Current Research in Translational Medicine 71 (2023) 103434

Contents lists available at ScienceDirect

Current Research in Translational Medicine

journal homepage: www.elsevier.com/locate/retram

GFCH 2023

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

ARTICLE INFO

Keywords
B-cell acute lymphoblastic leukemia
Cytogenetics
Cytogenomics
WHO classification
ICC classification

ABSTRACT

Cytogenetic analysis is mandatory at initial assessment of B-cell acute lymphoblastic leukemia (B-ALL) due to its diagnostic and prognostic value. Results from chromosome banding analysis and complementary FISH are taken into account in therapeutic protocols and further completed by other techniques (RT-PCR, SNP-array, MLPA, NGS, OGM). Indeed, new genomic entities have been identified by NGS, mostly RNA sequencing, such as Ph-like ALL that can benefit from targeted therapy.

Here, we have attempted to establish cytogenetic guidelines by reviewing the most recent published data including the novel 5th World Health Organization and International Consensus Classifications. We also focused on newly described cytogenomic entities and indicate alternative diagnostic tools such as NGS technology, as its importance is vastly increasing in the diagnostic setting.

1. Introduction

B-cell acute lymphoblastic leukemia (ALL) is a malignant proliferation of immature B lymphoid cells in bone marrow (BM). These leukemic cells (blast cells or blasts) can invade peripheral blood, lymphoid organs - lymph nodes, spleen, and/or the central nervous system (CNS). B-cell lymphoblastic lymphoma is the lymphoma counterpart of B-ALL: a prominent infiltration of lymphoid organs with only minimal BM invasion (less than 20 % blasts), a rare event.

While ALL is a rare disease (40 cases/year/million inhabitants), it represents the most frequent childhood cancer. Pediatric cases correspond to 75 % of ALL cases. B-ALL is more frequent than T-ALL, accounting for 85 % and 75 % of ALL cases in children and adults, respectively.

B-ALL is heterogeneous at the cytogenomic level. Numerous recurrent primary cytogenetic abnormalities (CAs) define specific entities recognized in the successive World Health Organization (WHO) classifications and in the recent International Consensus Classification due their prognostic and/or theranostic value [1,2].

B-ALL prognosis has greatly improved by risk-adapted therapy according to cytogenetics and measurable residual disease (MRD) which are the most powerful predictors of outcome. Five-year overall survival (OS) and event-free survival (EFS) reach 90 % and 85 % in children [3,4]. However, in adults, cure rates do not exceed 60 % [5].

Cytogenetics is thus required at diagnosis but chromosome banding analysis (CBA) in ALL can be hampered by poor chromosome morphology and/or low mitotic index and is therefore always complemented with FISH analysis and/or molecular investigations. Indeed, multiplex ligation-dependent probe amplification (MLPA), chromosomal microarray analysis (CMA) and optical genome mapping (OGM) can identify copy number variations (CNVs) including cytogenetically cryptic CNVs with prognostic value. Additionally, OGM and next generation sequencing (NGS) (RNA and DNA sequencing) can detect structural variants (SVs) such as fusion genes. Finally, NGS may identify mutations that are entity-defining or represent secondary genetic abnormalities with potential prognostic value [1,2].

Here, we propose guidelines for an optimal cytogenomic diagnosis in B-ALL based on a thorough review of the most recent data in literature, mainly focusing on cytogenetics as a cost-effective and validated method allowing a rapid diagnosis of most of the cytogenomic entities taken into account in the current therapeutic protocols.

2. Immunophenotypic features and their relationship with cytogenetic entities (Table 1)

B-ALL is characterized by expression of at least two early B cell markers CD19, CD22, cytoplasmic (c) CD79a. Expression of CD10 and cytoplasmic (c) immunoglobulin (Ig) μ chain allows the EGIL classification based on B-ALL blasts maturation degree [6]. Furthermore, correlations are observed between blasts immunophenotype and CAs [1].

Pro-B ALL (or EGIL B-I type) represents the most immature B-ALL stage, characterized by CD34 expression and no or low CD10 expression. Pro-B ALL frequently harbors t(v;11q23) with *KMT2A* rearrangements (-r) and less frequently *ZNF384-r* [1].

Common-B-ALL (pre-pre-B ALL or EGIL B-II type) with CD10 expression represents the next stage of B-ALL maturation and the largest phenotypic subgroup, associated with the two largest pediatric genetic subtypes, high hyperdiploidy (HeH) and t(12;21)(p13.2;q22.1)/*ETV6*::*RUNX1* and with the largest adult subgroup, the Philadelphia (Ph) positive ALL. In the two latest subgroups, aberrant expression of myeloid

https://doi.org/10.1016/j.retram.2023.103434
Received 9 July 2023; Received in revised form 20 October 2023; Accepted 22 November 2023
Available online 24 November 2023

markers (CD13 and/or CD33) can be observed [1].

Of note, co-expression of B-ALL and myeloid markers, such as cMPO or monocytic markers, can lead to a B/M mixed phenotype acute leukemia (MPAL). *KMT2A-r* and Ph-positive B/M MPAL are WHO recognized entities representing 15 % of B/M MPAL [1]. Other B-ALL defined genetic entities such as *ZNF384-r, DUX4-r* and *PAX5P80R* can present as B/M MPAL. These latter entities and the *KMT2Ar* B-ALL or B/M MPAL can switch to a myeloid or monocytic AML after therapy, especially after anti CD19 or CAR-T cells therapy [1].

Pre-B-ALL (or EGIL B-III type) is characterized by the cμ chain expression and frequently associated with t(1;19)(q23;p13)*/TCF3::PBX1* [1].

Of note, the Burkitt mature B-cell acute leukemia/lymphoma expressing cell surface Ig light chains (previously EGIL B-IV type) is no longer included among B-ALL [1]. This entity is characterized by *IG::MYC* juxtaposition but it is important to note that this CA can also be observed in pre-B or common B-ALL [7].

3. Genetic abnormalities

Cytogenetic abnormalities (CAs) and molecular abnomalities can be primary or secondary. Primary CAs are mainly specific for a subtype of B-ALL recognized as a classical or a newly described WHO entity [1]. The latter are often referred to as B-other ALL and include Ph-like ALL. Secondary CAs as additional CAs (ACAs) represent clonal evolution and can be part of a complex karyotype (CK).

3.1. Primary cytogenetic and molecular abnormalities (Table 1 and 2)

3.1.1. Classical cytogeneticabnormalities

3.1.1.1. Numerical abnormalities. Two main entities are recognized by the WHO classification [1] according to the blast cells modal chromosome number (MCN):

- High hyperdiploidy (HeH): MCN ranging from 51 to 67.
- Hypodiploidy encompassing two different entities:
  - Low-hypodiploidy (LH): MCN ranging from 30 to 40.
  - Near haploidy (NH): MCN ranging from 23 to 29.

HeH is the most frequent cytogenetic entity in pediatric B-ALL, accounting for 25–35 % of cases and associated with a favorable prognosis [3]. In adults, the frequency is much lower: 6–7 % of Ph negative B-ALL and 2–4 % of total B-ALL [8,9]. Classical HeH is defined by a MCN of at least 54, with a characteristic cytogenetic profile: gain of chromosomes X, 4, 6, 10, 14, 17, 18 and 21 in the absence of another stratifying cytogenetic abnormality such as Ph chromosome. Typically, HeH cases lack or present with non-specific structural abnormalities (mainly +1q and del(6q)). An elevated MCN as well as the presence of "favorable" trisomies such as trisomy 4, 10, 17 and 18 have been associated with a better prognosis [10,11]. Conversely, cases lacking trisomy 17 and 18 or displaying only one of these trisomies and trisomy 5 or 20 have been associated with a poorer prognosis [12]. On the other hand, mutations in the *SETD2* epigenetic regulator gene have been associated with a less favorable outcome [13].

LH and NH share some cytogenetic features: loss of chromosomes 2, 3, 4, 7, 9, 12, 13, 15 and 17 as well as the propensity to undergo endoreduplication leading to tetrasomies of retained chromosomes and uniparental disomies (UPD) of chromosomes that were lost in the hypodiploid clone. This doubling clone can be present at diagnosis either with the hypodiploid clone or alone as a "masked hypodiploid clone". The typical chromosomal profile is therefore very helpful to distinguish a masked hypodiploid clone (disomies and tetrasomies of characteristic chromosomes) from a HeH clone (mainly trisomies except for chromosome 21, frequently tetrasomic). Of note, the doubling clone in the LH entity leads to a near-triploid (60–80) modal number, thus LH is also named low hypodiploidy/near triploidy (Ho-Tr) (for review see Ref. [14]).

