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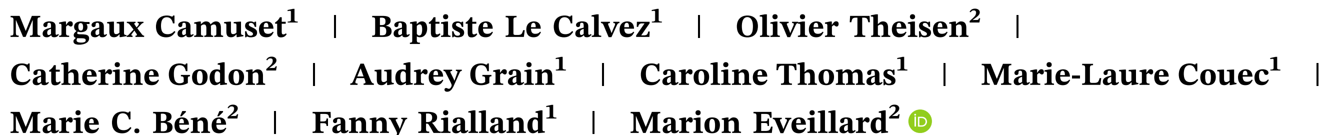
ORIGINAL ARTICLE

CANCER INNOVATION

Added value of molecular karyotype in childhood acute lymphoblastic leukemia



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Abstract

Background: Thanks to an improved therapeutic regimen in childhood B-cell precursor acute lymphoblastic leukemia (BCP-ALL), 5 year-overall survival now exceeds 90%. Unfortunately, the 25% of children who relapse have an initial poor prognosis, potentially driven by pre-existing or emerging molecular anomalies. The latter are initially and essentially identified by cytogenetics. However, some subtle alterations are not visible through karyotyping.

Methods: Single nucleotide polymorphisms (SNP) array is an alternative way of chromosomal analysis allowing for a more in-depth evaluation of chromosomal modifications such as the assessment of copy number alterations (CNA) and loss of heterozygosity (LOH). This method was applied here in retrospective diagnosis/relapse paired samples from seven children with BCP-ALL and in a prospective cohort of 38 newly diagnosed childhood cases.

Results: In the matched study, compared to the initial karyotype, SNP array analysis reclassified two patients as poor prognosis cases. Modulation during relapse was seen for 4 CNA and 0.9 LOH. In the prospective study, SNP reclassified the 10 patients with intermediate karyotype as 7 good prognosis and 3 poor prognosis. Ultimately, in all the children tested, SNP array allowed

Abbreviations: ALL, acute lymphoblastic leukemia; BCP-ALL, B-cell precursor-acute lymphoblastic leukemia; BM, bone marrow; CAN, copy number alterations; CAN-GR, CAN good risk; CBF-AML, core-binding factor acute myeloblastic leukemia; CGH, Ccomparative genomic hybridization; CNA-IR, CAN intermediate risk; CNS, central nervous system; CR, complete remission; DNA, deoxyribonucleic acid; EGIL, European group for immunophenotyping of leukemias; FISH, fluorescent in situ hybridization; FLT3, Fms-like tyrosine kinase 3; FRALLE, French acute lymphoblastic leukemia; GRAALL, group of research for adult acute lymphoblastic leukemia; iAMP21, intrachromosomal amplification of chromosome 21; LOH, loss of heterozygosity; MFC, multiparametric flow cytometry; MLPA, multiplex ligation-dependent probe amplification; MRD, minimal residual disease; OS, overall survival; PB, peripheral blood; PCR, polymerase chain reaction; RPMI, Rosswell Park Memorial Institute; RT, reverse transcriptase; SNP, single-nucleotide polymorphism; SNV, single nucleotide variant; TKI, tyrosine kinase inhibitor; WBC, white blood cells; WHO, World Health Organization.

Margaux Camuset and Baptiste Le Calvez contributed equally to this study and shared first authorship.

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to identify additional anomalies compared to conventional karyotype, refine its prognostic value and identify some druggable anomalies that could be used for precision medicine. Overall, the anomalies detected could be segregated in four groups respectively involved in B-cell development, cell proliferation, transcription and molecular pathways.

Conclusion: SNP therefore appears to be a method of choice in the integrated diagnosis of BCP ALL, especially for patients initially classified as intermediate prognosis. This complementary method of both cytogenetics and high throughput sequencing allows to obtain further classified information and can be useful in case of failure of these techniques.

KEYWORDS

BCP-ALL, karyotype, pediatric patients, prognosis, SNP array

1 | INTRODUCTION

Therapeutic progress in childhood B-cell precursor acute lymphoblastic leukemia (BCP-ALL) has resulted in a 5-year overall survival (OS) that now exceeds 90% [1]. Unfortunately, nearly a quarter of patients will relapse, partitioned into 60% of isolated medullary relapses, 20% of isolated central nervous system (CNS) relapses, 10% of combined relapses and 10% of isolated testicular relapses. In the event of a second cytological remission (CR) for these high-risk patients, 5-year OS is unfortunately not exceeding 30% to 40% [2].

Furthermore, in view of the excellent results obtained in pediatric BCP-ALL, therapeutic de-escalation should be offered to patients with good prognostic markers.

It seems therefore essential to stratify BCP-ALL children according to prognostic factors to best adapt therapy. Risk stratification, defined by the National Cancer Institute (NCI) is based on clinico-biological factors at diagnosis (including cytogenetics) and response to treatment determined by minimal/measurable residual disease (MRD).

