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Cytogenetics in the management of T-cell acute lymphoblastic leukemia (T-ALL): Guidelines from the Groupe Francophone de Cytogénétique Hématologique (GFCH)

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ABSTRACT

Molecular analysis is the hallmark of T-cell acute lymphoblastic leukemia (T-ALL) categorization. Several T-ALL sub-groups are well recognized based on the aberrant expression of specific transcription factors. This recently resulted in the implementation of eight provisional T-ALL entities into the novel 2022 International Consensus Classification, albeit not into the updated World Health Organization classification system.

Despite this extensive molecular characterization, cytogenetic analysis remains the backbone of T-ALL diagnosis in many countries as chromosome banding analysis and fluorescence in situ hybridization are relatively inexpensive techniques to obtain results of diagnostic, prognostic and therapeutic interest.

Here, we provide an overview of recurrent chromosomal abnormalities detectable in T-ALL patients and propose guidelines regarding their detection. By referring in parallel to the more general molecular classification approach, we hope to offer a diagnostic framework useful in a broad clinical genetic setting.

1. Introduction

T-cell acute lymphoblastic leukemia (T-ALL) arises when progenitor T-cells are blocked at an early maturation stage and start to accumulate in the peripheral blood, bone marrow and (non-)lymphoid tissues, including the thymus and central nervous system. In case of tissue invasion with limited bone marrow involvement (less than 20 % of blast cells), lymphoblastic lymphoma is the preferred term.

T-ALL is characterized by the presence of chromosomal abnormalities (CAs) which lead to kinase hyperactivation and/or ectopic expression of transcription factors (TFs). Rearrangements typically involve one of the T-cell receptor (TCR) genes, although many distinct partners or fusion genes have been described. Less frequently, intragenic alterations disrupt the normal gene function and lead to leukemia development [1,2]. In sporadic cases, erroneous V(D)J recombination causes deregulation of T-cell lineage associated genes by juxtaposing typical B-cell enhancers (e.g. *IGH, IGK, IGL*) [3].

T-ALL is a rare disorder (0.1–0.5/100 000 population), representing only 15–25 % of pediatric and adult ALL cases respectively [4,5]. While the cure rates in children are high in case of intensive treatment regimens (reaching 90 %), relapse cases and adult T-ALL patients fare significantly less well. The most important predictor of poor outcome is minimal residual disease detection [4,6–8].

In contrast to B-ALL, the impact of genetic aberrations on T-ALL prognosis is not clearly established and the existence of clinically relevant T-ALL subtypes is still under debate. Only early T-cell precursor lymphoblastic leukemia (ETP-ALL), a T-cell neoplasm with unique immunophenotypic characteristics, is considered a separate entity according to the novel 2022 World Health Organization (WHO) and International Consensus (ICC) classifications. No further subdivision for T-lymphoblastic leukemia/lymphoma not otherwise specified (T-ALL NOS) is provided by the WHO. The ICC however proposes 8 provisional categories based on the aberrant expression of the following TFs: *HOXA, SPI1, TLX1, TLX3, NKX2, TAL1/TAL2, LMO1/LMO2* and *BHLH*/other [2,9].

Despite the lack of apparent prognostic influence of CAs in T-ALL, cytogenetic analysis is recommended at diagnosis and may have therapeutic consequences. It is generally complemented with molecular investigations such as RNA-sequencing and/or Optical Genome Mapping (OGM) to detect otherwise cryptic variants [10–13]. These techniques are however relatively expensive and therefore not yet globally available.

Here, we offer guidelines regarding the cytogenetic analysis in T-ALL based on a thorough review of the most recent data in literature, while keeping the molecular classification methods at close hand. We also provide a brief overview of typical immunophenotypic characteristics and their cytogenetic correlates, hereby acknowledging the importance of flow cytometry in distinguishing ETP-ALL as well as other T-ALL subtypes.

2. Immunophenotypic characteristics

In the past, T-ALL has been subdivided based on immunophenotypic characteristics and TCR chain expression. The classification of the European Group for the Immunological Characterization of Leukemia (EGIL) is still used and distinguishes four T-ALL sub-groups, all $cCD3^+$, based on the physiological T-cell differentiation stage and dependent on the expression of the following cytoplasmic (c) and cell surface (s)

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Table 1
Cytogenetic abnormalities in T-ALL.

