



Acute lymphoblastic leukemia (ALL)

Status: July 2020

Continuous research and targeted examinations of blood and bone marrow result in various diagnostic recommendations for patients with acute lymphoblastic leukemia.

Diagnostic recommendation

Method	Anticoagulant	Recommendation
Cytomorphology	EDTA	mandatory
Immunophenotyping	EDTA or Heparin	mandatory
Chromosome analysis	Heparin	mandatory
FISH	EDTA or Heparin	mandatory
Molecular genetics	EDTA or Heparin	mandatory



Definition and characteristics

Acute lymphoblastic leukemia is characterized by a proliferation and accumulation of malignant lymphoid precursor cells of the B or T cell series. The incidence is 1/100,000 inhabitants per year. It is the most common malignant neoplasm in childhood. In adults, however, it accounts for only about 20% of acute leukemias.

Classification of ALL

WHO classification of ALL

According to the current WHO classification in 2017, ALL is classified together with lymphoblastic lymphoma as lymphoid precursor neoplasms of the B- or T-cell type. The detection of more than 25% blasts differentiates ALL from lymphoblastic lymphoma. A division into subgroups is made according to cytogenetic, molecular genetic and immunophenotypic criteria. Mature B-ALL or **Burkitt's leukemia/lymphoma** is classified as mature B-cell neoplasm. The differentiation of the mature cell Burkitt B-ALL is of high relevance, since the therapy of this subgroup differs significantly from the usual therapy regimens for B-precursor ALL.

Classification of ALL according to WHO 2017 (Swerdlow et al. 2017)

Precursor lymphoid neoplasms

- B-lymphoblastic leukemia/lymphoma, not further classified (NOS)
- B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities

t(9;22)(q34.1;q11.2); *BCR-ABL1*

t(v;11q23.3); *KMT2A* rearranged

t(12;21)(p13.2;q22.1); *ETV6-RUNX1*

Hyperdiploidy (hyperdiploid ALL)

Hypodiploidy (hypodiploid ALL)

t(5;14)(q31.1;q32.3); *IL3-IGH*

t(1;19)(q23;p13.3); *TCF3-PBX1*

B-lymphoblastic leukemia, *BCR-ABL1* like

lymphoblastic leukemia with *iAMP21*

- T-lymphoblastic leukemia/lymphoma

Early T-cell precursor lymphoblastic leukemia (ETP)

Pre-T-ALL

Pro-T-ALL

cortical T-ALL

mature T-ALL

- Natural killer (NK) cell lymphoblastic leukemia/lymphoma

Clinically relevant classification of lymphoblastic leukemia according to immunological subtypes

The classification according to immunological subtypes is of great importance in practice. The *German Multicenter ALL Study Group* (GMALL), for example, uses the EGIL classification, which classifies ALL primarily according to the degree of maturity of the leukemic cells into Pro-B-, common-, and pre-B-ALL and into Pro-T-, Pre-T-, cortical/thymic and mature T-ALL. The immunological subtypes of ALL are associated with specific clinical and cytogenetic or molecular genetic abnormalities (see Table 1).



Table 1: Classification of ALL (according G-MALL/EGIL)

Subgroup	Immunophenotyping (characteristic markers)	Cyto-/Molecular genetics		Incidence
		Common abnormalities	Molecular markers	
B-lineage-ALL	HLA-DR+, TdT+, CD19+ u/o CD79a+ u/o CD22+			76%
B-precursor-ALL				
Pro-B	CD10-	t(4;11)	ALL1-AF4	11%
Common	CD10+	t(9;22)	BCR-ABL1	49%
Pre-B	cyIgM+	t(1;19) t(9;22)	E2A-PBX1 BCR-ABL1	12%
T-lineage-ALL	TdT+,CD3+, CD7+			24%
Pro/Pre-T	sCD3-, CD1a-			6%
Pro-T	CD2-, CD5-, CD8-			
Pre-T	CD2+ u/o CD5+ u/o CD8+			
Cortical	sCD3+/-, CD1a+			12%
Mature T	sCD3+, CD1a-, TdT+/-			6%

Facts

≥60%

of adult ALL patients show cytogenetic abnormalities (Moorman et al. 2007, Graux et al. 2006)

Diagnostics of ALL

Cytomorphology

Cytomorphology and Cytochemistry: Standard Diagnostics for ALL

Cytomorphology, together with cytochemistry, is still part of the standard diagnostics in ALL. It is used to confirm the diagnosis of acute leukemia and to differentiate it from AML. In addition, it is an important method for assessing remission in follow-up examinations under therapy.

In ALL, morphologically undifferentiated blasts are found. In cytochemistry these show no relevant reaction with myeloperoxidase (<3%) or unspecific esterase. However, final assignment to the lymphoblastic line or reliable differentiation from AML requires immunophenotyping in all cases. The lymphoblasts of the T cell lineage resemble morphologically more mature lymphocytes. Immunophenotyping is also essential for the differentiation of mature T-cell leukemia.

If blasts with vacuolised and basophilic cytoplasm show up, the possibility of a mature B-ALL (**Burkitt leukemia/lymphoma**) should always be considered and the appropriate genetic diagnosis should be initiated immediately.

Immunophenotyping

Immunophenotyping is an important component of ALL diagnostics

Immunophenotyping enables a differentiation from AML beyond cytomorphology and thus ensures the diagnosis of ALL. Based on the immunophenotype a subtyping of ALL is performed, which is prognostically and therapeutically important. In addition, surface markers on the lymphoblasts are determined, which represent potential target structures for antibody therapy.

