Application of 2016 WHO classification in the diagnosis of paediatric high-grade *MYC*-negative mature B-cell lymphoma with Burkitt-like morphological features

Lei Zhang,[1] Laura E Brown,[1,2] Laurel M Bowen,[1] Laura C McCarthy,[3] Linda D Cooley,[1] Elena Repnikova,[1] Melissa A Gener,[1] Robert Garola,[1] Keith J August,[3] J Allyson Hays,[3] David L Zwick,[4] Weijie Li [1]

[1]Department of Pathology and Laboratory Medicine, Children's Mercy Hospitals and Clinics, Kansas City, Missouri, USA
[2]Department of Pathology and Laboratory Medicine, University of Kansas, Kansas City, Kansas, USA
[3]Division of Hematology/Oncology, Children's Mercy Hospitals and Clinics, Kansas City, Missouri, USA
[4]Department of Pathology, University of Texas Southwestern Medical Center, Dallas, Texas, USA

Correspondence to
Dr Weijie Li, Pathology, Children's Mercy Hospitals and Clinics, Kansas City, MO 64108, USA; wli@cmh.edu

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ABSTRACT

Aims Historically, there has been no consensus on the diagnostic classification of high-grade B-cell lymphoma (HGBCL) with morphological features of Burkitt lymphoma (BL) but no *MYC* gene rearrangement (*MYC*-negative). The 2016 WHO classification of tumours of haematopoietic and lymphoid tissues has shed some light on this field with the modification of the grey-zone lymphoma with features intermediate between BL and diffuse large B-cell lymphoma, and the creation of several new entities. The aim of this study was to investigate how the revised WHO classification affects our practice in diagnosing these lymphomas in children.

Methods We retrospectively reviewed cases of mature HGBCL diagnosed at our hospital between 2015 and 2018.

Results Among 14 mature HGBCL cases with BL morphological features, 11 showed *MYC* rearrangement consistent with BL and 3 were *MYC*-negative. Two *MYC*-negative cases showed regions of 11q gain and loss by microarray consistent with Burkitt-like lymphoma with 11q aberration (BLL-11q). The third *MYC*-negative case showed diffuse and strong MUM1 expression, translocation involving 6p25 by chromosome analysis and *IRF4* rearrangement by fluorescence in situ hybridisation analysis consistent with large B-cell lymphoma with *IRF4* rearrangement (LBL-IRF4). All patients were treated according to applicable chemotherapeutic protocols and achieved remission.

Conclusions BLL-11q and LBL-IRF4, two newly defined entities, should be considered in paediatric *MYC*-negative mature HGBCL cases. Accurate diagnosis needs careful histopathological examination and proper cytogenetic testing. Since they have unique cytogenetic features, specific treatments for them may emerge in the future. Therefore, accurate diagnosis based on the 2016 WHO classification is clinically significant.

INTRODUCTION

Burkitt lymphoma (BL) is a high-grade aggressive B-cell non-Hodgkin's lymphoma characterised by *MYC* rearrangement, most often in the form of t(8;14), resulting in the fusion of *IGH* and *MYC*. Morphologically, typical BL shows sheets of monomorphic medium-sized tumour cells with round nuclei, finely dispersed chromatin and multiple basophilic small to intermediate-sized nucleoli. The cytoplasm is moderately abundant and highly basophilic with multiple lipid vacuoles better visualised on Wright and/or Giemsa stained air-dried smears or imprint slides. There are many mitotic and apoptotic figures and numerous intermixed tingible body macrophages resulting in a so-called 'starry sky' pattern. The immunophenotype of typical BL is that of germinal centre B-cell (GCB) type, positive for IgM, CD19, CD20, CD22 and CD79a, CD10 and BCL6. BCL2 is usually negative. The proliferative index (Ki-67) is nearly 100%.[1]

Lymphomas with morphology resembling BL but showing more cytological pleomorphism or other atypical morphological or phenotypical features were classified as atypical BL/Burkitt-like lymphoma (BLL) by the 2001 WHO Classification of tumours of haematopoietic and lymphoid tissues. The name was then changed to B-cell lymphoma, unclassifiable, with features intermediate between BL and diffuse large B-cell lymphoma (DLBCL) by the 2008 WHO classification.[2] These lymphomas were thought to represent a continuum between BL and DLBCL. The newly revised WHO classification[1 3] has divided these lymphomas into two categories: high-grade B-cell lymphomas (HGBCLs) with *MYC* and *BCL2* and/or *BCL6* rearrangement (double-hit or triple-hit lymphomas), and HGBCLs, not otherwise specified (NOS). Both have very poor prognosis with no optimal therapeutic strategy.[4–6] Double-hit/triple-hit lymphomas are very rare in children,[4 5 7] and routine testing for them is not recommended in the paediatric population. HGBCLs, NOS, include cases with atypical BL features or blastoid morphology without double-hit or triple-hit cytogenetic findings, regardless of *MYC* status. This category seems not applicable for paediatric cases, considering the excellent prognosis of most paediatric high-grade B-cell non-Hodgkin's lymphoma cases and their distinct molecular features.[7–12]

