The genomic landscape of adult B-cell precursor acute lymphoblastic leukaemia

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Paysage génomique des leucémies aiguës lymphoblastiques B de l'adulte

B-cell precursor acute lymphoblastic leukaemia (BCP-ALL), oncogenesis, genetic alterations, next-generation sequencing

Abstract

Adult B-cell precursor acute lymphoblastic leukaemia (BCP-ALL) is usually classified based on the presence or absence of Philadelphia chromosome (Ph), although Ph- BCP-ALL is not a homogenous group in terms of genetic and clinical characteristics. In contrast to the long-standing role of cytogenetic classification in risk prediction and treatment stratification for paediatric BCP-ALL, genetic alterations and their prognostic relevance are poorly known in adult BCP-ALL. Recently, the development of high-throughput genome-wide analyses has allowed many recurrent alterations to be identified in children and adults with BCP-ALL. Ten years ago, the BCR-ABL1-like or Ph-like group was described in paediatric studies and the importance of this group in adults was subsequently recognised. Evaluation of new therapeutic options in this high-risk group is currently an important field in treatment development. More recently, genomic alterations have been identified, defining novel subtypes, such as DUX4/ERG, ZNF384, ME2D and PAX5^{P80R}. These multiple "classify-

Résumé

Les leucémies aiguës lymphoblastiques de la lignée B (LAL-B) de l'adulte se définissent classiquement par la présence ou l'absence du chromosome Philadelphie (Ph). Cependant, les LAL Ph-négatives ne représentent pas un groupe homogène tant sur le plan génétique que clinique. Si l'importance des anomalies cytogénétiques pour prédire le risque de rechute et stratifier le traitement des LAL-B est reconnue depuis longtemps chez l'enfant, chez l'adulte, le spectre des anomalies récurrentes et leur rôle pronostique sont relativement mal établis. Récemment, l'essor des techniques d'analyse pangénomique a permis d'identifier de nombreuses altérations récurrentes observées chez l'enfant et chez l'adulte. Ainsi, après la description initiale du groupe dit *BCR-ABL1-like* ou Ph-*like* dans des études pédiatriques, il y a près de dix ans, l'importance de ce groupe dans les LAL-B Ph-négatives de l'adulte a été reconnue et les opportunités thérapeutiques qu'il représente commencent à être évaluées. Plus récemment, plusieurs nouveaux sous-types ont été identifiés, tels que

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ing" alterations, together with many secondary alterations, have created a new genomic landscape for adult BCP-ALL of unexpected complexity. Understanding the biology of these leukaemias and analysing their clinical features and response to drugs are rapidly evolving fields that should speed up the development of more precise and effective treatments.

DUX4/ERG, *ZNF384*, *MEF2D* et *PAX5*^{P80R}. Ces altérations « classantes », ainsi que les nombreuses altérations secondaires qui peuvent s'y associer, dessinent un paysage génétique des LAL-B de l'adulte d'une complexité insoupçonnée. L'étude de la biologie de ces leucémies, de leurs caractéristiques clinicobiologiques et de leur réponse aux traitements, actuels et en développement, devrait permettre de progresser vers des traitements plus adaptés et une prise en charge optimisée

B-cell precursor acute lymphoblastic leukaemia (BCP-ALL) accounts for approximately 80% of ALLs. It is the most common cancer in children and is curable in almost 90% of cases [1]. In adults, on the other hand, it is a relatively rare cancer with a prognosis which remains guarded, despite significant improvements over the last ten years, as the result of:

– the use of tyrosine kinase inhibitors (TKIs) for Philadelphia chromosome (Ph) ALLs and
– the application of intensive paediatric-inspired chemotherapy protocols in young adults with Ph- ALLs.

Like all cancers, BCP-ALL is characterised by the sequential acquisition of genetic alterations that allow the emergence and maintenance of the leukaemic clone. A primary or founding abnormality is considered to initiate tumour transformation and results in a preleukaemic clone, which may acquire secondary or additional events, contributing to the tumour phenotype. The so-called "classifying" anomalies, generally assumed to be primary, make it possible to define groups that are homogeneous from a biological point of view [1, 2] but also in terms of epidemiology, from their clinical presentation, response to treatment and, finally, prognosis. In childhood BCP-ALL, several genetic subtypes have been identified and their prognosis is well established. Genetic typing of paediatric BCP-ALL thus constitutes a major component, alongside early response to treatment, of algorithms for risk assessment and therapeutic stratification. However, while most childhood BCP-ALL are characterised by a "classifying" abnormality identified by routine cytogenetic or molecular biological testing, more than half of adult BCP-ALLs fall into the so-called "B-other" category, in which no "classifying" abnormality is identified. The prevalence of different genetic types of BCP-ALL varies with age, and the most common types in children are rare in adults.

Over the past decade, emerging genome analysis technology has revealed a myriad of genomic alterations in BCP-ALL, revealing unsuspected heterogeneity and complexity. Studies based on comparative genomic hybridisation (CGH) or single-nucleotide polymorphism array (SNP-array) first revealed a multiplicity of microdeletions as a result of illegitimate recombination by recombination-activating gene (RAG) enzymes active in lymphoid precursors [3], leading to the inactivation of numerous tumour suppressor genes. In particular, this has made it possible to highlight recurrent deletions of *IKZF1* (Ikaros zinc finger protein 1), a secondary abnormality representing a marker of poor prognosis in many studies of both children and adults. More recently, the complete sequencing of the transcriptome (RNA-seq) has made it possible to highlight, in a large proportion of B-other cases, various gene fusions and products of chromosomal translocations that are most often not apparent on karyotyping and which define new clinical-biological entities.

This review will take stock of all the genomic alterations identified in adult BCP-ALL, focussing on their possible prognostic and therapeutic impact.

Classic cytogenetic groups

Different BCP-ALL entities are characterised by the identification of mutually exclusive recurring alterations (*table 1* and *figure 1A*). These primary alterations may involve major aneuploidies, deregulatory rearrangements of transcription factors often involved in normal haematopoiesis, or intracellular signalling factors such as tyrosine kinases.