Cytogenetically, recurrent chromosomal alterations such as aneuploidy or translocations identified by karyotype or fluorescence in situ hybridization (FISH) separate the ALL subtypes of the WHO classification [3]. High hyperdiploidy (>50 chromosomes) and *ETV6-RUNX1* fusion/t(12;21) and *DUX4*-rearrangement are associated with favorable outcome. *TCF3-PBX1* fusion/t(1;19), historically considered of high cytogenetic risk, is now associated with intermediate prognosis with appropriate therapy. Low hypodiploidy (<40 chromosomes) constitutes a poor prognosis factor, often associated with *TP53* mutation, as well as near-haploid cases (24–31 chromosomes) associated with *RAS*-activating mutations. In the same way, *KMT2A* gene rearrangements are most frequently found in infants (<1 year) and associated with poor prognosis. *BCR-ABL1* (Philadelphia chromosome) positive ALL, less common in children than in adults, were also originally associated with high-risk before the era of tyrosine kinase inhibitors (TKIs) which greatly improved their prognosis. Finally, intrachromosomal amplification of chromosome 21 (iAMP21), occurring in older children and associated with an adverse prognosis, had become a new provisional entity since the update of the 2016 WHO classification [3].

New technologies, including DNA microarray analyses and genomic sequencing, have led to the identification of other genetic anomalies, not detectable by conventional cytogenetics. Novel findings mainly concern «B-other ALL», a group of BCP-ALL with previously unknown genetic background, defined by the absence of the classifying aberrations described above and often associated with poor prognosis. They constitute a provisional WHO entity [3], also called Phi-like ALL, considered of poor prognosis. The Ph-like molecular signature comprises a variety of genetic alterations. It induces the activation of a small number of tyrosine kinase receptors (such as CRLF2, JAK2, or EPOR) or the activation of intracellular signaling pathways such as JAK/STAT, ABL, or MAPK. These alterations are possibly targeted by TKIs or JAK2 inhibitors. More recently, BCP-ALL with *DUX4*-, *MEF2D*-, *ZNF384*-rearrangements, or *ETV6-RUNX1*-like have been singled-out [4, 5].

These genetic anomalies, considered as initiating events, often require additional events to induce leukemogenesis, such as copy number alterations (CNAs) (deletions or gains) and sequence mutations, that affect genes involved in lymphoid differentiation, proliferation,

cell cycle and transcription [6]. The most frequently altered loci are *EBF1*, *IKZF1*, *PAX5*, *CDKN2A/B*, *ETV6*, *BTG1*, *RB1*, and *PAR1* (for the detection of the *P2RY8-CRLF2* fusion) [7], yet with variable prevalence and impact, *IKZF1* alterations, hallmark of Phi-like ALL, being associated with poor outcome [8].

CNAs can be explored by comparative genomic hybridization or single nucleotide polymorphism array (SNP-array). The latter, also dubbed "molecular karyotyping," complements usefully karyotyping analysis and FISH and can also detect loss of heterozygosity (LOH). SNP-array can be of major interest for the identification of new potential therapeutic targets. It can also help to monitor disease evolution such as the emergence of new sub-clones that may respond differently to treatment.

Here, we report on SNP-array analyses performed in two groups of childhood BCP-ALL. A first retrospective approach, in matched diagnosis/relapse samples from seven patients resulted in risk-reclassification that could have modified the therapeutic strategy and disclosed relapse-associated changes. Based on these results, SNP-array analyses were then prospectively performed in a cohort of 38 newly diagnosed BCP-ALL children, providing useful information for the management of potentially occurring relapses.

2 | MATERIALS AND METHODS

Seven patients, treated at Nantes University Hospital for BCP-ALL, at initial diagnosis and then at relapse, were enrolled retrospectively between March 2007 and September 2016. Then, 38 children, newly diagnosed for BCP-ALL from October 2016 to November 2018, were enrolled prospectively. The diagnosis was established according to the standard morphologic and immunophenotypic criteria [9, 10]. Patients, or their parents, were informed and had signed a written consent for inclusion in the research protocol that had been validated by the institutional ethics board.

Patient samples were analyzed extemporaneously during diagnosis and relapse using multiparametric flow cytometry, standard karyotype, and FISH. SNP-array (Affymetrix®) was performed on cryopreserved cells to determine CNA and LOH.

As mentioned above, multiparameter flow cytometry was performed according to recommendations by EGIL and the European LeukemiaNet [11]. Briefly, a method of stain-lyse-wash was used on 50 µL per tube of whole blood or bone marrow at diagnosis. Data were acquired using a Canto II® (BD Biosciences) instrument and analyzed with Diva (BD Biosciences) software and expressed as a percentage of blast cells expressing each individual marker. Blast cells themselves were gated on a CD45/side scattergram. An extensive panel was used to ascertain B-lineage markers expression, in the absence of T- and myeloid lineage antigens that would have oriented toward mixed phenotype acute leukemia. All the patients had *bona fide* BCP-ALL.

