of blasts in acute lymphoblastic leukemia. (A) L1 blasts; (B) L2 blasts; (C) "Starry sky" morphology on bone marrow biopsy in a case with L3 leukemia; (D) L3 blasts.

**(2) Cytochemistry and Immunophenotyping**

The key diagnostic cytochemical feature of ALL is the lack of myeloperoxidase (MPO) activity and negativity for nonspecific esterase (NSE) ***(Bennett J. et al. 1981).*** The functional MPO test using cytochemistry remains the gold standard for assessing MPO activity, but laboratories are increasingly using the chloroacetate esterase stain and immunostain, especially for detection by flow cytometry ***(Peffault de Latour R. et al. 2003).*** To distinguish ALL from increased peripheral blood or bone marrow blasts, fewer than 3% of blasts should express MPO activity ***(Bennett J. et al. 1981).*** Sudan black B (SBB) can also be used to confirm the presence of MPO granules in these cells ***(Schumacher H. 1998).*** Periodic acid-Schiff (PAS) staining is also positive in ALL lymphoblasts ***(Schumacher H. 1998).***

Negativity for MPO and NSE should raise the possibility of an ALL diagnosis, but further flow cytometric evaluation is necessary. Generally, the following markers

are useful and used by most laboratories: CD34, TdT, CD1a, CD2, CD19, CD3, CD7, CD4, CD8, CD10, CD13, CD14, CD22, CD33, CD64, CD117, cCD79a, and surface immunoglobulin (Ig)M. Cytoplasmic CD3 (cyCD3), cyIgM, and cyCD22 are usually helpful. Terminal deoxynucleotidyltransferase (TdT) expression along with CD19+ or cyCD79a and surface or cytoplasmic CD22+ are diagnostic for early precursor B-cell involvement, irrespective of CD13 and CD33 expression. The expression of CD10 (common ALL antigen, CALLA) in addition to the above markers is diagnostic for more mature (intermediate) precursor ALL. Although CD19 protein expression is diagnostic for B-cell lineage, it is detected in 80% of acute myeloid leukemia (AML) cases that carry the t(8;21) chromosomal abnormality ***(Kees U. et al. 2003)***. The diagnosis of precursor T-cell ALL is based on lack of expression of B-cell markers and expression of surface CD3 (sCD3) or cytoplasmic CD3 (cyCD3) in MPO-negative/NSE-negative blasts. However, approximately 10% of precursor T-cell ALL cases are TdT negative ***(Faber J. et al. 2000)*** .

| Subtype | Characteristic markers | Frequency in adult ALL |
|---|---|---|
| **Precursor B-cell** | **CD19+, CD22+, CD79a+ , cIg+/−, PAX5, sIgm−, HLA-DR+<br>CD20, CD34 – variable expression<br>CD45 – may be absent** | **~70–75%** |
| **Early precursor (pre -pre or pro-)-B cell** | **CD19+, cCD79a+, cCD22+, TdT+, CD10−** | **~10%** |
| **Common-B cell** | **CD10+** | **~50–65%** |
| **Pre-B cell** | **CD10+/−, c-μ+** | **~10%** |
| **Precursor T-cell** | **TdT+, CD7+, cCD3+(lineage specific); HLA-DR+/−, CD1a+/−, CD2+/−, CD4+/−, CD5+/−, CD7+/−, CD8+/−** | **~20–25%** |
| **Pro-T** | **cCD3+, CD7+, CD2−, CD1a−, CD34+/−CD4−, CD8−** | |
| **Pre-T** | **cCD3+, CD7+, CD2+, CD1a−, CD34+/−CD4−, CD8−** | |
| **Cortical T** | **cCD3+, CD7+, CD2+, CD1a+, CD34−CD4+, CD8+** | |
| **Medullary T** | **cCD3+, CD7+, CD2+, CD1a−, CD34−sCD3+; either CD4+ or CD8+** | |

**(Table-1):** shows immunophenotypes of ALL ***(Swerdlow S. et al. 2008).***

## Cytogenetic and Molecular Abnormalities

Cytogenetic analysis of each patient's ALL cells has become an essential component of diagnosis prior to treatment. It has furthered our understanding of leukemogenesis at a molecular level. Specific and well-characterized recurring chromosomal abnormalities facilitate diagnosis, confirm subtype classification, and have major prognostic value for treatment planning. ***(Le Beau M. and Larson R. 2000).***

Conventional cytogenetic analysis requires dividing cells, is technically difficult Therefore, alternative diagnostic methods have been sought, including fluorescence in situ hybridization (FISH). This method of analysis is more rapid, and in some cases more sensitive, than conventional cytogenetic analysis. FISH can be used to study nondividing cells ***(Le Beau M. and Larson R. 2000).***