LH and NH share the same frequency in children (around 0.5 % of ALL) with a median age of 11.5 years and 5 years, respectively [14]. In adults, the frequency of LH is 6–9 % of B-ALL [8,9] increasing with age. No cases of NH have been described after the age of 19 years.

Although LH and NH cases share a poor prognosis, their molecular profile is clearly distinct. The most striking molecular feature, present in 91 % LH cases, is a *TP53* mutation, being constitutional in half of the pediatric cases (see joint article) [15,16].

3.1.1.2. Classical structural abnormalities

t(9;22)(q34;q11)/BCR::ABL1 (Ph–positive ALL). Philadelphia (Ph) chromosome resulting from t(9;22)(q34;q11), the hallmark of chronic myeloid leukemia (CML), is relatively rare in pediatric B-ALL (3 %), but is the most frequent CA in adult B-ALL with an overall incidence of 20–25 %, increasing with age, reaching over 50 % in patients over 50. The phenotype is mostly common B-ALL with frequent expression of aberrant myeloid markers (CD13 and/or CD33).

The t(9;22)(q34;q11) involves the tyrosine kinase *ABL1* (9q34) and the *BCR* genes (22q11) with two main types of *BCR* breakpoints and *BCR::ABL1* transcripts, mBCR (66 %) and MBCR (33 %), both leading to ABL1 constitutive activation. Ph-positive B-ALL was previously associated with a poor prognosis but addition of tyrosine kinase inhibitors (TKI) to the therapeutic regimen has dramatically improved patients outcome [17]. ACAs are observed in 60–70 % of Ph positive B-ALL and include +der(22)t(9;22), +8, del(7p)/-7, del(9p)/-9 and high hyperdiploidy (51–65 chromosomes) [18–20]. The latest, with +2 being a hallmark, must be distinguished from good prognosis Ph-negative HeH.

The prognostic value of ACAs in the TKI era is not established. In adult Ph-ALL, presence of ACAs per se had either no prognostic [20–22] or a poor prognostic value [23,24]. Among ACAs, −9/del(9p) and/or +der(22), in the absence of high hyperdiploidy [25] and monosomal karyotype (MK) mainly due to −7 or −9 [20,23,24] have been associated with a poor prognostic value . More recent studies in adult Ph-ALL have focused on copy number alterations (CNA), mainly del(7p)/*IKZF1* (7p12) deletion and del(9p)/ *CDKN2A/B* (9p21) and *PAX5* (9p13) deletions, but their prognostic value is still discussed [9,26–28].

CBA and *BCR::ABL1* FISH are mandatory in B- ALL within one week after diagnosis in order to ensure rapid diagnosis of Ph-positive ALL and tailored TKI-based treatment. MRD evaluation based on *BCR::ABL1* transcript and *IG/TCR* quantification is mandatory. Recently, discrepancies between these two MRD techniques have led to the identification of a CML-like ALL subtype resembling CML blast phase with potential clinical implications [29–31].

t(v;11q23)/KMT2A-r. KMT2A rearrangements (*KMT2A-r*) represent around 10% of pediatric and adult B-ALL [32]. Half of cases occur in infants (80% of infant cases), then decrease in children and young adults (4 %) and peak again in older adults (15%). *KMT2A-r* ALL mainly present with high WBC counts, a pro-B phenotype with frequent aberrant myeloid markers and can present as a B/M MPAL [1]. More than one hundred translocation 3′partner genes have been identified [32]. Among *KMT2A*-r, t(4;11)(q21;q23)*/KMT2A::AFF1* is the most common (~50%), followed by t(11;19)(q23;p13.3)*/KMT2A::MLLT1* (22%), t(9;11)(p22;q23)*/KMT2A::MLLT3* (16%) and t(10;11)(p12;q23)*/KMT2A::MLLT10* (6%) [33]. Of note, deletion of the 3′part of *KMT2A* is detected by FISH in about 10% of *KMT2A-r* cases leading to an atypical FISH pattern [34].

KMT2A-r ALL present with low ACAs frequency, mainly +X [8] and del(9p21)*/CDKN2A/B* [9].

Interestingly, t(4;11) infants harbor a low mutational burden consistent with the known oncogenic potency of KMT2A fusion proteins and the very short disease latency in infants [35].

KMT2A-r ALL identifies a group of patients with poor prognosis

[8–10,33,36]. Adjunction of blinatumomab, a bispecific T-cell engager molecule targeting CD19, to standard chemotherapy has shown safety and efficacy in *KMT2A*-r ALL infants [37]. Moreover, *KMT2A-r* B-ALL blast cells are sensitive to BCL2 (Venetoclax), menin, DOTL1 and proteasome inhibitors, pointing to opportunities for targeted therapies [38–40].

t(12;21)(p13;q22)/ETV6::RUNX1. The t(12;21)(p13;q22) is a cryptic CA identified in ~25% of pediatric B-ALL cases, most frequently in young children, rarely in infants and exceptionally in adults after the age of 25 years. The characteristic immunophenotype is common B-ALL with frequent aberrant myeloid markers (CD13 and/or CD33) [1]. The translocation results in an in-frame fusion between 5′telomeric *ETV6* (12p13) and 3′centromeric *RUNX1* sequences (21q22) readily detectable by FISH or RT-PCR methods [1,2].

Although the t(12;21) originates *in utero,* gene fusion alone is not likely to cause overt leukemia, since around 1 % of healthy newborns are diagnosed with this translocation [41].

Patients with t(12;21) have a very good prognosis and excellent response to treatment [3,4].

ACAs are found in around 90% of cases, the most common being del (12p)/ETV6 deletion. Other ACAs include del(9p)*/CDKN2A/B* deletion, del(6q), +21 as duplication of the normal or the derivative 21, and tetraploidy [42] with a high diversity in subclones [43] but no prognostic impact [44] Conversely, secondary mutations in the *TBL1XR1* epigenetic regulator gene have been associated with a less favorable outcome [13].

Intrachromosomal amplification of chromosome 21: iAMP21. B-ALL with intrachromosomal amplification of chromosome 21 (iAMP21-ALL) is a distinct WHO entity [1] occurring in 2% pediatric and 1% adult B-ALL, mainly older children and young adults [45–47]. iAMP21 is a high risk form of B-ALL but intensive therapy can greatly improve patients' outcome [47]. The iAMP21 chromosome is an abnormal chromosome 21 generated *via* breakage-fusion-bridge cycles and chromothripsis (CTH) leading to amplification, inversion and deletion of multiple regions of chromosome 21q [48]. The classical feature of iAMP21-ALL is amplification of *RUNX1* (21q22.1) and FISH probes targeting *RUNX1* remain a reliable detection tool for iAMP21-ALL. The classical FISH pattern is at least 3 extra *RUNX1* copies on an abnormal chromosome 21 and/or, on nuclei, at least 5 *RUNX1* copies of which at least 4 are clustered [49]. The diagnosis is suspected on karyotype by the existence of an abnormal chromosome 21 or der(21) [47] and is confirmed by FISH and CMA showing the coexistence of amplified (*RUNX1, ERG*,...) and deleted (mainly subtelomeric) genes on chromosome 21 [49].

Recently, the COG group has shown the existence of atypical or "unusual" forms with extra copies of the *RUNX1* gene located either on a chromosome different from chromosome 21 or on the derivative 21 but in a lesser number [50]. CMA and/or FISH with an *ERG* (21q22.2) probe can be helpful for the detection of these rare cases. Indeed, recent studies have shown a 7.8 Mb common region of amplification including *RUNX1* and *ERG* genes [51].

ACAs are present in about 80 % iAMP21 cases, mainly within a CK. Main ACAs are +X, and structural abnormalities of chromosomes 1q, 7q, 9p, 11q and 12p [47,52]. CMA and NGS studies have shown frequent deletions involving *RB1, IKZF1, ETV6, CDKN2A/B*, and *SH2B3, P2RY8::CRLF2* fusion and a high burden of mutations mainly in the *RAS* signalling pathway [49,51,53,54].

Of note, children with a constitutional structural abnormality of chromosome 21 such as the der(15;21) Robertsonian translocation, ring (21) and isodicentric (21)(q22) are predisposed to iAMP21-ALL (see joint article) [48,50,55,56].

t(1;19)(q23;p13)/TCF3::PBX1. The t(1;19)(q23;p13) translocation fusing the 5′part of the *TCF3* gene (19p13.3) with the 3′ part of the *PBX1* gene (1q23.3) [57], accounts for 3 %−5 % pediatric and adult B-ALL, mainly pre-B ALL [1].