Conventional cytogenetics was performed from bone marrow or blood samples according to guidelines of the European LeukemiaNet-Workpackage Cytogenetics [12]. Briefly, the cells were grown for 17 and 24 h in RPMI supplemented with fetal calf serum, heparin, penicillin, and streptomycin according to the specifications of the French group of cytogenetics [13]. Mitoses were blocked with colchicin and band-R stained on glass slides. They were then photographed with an automated microscope linked to the classification and interpretation tool Cytovision® (Leica Biosystems) software. Results were reported in accordance with the recommendations of the ISCN 2016 [14].

All material was tested by FISH for *ETV6-RUNX1* and *BCR-ABL1* fusions and *KMT2A* rearrangements. Additionally, pre-B cases were tested for *TCF3-PBX1* rearrangement. FISH analysis on cell suspensions used Vysis (Vysis) and was analyzed on an Imager M2 fluorescence microscope (Zeiss) with images captured by Cytovision® image analysis system (Leica Biosystems). For each patient, at least 10 mitoses and 100 nuclei were analyzed.

SNP-Array allows to search for polymorphisms on the whole DNA. The percentage of blasts was recorded for each sample. DNA was extracted using standard methods (Qiagen) from bone marrow (BM) or peripheral blood (PB) cells that had been stored at −80°C or in fixative at −20°C. SNP array analysis was performed using the CytoScan® HD Array (Thermo Fisher Scientific) and analyzed with the Affymetrix's ChAS® (Chromosome Analysis Suite) software. This allowed for the detection of very small abnormalities throughout the genome (loss and gain of material, CNA, LOH). Genetic analysis of germline material was not performed in this study.

3 | RESULTS

3.1 | Patient cohorts

Clinico-biological characteristics of the patients are described in Table 1.

In the first retrospective cohort, seven patients was selected, for whom diagnosis and relapse samples were available. They were three boys and four girls with a median age of 6 and 10 years old at diagnosis and relapse respectively. The median time between diagnosis and

TABLE 1 Population characteristics in retrospective and prospective cohorts.

	Paired samples $n = 7$		Prospective study $n = 38$
	Diagnosis	Relapse	
Gender			
Female n (%)	4 (57.1)		1 (28)
Age (years)	6 (0–21)	10 (1–22)	4.4 (1.9–16.6)
Complete blood count			
Hemoglobin (g/dL)	8.1 (5.4-12.9)	11.9 (8.8–15)	7.7 (3.5–11.9)
Platelets (10^9/L)	40 (13–156)	110 (22–167)	61.5 (<5–381)
Leukocytes (10^9/L)	6.2 (2.3–82.8)	4.6 (1.3–22.2)	7.1 (1.9–105.5)
Peripheral blasts (10^9/L)	0.73 (0.2–68.8)	0.74 (0–19.1)	3.8 (0.1–98.1)
Peripheral blasts (%)	32 (3–87)	37.5 (0–86)	48 (4–98)
Medullary blasts (%)	95.8 (40.0–99.0)	90.0 (27.5–98.0)	96.0 (30.0–100.0)
CNS involvement n (%)	2 (2.8)	3 (4.3)	2 (5.2)
EGIL[a] classification n (%)			
B–I	2 (2.8)	2 (2.8)	1 (2.6)
B–II	3 (4.3)	3 (4.3)	34 (89.4)
B–III	2 (2.8)	2 (2.8)	3 (7.8)
Time to relapse (months)		38 (9–75)	

Note: Data are expressed as medians [min–max] except where specified.

[a]EGIL, European Group for Immunophenotyping of Leukemia.

relapse was 38 months (range 8–75 months). Two patients had CNS involvement at diagnosis and three at relapse (combined CNS and medullary relapses). As shown in Table 1, most patients ($n = 6$) presented with cytopenia at diagnosis versus five at relapse. At diagnosis, three patients were leucopenic whereas one patient presented with leukocytosis (white blood cell [WBC] count 82.84×10^9/L). At relapse, three patients were leucopenic and none of them had leukocytosis. The median percentage of medullary blasts was 95.75% (40.00%–99.00%) at diagnosis and 90% at relapse (27.5%–98%). According to the EGIL classification [10], the cohort included 2 B-I ALL, 3 B-II ALL, and 2 B-III ALL, without any changes between diagnosis and relapse.

Children were treated according to different therapeutic protocols at diagnosis: FRALLE ($n = 5$), GRAALL ($n = 1$), INTERFANT ($n = 1$). At relapse, alive patients received VANDA ($n = 1$), COOPRALL ($n = 2$), TACL ($n = 1$) and blinatumomab ($n = 2$). At last update, six of the seven patients have died, with a median time from relapse to death of 19 months (1–76 months). Six patients received hematopoietic stem cell transplantation, three before relapse and three after.