Cytogenetic abnormality*	Partner gene 1 (chromosome location)	Partner gene 2 (chromosome location)	Overexpression	Frequency and age class	Most frequently associated phenotype and clinical feature	Prognostic impact	Most frequently associated abnormalities	References
del(1)(p32p32)	*STIL* (1p32)	*TAL1* (1p32)	*TAL1*	25-30% (children) 10% (adults)	Mature and late cortical T-ALL	Undetermined	*PTEN* inactivation, 6q14-q16 deletion and *MYC* translocations *LMO* overexpression frequently associated with *TAL* and/or *LYL1* overexpression	[21,22,26,37,43,45,71,72]
t(1;14)(p32;q11)	*TRA* (14q11)			3%				
t(1;7)(p32;q34)	*TRB* (7q34)			< 1%				
t(7;9)(q34;q32)	*TRB* (7q34)	*TAL2* (9q32)	*TAL2*	1-2%				
t(11;14)(p15;q11)	*TRD* (14q11)	*LMO1* (11p15)	*LMO1*	1-5%	Mature and late cortical T-ALL			
t(11;14)(p13;q11)	*TRD* (14q11)	*LMO2* (11p13)	*LMO2*	21% (adults) 13% (children)	Immature	Undetermined		
del(11)(p13p13)	-							
del(11)(p13p12)	-							
t(7;10)(q34;q24)	*TRB* (7q34)	*TLX1* (10q24)	*TLX1*	20-30% (adults) 5-10% (children)	Cortical T-ALL (CD1a+)	Favorable (unless also *ABL1* amplification)	*CDKN2A/CDKN2B* deletion, *NOTCH1, PHF6, WT1, PTPN2* mutations, *BCL11B* inactivation *NUP214::ABL1*	[21–23,26,37,43,45,72–74]
t(10;14)(q24;q11)	*TRA* (14q11)							
t(5;7)(q35;q21)	*CDK6* (7q21)	*TLX3* (5q35)	*TLX3*	5-9% (adults) 20-25% (children)	Cortical T-ALL (CD1a+)	Undetermined		
t(5;14)(q35;q11)	*TRD* (14q11)							
t(5;14)(q35;q32)	*BCL11B* (14q32)							
inv(7)(p15q34)	*TRB* (7q34)	*HOXA* (7p15)	*HOXA*	3-5%	Immature, ETP or near ETP	More likely unfavorable especially if ETP-ALL	*NRAS, KRAS, BRAF* and *FLT3* mutations	[21,43,45,71,72,75]
t(7;7)(p15;q34)								
del(9)(q34q34)	*SET* (9q34)	*NUP214* (9q34)		1-3%				
t(9;9)(q34;q34)								
t(10;11)(p13;q14)	*PICALM* (11q14)	*MLLT10* (10p13)		5% (children) 10% (adults)				
t(v;11p15)	variable	*NUP98* (11p15)		<1%				
t(v;11q23)	variable	*KMT2A* (11q23)		3% (adults), 10% (children)				
t(1;11)(p36.1;p11)	*SPMN1* (1p36)	*SPI1* (11p11)	*SPI1*	<5%		Undetermined		[37,48]
t(5;11)(q31;p11)	*TCF7* (5q31)							
t(11;14)(p11;q32)	*BCL11B* (14q32)							
t(v;5q35.1)	variable	*NKX2-5* (5q35)	*NKX2*	<1% (adults), 5-7% (children)	Cortical T-ALL (CD1a+)	Favorable	*CDKN2A/CDKN2B* deletion, *NOTCH1* mutations	[21,23]
t(v;14q13.3)	variable	*NKX2-1* (14q13)						
t(v;20p11.22)	variable	*NKX2-2* (20p11)						
t(6;7)(q23;q34)	*TRB* (7q34)	*MYB* (6q23)	*MYB*	<5% (mostly children < 3 years)	Cortical T-ALL, high leucocytosis, CNS involvement	Refractory or late relapses described	*NOTCH1* and *FBXW7* mutations, *CDKN2A* deletion. *HOXA, TLX1/3* and *NKX* subgroup	[21,50,52,76,77].
t(7;9)(q34;q34)	*TRB* (7q34)	*NOTCH1* (9q34)	*NOTCH1*	<1%	/	Undetermined	/	[72]
t(7;19)(q34;p13)	*TRB* (7q34)	*LYL1* (19p13)	*LYL1*	<1%	Immature, pro T-ALL	? Unfavorable	/	[22,23,47,71,72]
t(14;21)(q11;q22)	*TRA* (14q11)	*OLIG2* (21q22)	*OLIG2*	<1%	/	? Unfavorable	/	[55,78]
t(9;12)(p24;p13)	*ETV6* (12p13)	*JAK2* (9p24)	*JAK2*	<1%	/	Undetermined	/	[78,79]
t(v;9q34)	variable	*ABL1* (9q34)	*ABL1*	8% (*NUP214::ABL1*: 6%; *BCR::ABL1*: <1%)	Sensitive to TKi	Intermediate	*NUP214::ABL1* as secondary abnormality in *TLX1/TLX3* subgroups, RAS-pathway mutations	[25,32,35,38]
t(v;8q24)	variable	*MYC* (8q24)	*MYC*	6% (children and adults equally)	High leukocytosis and poor response to treatment Cortical/mature T-ALL	Unfavorable	Associated with *TAL/LMO* subgroups and *PTEN* inactivation. Can be primary or secondary. Trisomy 6 and 7.	[25,58]
t(v;14q32)	variable	*BCL11B* (14q32)	*BCL11b*	1%	ETP-ALL	Undetermined	*FLT3, DNMT3A, TET2* and *WT1* mutations	[21,24,37]
del(17p)	/	/	/	2-3% (adults)	/	Undetermined	/	[80]
del(9p)	/	/	/	50-70% (children and adults equally)	/	No impact	Majority are cryptic deletions (only 10% is visualized with CBA). Typically biallelic loss.	[1,63,66]
del(6q)	/	/	/	10%	/	Undetermined	Higher median white blood cell count. Loss of *CASP8AP2* correlates with poor early treatment response.	[63,67]
Complex Karyotype (at least 5 abnormalities)	/	/	/	6-8% (adults)	/	Unfavorable	/	[63,80]