Burkitt-like lymphoma with 11q aberration (BLL-11q), a new provisional entity in the 2016 WHO classification,[1 3] comprises cases with morphological, phenotypical and gene expression resemblance to BL, but lacking *MYC* translocation (*MYC*-negative) and harbouring characteristic proximal 11q gains and distal 11q loss.[1 3 13 14] BLL-11q lymphomas have a mutational landscape different

from BL.[15] Previous studies[13 14] defined the typical gained region as 11q23.3 and the lost region as 11q24-qter. Collectively, their results suggest the upregulation of oncogenes *PAFAH1B2*, *USP2* and *CBL*, located in the gained regions of 11q23, and corresponding downregulation of tumour suppressor candidate genes *FLI1*, *ETS1*, *TBRG1* and *EI24*, located in the regions of 11q24-qter loss. Grygalewicz *et al*[16] identified two different types of 11q rearrangements seen in BLL-11q cases: a large duplication of 11q comprising more than 50 Mb with terminal deletion, and a small duplication of 11q comprising less than 20 Mb with an additional gain within the duplicated material as well as terminal deletion. BLL-11q cases occur over a wide age range but are more common in children and young adults. They are more frequently nodal than BL and tend to present as a single dominant mass or conglomerate mass.[14 16] Patients tend to present with limited disease without involvement of bone marrow or cerebrospinal fluid (CSF), and prognosis appears to be favourable, similar to classical BL.[1]

Large-B-cell lymphoma with *IRF4* rearrangement (LBL-IRF4), another newly recognised provisional entity by the 2016 WHO classification, most commonly affects children and young adults.[1 3] It mainly involves Waldeyer's ring and cervical lymph nodes and usually presents as a low-stage disease. Microscopically, the tumour cells are medium to large in size with finely clumped chromatin and small basophilic nucleoli. Mitotic figures are usually infrequent, and a starry sky pattern is absent, though the proliferation rate is usually high by Ki67 stain. These lymphomas may have a diffuse growth pattern consistent with DLBCL, follicular growth pattern consistent with grade 3 follicular lymphoma (FL), or follicular/diffuse pattern (composite FL grade 3/DLBCL). The tumour cells are positive for B cell-specific markers (CD20, CD79a and PAX5) and characteristically show strong expression of IRF4/MUM1 and BCL6. Over 50% of the cases are also positive for BCL2 and CD10. Despite the strong expression of IRF4/MUM1, these cases have a germinal centre signature by gene expression profiling. Most cases have a cytogenetically cryptic rearrangement of *IRF4* with an *IGH* locus. *BCL6* alterations may be detected in some cases, but essentially all cases lack *MYC* and *BCL2* rearrangement. Most cases have shown good response to chemotherapy.[17 18]

Both BLL-11q and LBL-IRF4 are rare, and only a limited number of cases have been reported. Studies of more cases are needed to better characterise these lymphomas and to determine if they are truly a distinct entity. Since these lymphomas have unique cytogenetic features, more targeted therapy may be on the horizon, and recognising these lymphomas may become clinically important.

To investigate how the revised WHO classification affects our pathological diagnosis in paediatric mature HGBCL cases, we retrospectively reviewed our cases diagnosed in a recent 3-year period and identified two paediatric BLL-11q cases and one paediatric LBL-IFR4 case. The detailed histopathological and cytogenetic findings of these cases and their clinical course have been described. The diagnostic features and proper testing strategy of these new entities have been discussed.

MATERIALS AND METHODS

We retrospectively reviewed 16 cases of high-grade mature B-cell non-Hodgkin lymphomas diagnosed at Children's Mercy Hospital (CMH) between 2015 and 2018. Eleven had *MYC* rearrangement, consistent with BL. Two were *MYC*-negative and morphologically consistent with DLBCL. Three were *MYC*-negative but showed BL morphological features. Clinical information, histological features, immunohistochemistry (IHC), multicolour flow cytometry (FCM), fluorescence in situ hybridisation (FISH), conventional cytogenetics, and microarray analysis previously performed on tumour tissue for these three cases were reviewed in detail. The study was approved by the CMH institutional review board.