Karyotype and FISH analysis [12, 13] classified two patients as high cytogenetic risk (iAM*P21* and *KMT2A* rearrangement respectively), three as good risk (hyperdiploidy $n = 1$, t(12;21) $n = 2$), and two as intermediate risk (normal karyotype). Three patients showed additional karyotype abnormalities at relapse (Table 2).

In the second cohort, 38 patients (including 11 girls) were enrolled prospectively from October 2016 to November 2018. Their median age was 4.4 years old (1.9–16.6 years old).

All but four patients were cytopenic at diagnosis. The median percentage of medullary blasts at diagnosis was 96.5% (30.00%–100.00%). Two patients had CNS involvement. The cohort included 1 B-I ALL (2.6%), 34 B-II ALL (89.4%), and 3 B-III ALL (7.8%).

All children were treated according to the CAALL therapeutic protocol. Two patients benefited from hematopoietic stem cell transplantation due to positive MRD. One patient relapsed early, less than 6 months after treatment completion. Another one relapsed 44 weeks after diagnosis. Two more had a delayed relapse 3 years after diagnosis. All patients are still alive, in complete remission (CR) and have completed treatment.

TABLE 2 Karyotype evolution between diagnosis and relapse in matched samples.

Patient	Diagnosis karyotype and FISH	Relapse karyotype
1	46.XX.der(4)t(4;8)(q3?2;q?11).add(4)(p16).del(6)(q15q25).-8.-9.-11.-15.+21.+21.+mar1.+mar2.inc[12]/46.XX[4] t(12;21) pos	Similar
2	47.X.-X.+10.+16[3]/48.idem.+mar[5]/45.XY[5] t(12;21)pos	Similar
3	46.XX[15]	45.XX.dic(9;20)(p13;q11)[4]/46.XX[16]
4	46.XY[20]	46.XY.del(6)(q21).del(9)(p21)[10]/46.XX[6]
5	No karyotype. *KMT2A* rearrangement	No karyotype
6	52.XY.+X.+6.+14.+18.+21.+21[12]/46.XY[8]	52.XY.+X.+6.+14.+18.+21.+der(21)add(21)(p11)[5]/46.XY[15]
7	46.XY.r(21)[p11q22][18]/46.XY[2]. ish r(21)(*AML1* amp)[5]	Similar

Karyotype and FISH analysis classified 25 patients as good cytogenetic risk (hyperdiploidy, $n = 17$; *ETV6/RUNX1*, $n = 8$), 10 as intermediate risk and three as high cytogenetic risk (hypodiploidy, $n = 1$; *RUNX1*, $n = 2$), according to the WHO 2016 classification.

3.2 | SNP study

In the first cohort, SNP array showed a mean of 11.7 CNA and 4 LOH at diagnosis with 4 CNA and 0.9 LOH modulations at relapse. Six of the seven patients presented modulation in CNA and LOH during evolution with a median of 4. Moreover, SNP showed that two patients acquired an *IKZF1* deletion at relapse (Figure 1). Some anomalies observed by cytogenetics were refined by SNP analysis, notably all chromosomal gains and losses were recovered and precisely located. Moreover, a t(4;8)(q32;q11) translocation identified by karyotype with one breakpoint on each chromosome was identified as a more complex rearrangement with over 10 breakpoints on each chromosome and a succession of deleted and duplicated segments for long arms of these two chromosomes. Patients with the most CNA and LOH also had a complex karyotype.

In the second cohort, a median of 4.6 CNA and 1.5 LOH were observed per sample. Again, all karyotyping anomalies were retrieved by SNP analyses in the prospective cohort. One *ETV6* deletion detected in FISH in a small portion of the nuclei (less than 15%) was not seen in SNP. All other FISH anomalies were retrieved, usually with additional SNP signals. Moreover, SNP analysis allowed to detect hyperdiploidy in two patients with a noncontributory karyotype. Data are summarized in Table 3, ordered by type of anomalies [15–24]. Most of these were CNA in hotspot regions. For genes involved in B-cell development, deletions were observed in *PAX5*, *ETV6*, and *IKZF1*. Of note, deletion