Table 1

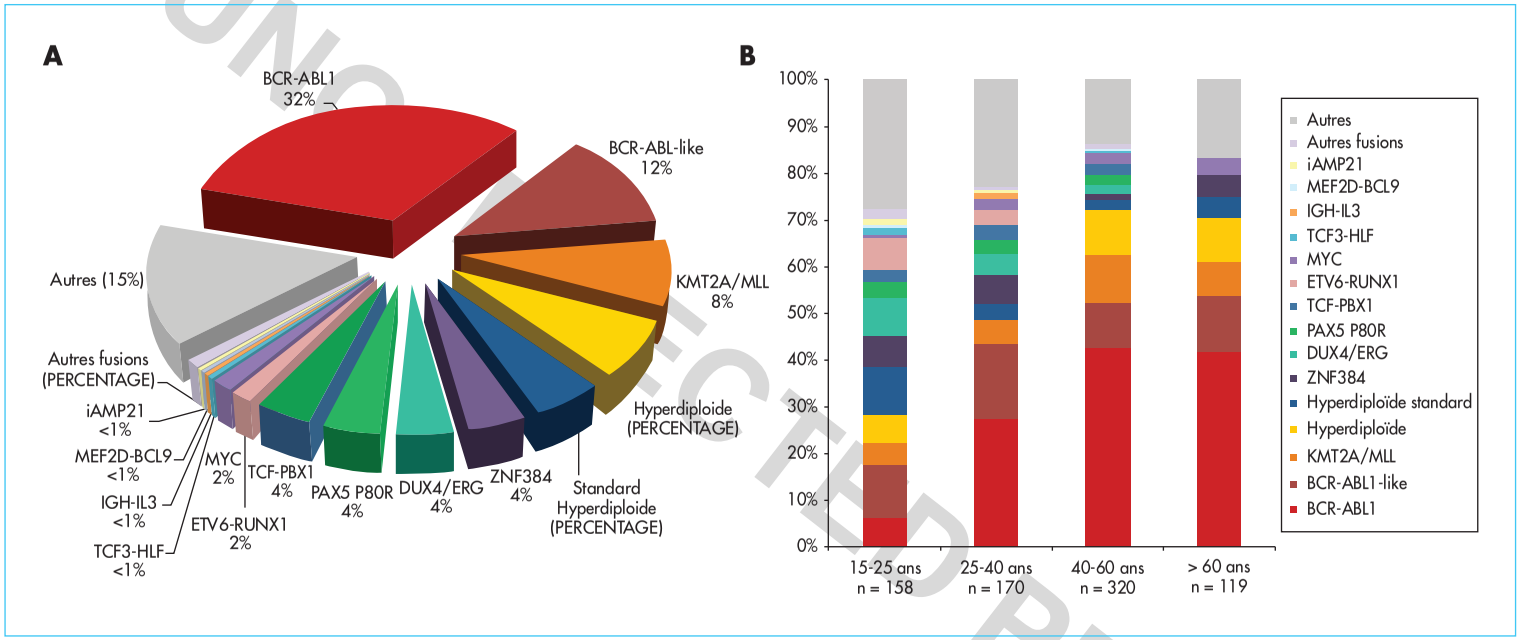
Prognosis associated with the different subtypes of BCP-ALL (data from the literature).

Subtypes	Prognosis
BCR-ABL1 t(9;22)(q34;q11.2)	Initially poor prognosis, improved since the introduction of TKIs [5, 6]
KMT2A (MLL) rearrangements	Poor prognosis [9, 10] High-risk criterion based on the GRAALL-2014 study.
BCR-ABL1-like	Unfavourable prognosis with conventional treatments [66]
Hypodiploidy < 40 chromosomes	Poor prognosis in children, less clear in adults [16, 17]
Standard hyperdipoidy > 50 chromosomes	Very good prognosis in children, less clear in adults [16, 18, 19]
DUX4/ERG	Good prognosis in children [75], no data in adults
ZNF384 fusion	Intermediate prognosis in children [77], few data in adults
TCF3-PBX1 t(1;19)(q23;p13.3)	Initially poor prognosis, improved by current intensive treatments [17, 23, 24]
$PAX5^{P80R}$	Good prognosis in adults [82]
ETV6-RUNX1 t(12;21)(p13;q22)	Very good prognosis in children, no data in adults
MEF2D fusion	Few data, appears to have a poor prognosis. [79]
PAX5 fusion	No data
TCF3-HLF t(17;19)(q22;p13)	Very bad [25]
iAMP21	Fairly poor prognosis in children, no data in adults
IGH-IL3	No data

Philadelphia-positive acute lymphoblastic leukaemia

The translocation, t(9;22)(q34;q11.2), is the most common "classifying" alteration in adult BCP-ALL (*figure 1A*). The relative proportion of this subtype varies greatly with age (*figure 1B*), ranging from about 5% in children to more than half of cases over the age of 60 in some cohorts [4]. Derivative 22 of this translocation results in the formation of a *BCR-ABL1* gene fusion, producing a fusion transcript and a chimeric protein, of which the ABL1 kinase part is observed in a constitutively active form. Depending on the location of the genomic breakpoints, the minor (m) or major (M) transcript is produced, leading to the p190 protein, present in the majority of Ph+ ALL cases, or to the p210 form, predominant in chronic myeloid leukaemia but rarer in Ph+ ALLs, respectively. The prognosis of Ph+ ALLs, which was initially very poor, has been transformed by the introduction of tyrosine kinase inhibitors [5, 6]. For example, in the first multi-centre GRAALL study, in which imatinib was combined with chemotherapy (GRAAPH-2003), overall survival at four years was 52%, compared with 20% in a previous cohort (the LALA-94 trial)

FIGURE 1



Distribution of oncogenetic subtypes of adult BCP-ALL. Data from the cohort of patients studied at the Saint-Louis laboratory between 01/01/2015 and 01/09/2018 (n = 767). **(A)** Pie chart for the entire cohort. **(B)** Age distribution.