IHC was performed using Leica's Bond Polymer Refine Detection system according to CMH institutional standard protocol with appropriate positive and negative controls. FCM of fresh specimen was performed by FACSCanto A (case 3) or FACSCanto 10-colour cytometer (case 2) and analysed by FACS Diva software (BD Biosciences) according to CMH leukaemia/lymphoma immunophenotyping protocol. No FCM was performed on case 1 due to sample limitations. The antibodies for IHC stains were obtained from Leica Biosystems, while the antibodies for FCM study were obtained from Becton Dickinson Biosciences.

FISH analysis was carried out using tumour touch imprints or tumour cells gathered from a cytogenetic direct harvest. Analysis was performed using Cytocell (Tarrytown, New York, USA) *MYC* triple-colour break-apart probes for all three cases: Cytocell *KMT2A* (11q23) break-apart probes for case 2, Cytocell *BCL2*/*IGH* dual fusion and *BCL6* break-apart probes for cases 2 and 3, and Leica (Buffalo Grove, Illinois, USA) *IRF4*/*DUSP22* break-apart probes for case 3. Chromosome analysis was performed on short-term cultured tumour cells in all three cases. Cytogenetic abnormalities were described according to the current International Standing Committee on Human Cytogenetic Nomenclature (2016).

Microarray analysis was carried out using formalin-fixed paraffin-embedded (FFPE) sample for case 1 and fresh tumour sample for cases 2 and 3 using Thermo Fisher Scientific OncoScanTM CNV Plus Platform (for the FFPE sample) and Cytoscan HD platform (for fresh tumour samples) according to manufacturer's instructions. Data were analysed using Thermo Fisher ChAS V.3.3 software with human genome build GRCh37(hg19).

RESULTS

The clinical information and key features of the three cases of *MYC*-negative mature HGBCLs with BL morphological features are summarised in table 1. The details are described separately for each case.

Case 1

A 4-year-old girl with no significant medical history presented with a 2-day history of anorexia, abdominal pain and fatigue. She was found to have a retroperitoneal and mesenteric conglomerate mass and a positron emission tomography (PET)-avid lymph node in the right thigh. She underwent an open biopsy of the abdominal mass, and histological examination of the tumour revealed sheets of medium to large-sized tumour cells with fine or vesicular chromatin, one or multiple prominent nucleoli, frequent mitosis and tingible body macrophages with 'starry sky' appearance. Tumour cells were diffusely positive for CD20, CD10, BCL6 and Ki67 (almost 100%), and mostly negative for BCL2 and MUM1 (figure 1A). Chromogenic in situ hybridisation (CISH) for kappa and lambda showed lambda restriction, and Epstein-Barr virus-encoded small RNA (EBER) was negative. FISH was negative for *MYC* rearrangement. Chromosome analysis revealed a complex karyotype with apparent duplication of a large portion of 11q (figure 1C), as well as loss of material from 4p and 6q. Microarray showed segmental gains (~56 Mb) from 11q13.1 to 11q23.3 (including *PAFAH1B2*) with a ~14 Mb adjacent terminal loss from 11q24.1 to 11qter (gain/

Table 1 Clinical, pathological and cytogenetic features of the three *MYC*-negative cases

