Genetic differences between paediatric and adult Burkitt lymphomas

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Summary

Dysregulation of MYC is the genetic hallmark of Burkitt lymphoma (BL) but it is encountered in other aggressive mature B-cell lymphomas. MYC dysregulation needs other cooperating events for BL development. We aimed to characterize these events and assess the differences between adult and paediatric BLs that may explain the different outcomes in these two populations. We analysed patterns of genetic aberrations in a series of 24 BLs: 11 adults and 13 children. We looked for genomic imbalances (copy number variations), copy-neutral loss of heterozygosity (CN-LOH) and mutations in TP53, CDKN2A, ID3 (exon 1), TCF3 (exon 17) and CCND3 (exon 6). Young patients displayed more frequent 13q31.3q32.1 amplification, 7q32q36 gain and 5q23.3 CN-LOH, while 17p13 and 18q21.3 CN-LOH were only detected in adult BLs. ID3 mutations were present in all adult samples, but only in 42% of childhood cases. CCND3 and ID3 double-hit mutations, as well as 18q21 CN-LOH, seemed to be associated with poorer outcome. For the first time, we report different genetic anomalies between adult and paediatric BLs, suggesting age-related heterogeneity in Burkitt lymphomagenesis. This may explain the poorer prognosis of adult BLs. Additional studies are needed to confirm these results in the setting of clinical trials.

Keywords: Burkitt lymphoma, childhood, SNP-array, ID3, CCND3.
Burkitt lymphoma/leukaemia (BL) is a germinal centre B-cell derived lymphoma. It is clinically characterized by its high aggressiveness, which has been overcome using short intensive pulse chemotherapy. The outcome remains poorer in adult BL patients than in paediatric patients with BL (Jacobson & LaCasce, 2014; Minard-Colin et al., 2015). Dysregulation of MYC through chromosomal translocation is the genetic hallmark of BL. It is also encountered in several other lymphomas, especially in diffuse large B cell lymphoma (DLBCL) and in B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL (DLBCL/BL) (Swerdlow et al., 2008). Although gene expression profiling (GEP) has identified a molecular signature that distinguishes BL from other aggressive B-cell lymphomas (Dave et al., 2006; Hummel et al., 2006), the unclassifiable grey-zone lymphomas remain challenging and the translation of GEP into daily practice is difficult.

MYC dysregulation alone seems to be insufficient to drive Burkitt lymphomagenesis: in particular, the pro-apoptotic properties of MYC must be counterbalanced. Several cooperating genomic and epigenetic changes have been identified: additional prognostic chromosomal aberrations (Poirel et al., 2009; Havelange et al., 2013); inactivation of the TP53 pathway, needed to prevent the TP53-dependent apoptosis induced by MYC deregulation (Olive et al., 2013); and over-expression of MIR17HG, which counteracts MYC-induced apoptosis and drives PI3 kinase signalling by reducing PTEN expression (Schmitz et al., 2014).

Recently, the mutational landscape of BL was decrypted by high throughput sequencing, which identified new deregulated pathways. Activating mutations of TCF3 (0–23%) and/or inactivating mutations of its negative regulator ID3 (34–68%) constitutively activate the TCF3 pathway, which is a master regulator of normal B-cell differentiation in the germinal centre (Love et al., 2012; Richter et al., 2012; Schmitz et al., 2012). TCF3 consequently promotes tonic B-cell receptor signalling and sustains BL survival by engaging the PI3Kinase pathway. CCND3 activating mutations (0–38%) drive proliferation by deregulating the G1-S cell-cycle transition (Love et al., 2012; Richter et al., 2012; Schmitz et al., 2012). Compared with other aggressive lymphomas, BL is not dependent on nuclear factor (NF)-κB pathway activation, but rather on PI3Kinase signalling (Sander et al., 2014).