* Cytogenetic aberrations in bold indicate primary events in T-ALL.

markers: TI or pro-T (CD7^{+}, CD2^{-}, CD5^{-}); TII, early or pre-T (CD7^{+}, CD2^{+}, CD5$^{+/-}$); TIII or cortical (CD1a^{+}) and TIV or mature (CD1a, sCD3^{+}) T-ALL [14].

A very immature category called ETP-ALL was first described in 2009 and became a provisional full-fledged entity in the WHO-2017 classification, which was later confirmed in the 2022 edition [2,9,15]. ETP-ALL corresponds to 5–17 % of pediatric cases and up to 22 % of adult forms [16–19]. This leukemia demonstrates a particular T-cell phenotype (cCD3^{+}, sCD3^{-}, CD1a^{-}, CD2^{+}, CD5^{dim}, CD7^{+}, CD4^{-}, CD8^{-}) associated with the expression of myeloid and stem cell markers such as HLA-DR., CD13, CD33, CD34 and CD117. A very heterogeneous genomic profile is observed with abnormalities in numerous genes (*FLT3, RUNX1, GATA, ETV6,*…), whereas a lower incidence of *NOTCH1* mutations and *CDKN2A* deletions is noted. Recently, chromothripsis was described as a frequent event in this subtype [20].

Initially, ETP-ALL has been associated with a poor prognosis and unfavorable response to standard intensive chemotherapy with high risk of relapse [15]. However, this seems to be more controversial in recent studies, especially when allogenic stem-cell transplantation is applied [18]. A "near-ETP" category has also been described which gathers T-ALL patients with similar characteristics yet whose CD5 expression is not low enough to meet the ETP-ALL definition [15].

There seems to exist a preferential association between certain gene expression profiles and the T-ALL immunophenotype, although the connection is not very strict. Indeed, T-ALL cases with *TAL* and/or *LMO* deregulation are more likely to be cortical or mature T-ALL with CD1a and sCD3 expression respectively. Conversely, *HOXA* positive and *BCL11b* positive T-ALL more often have an early immature phenotype or even an ETP presentation. Finally, leukemic cells with a *TLX1/TLX3* signature often present as early and cortical T-ALLs [2,21–24].

3. Cytogenetic and molecular diagnostics

See Table 1 for a detailed overview of the primary and secondary CAs in T-ALL. Primary CAs are considered driving events in the development of T-ALL that can be present as the sole aberration, while secondary CAs

typically appear later on during the course of the disease as additional events [21,25,26].

3.1. Primary chromosomal abnormalities in T-ALL

3.1.1. Aberrant TAL1/TAL2 expression

- Translocation t(1;14)(p32;q11)/*TRA::TAL1*
- Translocation t(1;7)(p32;q34)/*TRB::TAL1*
- Deletion del(1)(p32p32)/*STIL::TAL1*
- Translocation t(7;9)(q34;q32)/*TRB::TAL2*

TAL1 and *TAL2* are TFs involved in normal hematopoietic differentiation. Deregulation of *TAL1* expression is mostly due to a cryptic deletion (around 80–90 kb) that brings the *TAL1* coding region directly under control of the *STIL* enhancer. This del(1)(p32p32)/*STIL::TAL1* is present in 30 % of T-ALL patients [27]. Translocations involving one of the T-cell receptor genes and *TAL1* are rare, occurring in only 1 to 3 % of patients. Alternatively, a subset of patients acquires intergenic somatic mutations creating a so-called super-enhancer with constitutive activation of *TAL1* as a consequence [28]. In rare cases (1-2 % of T-ALL) a translocation is seen between *TRB* and a region downstream of *TAL2*. This t(7;9)(q34;q32)/*TRB::TAL2* is likely mediated by the V(D)J recombinase which recognizes an incidental recombination signal sequence on chromosome 9. Subsequent rearrangement of the locus leads to the typical *TAL2*/Jb2 junctions observed in T-ALL [29]. Both *TAL1* and *TAL2* rearrangements confer a poor prognosis [2].