Features	Case 1	Case 2	Case 3
Age (years)/sex	4/F	10/M	9/F
B symptoms	No	No	No
Clin. stage	III	II	I
LDH ↑	No	No	No
Site	Multiple retroperitoneal and mesenteric masses, involvement of the lymph node in the left thigh	Right palatine tonsil/adenoid with regional lymph node involvement above the diaphragm	Left posterior neck
Largest size (cm)	12.5 (retroperitoneal mass)	4.9	3.7
Histomorphology	Medium to large-sized tumour cells, fine or vesicular chromatin, one or multiple prominent nucleoli, frequent mitosis and tingible body macrophages, starry sky	Monotonous, medium to large-sized tumour cells, fine chromatin, distinct multiple small to intermediate nucleoli, frequent mitosis	Monotonous, medium-sized tumour cells, fine chromatin, multiple small nucleoli, frequent mitosis, tingible body macrophages, starry sky
Immunophenotype (IHC and FCM)	CD20+, CD10+, BCL6+, Ki67+ (almost 100%), BCL2−, MUM1−, lambda restriction by CISH, EBER−	CD20+, CD10+, BCL6+, Ki67+ (close to 100%), BCL2+ (weak, partial), MUM1+ (small subset), CD19+, CD38+, CD22+ (dim), CD45+, CD21+, kappa restriction by FCM, EBER−	CD20+, BCL6+, MUM1+, Ki67+ (close to 100%), CD79a+, CD19+, CD22+, CD45+, BCL2−, CD10−, kappa restriction by FCM, EBER−
FISH	Negative for *MYC* rearrangement	Negative for *MYC*, *BCL2* and *BCL6* rearrangements; positive for *KMT2A* amplification	Negative for *MYC*, *BCL2* and *BCL6* rearrangements; positive for *IRF4* and *IGH* rearrangements
Karyotype	46,XX,add(1)(p31),del(4)(p15.1p16),del(6)(q21q24),der(9)t(1;9)(p31;p13),dup(11)(q13q24)(7)/ 46,sl,-del(4),+4,der(5)t(5;11)(q22;q24),-dup(11),+der(11)dup(11)(q13q24)t(5;11)(q22;q24)(11)/ 47,sdl1,+X(2)	46,XY,der(11)del(11)(p13p15.1)dup(11)(q22.3q23.3)(11)/ 46,sl,del(6)(q21q25)(3)/ 46,XY,der(11)del(11)(p13p15.1)trp(11)(q22.3q23.3)(3)/ 46,XY(3)	46,XX,t(6;17)(p25;q24),inv(14)(q31q32),der(22)t(11;22)(q12.3;p11.2)(19)/ 46,XX(1)
Microarray-11q gain	11q13.1q23.3 (65 097 569–121 181 848) 53 Mb	11q22.3q23.3 (108 793 027–119 071 715) 7 Mb	11q12.3q25 (62 679 094–134 938 470) 72 Mb
Highest regions of 11q gain	11q13.1q13.2 (65 097 569–65 959 206)×5 862 kb 11q13.2 (65 978 452–68 292 930)×4 2.3 Mb	11q22.3q23.1 (108 739 027–110 493 792)×4 1.8 Mb 11q23.1 (110 493 848–111 861 921)×4.2 1.4 Mb	11q21q24.3 (96 556 567–128 017 406)×2.8 31 Mb 11q24.3q25 (130 057 989–134 938 470)×2.9 4.9 Mb
Microarray-11q loss	11q24.1q25 (121 206 487–4 938 847) 14 Mb	11q23.3q25 (119 075 428–134 938 470) 16 Mb	None
Microarray–other variants	Segmental losses in 4 p, 6q, 19 p Segmental gain in 9 p, 13q, 16q cn-LOH in 6 p, 16q, 17q, 19 p	Chromothripsis of 11 p Segmental losses in proximal 11q (11q12.1) Segmental loss in 12q24.33	Segmental gain within 17q22 cn-LOH of chromosome X
Diagnosis (2016 WHO classification)	BLL-11q	BLL-11q	Large B-cell lymphoma with *IRF4* rearrangement
Treatment	ANHL1131 group B plus rituximab	ANHL1131 group B	CCG-5961 group A
Response/FU (mo)	CR/24	CR/16	CR/36

BLL-11q, Burkitt-like lymphoma with 11q aberration; CISH, chromogenic in situ hybridisation; Clin. stage, clinical stage using the Murphy staging system; cn-LOH, copy neutral loss of heterozygosity; CR, complete response; EBER, Epstein-Barr virus-encoded small RNA; F, female; FCM, flow cytometry; FISH, fluorescence in situ hybridisation; FU, follow-up in months from end of therapy; IHC, immunohistochemistry; LDH ↑, lactate dehydrogenase elevated above the upper limit of normal; M, male.