The respective contribution of these pathways to the different categories of aggressive MYC–rearranged mature B-cell lymphomas, mainly BL and DLBCL/BL, remains unclear (Greenough & Dave, 2014; Schmitz et al., 2014). A recent study showed that the diagnostic grey zone between BL and DLBCL is also a grey zone of the mutational spectrum (Momose et al., 2015). Moreover, the differences that could explain the poorer prognosis of adulthood BL in comparison to childhood BL are not well established (Hoelzer et al., 2014; Jacobson & LaCasce, 2014; Minard-Colin et al., 2015). The impact of age on outcomes may reflect misclassification of DLBCL/BL, and/or increased treatment toxicity, and/or biological differences in the lymphomagenesis of older patients compared to children.

The aim of this study was to identify aberrant genomic patterns varying with age of onset in BL differences, using tools that are available in clinical practice. We looked for genome-wide copy number variations (CNV), copy-neutral loss of heterozygosy (CN-LOH) and mutations in the coding sequences of the TP53, CDKN2A, ID3, TCF3 and CCND3 genes.

Materials and methods

Selection of patients

We collected 24 BL samples (11 adults, 13 children), in collaboration with the Groupe Francophone de Cytogénétique Hématologique (GFCH) and the Belgian Cytogenetic Group for Haematoto-Oncology (BCG-HO) and following the guidelines of the local ethical committees. Patient characteristics are summarized in Table I.

MYC rearrangement was assessed by conventional karyotyping in 21 patients and by fluorescence in situ hybridization (FISH) in three cases with failed karyotype. Karyotypes and FISH results were performed according to standard procedures described according to the 2013 International System for Human Cytogenetic Nomenclature (Shaffer et al., 2013).

The pathological material with immunohistochemistry data (at least CD10 or BCL6, BCL2 and Ki67) for each case has been centrally reviewed by at least two haematopathologists and classified according to the most recent World Health Organization classification (Swerdlow et al., 2008). Leukaemic blast counts in bone marrow and blood samples were greater than 60%, detected by SNP array. BLs were treated according to lymphoma malignancy B (LMB) studies (Diviné et al., 2005; Minard-Colin et al., 2015). Genomic DNA was extracted from frozen samples using the Puregene® DNA extraction kit (Gentra Systems Inc., Minneapolis, MN, USA).

The study was authorized by the Ethics Committee of Cliniques universitaires Saint-Luc, Brussels, Belgium (ref: F/2005/13). In this case, the Ethics Committee waived the need for written informed consent.

Single-nucleotide polymorphism (SNP) array

Molecular karyotyping was performed with 100K-XbaI SNP-arrays according to the manufacturer’s instructions (Affymetrix, High Wycombe, UK). Results were analysed as previously reported (Amyere et al., 2013, 2014).

Aberrations involving at least five consecutive SNPs were considered for CNV analysis, and 30 neighbouring SNPs for CN-LOH analysis. The mean genotyping call rate was 95-6% (90–98.8%). Constitutional CNV polymorphisms were excluded based on comparisons with the Database of Genomic
Variants (http://dgv.tcag.ca/dgv/app/home). Rearrangements of the T-cell receptor and the immunoglobulin loci were excluded because they most likely represent somatic rearrangements that are clonotypic for the malignant lymphoid cells.

Fluorescence in situ hybridization (FISH)

Dual-colour FISH experiments were performed for confirmation of selected SNP-array anomalies, using either commercial probes [LSI-MYC, LSI-CBFB, LSI-BCL2, LSI-BCL6 (ABR), LSI-9p21/CEP 9, LSI-TP53/CEP17 from Abbott SA/NV (Wavre, Belgium); ON-MYC-TC, ON-TP53/MPO from Leica Microsystems Belgium BVBA (Diegem, Belgium); PAX5 from DAKO Belgium NV (Heverlee, Belgium)] or bacterial artificial chromosome (BACs) probes. The BAC clones were selected from public genome databases and were purchased from the Chori BACPAC Resources Center (Oakland, CA) (Table SI). Extraction, labelling and hybridization were performed as previously reported (Duhoux et al., 2012).