3.1.2. Aberrant LMO1/LMO2 expression

- Translocation t(7;12)(q34;p12) [*TRB::LMO3*]
- Translocation t(11;14)(p15;q11) [*TRD::LMO1*]
- Translocation t(11;14)(p13;q11) [*TRD::LMO2*]
- Deletion del(11)(p13p12) [*LMO2* rearrangement]
- Enhancer/promotor mutations

Rearrangements of *LMO1* and *LMO2* are seen in 5 and 10 % of T-ALL cases respectively, often accompanied by *TAL1* activation [2,21]. While translocations involving *LMO1/2* and the TCR genes are relatively rare (roughly 9 % of T-ALL), ectopic expression of *LMO* is seen in a large proportion of T-ALL patients due to cryptic abnormalities, e.g. del(11)(p13p12), and/or enhancer/promotor mutations [26,27,30]. A rare case with rearrangement of *LMO3* (12p12) has also been reported and likely represents a similar mechanism of action [31].

The prognostic significance of these changes is indeterminate.

3.1.3. Aberrant TLX1 expression

- Translocation t(10;14)(q24;q11) [*TRA::TLX1*]
- Translocation t(7;10)(q34;q24) [*TRB::TLX1*]

TLX1 rearrangements are seen in 5–10 % of childhood and up to 30 % of adult T-ALL cases. Both the t(10;14)(q24;q11)/*TRA/D::TLX1* as well as the variant translocation t(7;10)(q34;q24)/*TRB::TLX1* are associated with a favorable prognosis [2]. Additional CAs are observed in half of the cases, in particular *ABL1* amplification [25,32].

3.1.4. Aberrant TLX3 expression

- Translocation t(5;14)(q35;q11) [*TRD::TLX3*]
- Translocation t(5;14)(q35;q32) [*BCL11B::TLX3*]
- Translocation t(5;7)(q35;q21) [*CDK6::TLX3*]

In contrast to *TLX1, TLX3* rearrangements are more common in childhood (20–25 %) than in adult T-ALL (<5 %) [2]. Different partners that cause *TLX3* deregulation have been identified. *CDK6* is involved in cell cycle progression and regulatory regions of *CDK6* cause ectopic expression of *TLX3* upon translocation. Similarly, the cryptic translocation t(5;14)(q35;q32)/*BCL11B::TLX3* activates *TLX3* through potent enhancers located downstream of *BCL11B* at 14q32 [33,34].

TLX3 rearrangements mainly correlate with a favorable prognosis, unless when associated with *ABL1* amplification, a typical secondary event in both *TLX1* and *TLX3* positive T-ALL [2,21,35].

3.1.5. Aberrant HOXA expression

- Inversion inv(7)(p15q34) and translocation t(7;7)(p15;q34) [*TRB::HOXA10*]
- Translocation t(v;11q23) [*KMT2A* rearrangement]
- Translocation t(10;11)(p13;q14) [*PICALM::MLLT10*]
- Deletion del(9)(q34q34) or translocation t(9;9)(q34;q34) [*SET::NUP214*]
- Translocation t(v;11p15) [*NUP98* rearrangement]
- Rearrangements involving *HOXA13, ZFP36L2* and *ETV6*

Around 5 % of T-ALL patients experience overexpression of homeobox gene 10 (*HOXA10*) due to (usually cryptic) inversions or translocations affecting this locus on the short arm of chromosome 7 [36].

Other variants associated with aberrant *HOXA* expression include rearrangements of *KMT2A* [t(v;11q23)], *ZFP36L2* (2p21) or *ETV6* (12p13). In addition, t(10;11)(p12;q14)/*PICALM::MLLT10* and fusions involving 3′*NUP214* [del(9)(q34q34), t(9;9)(q34;q34)] leading to *SET::NUP214*, or *NUP98* (11p15) also cause ectopic activation of the homeobox genes. As a consequence, nearly 25 % of T-ALL patients suffer *HOXA* dysregulation [37].

Survival of these patients highly depends on the accompanying structural variant. Both del(9)(q34q34) and t(9;9)(q34;q34)/*SET::NUP214* for example are cryptic aberrations associated with corticosteroid and chemotherapy resistance, yet without impact on overall survival [38]. An intermediate prognosis is observed in patients carrying 11q23/*KMT2A* rearrangements. Although many different fusion partners of *KMT2A* have been described, the principal partner genes in T-ALL are *MLLT1*, located on 19q13.3, and *AFDN*, located on 6q27 [38].