At the time of diagnosis, a so-called "leukemia-associated immunophenotype" (LAIP) can also be determined. This serves to monitor the course of the disease under therapy ("measurable residual disease", MRD).

Antigen expression pattern determines ALL subtype

ALL is divided into B-precursor and T-precursor leukemias according to the immunophenotype. Further classification is done according to the degree of maturity into Pro-B, common and pre-B-ALL, and Pro-T, pre-T, cortical and mature T-ALL. In general, if the corresponding morphological findings (negativity for myeloperoxidase or MPO) are present, a B-precursor-ALL is diagnosed when cCD22, CD19 and TdT are expressed. A T-precursor-ALL is present when c/sCD3, CD7 and TdT are detected. Table 2 shows the antigen expression patterns of the respective subtypes.

If an acute leukemia is detected that has no more than one lineage-specific marker per line (Acute undifferentiated leukemia, AUL) or shows both specific markers of the lymphoblastic and myeloid cell series (mixed phenotype acute leukemia, MPAL), no ALL is diagnosed.



Table 2: Classification of B-precursor and T-precursor-ALL according to the immunophenotype according to EGIL

Antigen	B-precursor-ALL			T-precursor-ALL			
	Pro-B-ALL	c-ALL	Pre-B-ALL	Pro-T-ALL	Pre-T-ALL	cortical T-ALL	mature T-ALL
cCD22*	+	+	+	-	-	-	-
CD79 α	+	+	+	-	-	-	-
CD19	+	+	+	-	-	-	-
CD24	+/-	+	+	-	-	-	-
CD20	-/(+)	+/-	+/-	-	-	-	-
sIg*	-	-	-	-	-	-	-
cIgM*	-	-	+	-	-	-	-
cCD3*	-	-	-	+	+	+/-	-
sCD3*	-	-	-	-	-	-/+	+
CD7	-	-	-	+	+	+	+
CD5	-	-	-	-	+/-	+	+
CD2	-	-	-	-	+/-	+	+
CD1a	-	-	-	-	-	+	-
CD4	-	-	-	-	-/+	+/-	+/-
CD8	-	-	-	-	-/+	+/-	+/-
CD10	-	+	+/-	-/+	-/+	-/+	-
HLA-DR	+	+	+	-/+	-/+	-	-
CD34	+	+	+	-/+	-/+	-	-
TdT	+	+	+	+	+	+	+/(-)

*c: cytoplasmic; s: "surface", membrane phenotypic markers

Detection of the minimal/measurable residual disease (MRD)

MRD diagnostics is a highly sensitive method for assessing therapy response, which is the strongest independent prognostic factor in ALL (Brüggemann et al. 2006, Berry et al. 2017, O'Connor et al. 2017). In addition, MRD status is used for therapy management, planning and implementation of stem cell transplantation and for remission control. Immunophenotyping is suitable for the quantification of MRD in practically every patient with ALL. The sensitivity of this method reaches 10⁻³ to 10⁻⁴. The basis for MRD diagnosis during the course of the disease is the definition of the leukemia-associated immune phenotype (LAIP) at the time of diagnosis. For B-precursor LAIP, the coexpression of the antigens CD19 or CD10 is suitable for the detection of minimal residual disease. In addition, in many cases the aberrant coexpression of myeloid antigens and the expression of CD34 can be used. For T-precursor ALL, the coexpression of cCD3 and TdT can be used as LAIP.

Chromosome analysis

Chromosome analysis to determine the karyotype of leukemia cells is now standard diagnostic procedure for every patient with ALL. The karyotype is necessary for classification according to WHO; even more important, however, is the prognostic and thus therapeutic significance of the cytogenetic findings.

Chromosomal abnormalities of B-precursor ALL have prognostic and therapeutic relevance

Chromosomal abnormalities are detected in up to 80% of ALL patients (Moorman et al. 2007). Based on cytogenetics, ALL can be divided into two main groups: **ALL with structural aberrations** typically exhibit reciprocal translocations and associated leukemia-specific fusion genes (Table 3). ALL without leukemia-specific fusion genes can be divided into so-called **ploidy groups** based on chromosome number (Table 4).

The prognostic and therapeutic relevance of the detection of recurrent fusion genes is particularly evident in the example of t(9;22)(q34;q11)(BCR-ABL1). It is the most frequent translocation of ALL in adulthood and was associated with a adverse prognosis for a long time. However, the introduction of specific tyrosine kinase inhibitors has significantly improved this prognosis (Ravandi 2017). The detection of a translocation involving the KMT2A gene (formerly MLL gene) in chromosome band 11q23 is also of prognostic importance and has therapeutic consequences.