Sanger sequencing

TP53, CDKN2A locus (P14ARF, P16INKA), exon 1 of ID3, exon 6 of CCND3 and exon 17 of TCF3-isofrom E47 were sequenced (coding and flanking intronic sequences). Primer sequences are listed in Table SII. PCR products were purified

| UPN | SEX | AG (years) | FOLLOW-UP (years) | CNA (n) | CN-LOH (n) | 13qG/D | TP53 | CDKN2A | ID3 (exon 1) | CCND3 (exon 6) | TCF3 (exon 17) |
|-----|-----|-----------|------------------|--------|-----------|--------|------|--------|------------|-------------|--------------|
| B18 | F   | 4         | 7.5              | 2      | 3         |        |      |        |            |             |              |
| B4  | M   | 8         | 0.5              | 4      | 5         |        |      |        |            |             |              |
| B5  | F   | 11        | 5                 | 0      | 1         |        |      |        |            |             |              |
| B32 | M   | 5         | 9                 | 5      | 3         |        |      |        |            |             |              |
| B3  | M   | 8.5       | 11                | 2      | 7         |        |      |        |            |             |              |
| B2  | M   | 9         | 10                | 0      | 4         |        |      |        |            |             |              |
| B19 | M   | 11        | 3                 | 2      | 1         |        |      |        |            |             |              |
| B1  | M   | 14        | 10.5              | 3      | 2         |        |      |        |            |             |              |
| B10 | M   | 12        | 2                 | 3      |            |        |      |        |            |             |              |
| B36 | M   | 15        | 2                 | 5      |            |        |      |        |            |             |              |
| B12 | F   | 9         | 4                 | 9      | 4         |        |      |        |            |             |              |
| B34 | M   | 7         | 14                | 2      | 4         |        |      |        |            |             |              |
| B17 | M   | 7         | 3.5               | 0      | 2         |        |      |        |            |             |              |
| B9  | M   | 65        | 0.7               | 0      | 2         |        |      |        |            |             |              |
| B30 | M   | 27        | 12                | 1      | 1         |        |      |        |            |             |              |
| B7  | M   | 58        | 5.5               | 1      | 10        |        |      |        |            |             |              |
| B31 | M   | 22        | 0.5               | 2      | 3         |        |      |        |            |             |              |
| B6  | F   | 26        | 0.5               | 2      | 5         |        |      |        |            |             |              |
| B26 | M   | 37        | 0                 | 4      |            |        |      |        |            |             |              |
| B29 | M   | 38        | 1                 | 1      | 6         |        |      |        |            |             |              |
| B23 | M   | 31        | 1                 | 2      |            |        |      |        |            |             |              |
| B27 | M   | 54        | 1.5               | 0      | 2         |        |      |        |            |             |              |
| B39 | F   | 55        | 1                 | 4      |            |        |      |        |            |             |              |
| B41 | M   | 29        | 0                 | 11     |            |        |      |        |            |             |              |

Table I. Heat map representation of patients characterisitics and of the CNV, CN-LOH and mutational patterns.

UPN, unique patient number; M, male; F, female; BL, Burkitt lymphoma; DLBCL, Diffuse large B-cell lymphoma; DLBCL/BL, B-cell lymphoma, unclassifiable, with features intermediate between BL and DLBCL; CNA, copy number aberration; CN-LOH, copy neutral-loss of heterozygosity; 13qG/D 13q gain and/or 13q deletion; CDKN2A, mutations of P14ARF are in dark blue; P16INKA SS selective mutation of P16INKA (Splice site).

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with the ExoProStar (GE-Healthcare Life Sciences, Diegem, Belgium) and sequenced with the Big Dye v3.1 commercial kit (ThermoFisher Scientific, Waltham, MA, USA), according to the manufacturer’s instructions.

Statistical analyses

Data were analysed using the Fisher’s exact test for discrete variables and the Mann–Whitney test for continuous variables.