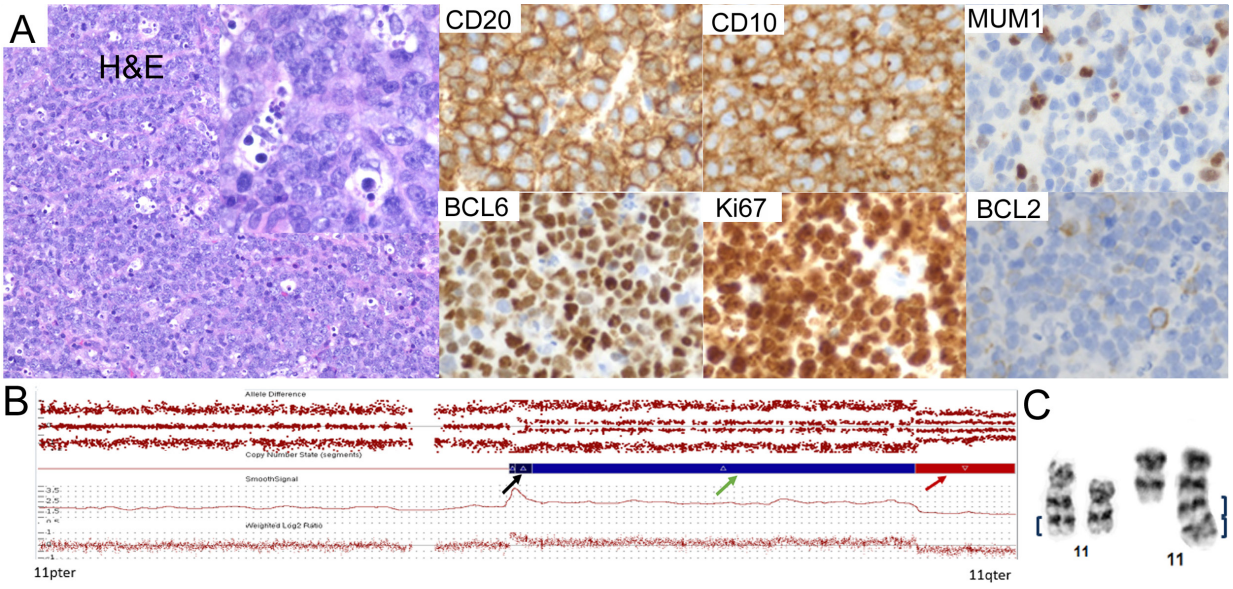

Figure 1 Histopathology, immunophenotype and cytogenetic findings in case 1. (A) Low-power and high-power views of the H&E-stained section revealing sheets of medium-sized to large-sized tumour cells with fine or vesicular nuclear chromatin, one or multiple prominent nucleoli, frequent mitosis and tingible body macrophages with 'starry sky' appearance. Tumour cells were diffusely positive for CD20, CD10, BCL6 and Ki67 (almost 100%), and mostly negative for BCL2 and MUM1. (B) Microarray analysis showing segmental gains within 11q with adjacent terminal loss. The solid blue bar represents the area of gain (green arrow); the solid red bar indicates the area of loss (red arrow); and the solid dark blue bar indicates higher level gains (black arrow). (C) Chromosome analysis showing an abnormal chromosome 11 with duplication/triplication of a large segment from 11q13 to 11q24 (indicated by blue brackets).

loss pattern). Three adjacent regions, 11q13.1q13.2, 11q13.2 and 11q13.2q23.3, showed different levels of gain, with the two proximal regions showing the highest levels (figure 1B). Other copy number changes included a ~30 Mb deletion from 4pter to 4p15.1 with 759 kb adjacent duplication, a ~22 Mb deletion from 6q15 to 6q21, a ~754 kb biallelic loss within 19p13.3p13.2, a ~993 kb duplication within 9p24.2, a ~674 kb four-copy gain within 13q31.3 involving *MIR17HG* and a ~11 Mb duplication from 16q23.1 to 16qter. There were also several large regions of copy-neutral loss of heterozygosity: 6pterp21.32 (33 Mb), the entire 16q and 17q, and 19pterp13.12 (~15 Mb), which encompassed the region of biallelic loss. Bone marrow biopsy and serial CSF examinations were negative for involvement by lymphoma. She was diagnosed with BLL-11q and treated per ANHL1131 group B protocol with cyclophosphamide, cytarabine, vincristine, prednisone, doxorubicin, methotrexate and rituximab. The treatment course was complicated by tumour lysis syndrome, superficial femoral vein thrombosis and mucositis. She nevertheless had a favourable response and has remained in remission for two years from the end of therapy.