Both balanced t(1;19) and der(19)t(1;19) occur, the unbalanced form being prevalent. Both forms which have been initially associated with poor prognosis and increased risk of CNS relapse, are now, due to treatment intensification, associated with a favorable to intermediate risk [3,8–10].

Most cases present with ACAs, mainly del(9p) frequently as an i(9)(q10) and 1q gains [8,9].

Of note, t(1;19) resembling t(1;19)(q23;p13) at the CBA level, but *TCF3::PBX1* negative at the molecular level, can occur either as a secondary abnormality in HeH and Ph-positive ALL or as a primary CA involving *MEF2D::DAZAP1* (see *MEF2D-r* chapter). Consequently, all t(1;19) cases have to be checked by FISH or another molecular method [57].

t(17;19)(q22;p13)/TCF3::HLF. This primary CA is difficult to detect by CBA and, in some cases, only the der(19)t(17;19) is observed. FISH using TCF3 break apart or *TCF3::HLF* probe or other molecular technique are necessary for identification [1,57,58].

This very rare WHO entity (<1 % B-ALL), associated with a dismal prognosis, is occurring mainly in adolescents and young adults (AYA) that present with high WBC, hypercalcemia, disseminated intravascular coagulation and CNS involvement [3,58–60]. *TCF3::HLF* positive cells have shown a striking sensitivity to the BCL2-inhibitor Venetoclax that can be combined with the bispecific anti CD19 monoclonal antibody (Blinatumomab) as a bridge to allogeneic HSCT [60,61].

t(5;14)(q31;q32)/IGH::IL3. In this very rare entity, the juxtaposition of the *IGH* locus (14q32) to the interleukin-3 (*IL3)* gene (5q31), induces an IL3 overproduction leading to an important eosinophilia that can impair the detection of blast cells and/or CA in the bone marrow. The outcome depends on both eosinophilia-induced damage and acute leukemia. Most patients are AYA, mainly males. *IKZF1* deletions are frequent and the prognosis is considered as intermediate [1,62].

3.1.2. *B-other and Ph-like ALL (Tables 1 and 2)*

3.1.2.1. Ph-like/BCR::ABL1-like ALL. Ph-like ALL represents approximately 12 % and 25 % of childhood and adult B-ALL cases, respectively, mainly found in AYA . This WHO subtype shares a « kinase-activated » gene expression signature with Ph-positive ALL but lacks the t(9;22)(q34;q22)/ *BCR::ABL1* fusion [63,64]. Over 70 genetic aberrations, CAs and mutations, targeting kinase genes have been identified. Like in Ph-positive ALL, *IKZF1* alterations are frequently found in this entity [63–65].

Two main subtypes of Ph-like ALL are currently recognized, based on expression pattern similarities and TKI sensitivity [66–68].

- Fusions involving *ABL*-class genes (mainly *ABL1/2, PDGFRA/B* and *CSF1R*).
- Alterations driving JAK-STAT signaling (including *CRLF2, JAK2* and *EPOR* alterations).

Ph-like ALL are associated with high MRD levels and a poor prognosis. However, TKI may be included in the therapeutic regimen thus improving patients' outcome, especially for ABL-class subtypes [66, 68–70].

ABL-class Ph-like ALL. ABL-class anomalies, including *ABL1* (9q34), *ABL2* (1q25), *CSF1R* (5q32), *PDGFRA* (4q12) and *PDGFRB* (5q32) alterations, represent 19 % of Ph-like ALL and are more frequently observed in children than adults [66,68]. Multiple fusions have been described, all of them preserving the kinase domain and the most frequent being *EBF1::PDGRB* arising from a cryptic del(5)(q32q33). FISH with ABL-class probes is considered the most powerful tool to detect these rearrangements that will be later fully characterized by RNA-seq [66–68]. These B-ALLs are are sensitive to imatinib and other ABL-class TKI.

JAK-STAT class Ph-like B-ALL. JAK-STAT class Ph-like B-ALL represent 70 % of Ph-like ALL and include B-ALL with *CRLF2* deregulation,

JAK2 fusions and *EPOR* rearrangements. Patients show a poor OS. These cases may be sensitive to ruxolitinib or other JAK inhibitors [9,66–68].

Xp22 or Yp11 abnormalities/CRLF2 deregulation. CRLF2 (Xp22/Yp11) deregulation/overexpression is the most frequently detected genetic abnormality in Ph-like ALL (50 % of Ph-like ALL). It usually results from *CRLF2-r* either as a translocation involving the immunoglobulin *(IG)* heavy chain (*IGH*) enhancer, t(X;14)(p22;q32) or t(Y;14)(p11;q32), or more frequently from a cryptic deletion of the pseudo-autosomal region (PAR1) on chromosome X or Y, leading to *CRLF2* juxtaposition to *P2RY8* promoter. *P2RY8::CRLF2* juxtaposition is frequently reported in Down syndrome B-ALL and as a secondary abnormality, mainly in iAMP21 and *PAX5*alt subgroups. While *CRLF2* alterations are cryptic at CBA level, they can be evidenced by FISH analysis using break-apart probes targeting the *P2RY8/CRLF2* locus [71].

CRLF2-r have been associated with *JAK2* non-V617F and, less frequently, with *JAK1* mutations. Associations with *IKZF1* or *SH2B3* mutations have also been reported [66,68].

t(9p24)/JAK2-r. At least 19 fusions involving the *JAK2* (9p24) locus have been described including the *PAX5::JAK2* rearrangement resulting from t(9;9)(p13;p24), inv(9)(p13p24) or del(9)(p13p24). The two latest forms are very difficult to detect with *PAX5* or *JAK2* FISH probes. In all cases, the JAK2 kinase domain is preserved, leading to constitutive activation of the JAK-STAT pathway [65,67].

t(19p13.2)/EPOR. CAs involving *EPOR* (19p13.2) are rare and mainly result from rearrangements with *IG* genes. They lead to a deregulated expression of *EPOR* and sustained JAK-STAT signaling. The most frequent is t(14;19)(q32;p13)*/IGH::EPOR*, mainly found in AYA and associated with an aggressive clinical course [54,65,67,72].

3.1.2.2. B-other ALL, non-Ph-like

Other IG abnormalities (14q32/IGH, 2p12/IGK, 22q11/IGL). IG CAs are found in around 5 % of B-ALL (up to 10 % if we include *DUX4-r* ALL), mainly in AYA [72–74]. *IG* translocations juxtapose an IG enhancer to a partner gene whose transcription is upregulated and thus constitute an important mechanism of oncogenic activation. These CAs can be detected by FISH with IG probes and/or probes targeting *IG* partner genes. However, the identification of the partner gene with IG probes can be challenging, even if metaphase FISH is used. Indeed, FISH with *IG* probes can identify *IG* 5′telomeric deletions related to the physiological V-D-J recombination occurring in B cells. As another important remark for clinical interpretation, *IG* translocations can be present as secondary CAs [7,75]. Besides *IG* CAs described above, we can consider:

ins(14;4)(q32;q35q35) or ins(14;10)(q32;q26q26)/IGH::DUX4 juxtaposition and ERG deletion. DUX4-rearranged (*DUX4*-r) ALLs represent 4% of pediatric and 6% of adult B-ALL, mainly found in AYA. *DUX4* gene is located within a subtelomeric D4Z4 repeat region on 4q35 and 10q26. *IGH::DUX4* rearrangement induces *DUX4* overexpression. Most cases result from a cytogenetically cryptic insertion of part of the *DUX4* gene into *IGH* [76–78]. As a unique phenomenon, DUX4 truncated protein binds to an intragenic region of the transcription factor *ERG* (21q22), resulting in ERG loss of function either by focal deletion (*ERG*-del) and//or by expression of an aberrant ERG isoform (ERG-alt) [77]. *DUX4*-r and *ERG*-del/ERG-alt cases share the same distinctive gene expression profile (GEP), peculiar immunophenotype (CD2+) and frequently deleted *IKZF1* but a good outcome [3,46,79,80]. FISH analysis can detect an abnormal *IGH* signal, as a split in the *IGH* break-apart probe or, more frequently, as a loss of the 5′ telomeric region (W. Cuccuini, personal communication). *ERG* deletion, detected by CMA in around 55 % of *DUX4-r* cases, is also a good surrogate marker. However, RNA-seq is the best tool for diagnosis [49].