Results

Genome-wide analysis by Karyotype and SNP-array

Results are summarized in the Table I and detailed in Table SIII (CNV), SIV (CN-LOH) and SV (Cytogenetics).

As expected, BL contained a low number of CNVs with a median of 1.5 [0–9]. The number of CN-LOH was higher, with a median of 3–5 [1–11] and 16/24 BLs had 3 or more CN-LOH.

Recurrent aberrations were defined as minimal modified region observed in at least three patients. They are listed in Table II.

Recurrent CNV

As expected, the subcentromeric 1q21 gain was the most frequent genomic imbalance, detected in 9/24 BL. At the karyotype level, it corresponded to the classical 1q duplication in five BL. It was cryptic at the karyotype level in three BLs.

A large terminal 7q32q36 gain (size of the minimal region: 31 Mb) was detected in four BL. The 7q gain was missed in one case with available karyotype.

Gain of 13q31.3–q32.1 was present in 5 BL. It was not detected by karyotype in two cases. The minimal gained region was restricted to 3.72 Mb with a higher gain (more than three copies) in two of these patients (Patients B4 and B10) (Fig S1). This gain was immediately followed by a large terminal deletion in two patients (Table II and Fig S1).

Characterization of 13q alterations by FISH using a contig of BAC clones (Table S1 and Fig S1b) showed a higher level of gains (ranging from 3 to more than 10 copies per cell) than expected by the number of CNVs (less than 4). This is explained by a subclonal pattern of 13q amplifications, suggesting it is a secondary event.

The minimal amplified region contains 10 coding sequences including the polycistronic MIR17HG cluster of six microRNAs. This cluster has a synergistic effect with deregulated MYC (Jin et al, 2013).

Recurrent CN-LOH

Six different recurrent CN-LOHs of varying size were detected in this series of BLs. As no matched constitutional DNA was available, we cannot exclude that these CNVs and CN-LOHs may have been constitutional. However, their absence in our local series of non-BL aggressive MYC-rearranged lymphomas (Poirel HA, unpublished observations) is an argument in favour of non-random acquired genomic aberrations.

The 1p31 CN-LOH was present in five cases. The minimal modified region (0.6 Mb) targets at least the 5' part of a candidate tumour suppressor gene NEGR1, which encodes a raft-associated extracellular protein that may regulate the cell adhesion required for normal cell growth and cell-to-cell communication. NEGR1 expression is reduced in several cancers (Kim et al, 2014).

The five other CN-LOHs were detected in three different BLs (respective minimal modified regions in brackets): 1p35p36 (32 Mb), 5q23.3 (3.3 Mb), 17p13 (9 Mb), 7q31 (1 Mb) and 18q21.2 (0.4 Mb). The large and terminal 1p35p36 CN-LOH includes ID3 and TNSFRSF14, and the 17p13 CN-LOH contains the tumour suppressor gene TP53.

Table II. SNP-array results: non random copy number aberrations (CNV) and copy neutral – loss of heterozygosity (CN-LOH).

| Minimal gained region | Minimal deleted region | Minimal CN-LOH | Size (kb) | Children | Adults |
|-----------------------|------------------------|----------------|----------|---------|--------|
| 1p35-p36              | 886-727–32 815-594     | 31-929         | B3 B19   | B39     |
| 1p31                  | 72-174-384–72 769-476  | 595            | B3 B18   | B9 B29  B41 |
| 1q21                  | 144-567-450–151-060-327| 6–493          | B1 B3 B4 B12 | B2 B7 B29 B30 B31 |
| 5q23-3                | ~128-674-129–131-976-790| 3-303          | B3 B4 B34 |        |
| 7q31                  | 116-896-861–117-898-666| 1-002          | B36       | B26 B31 |
| 7q32-q36              | 127-153-872–158-246-990| 31-093         | B1 B4 B12 | B6     |
| 13q31.3–q32.1         | 90-511-230–94-233-126  | 3-722          | B4 B10 B12 B32 | B31 |
| 13q33–qter            | 101-041–186–113-203-258| 12-162         | B4 B10 B32 |        |
| 17p13                 | 451-209–9-512-325     | 9-061          | B1 B10   | B26     |
| 18q21.2               | 49-258-894–49 679-919 | 421            | B6 B9 B31 |        |

CNV or CN-LOH detected by 100K single nucleotide polymorphism array in at least three patients are reported according to the Human Genome 18 nomenclature (hg18; http://www.ncbi.nlm.nih.gov/assembly/GCF_000001405-12/).