Table 3: Recurrent chromosomal abnormalities in adult ALL

Chromosomal abnormalities	Phenotype	Gene	Incidence
t(1;19)(q23;p13)	Pre-B-ALL	TCF3-PBX1	3%
t(4;11)(q21;q23)	Pro-B-ALL	KMT2A-AFF1 (MLL-AF4)	6%
t(6;11)(q27;q23)	B-ALL/T-ALL	KMT2A-MLLT4	< 1%
t(8;14)(q11;q32)	Pre-B-ALL	IGH-CEBPD	< 1%
t(8;14)(q24;q32)	B-ALL/ Burkitt L.	IGH-MYC	5%
t(9;11)(p21;q23)	Pro-, Pre-B-ALL	KMT2A-MLLT3	< 1%
t(9;22)(q34;q11)	c-ALL	BCR-ABL1	25-30%
10p12/11q23	Pro-, Pre-B, T-ALL	KMT2A-MLLT10	< 1%
t(11;19)(q23;p13)	Pro-, Pre-B-ALL	KMT2A-MLLT1	1%
t(12;21)(p13;q22)	Pre-B-ALL	ETV6-RUNX1	< 1%
t(14;14)(q11;q32)	Pre-B-ALL	IGH-CEBPE	< 1%
t(14;19)(q32;q13)	Pre-B-ALL	IGH-CEBPA	< 1%
t(14;20)(q32;q13)	Pre-B-ALL	IGH-CEBPB	< 1%
t(17;19)(q22;p13)	Pre-B-ALL	TCF3-HLF	< 1%
9p	T-, Pre-B-ALL	CDKN2A	B-ALL15%, T-ALL 65-70%
6q	c-, Pre-B-, T-ALL	?	B-ALL 6%, T-ALL 20-30%
t(1;14)(p32;q11)	T-ALL	TAL1	3%
t(5;14)(q35;q11)	T-ALL	TLX3-TRAD	9%
t(7;10)(q34;q24)	T-ALL	TRB-TLX1	<1%
t(10;14)(q24;q11)	T-ALL	TLX1-TRAD	5-10%

Table 4: Ploidy Groups in ALL (according Heim & Mitelman 2015)

Ploidy group	Chromosome number	Phenotype
Near haploidy	25-29	c-, Pre-B-ALL
Low hypodiploidy	30-39	c-, Pre-B-ALL
High hypodiploidy	42-45	B-ALL/T-ALL
High hypodiploidy	51-65	c-, Pre-B-ALL

Secondary chromosomal abnormalities of the B-precursor ALL

Hyper- and hypodiploidy and leukemia-specific fusion genes are usually primary aberrations that define biologically independent subgroups. In addition, a large spectrum of additional cytogenetic aberrations has been described in ALL. The most frequent are deletions in 6q, 7p, 9p, 12p, 13q, losses of chromosomes 7, 9, 17 and gains of chromosomes 5, 8, 21 and the X chromosome (Lafage-Pochitaloff et al. 2017). Various studies suggest that monosomy 7, trisomy 8 and complex aberrant karyotypes, especially after stem cell transplantation, are associated with an unfavourable prognosis in BCR-ABL negative ALL (Lazaryan et al. 2020). Frequently, the prognostic effect of individual additional aberrations seems to vary depending on the primary aberration, the age group and the therapy strategy. Furthermore, the evaluation of prognostic effects of secondary aberrations within the genetic subgroups is difficult due to the small number of cases, especially in adult ALL.

Differentiation of mature B-ALL rearrangements of the MYC gene



The translocation t(8;14)(q24;q32) leads to a rearrangement involving the MYC gene and the locus of the heavy chain of immunoglobulins. Rarely - as variants of t(8;14)(q24;q32) - the translocations t(8;22)(q24;q11) or t(2;8)(p12;q24) involving the loci of the immunoglobulin light chains occur. These aberrations are associated with mature B-ALL (according to WHO 2017: Burkitt's leukemia/lymphomas), but rarely occur in common and pre-B-ALL. With current treatment protocols specifically designed for this subtype, the prognosis of Burkitt's leukemia/lymphoma has been significantly improved (Hoelzer et al. 2014).

Chromosomal abnormalities of T-ALL

In childhood ALL, T-ALL occurs at a frequency of 15% and in adult ALL patients at 25% and is characteristically more common in men than in women. Chromosomal abnormalities can be detected in 50 - 70% of patients with T-ALL. The most common cytogenetic alterations are translocations associated with aberrant expression of oncogenic transcription factors. These chromosomal rearrangements put the transcription factors under the control of T-cell specific enhancers, which are located in the *TCR β* - (7q34) or *TRa δ* - (14q11) loci, resulting in their abnormal expression in T-cell progenitor cells. The most common partner genes of the TCR loci are *TAL1* (1p32), *TAL2* (9q32), genes of the HOXA gene cluster (7p15), *TLX1* (10q24), *TLX3* (5q35), *LMO1* (11p15), *LMO2* (11p13), *NOTCH1* (9q34), *MYC* (8q24) and *TCL1* (14q32). In 30 - 50% of the cases these rearrangements are cytogenetically cryptic. About 3% of pediatric patients with T-ALL show aberrant expression of *TAL1* due to translocation t(1;14)(p32;q11) or an intrachromosomal deletion in chromosome 1 leading to the fusion gene *SIL-TAL1*. Other chromosomal abnormalities in T-ALL patients include deletions of 6q, 11q23(*KMT2A*) translocations (prognostically unfavorable), the gain of chromosome 8, monosomy 7 and cryptic deletions in 9q34, which lead to the fusion gene *SET-NUP214*. 8 % of patients also show a complex aberrant karyotype (\geq 5 chromosomal aberrations), which has been described as prognostically unfavorable (Marks et al. 2009). Deletions of 9p21 are observed in 65 - 70% of patients. Furthermore, 8% of T-ALL patients show translocations involving the *ABL1* gene, with t(9;9)(q34;q34) (*NUP214-ABL1*) being the most frequent of these rearrangements. The *NUP214-ABL1* fusion is usually in the form of a submicroscopic extrachromosomal amplification.

FISH

FISH supplements the classical chromosome analysis

By means of FISH the presence of a *BCR-ABL1* or *KMT2A* rearrangement can be proven within 24 hours. In addition, FISH is often used in addition to classical chromosome analysis to confirm detected abnormalities and to obtain a baseline result for follow-up examinations to detect residual disease under therapy.

The so-called "chromosome painting" with 1-3 or 24 colours (24-colour FISH) on metaphase chromosomes is performed in addition to the classical chromosome analysis, if the karyotype cannot be clearly clarified with chromosome analysis after Giemsa band staining. This is often the case with complex aberrant karyotypes.