Multiple fusion partners have also been described in *NUP98* positive T-ALL, including *NSD1* (5q35), *SETBP1* (18q12), *CCDC28* (6q24), *IQCG* (3q29), *ADD3* (10q25) and, most frequently, *RAP1GDS1* (4q21) [39]. *NUP98* rearrangements have a poor prognostic impact in T-ALL [40]. Similarly, adult (~10 %) and pediatric (4–8 %) patients suffering a translocation t(10;11)(p13;q14)/*PICALM::MLLT10* have unfavorable outcome, although this might in particular hold true for cases with more immature phenotypes [38,41–43]. Both the *PICALM::MLLT10* and *NUP98* fusion genes are recurrently observed in acute myeloid leukemia, indicating a common origin in multipotent hematopoietic progenitor cells.

More recently, novel translocations have been described in T-ALL where *HOXA* overexpression is due to the juxtaposition of active enhancers from the *BCL11B, ERG* or *CDK6* gene upstream of *HOXA13*. These CAs are correlated with worse overall survival in pediatric and young adult T-ALL patients and are mostly present in ETP-ALL [40,44]. Moreover, rearrangements in *ZFP36L2* and *ETV6* have also been associated with the development of T-ALL or mixed phenotype T/myeloid leukemia [45,46].

3.1.6. Aberrant SPI1 expression

- Translocation t(5;11)(q31;p11) [*TCF7::SPI1*]
- Translocation t(1;11)(p36.1;p11) [*SPMN1::SPI1*]
- Translocation t(11;14)(p11;q32) [*BCL11B::SPI1*]

Another rare (<5 %) yet recurrent translocation in T-ALL involves

the *SPI1* locus, located on the short arm of chromosome 11 (11p11). Typical fusion partners include *STMN1* (1p36.11), *TCF7* (5q31.1) or *BCL11B* (14q32.2). *SPI1* fusions are usually observed in pediatric leukemia cases where they seem to correlate with a dismal outcome, although more observations are needed to firmly establish their prognostic impact [47–49].

3.1.7. Aberrant NKX2 expression

- Translocation t(v;14q13.3) [*NKX2–1* rearrangement]
- Translocation t(v;20p11.22) [*NKX2–2* rearrangement]
- Translocation t(v;5q35.1) [*NKX2–5* rearrangement]

Deregulated expression of *NKX2* can be due to translocations involving 14q13/*NKX2–1*, 20p11/*NKX2–2* or 5q35/*NKX2–5*, but also to intergenic structural alterations and/or mutations creating unexpected enhancer regions. Rearrangements typically involve one of the TCR genes, although *IGH, BCL11B, CDK6* and *DIO2* have also been described as juxtaposed partners. *NKX2* overexpression is mostly described in children (<5 %) and seems to correlate with a favorable prognosis [21,26,37,49].

3.1.8. MYB overexpression

- Translocation t(6;7)(q23;q34) [*TRB::MYB*]

This rare translocation (<5 % of T-ALL) is usually observed in children below the age of 3, uncommonly young for T-ALL development [50]. Although the variant can be detected with chromosomal banding analysis (CBA) in case of high quality metaphases, Fluorescence In Situ Hybridization (FISH) analysis is preferred. The translocation causes *MYB* deregulation, disturbing the normal T-cell maturation process which requires knockdown of *MYB* to allow differentiation. Duplication and hence overexpression of the *MYB* oncogene has also been described in 10 % of T-ALL cases, as well as extra-chromosomal amplification and somatic *MYB* mutations (2-3 % of patients) [51,52].

3.1.9. NOTCH1 overexpression

- Translocation t(7;9)(q34;q34)/*TRB::NOTCH1*

Mutations affecting *NOTCH1* have been described in about 65 % of T-ALL patients. In contrast, the translocation t(7;9)(q34;q34)/*TRB::NOTCH1* is extremely rare, present in less than 1 % of cases. Constitutive *NOTCH1* activation blocks maturation of T-cell progenitors, while also acting as a driver of proliferation. *MYC* is known to play an essential role in the *NOTCH1*-induced transformation process [53].

3.1.10. Other CAs

- Translocation t(7;19)(q34;p13) [*TRB::LYL1*]
- Translocation t(14;21)(q11;q22) [*TRA::OLIG2*]

The translocation t(7;19)(q34;p13)/*TRB::LYL1* has been uniquely described in T-ALL. It is present in <1 % of the cases and seems to be associated with an unfavorable treatment response. The activation of *LYL1* is linked to a pro-T/stem cell-like signature ($CD34^+$) [23,47,54].