[7]. Current studies, using second- or third-generation TKIs, will most likely allow for even further significant improvements in patient survival.

Rearrangements of the lysine N-methyltransferase 2A gene: *KMT2A*.

Rearrangements of the *KMT2A* gene, previously known as MLL (mixed lineage leukaemia), have been demonstrated in a variety of haematological malignancies: B-cell or T-cell ALL as well as acute myeloid leukaemia and myelodysplastic syndromes. *KMT2A* rearrangements define the second most common genetic subtype of BCP-ALL in adults (*figure 1A*). There is an extremely large number of potential partner genes of *KMT2A*, but these are relatively specific to certain types of haemopathy [8]. In BCP-ALL, the most common *KMT2A* rearrangement is the t (4;11)(q21;q23) translocation, which results in *KMT2A-AFF1* (*MLL-AF4*) fusion and is present in more than 80% of adult cases [8]. In both children and adults, ALLs with *KMT2A* rearrangement are characterised by an often hyperleukocytic presentation and have a poor prognosis due to frequent relapses, usually at an early stage. The results of the UKALLXII/ECOG2993 trial [9] showed that the *KMT2A-AFF1* fusion identifies a group of patients with a poor prognosis despite the intensification of allograft treatment during initial complete remission. The results of the GRAALL-2003 and 2005 studies showed that *KMT2A* rearrangement retained a pejorative prognostic value, independent of the residual disease [10]. In the GRAALL-2014 study, the presence of *KMT2A* rearrangement is therefore a high-risk criterion. However, because the results are rather weak due to the low number of cases of this group in each study, specific issues will have to be addressed based on consortium studies, such as :

– the role of allograft in initial complete remission,
– the significance and role of residual disease,
– the prognosis of ALL with *KMT2A* rearrangements involving fusion partners other than *AFF1*.

Hypodiploidy

Hypodiploidy, defined by karyotypic chromosome loss, is a heterogeneous group of BCP-ALLs. It includes near haploidy (25-30 chromosomes), low hypodiploidy (31-39 chromosomes) and high hypodiploidy (40-44 chromosomes). Unlike the first two, the latter group does not represent a homogeneous biological entity [11-13]. Near-haploid ALLs are virtually unobserved in adults, while the incidence of low-hypodiploid ALL increases with age [11], and is probably underestimated. Indeed, the cytogenetic diagnosis of low-hypodiploid ALL is complicated by the fact that hypodiploid blasts can duplicate their chromosomal content by an endoreplication phenomenon, resulting in a clone with a near-triploid chromosomal state, which may resemble standard hyperdiploidy. When the duplicated clone is predominant and the only one detected by cytogenetic analysis, only the detection of a loss of heterozygosity affecting the lost chromosomes can reveal the masked hypodiploidy. No recurrent structural abnormalities or common fusion genes have been identified in these subgroups [12, 14], and aneuploidy appears to be the initiating event. A large gene expression profiling study has also shown that both major types of hypodiploidies were associated with relatively specific additional transcriptional signatures and constellations of genetic alterations, confirming their status as clinico-biological entities. In particular, it is important to note that low-hypodiploid ALLs very often have *TP53* mutations [15]. In paediatric studies, *TP53* mutations have been observed in non-tumour cells in half of the patients [14, 15], suggesting that low-hypodiploid ALL may be a manifestation of Li-Fraumeni syndrome. However, in adults, *TP53* mutations have only been identified in the somatic state [14].

Low-hypodiploid ALLs are a known poor prognostic entity, with an estimated overall five-year survival rate of only 22% based on the UKALLXII/ECOG2993 study [16]. In the GRAALL-2005 study, poor overall survival (38.6%) [17] was due not only to relapses but also to increased mortality related to older age of the patients.

Standard hyperdiploidy

Standard or high hyperdiploidy (>51 chromosomes) is the most common subtype of BCP-ALL in children (30-35% of cases), and is associated with an excellent prognosis. This hyperdiploidy, characterised by the non-random gain of certain chromosomes (X, 4, 6, 10, 14, 17, 18 and 21), is much rarer in adults. As with the major hypodiploidies, the pathogenic event initiating leukaemogenesis appears to be hyperdiploidy itself, occurring within a single mitotic event [18]. The prognosis of this subtype in adults is not clearly established, probably in part due to possible confusion with near triploidy, hypodiploidy masked by duplication [19], or clustering with non-standard hyperdiploidies [16, 18].

ETV6-RUNX1 fusion

Translocation t(12;21)(p13;q22) is a cryptic rearrangement of the karyotype and is investigated using fluorescence *in situ* hybridisation (FISH) or RT-PCR of the *ETV6-RUNX1* fusion transcript (formerly *TEL-AML1*). This abnormality defines the second most frequent subtype in children (25% of cases with a peak frequency at three years of age), associated with an excellent prognosis, whereas it is very rare in adults [20, 21].

Fusions of the transcription factor 3 gene: *TCF3.*

TCF3, a gene involved in B cell differentiation, is the partner of two recurrent fusions. The most common is related to t(1;19)(q23;p13.3), which leads to the formation of the fusion transcript *TCF3-PBX1* and is observed in approximately 5% of BCP-ALL cases in both children and adults. An invasion of the central nervous system is more frequently observed in this group [17], and a greater risk of meningeal relapse has been reported [22]. Initially associated with a poor prognosis, this entity is now treated with contemporary intensive chemotherapy treatments, and the prognosis has become standard or even favourable, even in adults [17, 23, 24].

A variant of the translocation t(1;19) is t(17;19)(q23;p13), which leads to the *TCF3-HLF* fusion transcript. This defines a very rare entity, which is more likely to affect children or young adults and is associated with a disastrous prognosis [25].

14q32 abnormalities/rearrangements of immunoglobulin heavy chain genes

Illegitimate rearrangements of genes encoding immunoglobulin heavy chains (*IGH* locus) can lead to an overexpression of an oncogene under the control of regulatory sequences of the *IGH* locus, similar to the *IGH-MYC* translocation in Burkitt lymphoma. This type of rearrangement has been described with various genes in BCP-ALL and therefore does not define a homogeneous oncogenic group. However, studies based on cytogenetic classification tend to group them together and indicate an unfavourable prognosis [26].