Case 2

A 10-year-old boy with a medical history of growth hormone deficiency presented with a 2-month history of a right neck mass. CT revealed a cervical chain lymph node conglomeration, and PET–CT scan suggested additional involvement of the tonsils and thymus. Fine-needle aspiration was performed, which was reported as suspicious for lymphoma. He subsequently underwent excisional biopsy, and histological examination of the lymph node revealed sheets of monomorphic medium to large-sized tumour cells with fine chromatin, distinct multiple small to intermediate nucleoli, and frequent mitosis. Tumour cells were diffusely positive for CD20, CD10, BCL6 and Ki67 (close to 100%), with a small portion positive for BCL2 (weak) or MUM1. IHC revealed a germinal centre phenotype (figure 2A), and CISH was negative for EBER. FCM revealed a kappa light chain-restricted B-cell population positive for CD19, CD20, CD10, CD38, CD22 (dim) and CD45 (figure 2B), and negative for CD5 or CD34. Bone marrow and CSF studies were negative for lymphoma involvement. FISH was negative for *MYC*, *BCL6* and *BCL2* rearrangements. Chromosome analysis revealed 11q abnormality with apparent regional duplication in the majority of cells and triplication in a few cells, and a deletion in 11p within chromosome bands 11p13p15.1 (figure 2D). A subclonal abnormality with 6q21q25 deletion was detected. Metaphase FISH showed *KMT2A* amplification in the duplicated and triplicated regions of 11q. Microarray revealed 11q segmental gain (~10.3 Mb) from 11q22.3 to 11q23.3 with a ~16 Mb adjacent terminal loss from 11q23.3 to 11qter (gain/loss pattern). Three adjacent regions, 11q22.3q23.1, 11q23.1 and 11q23.1q23.3, showed different levels of gain with the two proximal regions showing the highest levels (figure 2C). Chromothripsis is seen between 11p15.1 and 11p13 with seven segmental losses; two adjacent losses are within 11q12.1 (~607 and ~889 kb); and a ~234 kb loss is seen within 12q24.33. The subclone with 6q deletion detected by chromosome analysis was not visible by microarray analysis. He was diagnosed with BLL-11q and treated per Children's Oncology Group (COG) ANHL1131 group B protocol with cyclophosphamide, cytarabine, vincristine, prednisone, doxorubicin and methotrexate, complicated by one episode of fever without identified cause. He achieved remission and remained well for 16 months from the end of therapy.

Case 3

A 9-year-old girl presented with a 6-week history of persistent cervical lymphadenopathy. Ultrasound revealed a mass, which was excised. Histological examination of the excisional biopsy revealed sheets of monotonous medium-sized tumour cells with fine chromatin, multiple small nucleoli, frequent mitosis and