t(v;14q32)/IGH::CEBP family juxtaposition. CEBP genes are involved as *IG* partner genes in around 1 % of B-ALL and 11 % of *IGH*-r cases [74,81]. *CEBPA* and *CEBPG* (both located at 19q13), *CEBPB* (20q13), *CEBPD* (8q11) and *CEBPE* (14q11) are juxtaposed to *IGH via* t(14q32) and/or inv(14)(q11q32) for *CEBPE* [72,74,75,81,82]. Of note, *IG::CEBP* have been described as secondary CAs [75]. Interestingly, *IGH::CEBPE* cases cluster with *ZEB2* H1038R mutation cases in a small specific GEP subgroup [46,83].

t(6;14)(p22;q32)/IGH::ID4 juxtaposition. 1 % of B-ALL display the t(6;14)(p22;q32)*/IGH::ID4* juxtaposition, which is mainly found in AYA and associated with an intermediate prognosis [54,72,74]. Most cases present with numerous ACAs including +5 and del(9p) leading to *PAX5* and *CDKN2AB* deletions [54,72].

t(8;14)(q24;q32)/ IGH::MYC and other IG::MYC. A recent international study analyzed 90 cases of adult and pediatric B-ALL harboring an *IG::MYC*-r, representing around 0.5 % of B-ALL [7]. *IG::MYC*-r occurs as a secondary CA in 16 % of cases, the primary CA being *KMT2A-r, DUX4-r* or rarely iAMP21 or *BCR::ABL1* fusion. In two-thirds of cases I*G::MYC*-r is the only defining feature. In the remaining cases (16 %), it co-occurs with a *BCL2* (18q21) and/or more rarely a *BCL6* (3q27) rearrangement. Of note, as described in Burkitt lymphoma, +1q is a prevalent ACA in this B-ALL subtype [7]. Children with *IG::MYC-r* as a primary CA without *BCL2/BCL6*-r had a 3-year EFS and OS of 47 % and 60 %, respectively, thus representing a high-risk BCP-ALL.

t(14;18)(q21;q32)/IGH::BCL2 and t(3;14)(q27;q32)/IGH::BCL6. In B-ALL, t(14;18)(q32;q21)/I*GH::BCL2* and the very rare t(3;14)(q27;q32)*/IGH::BCL6* most often co-occur with an *IG::MYC-r* as in double or triple-hit NHL (see joint article). These subtypes represent 1 to 3 % of adult and young adult B-ALL, respectively, are very rare in children and correlate with a poor prognosis [7,8,84].

t(12p13.3)/ZNF384-r. ZNF384-r leukemias represent a specific B-ALL subtype, mostly with a B-I phenotype (CD10 negative or weak) and aberrant myeloid antigens (CD13 and/or CD33) or B/M MPAL [85–87]. *ZNF384*-r are observed in ~4 % of childhood,~2 % of adult B-ALL, and 48 % of childhood B/M MPAL [9,46,83,84,86]. In t(12p13.3)*/ZNF384-r*, the 3′ telomeric part of *ZNF384* is translocated to create a *v::ZNF384* fusion gene. More than twenty 5′ partner genes have been described, the most frequent being *EP300* (22q13.2), *TCF3* (19p13.3), *TAF15 (17q12)* and *CREBBP* (16p13.3) [83,84,86].

The prognosis of B-ALL with *ZNF384-r* is considered intermediate in children but could depend on the partner gene as *EP300::ZNF384* present with a lower risk of relapse [86]. In adults, it is considered as relatively good [9] to intermediate [46]. Most of *ZNF384-r* are cryptic, being identified by RNA-seq studies [83,84,86]. Of note, *ZNF384-r* can be assessed by FISH with specific break-apart probes [86].

t(1q22)/MEF2D-r. t(1q22)*/MEF2D-r* mainly affects female teenagers and AYA patients and are evidenced in 2–4 % of pediatric and 2 % of adult B-ALL [46,83,88,89].

MEF2D-B-ALLs have been associated with two distinct immunophenotypes either pro-B or pre-B [88–90]. Numerous 3′partner genes have been described, the most frequent being *BCL9* (1q21) and *HNRNPUL1* (19q13.2). Other 3′partners are *DAZAP1 (19p13), CSF1R (5q32), FOXJP1* (12p13) and *SS18 (18q11)* [3,46,49,83,89,90]. Cases with t(1;5)(q22;q32)*/MEF2D::CSF1R* are very rare but present with a Ph-like gene expression and can benefit from *ABL*-class TKI [90,91].

Prognosis is intermediate to poor and could depend on the partner gene and/or expression of CD10 which is more frequent in t(1;19)*/MEF2D::HNRNPUL1* cases [46,89].

Of note, del(1)(q21q22)*/MEF2D::BCL9* is a cryptic CA that can be detected by CMA. Importantly, at the CBA level, t(1;19)*/MEF2D::HNRNPUL1* and t(1;19)*/MEF2D::DAZAP1* are very similar to t(1;19)*/TCF3::PBX1*, therefore FISH analysis with a break-apart probe targeting *TCF3* should be performed for all B-ALL cases harboring a t(1;19). Most *MEF2D*-r cases present with del(9p)*/CDKN2AB* deletion [89,90] .

t(15q14)/ NUTM1 fusions. Fusions of *NUTM1* (15q14) define a novel B-ALL subtype occurring only in pediatric cases, more especially in infants (1 % and 4 % of pediatric and infant cases, respectively) and associated with a favorable outcome [92]. *NUTM1-r* cases are mainly associated with a common or pre-B ALL but *BRD9::NUTM1* cases consistently present with a pro-B ALL [92]. At least ten 5′ partner genes that drive aberrant *NUTM1* expression have been described, the most

Table 1
Primary cytogenetic abnormalities.