The partner gene most frequently involved in *IGH* rearrangements detected by cytogenetic testing is the gene encoding the cytokine receptor-like factor 2 (CRLF2) (see below: BCR-ABL1-like alterations). Other partners include the transcription factors of the CCAAT/enhancer-binding protein (CEBP) family: *CEBPA*, *CEBPB*,

CEBPD, *CEBPE* and *CEBPG* [27], the oncogene *MYC*, the anti-apoptotic factor *BCL2*, and the differentiation inhibitor *ID4*.
In addition, the translocation t(5;14)(q31;q32) has also been reported, which deregulates expression of the interleukin-3 (IL-3) gene [28, 29]. This is a very rare abnormality [30], characterised by proliferation of eosinophilic polynuclear cells due to their paracrine stimulation by IL-3-secreting leukaemic cells. The mechanism of deregulating intracellular signalling, downstream of the IL-3 receptor, is similar to a BCR-ABL1-like alteration [31].
Finally, it should be noted that other rearrangements involve the *IGH* locus but are most often undetected on karyotyping, even with FISH. These include *IGH-DUX4* and *IGH-EPOR* (for erythropoietin receptor) rearrangements, which are discussed below.

Intrachromosomal amplification of chromosome 21

Intrachromosomal amplification of chromosome 21 (iAMP21) defines a rare subtype that mainly concerns older children. Its rather unfavourable prognosis is improved by intensifying therapy [32].

Paired box 5 fusions

The paired box 5 (*PAX5*) gene encodes a transcription factor essential for the differentiation of B lymphoid cells. Fusions of this gene with multiple partners have been identified [33-35]. These abnormalities are considered to be initiators of leukaemia [35, 36] but, probably due to their rarity and the heterogeneity of the fusion partners, the biological, clinical and prognostic characteristics of this group have not been established.

Secondary anomalies

About ten years ago, techniques in whole-genome copy number analysis (CGH-array and SNP-array) revealed the existence of numerous additional alterations in BCP-ALL, indicating a previously unsuspected complexity and multiplicity of oncogenic events [37, 38]. Indeed, screening of the BCP-ALL genome often reveals multiple microdeletions, produced by illegitimate recombination by RAG recombinase, an active recombinase during the maturation process of lymphoid precursors [3]. These alterations lead to the inactivation of a large number of genes, encoding [39-42]:

– transcriptional regulators of B lymphoid development such as *PAX5*, *IKZF1*, *LEF1* or *EBF1*,
– proteins involved in cell cycle regulation and non-specific tumour suppressors, such as cyclin-dependent kinase inhibitors 2A (CDKN2A) and 2B (CDKN2B), the retinoblastoma gene *(RB1*) and TP53,
– signalling channel controllers such as PTPN11, NF1, and SH2B3,
– epigenetic regulators such as *c*-AMP response element-binding protein binding protein (CREBBP) and lysine demethylase 6A (KDM6A) *(table 2*),
– or other targets involved in response to treatment such as nuclear receptor subfamily 3 group C member 1 (*NR3C1*), which encodes glucocorticoid receptor, and 5'-nucleotidase, cytosolic II (NT5C2), encoding a nucleotidase involved in purine metabolism.

These same genes can often also be targeted by inactivating mutations. A final category of additional abnormalities can be distinguished corresponding to activating mutations in genes encoding signalling factors such as Fms-like tyrosine kinase 3 receptor (FLT3), IL-7 receptor (IL7R) and actors in the Janus kinase signal transducers and activators of transcription (JAK-STAT) and Ras pathways.

Table 2

Main additional recurring BCP-ALL anomalies.

		Frequency (%)	Comments
Cell cycle and tumour suppressors			
CDKN2A/CDKN2B	Deletion	30-40	Associated with del(9p) and -9.
TP53	Deletion, mutation	5	Associated with hypodiploidies (*low hypodyploidy*) [14]
RB1	Deletion	5-10	Associated with hypodiploidies (*low hypodyploidy*) [14]
Haematopoietic and lymphoid development			
IKZF1	Total, intragenetic deletion, mutation	30	Associated with Ph+ and *Ph-like* ALLs [12] High-risk criterion based on GRAALL-2014 [10]
PAX5.	Deletion, mutation	20	Excluding $PAX5^{P80R}$ which defines an oncogenetic subtype
ETV6	Deletion	10-15	Associated with subtype *ETV6-RUNX1*
EBF1	Deletion	5-10	[37]
ERG	Intragenic deletion	5	Associated with DUX4 subtype [74] Good prognosis in children [75], no data in adults
Signalling			
JAK1/JAK2	Mutation		Associated with the rearrangement of *CRLF2* [31, 63]
Ras pathway	Mutation or deletion of NRAS, KRAS, NF1 and PTPN11		Selected during processing [57]
IL7R	Mutation		Associated with the *Ph-like* profile [31, 63]
SH2B3	Deletion		Associated with the *Ph-like* profile [31, 63]
Epigenetic regulators			
CREBBP	Deletion and mutation		Resistance to glucocorticoids [42]
EP300	Mutation		*CREBBP* paralogue [39]
Other			
NR3C1	deletion, mutation		Glucocorticoid receptor
NT5C2	Mutation		Resistance to purine analogues [40]

The prevalence of these different secondary abnormalities varies considerably within the different genetic subtypes of BCP-ALL, suggesting significant functional cooperation and selective constraints. The most important alterations in terms of frequency and/or clinical impact are outlined below.

Ikaros zinc finger protein 1

Among these abnormalities, deletions of *IKZF1* are reported in 30% of adult Ph- BCP-ALL cases [10] and about 80% of Ph+ ALLs [43]. *IKZF1* encodes a transcription factor essential for lymphoid differentiation. The most frequent alterations of *IKZF1* are intragenic focal deletions that are RAG-mediated (deletion of exons 4 to

7), leading to the expression of dominant negative or non-functional isoforms. Complete deletions of *IKZF1* are also observed, notably for monosomy 7 or deletion of the short arm of chromosome 7, leading to haploinsufficiency. Rarer point mutations are also described.