A FISH screening for the most frequent abnormalities is useful in cases where chromosome analysis does not yield a valid result. Also in normal karyotypes an extended FISH screening is recommended to detect aberrations that may not have been detected *in vitro* due to insufficient proliferation of ALL blasts. Here the probes can be selected depending on the immunophenotype. In addition, FISH is also used to detect cytogenetically cryptic chromosomal alterations: By detecting a translocation t(X;14)(p22;q32)(*IGH-CRLF2*) a Philadelphia-like ALL can be diagnosed. ALL with t(12;21)(p13;q22)(*ETV6-RUNX1*, formerly: *TEL-AML1*) form an independent ALL subtype according to WHO 2017 (Swerdlow et al. 2017). Both translocations are cytogenetically not visible due to the small size of the exchanged chromosome segments and can only be detected by FISH or molecular genetic methods. Furthermore, deletions of 9p21, which result in a deletion of the *CDKN2A* gene, are detected in about 30% of B-precursor ALL and 70% of T-ALL (Mullighan & Downing 2008). These deletions can be caused by monosomy 9 or by a total or partial loss of 9p. Some of these deletions are so small that they are cytogenetically cryptic and can only be detected by FISH.

Detection of the residual disease by FISH

Fluorescence in situ hybridization can also be used in the course of therapy to detect residual disease. It is more sensitive and specific than cytomorphology, but less sensitive than PCR and immunophenotyping.

Molecular genetics

Molecular genetics is used to detect prognostically and therapeutically relevant fusion transcripts and mutations. Furthermore, quantitative real-time PCR is a very sensitive method for the detection of MRD. It can be performed on the basis of fusion transcripts or clonal immunoglobulin and T-cell receptor (TCR) gene rearrangements.



Acute lymphoblastic leukemia of the B cell lineage

Molecular diagnostics of B cell lineage ALL first aims to identify patients from the high-risk groups with t(9;22)(q34;q11) and t(4;11)(q21;q23). Here, in addition to cytogenetics, an RT-PCR (reverse transcriptase polymerase chain reaction) is performed to detect a *BCR-ABL1* or *KMT2A-AFF1* (formerly *MLL-AF4*) fusion transcript. Especially in paediatric patients, an investigation for the detection of the cytogenetic cryptic translocation t(12;21)(p13;q22) and the associated *ETV6-RUNX1* rearrangement (previously: *TEL-AML1*) is of particular importance, since this can only be detected at the molecular level and/or by means of FISH. In addition, depending on the result of the chromosome analysis by RT-PCR, further fusion transcripts are detected which can be used as markers for MRD diagnostics. In ALL patients >50 recurrent deletions, amplifications and mutations in genes that play a role in various cellular processes were detected. For example, in 15% of infantile and 30% of adult B-precursor ALL deletions in the *ICZF1* gene were described. These are associated with an unfavourable prognosis. In the group of ALL with *BCR-ABL1* rearrangement as well as in Philadelphia-like ALL, *ICZF1* deletions are more frequent (about 70%). In these prognostically unfavourable subgroups *ICZF1* deletions lead to an additional deterioration of the prognosis (Mullighan et al. 2009, Roberts et al. 2014). *TP53* mutations are most frequently observed in ALL with low hypodiploid chromosome set or with *MYC* rearrangements. They are associated with an unfavourable prognosis, especially when both alleles are affected - either by two mutations or by mutation of one allele and deletion of the second allele (Mühlbacher et al. 2014, Stengel et al. 2014).

Table 5: Common recurrent fusion genes in B-precursor ALL

Cytogenetic	Fusion gene	Subtype	Frequency	
			Children	Adults
t(1;19)(q23;p13)	<i>TCF3-PBX1</i>	Pre-B-ALL	5-6%	3%
t(4;11)(q21;q23)	<i>KMT2A-MLL2</i> (<i>MLL-AF4</i>)	Pro-B-ALL	2%	6%
t(11;19)(q23;p13)	<i>KMT2A-MLL1</i>	Pro-B-ALL, Pre-T-ALL	< 1%	< 1%
t(9;22)(q34;q11)	<i>BCR-ABL1</i>	c-, Pre-B-ALL	2-5%	25-30%
t(12;21)(p13;q22)	<i>ETV6-RUNX1</i>	c-ALL	10-20%	< 1%

Table 6: Common recurrent fusion genes in T-ALL

Cytogenetic	Fusion gene	Subtype	Frequency
1p32 Deletion	<i>STIL-TAL1</i>	T-ALL	9%-26%
del(9)(q34q34)/t(9;9)(q34;q34)	<i>SET-NUP214</i>	T-ALL	5%
del(9)(q34q34)/t(9;9)(q34;q34)	<i>NUP214-ABL1</i>	T-ALL	6%
t(10;11)(p13;q14)	<i>PICALM-MLL10</i>	T-ALL	5-10%

Acute lymphoblastic leukemia of the T cell lineage

Molecular genetic analyses can be used to detect fusion genes that play a major role in the formation of T-ALL (Table 6). In particular, an examination to detect a *STIL-TAL1* fusion (del(1)(p32p32)) or a *SET-NUP214* fusion (del(9)(q34q34),t(9;9)(q34;q34)) is of particular importance, since these are cytogenetically cryptic changes which cannot be detected by conventional chromosome analysis. Furthermore, the fusion gene *NUP214-ABL1* can be detected by RT-PCR, which is usually present in the form of a submicroscopic extrachromosomal amplification. Although *NUP214-ABL1* positive cells react sensitively to tyrosine kinase inhibitors, the development of resistance is currently a clinical challenge.