Primary cytogenetic abnormalities	Modal chromosome number or genes	Typical chromosome gains/losses and gene location[a]	Frequency in B-ALL by age class	Clinical and biological features	Prognosis	Refs.
Classical numerical abnormalities						
• High hyperdiploidy (HeH)	51 to 67	Trisomies 4, 6, 10, 17, 18, 21	25–35 % (C), 2–4 % (A)	B-II (CD10+)	Favorable[b]	[11,12]
• Near haploidy (NH)	23 to 29	Loss of all chromosomes except X, 14, 18 and 21[c]	0.5 % (C)	*NF1* and *RAS* aberrations	Unfavorable	[14,15]
• Low hypodiploidy (LH) /Near-triploidy (Ho/Tr)	30 to 40/60 to 78	Loss of chromosomes 3, 7, 9, 13 and 17[c]	0.5 % (C),6–9 % (A)	*TP53* mutation (constitutional or somatic)	Unfavorable	[14,15]
Classical structural abnormalities						
•t(9;22)(q34;q11)	*BCR::ABL1*	*BCR***(22q11)**, *ABL1*(9q34)	3 % (C), 30 % (A), 50 % after age 50	B-II (CD10+) with aberrant myeloid markers and B/M MPAL, *IKZF1* intragenic deletion[d], TKI indication, frequency increasing with age	Unfavorable before TKI era	[18,20]
•t(4;11)(q21;q23) and other t (v;11q23)e */KMT2A-r*	*KMT2A::AFF1* *KMT2A::MLLT3* *KMT2A::MLLT1*	*KMT2A***(11q23)**, *AFF1* (4q21), *MLLT3* (9p22) *MLLT1* (19p13.3)	5–10 % (C), 5–10 % (A) 80 % (I)	B-I (CD10-) and B/M MPAL. Switch to AML possible after therapy	Unfavorable	[32,33,36]
•t(12;21)(p13;q22)[e]	*ETV6::RUNX1*	*ETV6*(12p13.2), *RUNX1* **(21q22)**	25 % (C),1 % (A) exceptional >25 years	B-II (CD10+) frequently CD13+ or CD33+,	Favorable	[42,44]
• iAMP(21)	Chromothripsis of 21q At least 3 extra copies of *RUNX1* on a der(21) which are clustered on nuclei	*RUNX1* **(21q22.1),** *ERG* **(21q22.2)**	2 % (C), 1 % (A)	Atypical forms (see text) Constitutional structural abn. of chromosome 21 are predisposing factors Frequent *CRLF2* deregulation and *RB1* deletion	Unfavorable (intermediate if intensive treatment)	[47, 49–51]
• t(1;19)(q23;p13)/der(19)t(1;19)(q23;p13)	*TCF3::PBX1*	*TCF3* **(19p13.3),***PBX1* (1q23.3)	3–5 % (C), 3–5 % (A)	B-III (intracytoplasmic m+)	Intermediate	[3,9,57]
• t(17;19)(q22;p13)[e]	*TCF3::HLF*	*TCF3***(19p13.3)**, *HLF* (17q22)	less than 1 % (C and A)	Teenagers and AYA, DIC, hypercalcemia	Unfavorable	[59–61]
• t(5;14)(q22;q14)	IGH::*IL3*	*IGH* (14q32), *IL3* (5q32)		Eosinophilia	Intermediate	[62]
Ph-like (*BCR::ABL1*-like) abnormalities			25 % (C), 12 % (A) 25–30 % (AYA)	Frequent *IKZF1* intragenic deletion [d]		
• *ABL* class abnormalities			15–20 % of Ph-like ALL	Sensitivity to ABL class TKI as imatinib or dasatinib	Unfavorable before TKI era	[63–65, 70]
ABL1-r/ t(v;9q34) and t(1;9)(q24;q34)	*v::ABL1and RCSD1::ABL1*	*RCSD1* **(1q24)**, *ABL1* (9q34)				
ABL2 -r/ t(v;1q25) and t(1;1)(q24;q25)/inv(1)(q24q25)	*v::ABL2 and RCSD1::ABL2*	*RCSD1* **(1q24)**, *ABL2* (1q25)				
CSF1-r/ t(v;5q32) t(5;5)(q14;q32)	*v::CSF1R SSBP2::CSF1R*	*SSBP2* **(5q14)**, *CSF1R* (5q32)				
t(1;5)(q22;q32)	*MEF2D::CSF1R*	*MEF2D***(1q22)**, *CSF1R* (5q32)				
PDGFRB/ t(v;5q32) and del(5)(q32q33)	*v::PDGFRB and EBF1::PDGFRB*	*EBF1* **(5q33),***PDGFRB* (5q32)				
PDGFRA-r/ t(v;4q12)	*v::PDGFRA*	*PDGFRA* (4q12)				
• *JAK-STAT* activating alterations			70 % of Ph-like ALL	Possible sensitivity to JAK inhibitors		
CRLF2 (Xp22/Yp11) deregulation t(X;14)(p22;q32)/t(Y;14)(p11;q32) del(X)(p22p22)/del(Y)(p11p11)	IGH::*CRLF2* P2RY8::CRLF2[f]	*IGH* (14q32), *CRLF2* (Xp22/Yp11) *P2RY8* (Xp22/Yp11), *CRLF2* (Xp22/Yp11)	50 % of Ph-like ALL	*CRLF2* deregulations frequently associated with DS and *JAK2* mutations *IGH::CRLF2*: mainly initiating event *P2RY8::CRLF2*: frequent secondary event in iAMP(21) and *PAX5* alt ALL	Depending on the primary CA	[71]
JAK2-r / t(v;9p24) and inv(9)(p24p13)/del(9)(p24p13)	*v::JAK2 and PAX5::JAK2* *SSBP2::JAK2* *BCR::JAK2*	*PAX5* **(9p13)**, *JAK2* (9p24) *SSBP2* (5q14) *BCR* (22q11)	7 % of Ph-like ALL		Unfavorable	[65]

(*continued on next page*)

Table 1 (*continued*)

Primary cytogenetic abnormalities	Modal chromosome number or genes	Typical chromosome gains/losses and gene location[a]	Frequency in B-ALL by age class	Clinical and biological features	Prognosis	Refs.
EPOR-r/ t(v;19p13) and t(14;19)(q32;p13) or ins(14;19)	*IG::EPOR and IGH::EPOR*	*IGH* (14q32), *EPOR* (19p13.2)	9 % of Ph-like ALL		Unfavorable	[65,74]
Other defined structural abnormalities						
• **Other *IG* abnormalities**		*IGH* (14q32), *IGK* (2p11), *IGL* (22q11)	10 %		Depending on the IG partner gene	[72,74]
DUX4-r as ins(14;4)(q32;q35q35)/t(4;14)(q35;q32)[e]	*IGH::DUX4*	*DUX4* (4q35)	4 % (C), 6 % (A)	Frequent *ERG* and *IKZF1* intragenic deletion[d]	Favorable (even with *IKZF1* deletion)	[46, 76–78]
CEBP family genes rearrangements including t(14;14)(q11;q32)/inv(14)(q11q32)	*IGH::CEBPA* *IGH::CEBPB* *IGH::CEBPD* *IGH::CEBPE* *IGH::CEBPG*	*CEBPA* (19q13) *CEBPB*(20q13) *CEBPD* (8q11) *CEBPE* (14q11) *CEBPG*(19q13)	1 %	Mainly AYA Primary or secondary CA	Intermediate (depending on the primary CA if secondary CA)	[72,74,75, 81,82]
t(6;14)(p22;q32)	*IGH::ID4*	*ID4* (6p22)	1 %	Mainly AYA	Intermediate	[54,74]
MYC-r	*IG::MYC*	*MYC* (8q24)	1–3 % (A)	Exclude Burkitt lymphoma (IgS+), primary CA and search for *BCL2* (18q22) and *BCL6* (3q27) associated rearrangements	Unfavorable	[7]
BCL2-r and *BCL6-r*	*IG::BCL2* *IG::BCL6*	*BCL2* (18q21) *BCL6* (3q27)	1–3 % (A)		Unfavorable	[7]
• t(v;12p13)/ *ZNF384-r* [e]	*EP300::ZNF384* *TCF3::ZNF384* *TAF15::ZNF384* *CREBBP::ZNF384*	*ZNF384* (12p13.3), *EP300* ***(22q13.2)*** *TCF3* **(19p13.3)** *TAF15* **(17q12)** *CREBBP* **(16p13.3)**	~4 % (C), ~3 % (A), ~4 % (AYA), 48 % of pediatric B/M MPAL	B-I (CD10- or weak) frequently CD13+ and/or CD33+ and B/M MPAL. Switch to AML possible after therapy. Mostly cryptic translocations	Intermediate (better for *EP300::ZNF384*)	[9,46, 83–87]
• t(1q22;v) and del(1)(q21q22)e/ *MEF2D-r* [g]	*MEF2D::BCL9* *MEF2D::HNRNPUL1* *MEF2D::DAZAP1*	*MEF2D* **(1q22)**, *BCL9* (1q21) *HNRNPUL1* (19q13) DAZAP1 (19p13.3)	4 % (C), 2 % (A)	B-I (CD10- or weak) or B-III(CD10+, cμ chain+), CD5+ Teenagers and AYA, mainly female patients	Intermediate depending on CD10 expression and partner gene	[46,83, 88–90]
• t(v;15q14) / *NUTM1* fusions	*ACIN1::NUTM1* *CUX1::NUTM1* *BRD9::NUTM1* *ZNF618::NUTM1*	*NUTM1* (15q14), *ACIN1* ***(14q11)*** *CUX1* **(7q22)** *BRD9* **(5p15)** *ZNF618* **(9q32)**	1 % (C) 4 % (Infants)	Only infants and children; hyperexpression of *NUTM1*	Favorable	[83,84,92, 93]
• t (9p13) and dic(9p13)/ *PAX5* fusions /*PAX5* alt [h]			3 % (C), 1 % (A)	*CRLF2* deregulation is a frequent secondary event	Intermediate	[46,83,84, 94,95]
dic(9;12)(p13;p13) [i]	*PAX5::ETV6*	*PAX5* **(9p13)**, *ETV6* (12p13)				
dic(9;20)(p13;q11) [i]	*PAX5::NOL4L*	*NOL4L*(20q11)				
t(3;9)(p14;p13)	*PAX5::FOXP1*	*FOXP1*(3p14)				
t(7;9)(q11;p13)	*PAX5::AUTS2*	*AUTS2*(7q11)				
t(7;9)(q11;p13)	*PAX5::POM121*	*POM121* (7q11)				
t(7;9)(q11;p13)	*PAX5::ELN*	*ELN* (7q11)				

Abbreviations: A: adults; AYA: adolescents and young adults; C: children; DIC: disseminated intravascular coagulation; DS: Down Syndrome; I: infants; -r: rearrangement; TKI: tyrosine kinase inhibitors.

[a] main location of the critical fusion gene is indicated in bold. it does not apply to gene juxtapositions such as *IG::v* juxtapositions and to insertions.

[b] cases with trisomies 4, 10, 17, 18 and modal chromosome number higher than 53 are associated with an even better prognosis.

[c] can present as duplicated clone (see text).

[d] detected by PCR.

[e] cryptic or possibly cryptic translocation, FISH or RT-PCR must be performed.