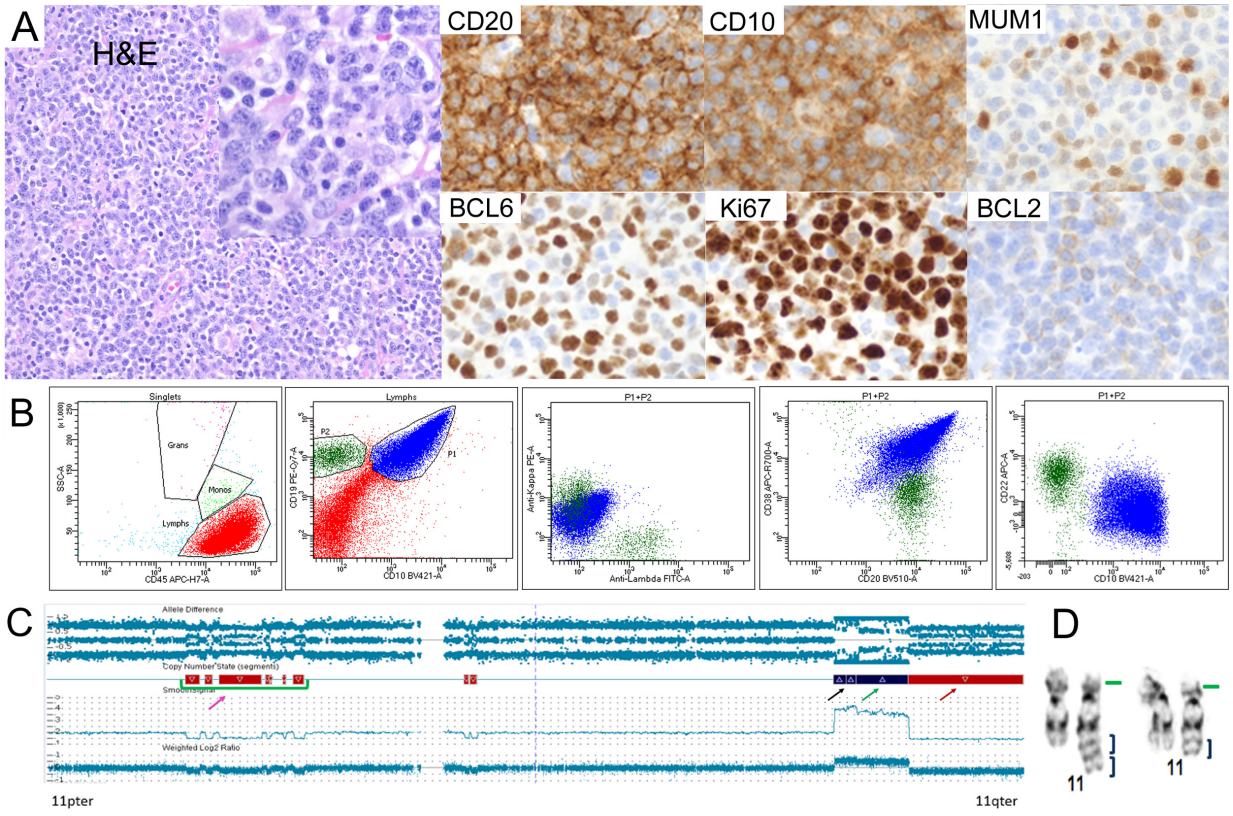

Figure 2 Histopathology, immunophenotype and cytogenetic findings in case 2. (A) Low-power and high-power views of the H&E-stained section revealing sheets of monomorphic medium-sized tumour cells with fine chromatin, multiple small to intermediate basophilic nucleoli and frequent mitosis. Tumour cells diffusely positive for CD20, CD10, BCL6, and Ki67 (close to 100%), with small portion positive for BCL2 (weak) and MUM1. (B) Tumour cells (blue) positive for CD19, CD20, CD10, CD38, CD22 (dim), kappa (dim) and CD45, and negative for lambda by multicolour flow cytometry analysis. Cells in green were reactive B cells. SSC,side scatter; APC, Allophycocyanin;FITC, Fluorescein isothiocyanate; PE, Phycoerythrin; BV, Brilliant Violet. (C) Microarray analysis showing segmental gains within 11q with adjacent terminal loss. Solid blue bar representing area of gain (green arrow) and amplification (black arrow), and solid red bar indicating area of loss (red arrow) within chromosome 11; the green bracket outlining an area of chromothripsis on 11p (pink arrow). (D) Chromosome analysis showing abnormal chromosomes 11 with deletion of 11p13p15.1 (corresponding to area of chromothripsis; indicated by green line) and duplication/triplication of 11q from 11q22.3 to 11q23.3 (indicated by blue brackets).

tingible body macrophages with starry sky appearance. Tumour cells were diffusely positive for CD20, BCL6, MUM1 and Ki67 (close to 100%), and negative for BCL2 and CD10 (figure 3A). The neoplastic proliferation invaded the surrounding soft tissue. CISH was negative for EBER. FCM analysis revealed a kappa-restricted B-cell population positive for CD19, CD20, kappa (bright) and CD45, and negative for lambda and CD10 (figure 3B). Chromosome analysis showed an unbalanced translocation between 11q and 22p, resulting in a gain of 11q (figure 3D). Other chromosomal abnormalities included a t(6;17)(q25;q24) and inv(14)(q31q32). FISH showed no evidence of *MYC*, *BCL2* or *BCL6* rearrangement. FISH confirmed *IRF4* rearrangement (figure 3E) and *IGH* rearrangement; however, *IRF4*/*IGH* fusion was not demonstrated. Microarray analysis identified a gain of one extra copy of 11q12.3q21 and two extra copies of 11q21 to 11qter with relative loss of a 2 Mb region within 11q24.3 (figure 3C) that contains *FLI1* and *ETS1*. There was also copy-neutral loss of heterozygosity of the entire chromosome X. Workup for disseminated disease was negative, including bone marrow and CSF examinations. Following complete surgical resection and well-tolerated treatment according to CCG-5961 group A protocol for DLBCL with vincristine, cyclophosphamide, doxorubicin and prednisone, she achieved remission and remained well at 36-month follow-up.

DISCUSSION

The two BLL-11q cases described here show features consistent with those reported in the literature except for some additional cytogenetic findings (table 1). Both cases showed sheets of medium to large-sized tumour cells with high proliferation index, GCB phenotype, surface light-chain restriction and negative for EBV infection. Both cases showed some atypical morphological features. Case 1 showed more nuclear pleomorphism, while case 2 showed mild nuclear pleomorphism and sparse tingible body macrophages with no overt starry sky appearance. Cytogenetically, these two cases show the previously reported abnormal pattern of 11q proximal gain with telomeric loss.[13 16] Case 1 represents the type of large duplication, while case 2 represents the small duplication as noted in the study by Grygalewicz *et al*.[16] The duplications/deletions in both cases encompass the minimal gain region, containing the candidate oncogene *PAFAH1B2*, and the minimal loss region, containing candidate genes, *FLI1* and *ETS1*, as reported.[13 16] Both cases show 6q deletion, the most common recurrent copy number abnormality other than those of 11q.[19] The additional cytogenetic abnormalities not previously described for this entity include focal higher level 11q gain/amplification segments shown in both cases, a gain of 13q31.3 in case 1, a biallelic loss at 19p13.3p13.2, distal 4p loss and distal 16q gain in case 1, and several large regions of copy-neutral loss