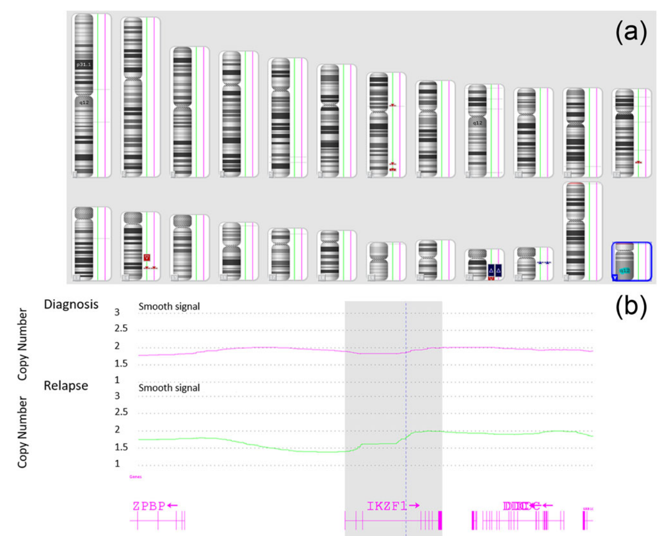

FIGURE 1 Example of copy number alterations (CNA) modulation between diagnosis and relapse for one patient. with an IKZF1 deletion acquired at relapse. (a) CNA (deletions in red and gains in blue) for 1 patient with RUNX1 amplification at diagnosis (pink line) and relapse (green line). At relapse. Six additional CNAs were acquired (only deletions). (b) Zoom on the 7p12 locus showing an acquired deletion encompassing part of the IKZF1 gene at relapse.

of the *IKZF1* gene could be established in three patients for whom molecular analysis was not conclusive. Hampered proliferation control was suggested either by deletion of *CDKN2A/2B* or LOH in *TP53*. A gene fusion between *ZNF384* and *CREBBP* (translocation t[12;16]), that could not be seen in conventional cytogenetics, was found in one patient (Figure 2). Moreover, an amplification of *RUNX1* was retrieved in two patients. Both these anomalies are liable to alter a number of transcription pathways. Similarly, the

TABLE 3 Genes involved in alterations observed in the two cohorts studied together with their function. incidence and thernostice potential.

Role	Gene	Type of anomaly	Diagnosis $n = /7$	Relapse $n = /7$	Prospective patients $n = /37$*	Indicence (%)	Druggability
B-cell development	*PAX5*	CNA (gain or loss)	4	5	4	10–35	
B-cell development	*ETV6*	CNA (loss)	3	3	6	20–22	
B-cell development	*IKZF1*	CNA (loss)	0	2	5	15	
Proliferation control	*CDKN2A/2B*	CNA (loss)	4	5	7	8–28	TKI. Bcl2 inhibitors
Proliferation control	*TP53*	LOH	0	0	4	2.4	APR 246
Transcription factor	*RUNX1*	CNA (loss)	1	1	2	3	
Transcription factor	*ZNF384/ CREBBP*	CNA** (loss)	0	0	1	2.5	FLT3 inhibitors. HDAC inhibitors
RAS pathway	*N-RAS*	LOH	1	1	0	16	Selumetinib
	K-RAS	LOH. CNA (loss)	0	0	4		
JAK pathway	*JAK/STAT*	CNA (loss)	2	3	1	0–12.5	Ruxolitinib
Other hematopoietic pathways	*PAR1 (CRLF2/ P2RY8)*	CNA (loss)	0	0	2	4	TKI

Note: *One patient with severe hypodiploidy (28 chromosomes) was removed from this table ** by unbalanced translocation. This table is based on references [13–22].

RAS/MAPk and JAK pathways were targeted by LOH and CNA respectively of *N-RAS*, *JAK2*, and *JAK/STAT*. Finally, activation could be impaired by the deletion of *PAR1* related to the *CRLF2-P2RY8* fusion observed in one case. Finally, a constitutional deletion of the *PCDH19* gene was found in one patient. This gene is involved in the EFMR syndrome (epilepsy, female restricted, with mental retardation) and, following this observation and subsequent re-evaluation, it appeared that the patient indeed suffered from epilepsy. This anomaly was confirmed on a germline sample.

3.3 | Impact of SNP analysis on classification

Using the genetic classification of Hamadeh et al. [25]. based on SNP array for eight genes at diagnosis (*IKZF1, CDKN2A/2B, PAR1, BTG1, EBF1, PAX5, ETV6*, and *RB1*), SNP reclassified patients of the first cohort (Table 4) as three of good prognosis (green) and four as poor prognosis (red), with a median of two CNA. The two patients with cytogenetic intermediate risk should thus probably have been considered for a more intense therapeutic regimen, that is, allogeneic stem-cell transplantation. Among the two patients reclassified as high risk, one patient achieved MRD-negative CR at the end of induction therapy. The other had active disease with extra-medullary lesions at the end of induction. These two patients died from relapse.