Another rare and presumably unfavorable translocation is the one between 14q11/*TRA* and 21q22/*OLIG2*. It is believed that *OLIG2* acts as a functional inhibitor of TCF3-mediated transcription activation, required for normal T-cell differentiation [55,56].

3.2. Secondary chromosomal abnormalities and complexity in T-ALL

3.2.1. ABL1 overexpression

- Translocation t(9;22)(q34;q11) [*BCR::ABL1*]
- Episomes/homogeneously stained region hsr [*NUP214::ABL1*]
- Translocation t(9;12)(q34;p13) [*ETV6::ABL1*]
- Translocation t(9;14)(q34;q32) [*EML1::ABL1*]

ABL1 (9q34) fusions are present in 8 % of T-ALL cases and should be investigated routinely due to their responsiveness to targeted kinase inhibitors. They typically represent secondary events with the exception of translocation t(9;22)(q34;q11) [*BCR::ABL1*]. Philadelphia chromosomes however are very rare (<1 %) in T-ALL and often indicative of chronic myeloid leukemia evolution.

ETV6 and *EML1* have been described as additional, yet extremely rare, fusion partners for *ABL1* in T-ALL.

The most common alteration in this group is the *NUP214::ABL1* fusion gene (6 %), a secondary event almost exclusively detected in *TLX1* or *TLX3* positive T-ALL. FISH analysis in these cases reveals a multitude of extra-chromosomal *ABL1* signals. The additional signals or so-called episomes are the result of the excision of the 9q34 region between the *ABL1* and *NUP214* breakpoints followed by circularization of the fragment. These episomes are further amplified during subsequent cell division. Reinsertion of the fusion into the genome can occur, appearing as homogeneously staining regions with CBA [32,57].

3.2.2. MYC overexpression

- Translocation t(v;8q24) [*MYC* rearrangement]

MYC rearrangements are observed in about 6 % of T-ALL patients, typically as secondary events in cases that express *TAL1* or *LMO*. Rearrangements which involve the TCR loci as well as other partner genes have been described [58].

In general, patients with *MYC* alterations present with a particular molecular profile (loss of *PTEN* and no *NOTCH1* abnormalities) and tend to have a more aggressive course of their disease [58–60].

3.2.3. BCL11B overexpression

- Translocation t(2;14)(q22;q32)/*ZEB2::BCL11B*
- Translocation t(6;14)(q25;q32)/*ARID1B::BCL11B*
- Translocation t(7;14)(q21;q32)/*CDK6::BCLL11B*
- Translocation t(8;14)(q24;q32)/*BENC::BCLL11B*

The four translocations mentioned above are *BCL11B* deregulating events caused by juxtaposition of a heterologous enhancer near the *BCL11B* locus. Although *BCL11B* is a well-known partner gene in some of the previously described T-ALL entities, it was recently suggested to act also as a secondary event in mixed-lineage leukemia expressing both myeloid and T-cell markers, including ETP-ALL. The 3-hit model proposed by Di Giacomo *et al.* suggests that epigenetic mutations (*WT1, DNMT3A, TET2*) cause chromatin opening in a totipotent progenitor cell, followed by rearrangements at 14q32 (*BCL11B*) to be completed with a subsequent *FLT3* hit that further supports leukemic proliferation [24].

The complementary of this entity to other T-ALL categories described here still needs to be deciphered. Especially since all reported cases seemed to express high *SPI1* levels.

3.2.4. No characteristic chromosomal abnormality

In 2019, Olshanskaya *et al.* determined that T-ALL patients with normal cytogenetic test results (CBA and FISH) experience a more favorable prognosis. Abnormal karyotypes are however present in more than half (50–60 %) of the T-ALLs and the correlation between specific

Table 2
GFCH recommendations for cytogenetic analysis in T-ALL.

<table>
<tr><th colspan="2">Karyotype (CBA) result</th><th>FISH
BCR::ABL1 is mandatory for all cases</th></tr>
<tr><td rowspan="6">Informative with recurrent primary abnormality</td><td>t(9;22)(q34;q11)/Ph chromosome</td><td>BCR::ABL1</td></tr>
<tr><td>t(10;14)(q24;q11) or t(7;10)(q34;q24)</td><td>TLX1*</td></tr>
<tr><td>t(v;14q11)</td><td>TRA/D*</td></tr>
<tr><td>inv or t(7q34;v)</td><td>TRB*</td></tr>
<tr><td>t(10;11)(p12;q14)</td><td>PICALM::MLLT10*</td></tr>
<tr><td>t(v;11q23)</td><td>KMT2A*</td></tr>
<tr><td rowspan="4">Normal or non-informative (including abnormal karyotypes without recurrent primary abnormality)</td><td>Insufficient sample or failure: ask for a new BM or PB sample</td><td rowspan="4">First round of FISH
BCR::ABL1
KMT2A (highly recommended)**