[f] resulting from juxtaposition of *IGH* enhancer or *P2RY8* promotor at the vicinity of *CRLF2* gene.

[g] t(1;5)(q22;q32) */MEF2D::CSF1R* is a Ph-like *ABL-class* ALL.

[h] Most *PAX5* fusions are primary CA belonging to the *PAX5* alt subgroup; inv(9)(p24p13)/del(9)(p24p13)/*PAX5::JAK2* belongs to the Ph-like subgroup; del(9)(p13p13)/inv(9)(p13p13)/ *PAX5:: ZCCHC7* is a cryptic secondary CA in B-ALL.

[i] dic(9p13;v) can also present as a secondary CA in *PAX5 P80R B* ALL as a mechanism of deletion of the wild type *PAX5* allele.

frequent being *ACIN1* (14q11*), CUX1* (7q22*), BRD9* (5p15*)* and *ZNF618* (9q32) [83,84,92,93]. The resulting translocations can be detected on CBA and confirmed by FISH with *NUTM1* probe and RNA sequencing [92,93].

t(9p13) and dic(9p13) /PAX5 fusions. PAX5 gene (9p13.2) is frequently abnormal in B-ALL. Translocations and dicentric chromosomes leading to *PAX5*-r and in frame fusion genes are present in ~3 % pediatric and ~1 % adult B-ALL, as a primary CA, mainly within simple karyotypes [94,95]. More than twenty 3′partners have been identified. The most frequent are *ETV6* (12p13) and *NOL4L* (20q11) resulting from dic(9;12)(p13;p13) and dic(9;20)(p13;q11) respectively. Of note, this latter CA can be difficult to detect, but an apparent monosomy 20 should prompt attention [96]. Furthermore, both dicentric chromosomes are heterogeneous at the molecular level and can be also found as secondary CAs leading to complete loss of one *PAX5* allele [95].

Other frequent *PAX5-r* are *PAX5::AUTS2* or *PAX5::ELN* fusions resulting from t(7;9)(q11.2;p13.2) or fusion with genes leading to a *PAX5*-altered (*PAX5*-alt) subgroup GEP (see below) [83,84]. Of note, cases with t(9;9)(p13;p24)/inv(9)(p13p24)/del(9)(p13p24) inducing *PAX5::JAK2* fusion cluster with Ph-like B-ALL whereas the cryptic inv(9)(p13p12)/del(9)(p13p13) inducing *PAX5::ZCCHC7* fusion has been identified as a secondary CA in *ETV6::RUNX1* or *IGH::DUX4* B-ALLs [84].

B-ALL with other defined molecular abnormalities (Table 2). Recent gene expression studies using whole transcriptome sequencing (WTS) have identified new B-ALL subtypes that do not or partially match with a specific primary CA but are clinically relevant [13,46,78,83,84,97–99].

The *PAX5*-alt subgroup represents 3–10 % and 7–12 % of pediatric and adult B-ALLs, respectively. It is defined by a specific GEP with diverse *PAX5* abnormalities: rearrangements leading to in frame fusion genes (see above), *PAX5* non P80R mutations and *PAX5* focal intragenic amplification (*PAX5*amp) [46,83,84,98,99].

PAX5 amp or *PAX5* intragenic tandem duplications (*PAX5*-ITD) were previously defined as B-ALL cases with 3 or more partial *PAX5* copies lacking a classical primary CA. *PAX5*-ITD represents 1 % of pediatric B-ALL and is associated with a higher relapse rate [100]. Frequent ACA are del(9p) (26 %)/*CDKN2A/B* deletion (82%), +5 (23%) and −7 (12%)/*IKZF1* deletion (13%).

The most frequent ACA/additional molecular abnormality (AMA) in the *PAX5*-alt subgroup are visible or cryptic del(9p) leading to *PAX5, CDKN2A/B and MTAP* deletions [46,83,84,98,99].

PAX5-alt B-ALL is associated with an intermediate prognosis [46,83,84,99].

The *PAX5* P80R subgroup represents 2–3 % of childhood and adult B-ALL [46,83,84,101,102]. *PAX5* P80R mutation results in PAX5 loss of function. Indeed, both alleles are inactivated as mutations are usually hemizygous mainly due to dic(9;20)(p11;q11) and dic(7;9)(p11;p11) leading to loss of wild-type *PAX5* and *CDKN2A/B* and, for dic(7;9) cases, loss of *IKZF1* [101]. Mutations in *RAS* or *JAK2* signaling pathway genes are frequent [83,84,101]. *PAX5* P80R subgroup is associated with an intermediate to favorable outcome [46,84,101,102].

The *IKZF1 N159Y* subgroup has been identified in rare cases (<1 %) of pediatric and adult B-ALL. Interestingly, most of these patients harbored a +21 [84].

The *ETV6::RUNX1*–like subgroup clusters with the t(12;21)(p13;q22)/*ETV6::RUNX1* subgroup but lacks *ETV6::RUNX1* transcript thus representing a phenocopy subgroup [78]. Interestingly, it also shares the same immunophenotype [97] and cytogenetic profile with +21 and *ETV6* deletions [84]. Conversely, it is also characterized by co-occurrence of *ETV6* and *IKZF1* abnormalities such as fusion genes and deletions, a rare finding in other B-ALL subtypes [78,83,84]. *ETV6::RUNX1*–like ALL represents 3% of pediatric, but only 0.5–1% of adult B-ALL cases, mainly young adults [46,78,84] and confers a relatively favorable prognosis [46,83,99].

Other WHO/ICC phenocopy subgroups such as *KMT2A*-like and *ZNF384*-like (mainly harboring *ZNF362-r*) subgroups are rare and not fully characterized [83,84].

The recently reported *CDX2/UBTF* subgroup is defined by a unique gene expression profile and characterized by the association of two microdeletions: one located at 13q12.2 leading to *CDX2* overexpression and the other at 17q21.3 leading to *UBTF::ATXN7L3* in-frame fusion. This subtype mainly found in AYA, represents 0.2% of children (all cases older than 10) and 2% of adult B-ALL, mainly female patients. It is associated with a low WBC and a pro-B phenotype. 1q gain is the most frequent ACA. The outcome is poor due to higher risk of induction treatment failure, higher post-induction MRD level and higher cumulative incidence of relapse [103,104].

3.2. Secondary cytogenetic and molecular abnormalities

3.2.1. Secondary CAs, complex karyotype and monosomal karyotype

Secondary CAs detectable at the CBA level and/or at the CMA/MLPA level are frequently characteristic of B-ALL primary subtypes (see above). They are mainly unbalanced CAs: deletions and monosomies such as del(6q), del(7p)/-7, del(9p)/-9, del(12p), del(13q)/-13, del(17p)/-17), and chromosomal gains such as +X and +21 [8,10,105].

In adult Ph-negative ALL, CK has been defined by the MRC-ECOG group as cases lacking an established translocation and presenting

Table 2
Comparison of WHO-HAEM5 [1] and ICC 2022 [2] classifications for B "other" ALL

WHO-HAEM5 classification	ICC 2022 classification
B-ALL with *BCR::ABL1*-like features	**B-ALL, *BCR::ABL1* like**, ABL-class rearranged
	B-ALL, *BCR::ABL1* like, JAK-STAT activated
	B-ALL, *BCR::ABL1* like, NOS
B-ALL with other defined genetic abnormalities§**:**	**New entities in B ALL:**
B-ALL with with *DUX4* rearrangement§	B-ALL with with *DUX4* rearrangement
B-ALL with *MYC* rearrangement§	B-ALL with *MYC* rearrangement
B-ALL with *ZNF384* rearrangement§	B-ALL with *ZNF384 (or ZNF362)* rearrangement
B-ALL with *MEF2D* rearrangement§	B-ALL with *MEF2D* rearrangement
B-ALL with *NUTM1* rearrangement§	B-ALL with *NUTM1* rearrangement
B-ALL with with *PAX5* alterations§	B-ALL with *PAX5* alterations§
B-ALL with PAX5 P80R mutation§	B-ALL with *PAX5* P80R mutation
B-ALL with *ETV6::RUNX1*-like features§	B-ALL, *ETV6::RUNX1*-like§
	B-ALL with *IKZF1* N159Y mutation
	B-ALL, *CDX2/UBTF*
	B-ALL with *ZEB2B* mutation/*IGH::CEBPE*§
	B-ALL, *ZNF384*-rearranged like§
	B-ALL, *KMT2A*-rearranged like§
B-ALL, NOS	**B-ALL, NOS**

Abbreviations: B-ALL: B-lymphoblastic leukemia/lymphoma; NOS: not otherwise specified
§ Provisional entity

with at least 5 unrelated CAs within the 40–50 MCN range [105]. This CK (CK5) is mainly due to combinations of classical ACAs. CK5 is found in 5–6 % of adult Ph-negative B-ALL cases. The prognosis of CK5 in B-ALL is still a matter of debate [8,9,105].