In the second cohort, six patients presented with high-risk CNA (CNA-HR), five with intermediate risk (CNA-IR) and 27 with good risk-CNA (CNA-GR). For both cohort cytogenetic reclassification could be achieved by SNP analysis, as shown in Table 5. Three patients with intermediate risk karyotype were reclassified as poor prognosis. Of these 3 patients, 2 patients were MRD-negative post-induction negative MRD (<0.001%) on molecular biology and 1 patient had very high residual disease at the end of induction (1%) and benefited from treatment intensification. Of the 2 MRD-negative patients, 1 relapsed early and died. One had deletions of *IKZF1* and *CDKN2A/B* and the second deletions of *CDKN2A/B* associated with *PAX5* gain. Similarly, the seven other patients with intermediate risk-karyotype had CNA-GR allowing reclassification in the good prognosis group. Finally, combining conventional technologies with SNP, patients were reclassified as 32 good prognosis and 6 poor prognosis. Among the 4 relapses of the prospective cohort, 3 patients had poor risk features according to SNP results (2 patients carrying *CDKN2A/B* deletion associated with *PAX5* gain,

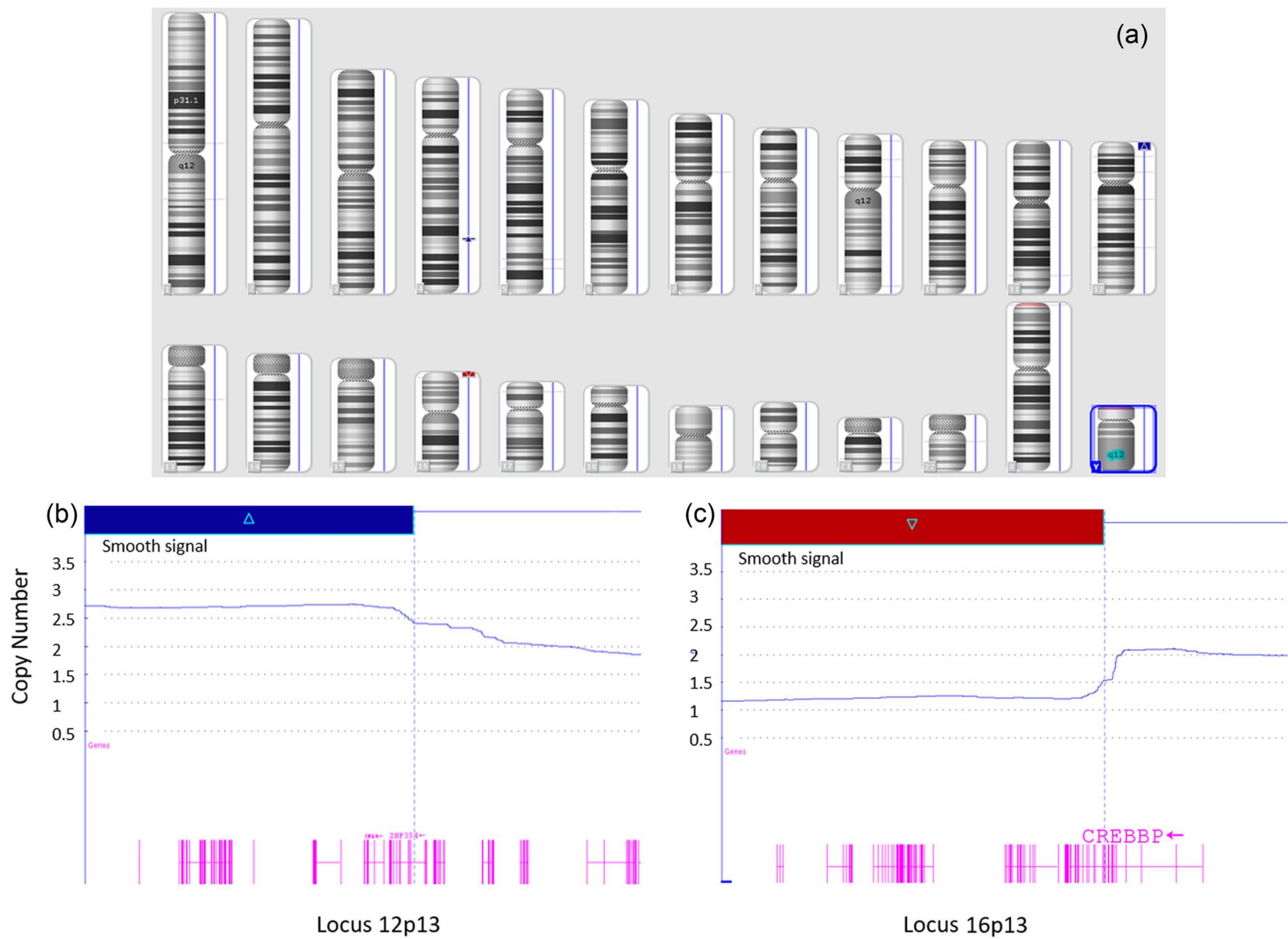

FIGURE 2 Gene fusion between ZNF384 and CREBBP (translocation t[12;16]). (a) Copy number alterations (CNA) (deletion in red and gain in blue) for one patient from the prospective cohort with normal standard karyotype shows three CNA. especially a duplication in 12p13 associated with a deletion in 16p13. (b) In the 12p13 locus. there is a breakpoint in the *ZNF384* gene with duplication from the proximal segment before this breakpoint. (c) In the 16p13 locus, there is a breakpoint in the *CREBBP* gene with a deletion from the proximal segment before this breakpoint. Duplication 12p13 with a breakpoint in the *ZNF384* gene (b) and deletion 16p13 with a breakpoint in the *CREBBP* gene (c) could correspond to an unbalanced translocation t(12;16)(p13;13).