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The 18q21.2 CN-LOH does not involve two genes that are well known for their role in B-cell lymphomagenesis, MALT1 and BCL2 (located telomeric to both 18q21 alterations). The minimal region of 0.4 Mb affected the 3’ end of the DCC gene, which corresponds to the intra-cellular domain of this netrin receptor. The BLs with 18q21 CN-LOH displayed a complex karyotype. Karyotype only identified a 18q21 chromosomal rearrangement in B6, not involving the BCL2 locus by FISH. It is noteworthy that the 3 patients with CN-LOH died within 8 months on-therapy.

Genomic aberrations according to the age at onset in BL
Within our BL cohort, there was no statistical difference between adult and childhood subgroups with regard to the median number of CNVs (1 [0–2] vs. 2 [0–9] respectively) and of CN-LOHs (4 [1–11] vs. 3 [1–7] respectively).

Two gains appeared more frequently in younger patients: 4/5 BL cases with 13q31.3–q32.1 gain were less than 18 years old and the four BL cases with 7q32q36 gain were less than 26 years old.

The 5q23.3 CN-LOH was only detected in children, while the 18q21.2 CN-LOH was only found in adult BLs.

Mutation analyses

P14ARF/TP53 pathway. Deleterious mutations involving the P14ARF/TP53 pathway were detected in 19/24 BLs (79%) (Tables I and SV). TP53 was mutated in 14 BL (58.3%). TP53 inactivation was bi-allelic in 10 BL (homozygous in 3 cases due to CN-LOH, composite heterozygous in 7 cases). The mutations were all located in the DNA binding domain (exons 5–8) as previously reported (Schmitz et al., 2012).

The 2 different genes belong to the CDKN2A locus that encode P14ARF and P16INKA were studied. Heterozygous mutations targeted exclusively P14ARF in five cases of BL including one case with a variant of unknown significance (VUS). Both genes encoding for P14ARF and P16INKA were deleted in two BLs while one mutation selectively altered splice site of P16INKA was found in one BL.

TP53 and P14ARF were mutually exclusively in BLs (except for Patient B32, who had a VUS in P14ARF in addition to a TP53 mutation).

ID3/TCF3/CCND3 pathway. ID3 and CCND3 mutations were present in 20/23 BLs (87%) (Tables I and SV): ID3 mutation was the sole mutation in 22% cases, CCND3 mutation was the sole mutation in 22% cases, while 44% cases had concomitant ID3 and CCND3 mutations. Nearly all of the mutations were previously reported for both genes (Love et al., 2012; Richter et al., 2012; Schmitz et al., 2012). CN-LOH of 1p36 including the ID3 locus was detected in three BL cases without ID3 mutations. ID3 or CCND3 may be either mutated in another exon or modified by an epigenetic mechanism. No TCF3 mutations were detected in our series.

Mutation patterns according to the age at onset in BL. P14ARF mutations were mainly detected in BL patients younger than 27 years old (P = 0.05, test U Mann–Whitney) while TP53 mutations were equally present in adults and children.

ID3 mutations occurred more frequently in adult than in paediatric BLs, while the CCND3 mutations were equally detected in both categories. The median age for patients with ID3 mutations was 27 [4–65] years old vs. 8.75 [7–14] without (P = 0.002, test U Mann–Whitney). All 10 (100%) informative adult BLs had an ID3 mutation compared to only 5/13 (38-5%) paediatric BL (P = 0.0027, Fisher exact test).