The NOTCH signalling pathway plays a crucial role in the development of T cells. The aberrant NOTCH1 signaling pathway in T-ALL was originally discovered by translocation t(7;9)(q34;q34.3), which leads to the expression of a truncated and constitutively active form of *NOTCH1*. However, the central role of NOTCH1 in T-cell transformation was not recognized until activating mutations in the *NOTCH1* gene were identified, which are present in over 50% of T-ALL cases. In addition, *FBXW7* mutations, present in about 15% of T-ALL cases, contribute to NOTCH activation by inhibiting the proteasomal degradation of activated *NOTCH1* in the cell nucleus. Other genes frequently mutated in T-ALL patients are *PHF6* (16% of children, 40% of adults with T-ALL), *DNMT3A* (18% of adult patients), *JAK1* (18% of adult patients), *NRAS* (14% of children, 9% of adults), *RUNX1* (16% adult patients), *PTEN* (6 - 10%), *IL7R* (12% adult patients), *CDKN2A* (4% adult patients), *FLT3-ITD* (2 - 4%) and *FLT3-TKD* (1% adult patients). Mutations in the genes *RUNX1* and *DNMT3A* were described as prognostically unfavourable, mutations in *NOTCH1* and *FBXW7* were described as prognostically favourable. However, additional *RAS/PTEN* mutations may be associated with a less favourable prognosis (Trinquand et al. 2013).



Detection of measurable residual disease (MRD)

By means of quantitative real-time PCR, reciprocal fusion transcripts with a sensitivity of 10^{-4} to 10^{-5} can be detected, e.g. in ALL with *BCR-ABL1* or *KMT2A* rearrangement. In addition, patient-specific B cell receptor and T cell receptor rearrangements can be used as diagnostic targets. Thus, at least one suitable molecular MRD marker is available for practically every patient with ALL, which allows an assessment of the therapeutic response beyond the cytomorphological detectability limit.

Prognosis of ALL

There are internationally accepted prognostic factors for adult ALL, whereby the various study groups use different criteria for risk stratification. The GMALL studies (see Table 7) define the high-risk group at initial diagnosis of ALL based on leukocyte count, immunophenotype and cytogenetics or molecular genetics (at least one unfavourable prognostic factor present, see Table 7). In addition, the response to therapy is monitored, especially by MRD control, and taken into account as an individual prognostic factor.

Table 7: Unfavourable prognostic factors in adult ALL
(GMALL-trial 08/2013)

High leucocyte count	> 30.000/ μ l in B-precursor-ALL
Subtype	Pro-B, ETP, mature T
Late CR	> 3 weeks (after induktion II)
Cytogenetic / Molecular abnormalities	<ul style="list-style-type: none"> • t(9;22) - <i>BCR-ABL1</i> • t(4;11) - <i>KMT2A-AFF1</i>
Minimal residual disease	Molecular failure after consolidation I (=MRD $>10^{-4}$) Molecular relapse (MRD $>10^{-4}$ after previous CR)

Importance of the minimal/measurable residual disease

In several randomized studies, the detection of minimal residual disease beyond the cytomorphological detectability limit was shown to be a highly significant independent prognostic factor in both children and adults. MRD status during and after therapy influences both event-free survival and overall survival (Brüggemann et al. 2006, Berry et al. 2017, O'Connor et al. 2017). Therefore, MRD is now found in almost all clinical protocols for re-evaluation of individual risk assessment and optimal therapy control. In addition, continuous MRD monitoring of patients with negative MRD allows early detection of preclinical relapses and thus a rapid adaptation of the therapy strategy.

In ALL, MRD diagnostics can be performed during the course of the disease using both molecular genetic analyses and immunophenotyping. Molecular methods are based on the detection of leukemia-specific B-cell receptor and T-cell receptor rearrangements as well as leukemia-specific fusion transcripts. The sensitivity of MRD diagnostics using molecular genetics is 10^{-4} to 10^{-5} . While the detection of typical fusion transcripts is very specific, clonal B-cell receptor and T-cell receptor rearrangements are not directly involved in oncogenesis. If these are altered by clonal evolution, false negative MRD results can occur. False positive results, however, are possible due to unspecific primer attachment in cases of high-grade bone marrow regeneration after therapy (Brüggemann & Kotrova 2017).

Immunophenotyping is used to define an individual leukemia-associated immunophenotype (LAIP) for each patient, which allows a quantification of MRD in the course of the disease. The sensitivity is about one log level below the sensitivity of molecular genetic methods. Like the specificity, it depends on the similarity of the leukemic blasts and the physiological precursor cells. In addition, phenotypic shifts are frequently observed both in MRD cells and in normal cells, which make the detection of leukemic cells more difficult, especially in antibody therapy (Brüggemann & Kotrova 2017).

Common entities of ALL of the B cell series

t(9;22)(q34;q11), *BCR-ABL1* (WHO entity)

The translocation t(9;22)(q34;q11), which leads to a *BCR-ABL1* rearrangement, represents the most frequent abnormalities in adult patients with ALL (25%). In children, the so-called Philadelphia+ ALL occurs in only 3% of patients (Pui et al. 2004).