TABLE 4 Genetic reclassification in the retrospective study.

		Cytogenetic risk		
		GR $n=3$	IR $n=2$	HR $n=2$
CNA-risk	GR $n=4$	3	0	1
	IR $n=3$	0	2	1
	HR $n=0$	0	0	0

Note: Green: reclassification in good prognosis. Red: reclassification in poor prognosis.

Abbreviations: GR, good risk; HR, high risk; IT, intermediate risk.

and one with *RUNX1 amplification* and *IKZF1* deletion) while the last patient had good risk features (high hyperdiploidy).

Considering both the retrospective and prospective cohorts that encompass 45 patients at diagnosis, the ultimate input of SNP assessment is that five patients (11%) were reclassified as poor risk (Table 6). Three of these indeed relapsed versus only one in the good risk cohort. Of course, these fortunate low relapse levels impair any statistical analysis.

4 | DISCUSSION

In this study, SNP array allowed to detect additional abnormalities not identified by standard karyotype in all BCP-ALL children tested. A clonal evolution was identified in most patients at relapse, with a median of four CNA modifications. In the relapsed cohort, children expectedly presented more anomalies at diagnosis than in the prospective cohort. Moreover, SNP led to change the prognostic value of karyotypic anomalies at diagnosis

TABLE 5 Genetic reclassification in the prospective study.

		Cytogenetic risk		
		GR $n = 25$	**IR $n = 10$**	**HR $n = 3$**
CNA-risk	GR $n = 27$	19	7	1
	IR $n = 5$	3	2	0
	HR $n = 6$	3	1	2

Note: Green: reclassification in good prognosis. Red: reclassification in poor prognosis.

Abbreviations: GR, good risk; HR, high risk; IT, intermediate risk.

TABLE 6 Global genetic reclassification of the whole cohort of 45 patients at diagnosis.

		Cytogenetic risk		
		GR $n = 28$	**IR $n = 12$**	**HR $n = 5$**
CNA-risk	GR $n = 31$	22	7	2
	IR $n = 8$	3	4	1
	HR $n = 6$	3	1	2

Note: Green: reclassification in good prognosis. Red: reclassification in poor prognosis.

Abbreviations: GR, good risk; HR, high risk; IT, intermediate risk.

in all patients with intermediate karyotype in both cohorts. Indeed, signals rising the awareness of clinicians as for potential relapse risk can be drawn from these explorations which conversely convey reassuring information when no additional alarming feature is detected.

For instance, findings observed only in SNP, such as 3/5 *IKZF1* CNA could have led to upgrade the risk group for these patients according to the CAALL protocol. Similarly, the two patients for whom *CRLF2/P2RY8* CNA were detected could have benefited from treatment with TKI. All in all, SNP analysis in this cohort of 45 patients allowed for a reclassification of 26% of the patients and led to 84% of GR and 16% of PR, slightly better than in the study by Moorman et al. [7].

By comparison with other pediatric cancers, BCP-ALL display a rather low rate of anomalies. In a study by Gröbner et al. [23], BCP-ALL rank 10 out of 20 types of tumors ordered by increasing genome instability and 6/24 when ordering by increasing coding single nucleotide variant (SNV) per megabase. Data from the present cohort of 45 patients are comparable to this report.

Considering the role of molecular alterations, four groups could be segregated, respectively perturbing B-cell development, cell proliferation, transcription or molecular pathways (Table 3). Characteristics of the genes involved are summarized below, with a special focus on potential therapeutic targets or loss thereof.

Deletions of B-cell development genes have been reported to be associated with high risk ALL [15]. *PAX5* deletion results in loss of the tumor repression function of this molecule together with alterations in B-cell precursor differentiation. To date, there does not seem to be any specific drug to target this deficiency. However, because of the important role of *PAX5* in the control of B-cell physiology, its loss might result in the disappearance of targets for bispecific antibodies or CAR-T cells [24].

ETV6 deletions, frequent in hematological malignancies have been shown to be associated to t(12;21) in childhood BCP-ALL [17]. This was the case here in 3/5 patients. Deregulation of this important hematopoiesis factor is likely to impair differentiation. Again, no drug to date appears to target this deletion.

IKZF1 alterations modify cell adhesion and are associated with resistance to both chemotherapy and TKI. *IKZF1* deletions are historically associated with poor prognosis although their impact is less clear in the era of modern therapies. Some mouse models and trials have shown that this can be reversed by retinoids and focal adhesion kinase (FAK) inhibitors [15].