### (1) Hyperdiploidy

Hyperdiploidy, defined as the gain of additional chromosomes, is one of the most frequent cytogenetic abnormalities in ALL (25% of pediatric ALL and 5% of adult ALL). This increase in chromosomes is not random: the most commonly gained chromosomes are 4, 8, 10, and 21, followed by chromosomes 5, 6, 14, and 17. Most hyperdiploid cells contain either 47 to 50 chromosomes or 51 or more. Patients with ALL and high hyperdiploidy have longer response and survival durations after therapy, particularly those with more than 50 chromosomes. In general, hyperdiploid cells are more sensitive than nonhyperdiploid cells to chemotherapy and show more rapid induction of apoptosis ***(Pui C. et al. 2004).***

### (2) Hypodiploidy

Hypodiploidy, or the presence of fewer than 46 chromosomes, is seen in 5% of ALL and this abnormality is generally considered a poor prognostic factor. However, near haploidy (23–29 chromosomes) is particularly associated with poor outcome ***(Raimondi S. et al. 2003).***

**(3) Philadelphia Chromosome**

The Philadelphia translocation is one of the most frequently identified chromosome abnormalities among adults with ALL (25%), while only 3% of pediatric ALL is Philadelphia chromosome positive. The presence of the Philadelphia chromosome is associated with uniformly poor outcomes ***(Faderl S. and Albitar M. 2000)*** Philadelphia chromosome results from a reciprocal translocation between the long arms of chromosomes 9 and 22 [t (9; 22) (q34; q11)], which moves the ABL gene from 9q34 into the BCR (breakpoint cluster region) region of chromosome 22q11. The resulting BCR-ABL fusion gene encodes a tyrosine phosphokinase that is constitutively active and leads to downstream activation of several proteins. ***(Pui C. et al. 2004).***

**(4) 12p12.3 Abnormalities**

Translocation t (12; 21) (p12; q22), which results in the ETV6 (TEL)-AML1 (CBFA2) fusion protein, is detected in 20–25% of children with B-cell precursor ALL. This is the most common cytogenetic–molecular abnormality in childhood ALL, but is relatively uncommon in adult ALL (<5%). The ETV6-AML1 fusion protein in children with ALL is associated with an excellent prognosis, with longer event-free and overall survival ***(Pui C. et al. 2004).***

**(5) 11q23 Abnormalities**

The MLL (mixed lineage leukemia) gene, located at the 11q23 locus, is involved in translocations onto other chromosomes as well as duplication. The most common MLL translocations in ALL are t(4;11)(q21;q23), t(9;11) (p21;q23), t(11;19)(q23;q13.3), and t(3;11)(q22;q23), which are associated with poor outcomes and a high incidence of myeloid marker expression ***(Rubnitz J. et al. 1996).***

**(6) 19p13.3 Abnormalities**

The E2A gene is located on chromosome 19p13.3. Translocation t (1; 19) (q23; p13) forms the E2A-PBX1 fusion gene, leading to expression of the E2A-PBX1 fusion protein. This abnormality is seen in precursor B-cell ALL and is detected in approximately 5% of pediatric and 3% of adult ALL cases. A similar translocation t

(17; 19) (q22; p13) involving the E2A gene results in expression of the fusion protein E2A-HLF. The E2A-PBX1 and E2A-HLF fusion proteins are associated with poor outcomes ***(Uckun F. et al. 1998).***

**(7) 5q35 Abnormalities**

A recently described cryptic translocation in T-cell ALL, t(5;14)(q35;q32), moves the HOX11L2 (HOX11like2)(RNX; TLX3) homeobox gene into close proximity of the CTIP2 gene on chromosome 14. This translocation, which can be detected with FISH or RT-PCR, leads to over expression of HOX11L2. Translocation t (5; 14) appears to be restricted to T-lineage ALL and is more common in children than adults (22% vs. 13%) ***(Cave H. et al. 2004)***

**(8) 9p21 Abnormalities**

Abnormalities of the short arm of chromosome 9 at band p21 occur in up to 15% of patients with ALL. These patients, mainly children, tend to present with unfavorable clinical characteristics (high white blood cell and blast counts and organomegaly) and predominantly T-cell immunophenotype. Clinical outcome is characterized by high relapse rates and short overall survival ***(Faderl S. et al. 1999).***

**(9) NOTCH1**

NOTCH1 point mutations, insertions and deletions producing aberrant increases in NOTCH1 signaling are frequently present in T-cell ALL. NOTCH1 signaling was shown to be required for sustained growth and, in a subset of cell lines, for survival **(Beverly L. and Capobianco A. 2004).**