In contrast to CML, the breakpoint in the *BCR* gene is found in 70% of *BCR-ABL1*+ ALL patients in the m-*BCR* region (minor) and only in 30% of patients in M-*BCR* (major). Additional cytogenetic abnormalities occur in 41-86% of *BCR-ABL1*+ ALL patients. The most frequent findings are gains of a derivative chromosome 22, a chromosome 8 and an X chromosome, the loss of a chromosome 7 and an isochromosome 8q, 9p deletions and hyperdiploidy (Moorman et al. 2007). Furthermore, in more than 60% of the *BCR-ABL1*+ B-ALL deletions of *ICZF1* have been described, which are associated with an additionally unfavorable prognosis (Mullighan et al. 2009, van der Veer et al. 2014, Slayton et al. 2018).

Overall, there is a correlation of the translocation t(9;22)(q34;q11) with a higher age of the patients, with higher leukocyte values and with an unfavorable prognosis (Moorman et al. 2007). The treatment of *BCR-ABL1*+ ALL patients with a combination of chemotherapy and tyrosine kinase inhibitors currently leads to a significant improvement in the prognosis, but long-term observations indicate problems with the development of drug resistance (Ravandi 2017). Mutations that lead to resistance to tyrosine kinase inhibitors can be detected using next generation sequencing. Furthermore, the response to therapy can be quantitatively determined by MRD measurement.

11q23 (*KMT2A*, formerly *MLL*) arrangements (WHO entity)

Chromosomal abnormalities involving 11q23 are observed in about 10% of ALL patients in adulthood. In infants *KMT2A* rearrangements occur in 80% of ALL (Pui et al. 2004). Typically, a Pro-B-ALL phenotype is detected.

The most frequent translocations involving 11q23 are the t(4;11)(q21;q23) (*KMT2A-MLLT2*), t(6;11)(q27;q23) (*KMT2A-MLLT4*), t(9;11)(p22;q23) (*KMT2A-MLLT3*), t(10;11)(p12;q23) (*KMT2A-MLLT10*) and t(11;19)(q23;p13) (*KMT2A-MLLT1*), wherein more than 100 partner genes of the *KMT2A* gene are known. *KMT2A* rearrangements are associated with an unfavorable prognosis in B-precursor ALL; in particular, the translocation t(4;11)(q21;q23) (*KMT2A-MLLT2*) is considered a high-risk abnormality (Moorman et al. 2007, Moorman et al. 2010, Pullarkat et al. 2008).

t(12;21)(p13;q22), *ETV6-RUNX1* (WHO entity)

Approximately 25% of children and 2% of adults with B-precursor ALL have a translocation t(12;21)(p13;q22) that leads to an *ETV6-RUNX1* rearrangement and is considered prognostically favorable (Pui et al. 2004, Bhojwani et al. 2012).

t(1;19)(q23;p13), *TCF3-PBX1* (WHO entity)



The translocation t(1;19)(q23;p13) occurs in about 5% of children and adults with ALL (Pui et al. 2004); often an unbalanced rearrangement with two cytogenetically inconspicuous chromosomes 1 and a derivative chromosome of (19)t(1;19)(q23;p13) is present. *TCF3-PBX1* rearrangements were associated with an unfavorable prognosis, which could be improved by the application of more intensive chemotherapy regimens according to the ALL-BFM protocol, so that the t(1;19)(q23;p13) in children can currently be classified as prognostically favorable (Kager et al. 2007). This subtype is also no longer associated with an unfavourable prognosis in adults (Moorman et al. 2007, Burmeister et al. 2010, Lafage-Pochitaloff et al. 2017). The translocation t(17;19)(q22;p13) is a variant of t(1;19)(q23;p13) and leads to a *TCF3-HLF* rearrangement at the molecular level. According to the data available so far, it is associated with an unfavorable prognosis in children and adults (Moorman et al. 2012). ALL with t(17;19)(q21;p13) are not further classified as B-lymphoblastic leukemia/lymphoma according to the WHO classification 2017 (NOS).

High Hyperdiploidy (WHO entity)

ALL with a highly hyperdiploid set of chromosomes are observed in 25% of child ALL and 7% of adult ALL patients (Pui et al. 2004).

The chromosome sets have more than 50 chromosomes and usually less than 66 chromosomes (Heim et al. 2015). Characteristic for this ALL subgroup is a pattern of gains on chromosomes 4, 6, 10, 14, 17, 18 and 21 and the X chromosome. More rarely, gains of chromosomes 5 and 8 can be detected. The chromosomes are usually present as trisomies, with chromosome 21 being the most frequently gained; in 90% of cases it is present in three or more copies. In addition, 50% of the highly hyperdiploid chromosome sets show structural changes, mainly partial gains of 1q, deletions of 6q and the isochromosomes i(7q) and i(17q) (Moorman et al. 2003, Paulsson & Johansson 2009). Children with a highly hyperdiploid set of chromosomes often have mutations and deletions in the *PAX5* gene (Mullighan et al. 2007). In addition, mutations in *FLT3*, *NRAS*, *KRAS* and *PTPN11* have been detected (Case et al. 2008).

In children, a highly hyperdiploid set of chromosomes is associated with a good prognosis, especially in the presence of the so-called "triple trisomy" with gains of one chromosome 4, 10 and 17 each (Paulsson et al. 2013). This subtype can also be considered prognostically favourable in adults (Moorman et al. 2007). However, if one of the recurrent translocations t(9;22)(q34;q11), t(1;19)(q23;p13) or an 11q23 translocation occurs, its prognostically unfavourable effect is valid.

Hypodiploidy (WHO entity)

Hypodiploid chromosome sets have less than 46 chromosomes. In both children and adults they occur in 1 - 5% of ALL (Pui et al. 2004, Harrison et al. 2004).