CDKN2A is an important cell-cycle regulator, the second most commonly inactivated gene in cancer after *TP53*. Located on (9p), it is often deleted together with *PAX5* and *JAK2*. It is targetable by TKI and BCL2 inhibitors [21].

TP53 anomalies, rather rare in ALL, are usually associated to hypodiploidy observed here (LOH) in the prospective cohort only. This tumor-suppressor gene, altered in about 50% of cancers, is crucial in controlling the cell cycle in response to DNA damage. The recently developed small molecule APR-246 can induce cell apoptosis in p53-deficient cells and has been tested successfully in a clinical trial of childhood ALL with mutated *TP53* in addition to chemotherapy [22].

RUNX1 is involved in the poor prognosis iAMP entity associated to chromotrypsis but is not yet druggable [12].

The *CREBBP-ZNF384* fusion and unbalanced translocation have been confirmed by RT-PCR to be a potential therapeutic target for histone deacetylases [26]. Moreover, this alteration, also shared by mixed phenotype acute leukemias, is characterized by Fms-like tyrosine kinase (FLT3) overexpression and can thus be targeted by FLT3 inhibitors.

Abnormalities in the *JAK/STAT* and *RAS* signaling pathways, frequent in many cancers [23] can be targeted by specific TKI such as selumetinib or ruxolitinib [15].

Finally, the pseudo-autosomal region (*PAR1*) is deleted on chromosomes X or Y upon *P2RY8/CRLG2* fusion. This results in an overexpression of the cytokine receptor-like factor CRLF2 and spontaneous activation of

the JAK/STAT and AKT-mTOR pathways [21]. TKI can be indicated in such cases, as this alteration belongs to Phi-like molecular anomalies.

This study confirms the interest of SNP array, combined to conventional cytogenetics in an integrated diagnostic approach possibly extended to whole exome DNA or RNA sequencing. Although a strong correlation was retrieved between the number of karyotypic abnormalities and CNA/LOH in SNP-array the complementarity of these two approaches was confirmed here. Moreover, the sensitivity of SNP assessment appears to be superior to other molecular techniques such as multiplex ligation-dependent probe amplification (MLPA) that was available for some of the patients of this cohort (data not shown). Sample blast-infiltration should be at least 20% for SNP versus at least 50% for MLPA. Here, SNP was informative for 3 patients without possible MLPA interpretation. Of note, SNP disclosed a deletion of *IKZF1* in two patients with MLPA failure. It has also been reported that SNP can be performed on altered DNA [27].

SNP-array moreover provides precise information, in terms of CNA, not detectable by conventional methods, but present on genomic DNA. As previously mentioned, this enables patients to be reclassified in terms of genetic risk and therefore potentially change their prognosis, as already shown and validated in the literature [25, 28, 29]. This interest of SNP-array has also been proven in other hematological malignancies. In multiple myeloma, molecular karyotyping by SNP revealed strong prognostic factors and changed risk stratification algorithms [30–32]. More recently, SNP-array analysis in acute myeloblastic leukemia disclosed new prognosis CNAs, as well as recurrent genetic aberrations, notably in CBF-AML where lesions with tyrosine kinase signaling were highlighted [33–35].

5 | CONCLUSION

Taken together, SNP-array molecular karyotyping, combined with classical analyses at diagnosis, might modify therapeutic options in childhood BCP-ALL, especially in the intermediate karyotype subgroup, and detect druggable lesions that might be targeted in case of poor response to treatment or relapse. Current therapeutic protocols, either national or international, although highly efficient in yielding high levels of sustained CR and allowing for an excellent management of relapses [36], could benefit from the additional information provided by SNP-driven chromosomal analysis.

AUTHOR CONTRIBUTIONS

Margaux Camuset: Investigation (equal); writing—original draft (equal). **Baptiste Le Calvez**: Investigation (equal); writing—original draft (equal); writing—review and editing (equal). **Olivier Theisen**: Conceptualization (equal); investigation (equal); methodology (equal); writing—original draft (equal). **Catherine Godon**: Conceptualization (equal); investigation (equal); methodology (equal). **Audrey Grain**: Resources (equal). **Caroline Thomas**: Resources (equal). **Marie-Laure Couec**: Resources (equal). **Marie C. Béné**: Conceptualization (equal); methodology (equal); writing—original draft (equal). **Fanny Rialland**: Conceptualization (lead); funding acquisition (lead); investigation (equal); writing—original draft (equal). **Marion Eveillard**: Conceptualization (lead); funding acquisition (lead); investigation (equal); methodology (lead); project administration (lead); writing—original draft (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study protocol was approved by the Ethics Committee of Nantes University Hospital direction of research as the MEFRALL study (RC_144) and it was compliant with the Helsinki Declaration of 1975, as revised in 2008.

INFORMED CONSENT

All patients or patient parents provided informed consent for the valorization of biological data related to their management.

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