Genomic alterations in *IKZF1* have been described in numerous adult and paediatric studies with a poor prognosis [10,44,45]. The presence of *IKZF1* deletion is now a criterion for therapeutic stratification in some current studies, including the GRAALL-2014 study, in which intragenic deletions were one of the high-risk criteria. The prognostic role of the different types of deletions (complete, intragenic, or affecting exons 4-7) is still the subject of debate and appears to differ depending on the population being studied (paediatric or adult) and the treatment protocol [10, 45, 46].

Other members of the Ikaros family of transcription factors are altered in BCP-ALL, such as *IKZF2* and *IKZF3* in low-hypodiploid and near-haploid ALLs, respectively [14].

PAX5

Genomic deletions of *PAX5*, located on chromosome 9p, as well as point mutations are common abnormalities in BCP-ALL [37, 47]. Unlike fusion genes involving *PAX5*, deletions and mutations have been described as additional oncogenic events (with the exception of the P80R mutation, *see below*).

TP53

Although *TP53* alterations, mutations and deletions are associated with treatment resistance and poor prognosis in many cancers, relatively few data are available for BCP-ALL. A study of a heterogeneous cohort of ALL (B and T in adults and children) reported a frequency of 16%, increasing with age [48]. In paediatric BCP-ALL cohorts during a first relapse, changes in *TP53* are associated with a higher risk of further relapse and reduced survival [49, 50].

In adults, the prognostic impact of *TP53* alterations remains controversial, particularly due to the association with older age, a low-hypodiploid karyotype, and *MYC* rearrangements, which represent confounding factors [48, 51-53].

RAS pathway

Alterations in the genes encoding essential components of the RAS pathway, *NRAS*, *KRAS* and its regulators such as *PTPN11* phosphatase (for protein-tyrosine phosphatase non-receptor type 11) and ubiquitin ligase c-CBL (for Casitas B-lineage lymphoma), are common in BCP-ALL based on published data, mainly on paediatric cohorts. These alterations are particularly frequent, and around 50% are reported in some subtypes such as BCP-ALLs with rearrangements of *KMT2A* and *iAMP21* [54-56]. The presence of these mutations has been shown to be associated with reduced sensitivity to chemotherapy and an increased risk of relapse [57-59], particularly in ALLs with standard hyperdiploidy [60]. The sensitivity of cells to MEK inhibitors (mitogen-activated extracellular signal-regulated protein kinase) in different *in vitro* and *in vivo* models [57, 61] suggests a therapeutic opportunity that should be explored.

New entities

Until recently, almost 50% of adult BCP-ALLs, the so-called “B-others”, could not be assigned to a genetic subtype because no “classifying” abnormality was revealed through standard cytogenetic and molecular biological testing. Analyses of large-scale gene expression profiles have shown that homogeneous subtypes of leukaemia remain genetically unresolved. In recent years, several studies, based, in

particular, on transcriptome sequencing (RNA-seq), have identified cryptic genomic fusions leading to new BCP-ALL entities.

BCR-ABL1-like

The BCR-ABL1-like, or Ph-like group was originally described based on expression profiling studies in paediatric BCP-ALL cohorts as a group of patients presenting a transcriptional signature similar to Ph+ BCP-ALL but without a BCR-ABL1 fusion transcript [62-64]. This group has subsequently been observed in adults, with a prevalence that increases with age and reaches over 20% in some cohorts [65]. It is characterised by poor sensitivity to chemotherapy and a poor prognosis, similar to Ph+ ALLs in the pre-ITK era [66]. Like Ph+ ALL, this subtype of BCP-ALL is associated with a high frequency of intragenic deletions of *IKZF1* as well as other lymphoid factor alterations. The so-called BCR-ABL1-like genomic alterations are particularly varied [67].

A first group, representing about 12% of BCR-ABL1-like cases, consists of fusions very similar to *BCR-ABL1,* involving Abelson class (ABL) kinase genes (*ABL1*, *ABL2*, platelet-derived growth factor alpha [*PDGFRA*] and beta [*PDGFRB*] receptors or colony stimulating factor 1 receptor [*CSF1R*]) as well as many other different fusion partners that appear to lead to a mechanism that closely resembles constitutive activation [31, 65]. ABL class kinase fusions are generally sensitive to imatinib-like ITKs [68], which led to the recommendation of a combination of imatinib and first-line chemotherapy for all patients with this type of alteration based on the GRAALL-2014 study.

Another class of fusions, BCR-ABL1-like, involve *JAK2* and a large number of fusion partners (7% of BCR-ABL1-like cases). These alterations result in constitutive activation of JAK2 kinase and are sensitive *in vitro* to JAK2 inhibitors such as ruxolitinib. However, the clinical interest in this type of inhibitor remains to be assessed.

Finally, some less frequently described fusions target other kinases such as fibroblast growth factor receptor type 1 (FGFR1), tyrosine kinase 2 (TYK2) and neurotrophic tyrosine kinase receptor type 3 (NTRK3).

Other BCR-ABL1-like alterations target cytokine receptors or downstream signalling factors. Half of BCR-ABL1-like ALLs show deregulation of the *CRLF2* gene. *CRLF2* encodes cytokine receptor-like factor 2 which forms a heterodimeric receptor with the alpha chain of the IL-7 receptor, of which the ligand is thymic stromal lymphopoietin (TSLP). The intracellular part of this receptor interacts with JAK1 and JAK2 kinases and activation of the receptor leads to activation of the JAK-STAT, phosphoinositide 3 kinase (PI3 kinase)-protein kinase B (AKT) and Ras-mitogen-activated protein (MAP) kinase pathways. *CRLF2* can be deregulated by translocation into the *IGH* locus *(IGH-CRLF2*) or by focal deletion upstream of *CRLF2* (deletion of the pseudo-autosomal region, PAR1), resulting in the formation of a *P2RY8-CRLF2* fusion transcript. *JAK2* activating mutations are observed concomitantly in half of the cases of BCP-ALL with *CRLF2* rearrangements, and less frequently with *IL7R* mutations and *CRLF2* activating mutations (F232C) [69].