According to the WHO 2017 classification, a further subdivision is made into nearly haploid (23 - 29 chromosomes), low hypodiploid (33 - 39 chromosomes) and high hypodiploid (40 - 43 chromosomes) (Swerdlow et al. 2017), whereby high hypodiploid chromosome sets represent a very heterogeneous group and differ prognostically from karyotypes with <40 chromosomes (Harrison et al. 2004).

Patients with a low hypodiploid karyotype typically show losses of chromosomes 3, 7 and 17 from a diploid chromosome set. In addition, monosomes of chromosomes 13, 15 and 16 are usually present, somewhat less frequently losses of chromosomes 4, 9, 12 and 20. As a rule, both chromosomes 21 are retained. The set of chromosomes is often doubled (so-called hypotriploid chromosome set, biologically very low tetraploid chromosome set). This results in a typical pattern of two or four chromosomes, which allows a distinction between hypodiploid ALL with doubled chromosome set and highly hyperdiploid ALL (Charrin et al. 2004, Mandahl et al. 2012). In more than 90% of patients with low hypodiploid chromosome set a mutation in the *TP53* gene was detected (Moorman et al. 2007, Holmfelt et al. 2013, Mühlbacher et al. 2014, Stengel et al. 2014). Furthermore, alterations of the *RB1* gene as well as deletions of the *ICZF2* gene are frequently found, whereby the latter are biallelic due to aneuploidy (Holmfelt et al. 2013).

The pattern of chromosomal losses of the nearly haploid ALL is very similar to that of the low hypodiploid ALL. Especially the sex chromosomes as well as chromosomes 14 and 18 are mostly preserved. Chromosome 21 is also typically present in two copies. While about 95% of patients with a high hypodiploid karyotype have a complex aberrant chromosome set, structural abnormalities are rare in karyotypes with <40 chromosomes, especially in nearly haploid chromosome sets (Pui et al. 1990, Charrin et al. 2004, Harrison et al. 2004). In contrast, a doubling of the nearly haploid chromosome set is frequently observed. In contrast to the low hypodiploid ALL, *TP53* mutations are rarely detected in the nearly haploid ALL. This subtype is characterized by deletions, amplifications or sequence mutations in genes of the RTK and Ras signaling pathway and the *ICZF3* gene (Holmfelt et al. 2013).

Overall, the prognosis for patients with hypodiploidy is unfavourable, although it has been described as particularly unfavourable in the presence of less than 40 chromosomes in both children and adults (Harrison et al. 2004, Moorman et al. 2007, Moorman et al. 2012).

Philadelphia-like ALL (provisional WHO entity)

The Philadelphia-like ALL comprises a group of the B-precursor ALL, which shows a similar gene expression profile to the Philadelphia+ ALL, but does not have a *BCR-ABL1* rearrangement. Similar to *BCR-ABL1+* ALL, the incidence increases with age. It occurs in less than 10% of children and about 25% of adults with B-precursor ALL (Roberts et al. 2014, Herold & Gökbuget 2017).

In about 90% of Philadelphia-like ALL rearrangements have been shown to lead to activation of tyrosine kinase and cytokine receptor-mediated signaling pathways (Roberts et al. 2014). These include fusions of genes of the *ABL* class (*ABL1*, *ABL2*, *CSF1R*, *PDGFRA*, *PDGFRB*), rearrangements of the genes *CRLF2*, *EPOR* and *JAK2*, respectively, as well as other fusions and mutations that activate the *JAK-STAT* signaling pathway (*TSLP*, *TYK2*, *FLT3*, *IL7R* and *SH2B3*) (Den Boer et al. 2009, Roberts et al. 2014).

Gene expression analyses are not yet part of the standard diagnostics of ALL. However, since the definition of this subtype is based on the typical gene expression profile and the underlying genetic changes are very heterogeneous, the diagnosis of Philadelphia-like ALL is difficult. However, immunophenotyping can detect *CRLF2* overexpression in about half of the cases (Iacobucci & Mullighan 2017); the mostly underlying and cytogenetically cryptic *CRLF2* rearrangements can be confirmed by fluorescence in situ hybridization. In addition, the karyotype and subsequent FISH analysis can be used to identify a Philadelphia-like ALL by detecting rearrangements of the *JAK2* and *EPOR* genes and *ABL* class genes. A further genetic characterization is possible by molecular genetic analysis, e.g. RNA sequencing (Harvey & Tasian 2020).

The Philadelphia-like ALL is associated with an unfavourable prognosis (Roberts et al. 2014, Harvey et al. 2020). *In vitro* and *in vivo* studies could show a sensitivity of ALL blasts to tyrosine kinase inhibitors (Tasian et al. 2017, Roberts et al. 2017). Several clinical studies are currently evaluating the therapeutic benefit of tyrosine kinase inhibitors in Philadelphia-like ALL.

iAMP21 (provisional WHO entity)

Intrachromosomal amplification of chromosome 21 is detected in about 3% of children with B-precursor ALL, in adults this subtype is very rare (<1%) (Gu et al. 2019). The prognostic significance of this abnormality remains controversial. On the one hand, there are indications that iAMP21 should be assigned to high-risk abnormalities. On the other hand, the MRD status of these patients is possibly a stronger marker on which therapeutic strategies should be based (Moorman et al. 2016).

B-lymphoblastic leukemia/lymphoma, not further classified (NOS)

This group of B-precursor-ALL shows none of the genetic abnormalities defined by WHO 2017 that allow an assignment to an entity. However, rare recurrent genetic abnormalities are also found here.