Second round of FISH**
TLX1, PICALM::MLLT10, TLX3
ABL class probes ***

Third round of FISH (optional)
MYC, TP53, CDKN2A/B
TRAD, TRB , STIL::TAL1</td></tr>
<tr><td>Secondary abnormalities
(del(6q), del(9p), del(12p), del(17p)...)</td></tr>
<tr><td>Nonspecific ploidy abnormalities
(hypodiploidy 44-45,
hyperdiploidy 47-50, tetraploidy)</td></tr>
<tr><td>Nonspecific structural abnormality
(chromosomal breakpoint suggesting a variant translocation)</td></tr>
</table>

*Confirmation of the primary CA (FISH or other informative technique; whenever possible)
** FISH or other informative technique
*** ABL-class probes: if presence of an evocative chromosomal breakpoint or if refractory /relapse case (*ABL1, ABL2, PDGFRB/CSF1R*)

cytogenetic events and patient outcome is often unclear [1,61].

Complex karyotype (CK) has consistently been associated with inferior outcome in adult patients, yet the number of CAs needed to define a CK (3 or 5) is still under debate. Depending on the definition, CK is observed in 8 to 16 % of adult T-ALLs [62,63].

Adult patients (with ETP-ALL) also seem to be at risk for chromothripsis, with chromosomes 1, 6, 7 and 17 being recurrently affected [20].

Secondary changes in the ploidy status have been described, including hyperdiploid (47–50 chromosomes), hypodiploid (44–45 chromosomes) as well as tetraploid karyotypes. Near-tetraploidy (81–103 chromosomes, 1-2 % of T-ALLs) was recently associated with low-risk disease in pediatric patients [1,64,65].

While deletions are widespread in T-ALL, they can often only be visualized using FISH or molecular analysis. Up to 70 % of T-ALL cases show mono- or bi-allelic loss of 9p21/*CDKN2A*, typically as a secondary event without any correlation with leukemia survival [1,43,63,66]. Another commonly deleted region with undefined prognostic implication is 6q, present in 10 % of T-ALL patients [63]. Of note, deletion of 6q15–16.1 encompassing the *CASP8AP2* apoptotic regulator, mainly found in the *TAL/LMO* subgroup, has been associated with poor early treatment response [21,67].

3.3. Molecular aberrations in T-ALL

The mutational profile of T-ALL will not be discussed in detail. However, mutations in different signaling pathways can be linked to distinct maturational stages or CAs. *HOXA* deregulated leukemia for example is often accompanied by *JAK3* or *STAT5B* alterations, while *TLX1* and *TAL1* positive cases typically present with *PTPN2* or *PIK3R1/PTEN* mutations respectively. On the other hand, mutations in *NRAS* and *FLT3* are more common in immature T-ALL [45], with *FLT3* changes even being detected in over 80 % of ETP-ALL patients. Concurrent, inactivating mutations in *EZH2* and *RUNX1* have been implied as primary events in ETP-ALL murine models [68].

Furthermore, some mutations have been suggested to have an impact on T-ALL prognosis. Variants in *NOTCH1* and *FBXW7* have been associated with a more favorable outcome, at least in the absence of *RAS* or *PTEN* alterations. More recently, PI3K-pathway mutations were linked to a more dismal survival. In the future, the prognostic and therapeutic importance of the T-ALL mutational profile will become more evident [43,69,70].

4. Recommendations

We propose the following recommendations for the cytogenetic analysis of T-ALL patients, based on the review of the literature as well as on our experience with recommended cytogenetic tests in (inter)national study protocols:

General remarks:

- The diagnosis of T-ALL and ETP-ALL is made based on specific morphologic and immunophenotypic features. The GFCH kindly refers to the WHO, ICC and EGIL criteria for more details [2,9,14].
- The frequencies of the different CAs with their molecular counterpart are provided in Table 1, where possible supplemented with an age distribution as well as additional patient/sample characteristics.
- The recommendations take into account the presence of cryptic aberrations [e.g. t(5;14)(q35;q32)/*BCL11B::TLX3*] and of CAs with prognostic (e.g. CK) or therapeutic importance [e.g. t(v;9q34)/*ABL1*

rearrangement]. They also allow for non-cryptic variants that can be difficult to diagnose in case of poor quality metaphases [e.g. t(v;11q23)/*KMT2A* rearrangement].