Genomic rearrangements involving the gene encoding the erythropoietin receptor (EPOR) represent another class of BCR-ABL1-like alterations (3% of cases). *EPOR* rearrangements comprise reciprocal translocations with the *IGH* locus or cryptic insertions of part of *EPOR* into the *IGH* locus or other loci, which cause over-expression of the receptor and truncate its cytoplasmic tail, resulting in activation of JAK-STAT signalling [64,70].

Finally, various alterations of other signalling factors or receptors have been identified, some of which target the JAK-STAT pathway (activating mutations of *JAK1*, *JAK2* or *JAK3*, and deletions or inactivating mutations of *SH2B3*), the Ras

channel (activating mutations of *NRAS*, *KRAS* or *BRAF*, and inactivating mutations or deletions of *NF1* or *PTPN11*), or the FLT3 or IL7R receptors [31, 65, 71].
All of these BCR-ABL1-like alterations may lead to identification of potential therapeutic targets, which is currently being evaluated clinically using different inhibitors, including dasatinib (NCT02883049, NCT03564470, NCT03117751, NCT02420717), ruxolitinib (NCT02723994, NCT03571321, NCT02723994, NCT02420717, NCT03117751) and selumetinib (NCT03705507).

The *DUX4/ETS* transcription factor gene

Another recently identified BCP-ALL entity is linked to genomic rearrangements of *DUX4* associated with ERG (avian erythroblastosis virus E26 homologue [ETS] transcription factor gene) deregulation [72-74]. Initially, a form of BCP-ALL representing approximately 4% of paediatric cases was described, characterised by the presence of recurrent intragenic deletions of the *ERG* gene excluding other "classifying" abnormalities. This group exhibited cytological and immunophenotypic peculiarities; frequent expression of CD2 and/or CD56 and the presence of monocytoid cells [75, 76], as well as a high frequency of intragenic deletions of *IKZF1*. Survival of this group in several paediatric studies was surprisingly good, with no adverse effects associated with the *IKZF1* deletions [75, 76]. More recently, based on RNA-seq studies, the initiating abnormality and associated molecular mechanism has been described; a rearrangement of the *IGH* locus involving the *DUX4* gene, leading to its ectopic over-expression. *DUX4* encodes a homeodomain transcription factor located in the subtelomeric 4q region of the *D4Z4* macrosatellite repeat. The overexpressed DUX4 protein binds to an intragenic region of *ERG* and induces the expression of alternative *ERG* transcripts and the production of truncated protein isoforms that have a transforming effect [74]. Binding of DUX4 at the *ERG* locus also leads to an opening in the locus and accessibility to RAG recombination enzymes responsible for illegitimate rearrangements that cause the recurrent deletions observed [74].
DUX4 rearrangements are cryptic based on cytogenetics, and testing for intragenic *ERG* deletions, present in about two thirds of BCP-ALL DUX4/ERG cases, is the only simple diagnostic technique to identify those cases that would otherwise require integrated analysis through gene expression profiling. The prognosis for this group of adult BCP-ALL has yet to be reported.

Zinc finger 384 fusions

Fusions involving the zinc finger *384* gene *(ZNF384*) define another new subtype of BCP-ALL, accounting for about 7% of adult cases [77]. Many partners, usually transcriptional or chromatin regulators (transcription factor 3 [*TCF3*], histone acetyltransferase p300 [*EP300*], *CREBBP*, TATA-box binding protein associated factor 15 [*TAF15*], synergin gamma [*SYNRG*], Ewing sarcoma breakpoint region 1 [EWSR1], AT-rich interaction domain 1B [*ARID1B*], bone morphogenic protein 2 inducible kinase [*BMP2K*], and switch/sucrose non-fermentable-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 2 [*SMARCA2*]) may be involved in fusions that do not affect the entire coding part of *ZNF384*. Rearrangements of *ZNF384* are frequently observed in BCP-ALLs that do not express or poorly express CD10 and co-express myeloid markers, as well as acute mixed B/myeloid phenotype leukaemia [78]. The prognosis for this group of BCP-ALLs is not yet clear.

Myocyte enhancer factor 2D fusions

Fusions involving the myocyte enhancer factor 2D (*MEF2D*) gene have also been reported recently in children and adults. So far, seven partners have been described: *BCL9, CSF1R,* deleted in azoospermia-associated protein 1 (*DAZAP1*),

forkhead box J2 (*FOXJ2*), heterogeneous nuclear ribonucleoprotein H1 (*HNRNPH1*), heterogeneous nuclear ribonucleoprotein U like 1 (*HNRNPUL1*) and *SS18* [79-81]. Regardless of the partner, the fusion preserves the MADS-box domain of *MEFD2* that binds to DNA, and leads to over-expression of *MEF2D*, which has been reported to have leukaemogenic effects *in vitro* and *in vivo* [79]. This subtype is associated with advanced age and a particular immunophenotype; CD10- and CD38+. The prognosis associated with *MEF2D* fusions appears to be rather unfavourable [79], however, this needs to be confirmed with larger cohorts and in adults. Deregulation of *MEF2D* could be of interest therapeutically as one of the target genes of *MEF2D* is *HDAC9,* and significant sensitivity to panobinostat (histone deacetylase inhibitor) has been described in xenograft models [79].