Translocations of the *CEBP* family

In about 1% of B-ALL patients, transcription factors of the *CEBP* family are involved in a rearrangement with the *IGH* locus. These include the translocations t(8;14)(q11;q32) (*IGH-CEBPD*), t(14;14)(q11;q32) (*IGH-CEBPE*), t(14;19)(q32;q13) (*IGH-CEBPA*) and t(14;20)(q32;q13) (*IGH-CEBPB*). *IGH* translocations are associated with an unfavourable prognosis (Moorman et al. 2016, Lafage-Pochitaloff et al. 2017).

ZNF384 rearrangements

ZNF384 rearrangements are detected in about 3% of pediatric and 7% of adult B-precursor ALL patients, with different translocation partners described. Typically, a pro-B-ALL with expression of myeloid antigens or a mixed phenotype acute leukemia (MPAL) is diagnosed. According to the current state of knowledge, the prognosis in children and adults can be classified as intermediate (Iacobucci & Mullighan 2017, Gu et al. 2019).

Rare genetically defined subgroups

In various research projects, RNA sequencing was used to identify further subtypes that differ from each other in their genetic characteristics. These include ALL with *DUX4* rearrangements associated with a favourable prognosis, *MEF2D* rearrangements and *PAX5* alterations (Iacobucci & Mullighan 2017, Gu et al. 2019, Mullighan et al. 2019). With the *PAX5* P80R mutation, a subtype was identified for the first time that is defined by a point mutation. In addition, groups were detected that exhibit an *ICZF1* N159Y mutation or rearrangement involving the genes *BCL2/MYC*, *HLF* (usually *TCF3/TCF4-HLF*) or *NUTM1* and that show distinct gene expression profiles. For further cases that have not been classified so far, it could be shown that they correspond to already existing phenotypes without showing the respective characteristic genetic change (*ETV6-RUNX1*-like, *KMT2A*-like, *ZNF384*-like ALL) (Iacobucci & Mullighan 2017, Gu et al. 2019, Mullighan et al. 2019). These results show the heterogeneity of acute lymphoblastic ALL.

Subgroups of ALL of the T cell series

Early T-cell precursor lymphoblastic leukemia (ETP) (according to WHO 2017)

In the WHO 2017 classification, ETP is listed as a separate entity. ETP-ALL occurs in about 11% of children and 7% of adults and is formed from thymus cells in the early differentiation stage of T-cell precursors (ETP). Due to their low differentiation potential and their similarity to haematopoietic stem cells and myeloid progenitor cells, their immunophenotype, besides the absence of CD1a, CD8 and the weak expression of CD5, shows positivity for one or more stem cell markers or myeloid antigens. The gene expression profile is also more similar to myeloid leukemias than T-cell leukemias. A lower incidence of *NOTCH1* mutations and a frequent occurrence of *FLT3*, *DNMT3A*, *IDH1* and *IDH2* mutations as well as mutations of the *RAS* gene family have been described (Zhang et al., 2012). Furthermore, ETP-ALL is associated with a significantly worse prognosis in children and young adults compared to other T-ALL/LBL subtypes (Coustan-Smith et al. 2009).

Molecular subgroups of the T-ALL

Gene expression studies, in addition to the classification of T-ALLs by their immunophenotype, have also identified molecular subgroups with unique gene expression signatures. The four subgroups are based on the overexpression of the transcription factors **TAL1**, **TLX1**, **TLX3** and the genes of the **HOXA** gene cluster. About 3% of pediatric patients with T-ALL show aberrant expression of **TAL1** due to translocation t(1;14)(p32;q11). Furthermore, the cytogenetic cryptic intrachromosomal deletion in the short arm of chromosome 1 leads to the fusion gene *STIL-TAL1*. Both mechanisms lead to an overexpression of *TAL1*. Another recently discovered mechanism shows changes in the chromatin structure near the *TAL1* gene, indicating the presence of a new enhancer region. Detailed analysis of this region led to the identification of mutations that result in a de novo binding site for *MYB*, which leads to the recruitment of additional transcriptional regulators and activation of *TAL1* expression (Navarro et al. 2015, Mansour et al. 2014).

In contrast, 8% of infantile T-ALLs and 20% of adult T-ALLs exhibit translocations involving the *TRA/D* locus (14q11) or the *TRB* locus (7q34) with the **TLX1** oncogene (t(10;14)(q24;q11), t(7;10)(q34;q24)), resulting in the overexpression of *TLX1*. The overexpression of *TLX1* seems to be associated with a more favourable prognosis and a low risk of recurrence (Ferrando et al. 2004).

Another subgroup is defined by the overexpression of the **TLX3** transcription factor, which is caused by the translocation t(5;14)(q35;q11) or t(5;14)(q35;q32), where *TLX3* is under the control of the *TRA/D* locus (14q11) or the *BCL11B* gene (14q32). In contrast to *TLX1*, the prognosis seems to be less favourable and recurrences have been described more frequently in patients with *TLX3* overexpression (Baak et al. 2008).

The fourth molecular subgroup shows an aberrant expression of the **HOXA** gene cluster (7p15). Cytogenetically recurrent alterations resulting in overexpression of genes of the *HOXA* cluster were described in 5% of infantile T-ALLs and 8% of adult T-ALLs. Inversion 7 (inv(7)(p15q34)) predominantly leads to overexpression of *HOXA9* and *HOXA10*. Cryptic deletions in 9q34 leading to the fusion gene *SET-NUP214* and the translocation t(10;11)(p13;q10) leading to the *PICALM-MLLT10* fusion gene have also been associated with the overexpression of *HOXA* genes.

References

You can find the corresponding references here:

<https://www.mll.com/en/diagnostic-offer/acute-lymphoblastic-leukemia-all/acute-lymphoblastic-leukemia-all.html#references>