Despite the small sample size (18 cases with a median follow-up of 6 years [1–14]), BL with concomitant ID3 and CCND3 mutations showed a significant poorer outcome: 4/9 with ID3 and CCND3 double-hit mutations died within 8 months of diagnosis versus 0/9 without (P = 0.024, Fisher exact test).

Discussion

Among BL patients, the outcome is poorer in older patients, suggesting heterogeneity within this well-defined entity (Jacobson & LaCasce, 2014). Past studies did not detect obvious differences between childhood and adulthood BL at the genomic or the transcriptomic levels (Klapper et al., 2008; Deffenablecher et al., 2012). Recent next generation sequencing (NGS) studies also did not pinpoint specific differences (Love et al., 2012; Richter et al., 2012; Schmitz et al., 2012). We chose to describe a series of well-characterized Burkitt lymphomas using tools that are available in clinical practice: SNP-array, FISH and sequencing of five selected genes.

For the first time, we report different patterns of genetic anomalies in BLs according to patient age. Children displayed more 13q amplifications, 7q gains and exclusive 5q CN-LOH, while 18q21 CN-LOH was only detected in adults. All adults had ID3 mutations compared to only 5/13 children. CCND3 had an added value to ID3 only in childhood BL in the present series (5/8 unmutated ID3 cases were CCND3 mutated). These differences suggest that aging influences the lymphoma biology. This has been already shown for DLBCL (Klapper et al., 2012). The question of the age cut-off for BL is yet undefined. Studies have shown that adolescent BLs older than 15 years of age had a poorer outcome compared to younger children but this was not confirmed in the international French-American-British/LMB96 study (Cairo et al., 2012).

A previous targeted genomic sequencing study (182 genes) of paediatric BL showed that the rate of mutations is lower in Epstein–Barr virus (EBV)-positive versus EBV-negative BL (Giulino-Roth et al., 2012). As EBV-encoded small RNA (EBER) expression was investigated in a subset of cases (n = 7) but not in the whole series, we could not assess if the presence of EBV could explain the different pattern of mutations between paediatric and adult patients. However,
Several arguments suggest that EBV expression may not explain the different patterns of genomic aberrations observed in paediatric versus adult BLs. Studies showed that the incidence of EBV expression does not differ between adult and paediatric sporadic BLs (Chuang et al, 2008; Pannone et al, 2014). Moreover, the incidence of CCND3 mutations did not vary between adult and paediatric BLs in our series (70% vs. 61% respectively) while Schmitz et al (2012) reported that the main mutational variation between sporadic BL (<30% EBV+) and endemic BL (>95% EBV+) was the much lower incidence of CCND3 mutations in the latter (38% vs. 1.8%, respectively).

Two genetic aberrations were associated with an adverse prognosis in BL: the ID3 and CCND3 double-hit mutations on the one hand, and the 18q21 CN-LOH on the other. Both alterations were mostly detected in adult patients. However, these observations need to be confirmed in a larger series, before claiming that it may be an explanation for the poorer prognosis of adult BL.

The double ID3 and CCND3 mutations may be more deleterious by acting simultaneously on two different pathways that cross-talk to activate the pro-survival PI3kinase pathway (ID3/TCF3) and to drive cell cycle progression (CCND3/CDK6).

The 18q21 CN-LOH targets the 3' part of DCC which encodes a receptor belonging to the superfamily of immunoglobulins. DCC is thought to act as a tumour suppressor gene due to its frequent deletion in colorectal cancer (Ma et al, 2010). The CN-LOH of its intracellular element may modify the downstream signalling (Ma et al, 2010).

In the setting of an international clinical trial, we previously showed that chromosome 13 aberrations were associated with an independent adverse prognosis (Poiré et al, 2009). This anomaly probably corresponds to the 13q gain, which targets the MIR17HG cluster frequently associated with a terminal 13q deletion. The relative small number of cases did not allow us to confirm the adverse prognostic impact associated with the MIR17HG amplification (Schiffman et al, 2011).