In adult Ph-negative B-ALL (after exclusion of the LH subtype), MK has no significant prognostic value [8].

3.2.2. Secondary molecular abnormalities

Alterations of genes involved in B cell development (such as *IKZF1, PAX5* or *EBF1*), and cell cycle regulators (such as *CDKN2A/B)* are frequent secondary molecular events [106,107]. These copy number alterations (CNA), mainly cryptic, can be easily evidenced by CMA, NGS or MLPA kits detecting the most frequent deleted loci: *EBF1* (5q33.3), *IKZF1* (7p12.2), *PAX5* (9p13.2), *CDKN2A/B* (9p21.3), *ETV6* (12p13.2), *BTG1* (12q21.3), *RB1* (13q14.2) and *PAR1* (Xp22/Yp11). Furthermore, these CNA, either as isolated or in unique combinations, are prognostically relevant, mostly in cases lacking a stratifying primary CA [108, 109].

Of note, 3′*EBF1 and PAR1* deletions can correspond to the formation of a *EBF1::PDGFRB* and *P2RY8::CRLF2* rearrangement, respectively.

IKZF1 intragenic deletion is observed in ~15 % of B-ALL and ~50 % of high-risk B-ALL. *IKZF1* deletions are mainly evidenced in Ph and Ph-like ALLs. However, it is important to note that *IKZF1* deletions are also frequent in patients with *DUX4*-r/*ERG*del cases without altering their favorable prognosis [3,46,79,80].

Complete or partial monoallelic *PAX5* deletions occur in 30 % of B-ALLs. Of note, 3′PAX5 deletion can reflect the formation of a *PAX5* gene fusion in a dicentric chromosome, mainly in the *PAX5*alt subgroup.

4. Novel technologies in ALL

Many of the newly discovered fusion genes and/or rearrangements in B-ALL have been recognized thanks to RNA sequencing (RNA-seq). For phenocopy subgroups, such as the *ETV6::RUNX1*-like good prognosis subgroup, it represents the best diagnostic technique [13,46,78,83,84, 110]. (Targeted) RNA-seq is therefore very likely to complement routine

Table 3
GFCH guidelines in a routine cytogenetic laboratory according to karyotype results.

Karyotype results (1)	FISH analyses (2)
Informative karyotype with recurrent primary CA	Confirm primary CAand/or check for *BCR::ABL1* (3)
t(9;22)(q34;q11)/Ph	*BCR::ABL1* (3)
t(4;11)(q21;q23) or t(v;11q32)	*KMT2A* (4)
t(1;19)(q23;p13)	*TCF3/TCF3::PBX1* (5)
t(17;19)(q22;p13)	*TCF3/TCF3::HLF* (5)
High hyperdiploidy (HeH)	*BCR::ABL1*
Hypodiploidy < 41 (Ho-Tr or NH)	*BCR::ABL1*
Non specific CA but evocative of a primary CA	**Search for a specific primary CA**
Chromosomal breakpoint suggesting a primary CA	FISH according to the chromosomal breakpoint
Abnormal chr. 21 suggesting an iAMP21	*ETV6::RUNX1*
Suspicion of Hypodiploidy:	Combination of probes for one lost chr (3, 7 or 17) and one retained chr (1 or 6 in Ho-Tr; 18 or 21 in NH)
Suspicion of High Hyperdiploidy:	chr 4, 10, 17 and 18 probes (7)
Suspicion of Atypical High Hyperdiploidy (7):	chr 17 and 18 probes and,if both negative, chr 5 and chr 20 probes (7)
Normal or non informative karyotype (1) or abnormal karyotype without recurrent primary abnormality:	**Sequential FISH analyses**
	First FISH round
Secondary abnormalities:	*BCR::ABL1*(3), *KMT2A* (4),*TCF3* (5), *ETV6::RUNX1* (6)
+X, +21, del(6q), del(9p), del(12p),…	
	Second FISH round
	(If first FISH is round negative and if atypical HeH)
Non specific ploidy abnormalities:	*ABL* class probes (*ABL1, ABL2, PDGFRB/CSF1R*)
Hypodiploidy 41–45	*MYC* and/or *IGH::MYC* (8)
Hyperdiploidy (47–50)	
Atypical High Hyperdiploidy (7)	**Third FISH round**
Tetraploidy	If *MYC-r*: *BCL2* and *BCL6* and possibly *IGH, IGK and IGL*(8)
	if *MYC* normal: *CRLF2/P2RY8, JAK2* (optional) (9)

Abbreviations: CA: chromosomal abnormality; CBA: chromosome banding analysis; chr: chromosome; HeH: high hyperdiploidy >50 chr.; r: rearrangement; Ho-Tr: hypodiploidy 30–39 chr./near triploidy 60–78 chr.; NH: near-haploidy 23–29 chr; OGM: Optical Genome Mapping.
(1) If insufficient sample or failure, ask for a new BM or PB sample.
(2) FISH or other informative technique. Whenever possible, confirm a stratifying CA by another technique. For structural CA leading to fusion transcripts: FISH, RT-PCR, multiplex RT-MLPA or OGM. For chr. gains and losses: FISH, SNP-array or NGS (both allowing UPD diagnosis in duplicated hypodiploid cases and chr 21 chromothripsis in iAMP21 cases). NGS identifies mutations in the targeted areas, especially *TP53* mutations in Ho-Tr ALL.
(3) Ph/*BCR::ABL1* positive ALL is the most frequent CA in adults. Pediatric and adult Ph-ALL and Ph-like ALL can present with a HeH pattern. Ph-ALL and ABL-class Ph-like ALL require ABL-class TKI based treatment.
(4) *KMT2A*: FISH allows the diagnosis of most *KMT2A*-r cases; metaphase FISH can help in the identification of the partner gene; t(4;11)/ *KMT2A::AFF1* is the most frequent t(11q23)/*KMT2A*r in B-ALL.
(5) t(1;19)(q23;p13)/*TCF3::PBX1* is very similar at the CBA level to t(1;19)(q22;p13)/*MEF2D::DAZAP*1 thus *TCF3*-r has to be confirmed; t(17;19)(q22;p13)/*TCF3::HLF* is a subtle CA at the CBA level and a very high-risk CA in B-ALL.
(6) t(12;21)(p13;q22)/*ETV6::RUNX1 is* present in around a quarter of pediatric B-ALL cases. Cryptic CA to be searched for in < 25 year-old patient; *ETV6::RUNX1* probe can detect multiple copies of the RUNX1 gene clustered (mostly on a der(21)) and in nuclei in iAMP21 cases or present as unique signals on multiple chr21 in HeH (trisomy or tetrasomy) or duplicated hypodiploid cases (tetrasomy).
(7) Classical HeH (54–59 chr) present with trisomy for chr 4, 10, 17, 18; Atypical HeH present with lack of trisomy 17 and 18 or lack of trisomy 17 or 18 and presence of trisomy 5 or 20, and/or 51–53 chr, and/or *IKZF1*del.
(8) *IG::MYC-r* detected in B-ALL as a primary CA are high-risk B-ALL; a Burkitt leukemia/lymphoma must be excluded (cf text); *BCL2-r* and/or a BCL6-r can be present in a subset of these *MYC-r* and could be DLCBL blast phase thus excluded.
(9) *CRLF2*-r (Xp22/Yp11) and *JAK2*-r (9p24) cases belong to JAK-STAT Ph-like ALL that could benefit from JAK-class TKI therapy.

genetic diagnostics. However, its role and importance in light of whole genome sequencing (WGS) and the upcoming optical genome mapping (OGM) that have been proven useful for the identification of both SVs and CNVs, remains to be determined (see joint dedicated article) [98,111]. Indeed, OGM is developed in many cytogenetic laboratories as a complement to karyotype and as a surrogate for multiple FISH analyses [112,113]. However, the technique is not suited for detection of mutations nor does it allow the design of primers necessary for molecular minimal residual disease (MRD) assessment as the identification of breakpoints is not sufficiently precise. Conversely, WGS could replace RNA sequencing as suggested by two recent studies [98,111].