PAX5^{P80R}

A study conducted in our team, in collaboration with the GRAALL investigators, has enabled us to describe a new group of adult BCP-ALLs [82]. In order to identify primary genomic alterations in unresolved adult BCP-ALL cases, we performed transcriptome sequencing of 170 B-other cases. An unsupervised clustering study of gene expression data combined with a search for fusions identified some recently described genetic groups, *ZNF384*, *ERG/DUX4*, BCR-ABL1-like as well as a cluster of cases with no identified genomic alterations. A single mutation of *PAX5*, c.239C>G, p.P80R, was found in all of these cases and was not identified in other groups, suggesting that this mutation defines a distinct group of BCP-ALLs. Screening of an additional cohort of adult BCP-ALL patients included in the GRAALL studies led to identification of additional cases and analysis of the characteristics of this new entity. The prevalence of the PAX5^{P80R} mutation was 5.3% in adult Ph- BCP-ALL and this mutation has never been observed in association with other "classifying" abnormalities, unlike other *PAX5* mutations and deletions. Inactivation of the second allele of *PAX5*, by deletion or secondary mutation, was consistently identified, as were additional alterations in the Ras pathway or the CRLF2/IL7R-JAK-STAT axis. Deletions of *CDKN2A* were also frequent, often in association with 9p deletions, as well as complete deletions of *IKZF1* by 7p deletion, while intragenic deletions of *IKZF1* were very rare. Finally, patients with this subtype of BCP-ALL had a better molecular response post-induction and improved survival (91% versus 47% for other patients, p= 0.036). This is the first oncogenic subtype of BCP-ALL defined by a point mutation as well as the first subtype reported with a favourable prognosis in adults, at least in the context of intensive paediatric chemotherapy.

Double hit: *MYC* and *BCL2*

MYC and *BCL2* translocations are recurrent abnormalities in B lineage lymphomas. Cases with these two translocations are referred to as "double hit" lymphoma and are associated with a poor prognosis. Rare cases of BCP-ALL may harbour this combination of *MYC* and *BCL2* rearrangements, which also appears to be associated with a poor prognosis [83]. A better understanding of the biology of this ALL entity is required along with an assessment of whether BCL2 inhibitors, which appear to be promising in the treatment of double hit lymphomas, might also be effective in these cases of ALL.

Other entities

Two studies describe a new entity, referred to as ETV6-RUNX1-like, defined by an expression profile similar to that of ETV6-RUNX1. To date, this profile has not been described in adults [73, 84]. A similar rare subtype in children, but not reported in adults, has also been reported based on fusions involving the NUT midline carcinoma family member 1 gene (*NUTM1*) [73, 79].

A prospective diagnostic strategy centralised at Saint-Louis Hospital

The haematology laboratory at the Saint-Louis Hospital in Paris, the reference laboratory for the GRAALL-2014 study of oncogenetics of Ph- BCP-ALLs, aims to characterise Ph- BCP-ALLs in adults using an exhaustive genome-based approach in order to determine prognosis associated with the various "classifying" anomalies and identify therapeutic targets. In this context, a diagnostic strategy has been implemented, combining a search for known recurrent genetic alterations and investigation of unsolved cases by next-generation sequencing (NGS) (*figure 2*). This strategy is applied prospectively in patients, in particular, with the aim of identifying abnormalities constituting therapeutic targets for TKIs, especially in patients who respond poorly to chemotherapy [68].

Conventional cytogenetics

Prior to the centralised oncogenetic assessment of BCP-ALL, the cytogenetic analysis performed in each clinical centre by a network of competent cytogeneticists remains fundamental. Not only does it enable the early detection of t(9;22)(q34;q11.2), an indication for treatment combining ITK and chemotherapy, but also allows the identification of aneuploidies as well as chromosomal translocations that define certain subtypes of BCP-ALL, such as *KMT2A* rearrangements, which represented a high-risk criterion in the GRAALL-2014 study.

Intragenic deletions of Ikaros zinc finger protein 1 and ETS transcription factor genes

The presence of intragenic deletions of *IKZF1* (which are cryptic based on cytogenetic testing), representing another high-risk criterion in the GRAALL-2014 study, may be systematically determined based on genomic breakpoint-specific multiplex fluorescent PCR [85]. This technique detects deletions with clustered breakpoints in the target regions of RAGs.

The presence of intragenic *ERG* deletions, which, like *IKZF1* deletions, are cryptic based on cytogenetic testing, may also be systematically tested using genomic breakpoint-specific multiplex fluorescent PCR [75]. It is possible to quickly and easily identify approximately two thirds of patients in the DUX4/ERG group due to the detection of intragenic *ERG* deletions.

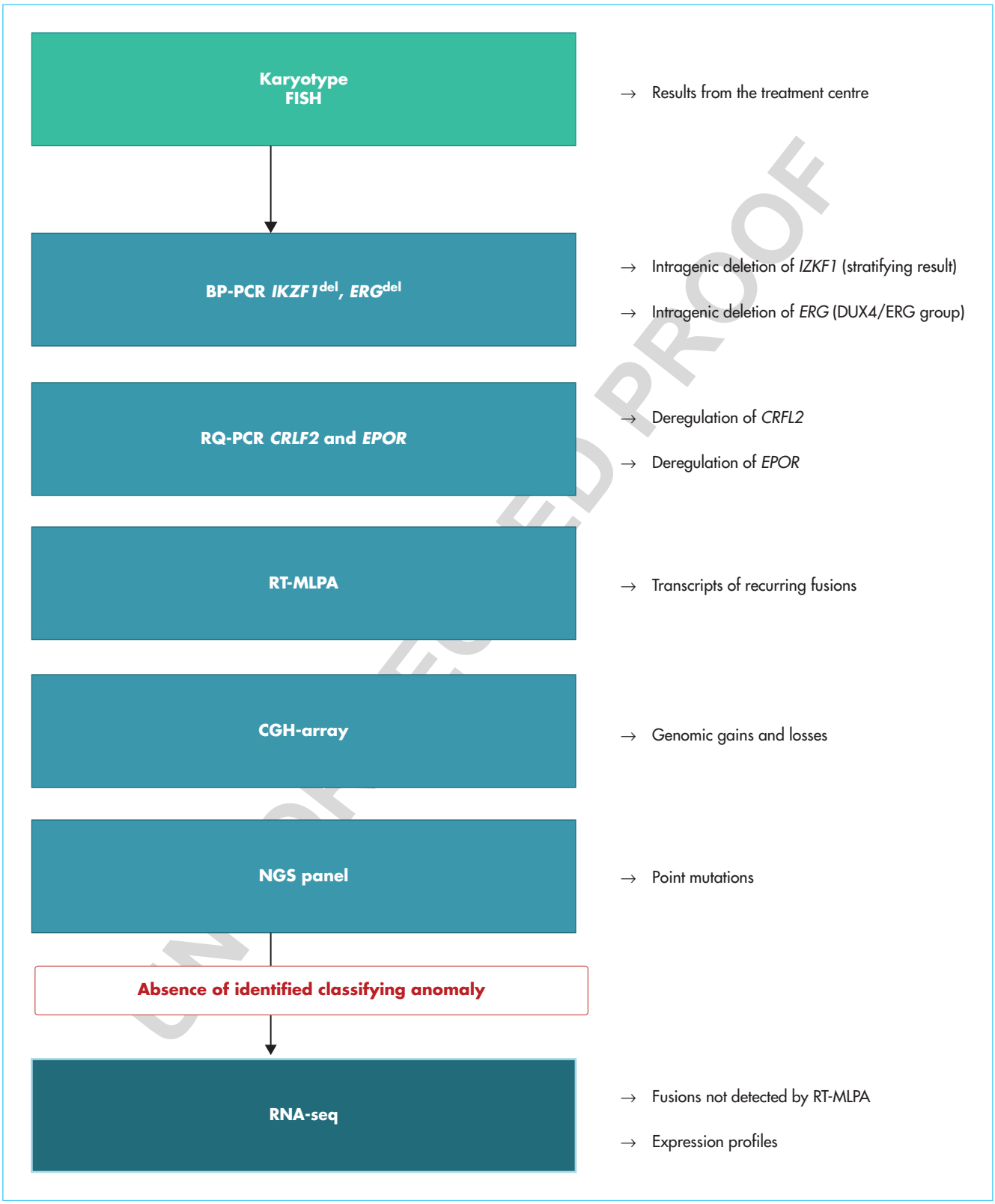
Expression of cytokine receptor like factor 2 and erythropoietin receptor genes