- The GFCH starts from the principle of an obligatory CBA and FISH *BCR::ABL1* for each T-ALL patient, completed by FISH tests that can be either (highly) recommended (required for therapeutic decision making, included in study protocols or advised for MRD detection) or optional. While the CBA provides a genome wide view, FISH tests can be tailored depending on the EGIL immunophenotype (e.g. t(v;14q32)/*BCL11B* rearrangements in ETP-ALL), patient age and therapeutic options.
- Molecular techniques provide important complementary information and depending on the in-house experience of the genetic laboratory RT-PCR, RNA sequencing or OGM is preferred. A detailed description of these novel applications is foreseen in a separate GFCH guideline (see joint article).
- In general, it is preferred to confirm decisive variants with 2 independent techniques (e.g. CBA and FISH or FISH and RT-PCR).

Our recommendations are detailed further in Table 2:

- For all T-ALL patients, CBA is highly recommended and FISH for *BCR::ABL1* mandatory. In case of amplification or fusions involving the *ABL1* locus tyrosine kinase inhibitors need to be associated to the regular treatment protocol.
- In case of informative CBA, the recurrent primary abnormality needs to be confirmed by FISH (or other informative technique). This is in particular true for t(10;14)(q24;q11)/*TRA::TLX1* or t(7;10)(q34;q24)/*TRB::TLX1* since *TLX1* overexpression is associated with a favorable prognosis. On the other hand, t(10;11)(p13;q14)/*PICALM::MLLT10* and t(5;14)(q35;q32)/*BCL11B::TLX3* with *ABL1* amplification require confirmation as they are associated with unfavorable outcome. *KMT2A* variants [t(v;11q23)] provide an intermediate risk.
- For patients with a normal or non-informative karyotype, FISH for *KMT2A* is highly recommended. We also recommend a second FISH round with *TLX1, PICALM::MLLT10, TLX3* and *ABL* class probes to exclude targetable or prognostically relevant abnormalities. The following probes are considered optional since detection of these CAs provides no direct diagnostic, prognostic nor therapeutic benefit: *MYC, TP53, CDKN2A/B, TRA/D, TRB, STIL::TAL1*. Of note, non-informative karyotypes also include abnormal karyotypes without a typical recurrent primary abnormality. The use of the FISH probes can then be tailored depending on the presence of suspicious chromosomal breakpoints by CBA.
- Patients who relapse are entitled to receive a new cytogenetic work-up consisting of CBA and FISH to confirm/exclude a specific diagnostic CA or to exclude appearance of secondary events with therapeutic implications (e.g. *ABL1* and *ABL* class probes).

5. Conclusion

Here, the GFCH provides an overview of the typical CAs detectable in T-ALL patients supplemented with patient characteristics and prognostic implications when possible. Connections between the CBA and FISH results on the one hand and the molecular classification on the other hand are specified to strengthen the bond between research and routine diagnostic settings. Our guidelines however indicate that correct T-ALL (cyto)genetic diagnosis is equally possible in settings with limited resources or restricted access to molecular facilities. This outline finally results in a set of recommendations that we hope will optimize and standardize cytogenetic testing in the field of hemato-oncology.

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Jolien De Bie[a], Julie Quessada[b,c], Giulia Tueur[d], Christine Lefebvre[e], Isabelle Luquet[f], Saloua Toujani[g], Wendy Cuccuini[h], Marina Lafage-Pochitaloff[b], Lucienne Michaux[a,i,*]

[a] *Center for Human Genetics, University Hospitals Leuven, Herestraat 49, Leuven 3000, Belgium*

[b] *Laboratoire de Cytogénétique Hématologique, Département d'Hématologie, CHU Timone, APHM, Aix Marseille Université, Marseille 13005, France*

[c] *CRCM, Inserm UMR1068, CNRS UMR7258, Aix Marseille Université U105, Institut Paoli Calmettes, Marseille 13009, France*

[d] *Laboratoire d'hématologie, Hôpital Avicenne, AP-HP, Bobigny 93000, France*

[e] *Unité de Génétique des Hémopathies, Service d'Hématologie Biologique, CHU Grenoble Alpes, Grenoble 38000, France*

[f] *Laboratoire d'Hématologie, CHU Toulouse (IUCT-O), Toulouse 31000, France*

[g] *Service de Cytogénétique et Biologie Cellulaire, CHU de Rennes, Rennes 35033, France*

[h] *Laboratoire d'Hématologie, Unité de Cytogénétique, Hôpital Saint-Louis, AP-HP, Paris 75010, France*

[i] *Katholieke Universiteit Leuven, Leuven 3000, Belgium*

[*] Corresponding author at: Center for Human Genetics, University Hospitals Leuven, Herestraat 49, Leuven 3000, Belgium.
E-mail address: lucienne.michaux@uzleuven.be (L. Michaux).