The incidence of ID3 in our series of BL (65%) seemed to be within the range of other studies (Love et al, 2012; Richter et al, 2012; Schmitz et al, 2012; Momose et al, 2015). Clinical data are not detailed enough in these different studies to be able to confirm our findings of higher incidence of ID3 mutations in adults. The incidence of TCF3 and CCND3 mutations varied according to the different series. The CCND3 incidence varied from 0% (Love et al, 2012) to 26% (Momose et al, 2015) and 38% of molecularly defined sporadic BLs (Schmitz et al, 2012) compared to 65% in our series. No TCF3 mutations were detected in our series and in 51 whole-exome sequenced BL (Love et al, 2012), compared to 6–18% of BL (Schmitz et al, 2012; Momose et al, 2015) and in 1/4 whole-genome sequenced BL (Richter et al, 2012).

These apparently discrepant results may reflect a sampling bias in the context of a relatively low frequency of TCF3 mutations in BL. First, the age of the studied cases varies in the different series. Richter et al (2012) screened mutations in four children in whilst two other studies (Love et al, 2012; Schmitz et al, 2012) as well as in our study included both adults and children. However, the median age in our study was younger (14–5 [4–65] years old with 54% <18 years old) compared to the series reported by Love et al (2012) (18–5 [3–82] years) and Schmitz et al (2012) (38–5% <18 years old and 23% >60 years old). The TCF3 mutations may be present in older adults (less represented in our series) and CCND3 in younger patients (more frequent in our series). Second, our cases were recruited based on classical WHO criteria (relying on morphology, immunohistochemistry and cytogenetics) while the NGS studies used to detect TCF3 mutations relied on a molecular definition of BL based on GEP (Richter et al, 2012; Schmitz et al, 2012). In Love et al (2012) performed GEP on selected cases only. If lymphomas with TCF3 mutations more frequently display some atypical morphological features for BL with a molecular BL signature, they may be selected by GEP but not by morphological analysis.

The CDKN2A mutations targeted preferentially the P14ARF isoform in 4/5 BL cases. Altogether, mutations or deletions within the TP53/P14ARF pathway were present in almost 80% BL in a mutually exclusive manner. Although not specific to BL, it confirms that inactivation of this pathway is a necessary event for counteracting the pro-apoptotic effect of MYC. In the five cases without detected mutations or deletions, another mechanism of inactivation might have occurred such as DNA methylation as reported for P14ARF/CDKN2A.

We detected six recurrent CN-LOH (1p31, 1p35p36, 5q23.3, 17p13, 7q31.2, 18q21.2) of various sizes in BL. CN-LOH is due either to mitotic illegitimate homologous recombination or to nondisjunction and may render a cell homozygous for a pre-existing abnormality, leading to clonal selection. It is an additional way to inactivate tumour suppressor genes, such as TP53 at 17p13 and, possibly, DCC at 18q21. The targeted genes in the other CN-LOHs remain to be determined.

In conclusion, our study allowed the identification of aberrant genomic patterns that vary with the age of onset among BL patients. It also pinpointed new potential genomic biomarkers. The association of ID3 and CCND3 double-hit mutations with a poorer survival on the one hand, and of 18q21 CN-LOH involving DCC on the other hand, especially in adult BL, should be confirmed in the setting of randomized clinical trials.

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 Genetic Differences Between Paediatric and Adult BL  

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Author contributions

VH, XP and GA performed the research; HAP designed the research study; IT and MR centrally reviewed the cases; ECB, CB, DP, EL, LM, FM and ND performed cytogenetics and provided materials; VH, MV, HAP analysed data; HAP wrote the manuscript.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. MicroRNA expression.
Table SI. BAC Probes.
Table SII. Primers.
Table SIII. Detailed CNV results per patients.
Table SIV. Detailed CN-LOH results per patients.
Table SV. Detailed cytogenetics and mutation results.
Fig S1. 13q gains.

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