5. Guidelines for the diagnosis of clinically relevant cytogenomic subgroups (Tables 3 and 4)

Keeping in mind that a clinically relevant cytogenomic abnormality must ideally be identified by two different techniques and that results must be given in a turnaround time (TAT) compatible with therapy, we propose guidelines for cytogenomic diagnosis in accordance with these assumptions.

5.1. Classical cytogenetics (Tables 3 and 4)

5.1.1. Chromosome banding analysis

In ALL, CBA must be performed on bone marrow. Peripheral blood can be used as a rescue, mainly in case of fibrosis, if it contains sufficient blast cells. Classical and optimal culture time is 1 day (overnight). A sufficient number of cells must be cultured (30–60 millions cells) in order to obtain a cytogenetic pellet allowing CBA and multiple FISH analyses. In case of karyotype failure, either due to poor sampling and/or poor chromosome morphology, the cytogeneticist should rapidly request a new sample [114,115]. CBA turnaround time (TAT) is 2–7 days; results should be optimally provided within 7 days.

5.1.2. FISH and other classical molecular techniques

In ALL, FISH and/or molecular techniques - according to the locally available facilities - are necessary as complementary methods to CBA in view of a correct assignment to stratifying CAs . Multiplex RT-MLPA can detect in a single analysis multiple targeted relevant transcripts whereas SNP-A is very efficient in the detection of chromosomal gains and losses and UPD allowing differential diagnosis between duplicated hypodiploid and high hyperdiploid cases [45,68].

FISH TAT is 1–3 days. FISH performed on the cytogenetic pellet allows metaphase and interphase FISH on the same slide. According to CBA results, we propose successive rounds of FISH (Table 3).

BCR::ABL1 FISH is mandatory in B-ALL in order to detect Ph-positive ALL that can rapidly benefit from TKI.

Other FISH probes can be used:

- To confirm a stratifying CA detected at the CBA level (informative karyotype).

Table 4
Available methods and performance for the detection of main chromosomal and genomic aberrations.

Main chromosomal / genomic aberrations	Detection methods						
	CBA	FISH	OGM	CMA	MLPA	RT-PCR	NGS
High Hyperdiploidy (HeH)	++ [a]	++ [b]	++	+++			+ [c]
Low Hypodiploidy (LH) and Near Haploidy (NH)	++ [a]	++ [b]	++	+++ [d]			+ [c]
t(9;22)(q34;q11)/ *BCR::ABL1*	++ [a,e]	+++	+++			+++	+++
t(v;11q23) / *KMT2A-r*	++ [a,f]	+++	+++			++ [g]	+++
t(12;21)(p13;q22)/ *ETV6::RUNX1*	¤ [h]	+++	+++	¤		+++	+++
iAMP21	¤ [i]	+++ [j]	+++	+++	++ [k]		++ [c]
t(1;19)(q23;p13)/ *TCF3::PBX1*	++ [a,l]	+++	+++	¤		+++	+++
t(17;19)(q22;p13)/ *TCF3::HLF*	+ [a,f]	+++	+++			+++	+++
ABL-class fusions (*ABL1, ABL2, CSF1R, PDGFRB*)	+ [a,f]	++	+++			++ [g]	+++
JAK-STAT class rearrangements (*CRLF2, JAK2, EPOR*)	+ [a,f]	++	+++			++ [g]	+++
IG rearrangements including *MYC*r	++ [a,f]	+++	++				+ [m]
Deletions (BTG1, CDKN2A/B, EBF1, ETV6, IKZF1, PAR1, PAX5, RB1)		++	+++	+++	+++ [k]		++ [c]
*Mutations (IKZF1, JAKs, PAX5,*and other relevant genes)							+++

Abbreviations.
CBA: chromosome banding analysis (karyotype); CMA: chromosomal microarray (SNP-array and array-CGH); iAMP21: intrachromosomal amplification of chromosome 21; OGM: optical genome mapping; MLPA: multiplex ligation-dependent probe amplification; RT-PCR: reverse transcription polymerase chain reaction including reverse transcription multiplex MLPA (RT-MLPA); NGS: next generation sequencing including RNA sequencing (RNASeq) and Whole Genome Sequencing (WGS); **r**: rearranged.
+: contributes to diagnosis, ++: good diagnostic tool, +++: sufficient tool for diagnosis.
¤: can suggest but does not identify the anomaly.

[a] Blast cells can present with a low mitotic index or poor chromosome morphology.

[b] A "FISH cocktail" with probes for common trisomies/tetrasomies (for high hyperdiploid or duplicated hypodiploid cases) and for common monosomies (for hypodiploid cases) can be used (see Table 3).

[c] Only when the technique allows CNV analysis (WGS).

[d] SNP arrays identify all numerical changes and also provide information on uniparental disomies (UPDs), which is valuable for identifying duplicated near-haploid/low hypodiploid clones.

[e] CBA can miss the translocation (due to e.g. cryptic insertions) and should not be used as the sole screening method, always confirm with a second technique.

[f] CBA can miss subtle and cryptic rearrangements and should not be used as the sole screening method.

[g] RT-PCR alone insufficient.

[h] The *ETV6::RUNX1* fusion is cytogenetically cryptic. Only the characteristic secondary chromosomal abnormalities can be detected.

[i] A structurally abnormal chromosome 21 is evocative of an iAMP21.

[j] When interphase FISH analysis suggests iAMP21, this must be confirmed by metaphase FISH in order to verify that the extra *RUNX1* signals are clustered and located on one abnormal chromosome, mostly chromosome 21.

[k] Detected only with dedicated MLPA probes.

[l] *TCF3::PBX1* fusion must be identified with a second technique.

[m] *IG* rearrangements are inconstantly detected by RNASeq techniques.

- To search for a stratifying CA not detected at the CBA level (non-informative or insufficiently informative karyotype).

FISH results should be available within optimal delays after diagnosis according to therapeutic implications and in order to potentially launch successive FISH rounds or other complementary techniques (Table 3):

- First FISH round: *BCR::ABL1* and other classical primary CAs.
- Second FISH round: *ABL*-class CAs in B-ALL cases lacking classical primary CAs (B "other" ALL).
- Third FISH round: *MYC* rearrangements in B-ALL with an 8q24 CA or in cases with CBA failure.

5.2. New technologies (Table 4)

5.2.1. Optical genome mapping (OGM)

OGM has a turnaround time of 4–7 days. It detects prognostically relevant CNVs such as those observed in the iAMP21 entity and those included in the UKALL chromosome number abnormality classifier and detected by the dedicated MLPA kit assays (CNVs involving the PAR1 region and the *EBF1, IKZF1, CDKN2A/B, PAX5, ETV6, BTG1* and *RB1* genes).

OGM can also detect most fusion genes with a relevant prognostic and theranostic value, potentially avoiding multiple FISH analyses.

However, the technique is still very costly and incapable of detecting mutations and robertsonian translocations (which may be present in iAMP21 patients). The ploidy assessment is not always reliable and minor (sub)clones are also easily missed [112,113].

5.2.2. Next generation sequencing (NGS)

NGS including Whole Transcriptome Sequencing and Whole Genome Sequencing (WGS) are very efficient in ALL, yet also very costly with long turnaround times (reviewed in Ref. [116,117]). Targeted RNA and DNA sequencing focused on specific genes of interest are less costly and could be more effective in terms of detectability and turnaround time.

6. Conclusion

The identification of primary genetic abnormalities is critical in the management of B-ALL patients. Classical cytogenetic techniques (karyotype and FISH) following well-established guidelines remain essential in ALL patients' management, especially in low-income countries, as they are low-cost techniques, ensuring on-time stratifying and theranostic results. New technologies such as WGS are likely the most informative techniques, allowing the classification of virtually all B-ALL patients and the identification of secondary mutations that could be taken into account in future therapeutic protocols. Despite their cost and extensive turnaround times, WGS technologies are likely to take a vastly increasing place in the cytogenomic diagnosis of ALL in the future, as they become more widely available.

CRediT authorship contribution statement

Giulia Tueur: Writing – original draft. **Julie Quessada:** Writing – original draft. **Jolien De Bie:** Writing – original draft, Writing – review & editing. **Wendy Cuccuini:** Writing – original draft. **Saloua Toujani:** Writing – original draft. **Christine Lefebvre:** Writing – original draft, Software. **Isabelle Luquet:** Writing – original draft. **Lucienne Michaux:** Writing – review & editing, Writing – original draft. **Marina Lafage-Pochitaloff:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Conceptualization.

Acknowledgments

We thank Hélène Guermouche and Dominique Penther for fruitful discussions and helpful comments.

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