In order to identify certain patients in the Ph-like ALL group, the expression of *CRLF2* and *EPOR* is quantified by RQ-PCR in order to detect any anomalies that may result from genomic rearrangements such as *IGH-CRLF2* and *IGH-EPOR*. For *CRLF2*, the difference in expression is measured between *CRLF2* and a reference gene, *ABL1*, with an over-expression threshold validated using FISH based on cases with known rearrangement. For *EPOR*, over-expression of the normal transcript or its truncated form is determined by measuring expression of two exons using two PCR systems.

Reverse transcription-multiplex ligation-dependent probe amplification

The search for fusion transcripts already described in the literature was carried out using reverse transcription-multiplex ligation-dependent probe amplification (RT-MLPA), which is based on a mixture of a very large number of probes corresponding to the potentially fused sequences. This technique, which was developed to detect transcripts of fusions from different types of acute leukaemia [86], has been adapted and enriched for the very large number of fusions recently described in BCP-ALLs. In particular, it enables rapid identification of previously reported fusions involving ABL class genes in accordance with the new therapeutic

FIGURE 2



recommendation from the GRAALL-2014 study, advocating the combination of imatinib and first-line chemotherapy from the consolidation phase for all patients with this type of alteration.

Comparative genomic hybridisation

Chromosomal analysis using CGH-array is also performed to highlight the many additional microdeletions that characterise the different subtypes of BCP-ALL. This can aid the differential diagnosis between duplicated hypodiploidy and hyperdiploidy. In certain cases, CGH-array may reveal unbalanced translocations which would not have been detected by karyotyping or highlight alterations in case of karyotype failure.

Search for mutations: a new-generation sequencing panel

For an even more complete characterisation of the genetic alterations of BCP-ALL, we established high-throughput sequencing of a panel of 160 genes selected on the basis of their known or suspected involvement in the pathogenesis of BCP-ALL, based on the literature and CGH-array data obtained in the laboratory. Two types of analysis are performed on the data obtained: an analysis of variants (which allows point mutations to be identified) and copy number analysis (which allows genomic gains and losses to be identified). This sequencing allows us to reveal $PAX5^{P80R}$ mutation [82].

Transcriptome sequencing

In the absence of a "classifying" anomaly based on the analyses described above, transcriptome sequencing (RNA-seq) is performed. The data obtained are first analysed to identify fusions that are not detected by karyotyping or RT-MLPA. This is the case for some *IGH* fusions (for example, *IGH-DUX4*) and fusion transcripts that are not reported due to significant heterogeneity of fusions in cases of BCP-ALL. Global expression analysis by hierarchical clustering of the RNA-seq data then makes it possible to identify groups of patients with a similar gene expression profile.

Conclusion and outlook

Recently, the development of genome-wide analysis techniques has led to the identification of many new recurrent alterations in paediatric and adult cohorts of BCP-ALL. These "classifying" alterations, as well as the numerous associated secondary alterations, form a very heterogeneous and complex adult BCP-ALL genetic landscape. Indeed, with the exception of Ph+ ALL, no single genetic subtype accounts for more than 10% of adult B-ALLs. To further delineate prognosis, clinico-biological characteristics of the different entities, as well as their response to treatment should be investigated in large cohorts of patients treated in a homogeneous manner.

In addition, the characterisation of genetic alterations may make it possible to identify therapeutic targets, such as ABL class fusions in Ph-like ALLs, which may justify an in-depth molecular diagnostic approach within a timeframe compatible with adaptable therapeutic management. Numerous clinical trials are under way to evaluate the contribution of TKIs in chemotherapy, which will also be evaluated as part of an investigation of patients treated according to the GRAALL-2014 recommendation.

Alongside the evolution of techniques that allows the entire genome to be investigated, considerable developments in the treatments available for BCP-ALL has taken place in recent years, with the development of monoclonal antibodies and CAR-T cells. New classes of pharmacological inhibitors already used for other

diseases may also be of interest in BCP-ALL. It will therefore be important to analyse the response of different types of leukaemia to these new treatments, in order to identify the most appropriate treatments for each patient and move towards optimised management.

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