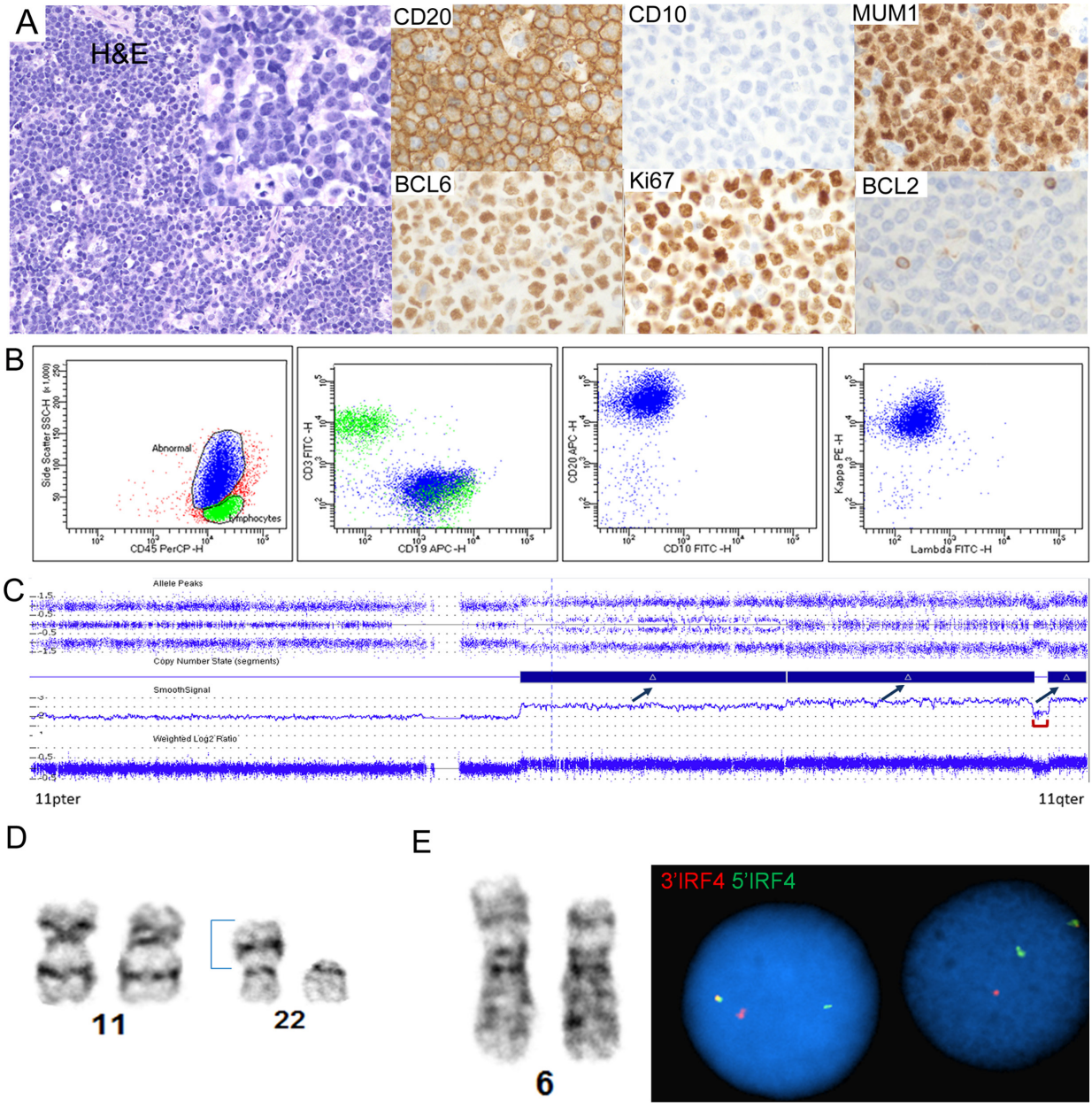

Figure 3 Histopathology, immunophenotype and cytogenetic findings of case 3. (A) Low and high-power view of H&E stained section revealing sheets of monomorphic medium-sized tumour cells with fine chromatin, multiple basophilic small nucleoli, frequent mitosis and tingible body macrophages with 'starry sky' appearance. Tumour cells were diffusely positive for CD20, BCL6, MUM1 and Ki67 (close to 100%), and negative for BCL2 and CD10. (B) Tumour cells (blue) positive for CD19, CD20, kappa (bright) and CD45, and negative for lambda and CD10 by multicolour flow cytometry analysis. PerCP, Peridinin-Chlorophyll-Protein; PE, Phycoerythrin; APC, Allophycocyanin; FITC, Fluorescein isothiocyanate. (C) Microarray analysis showing segmental gains within 11q. Solid blue bars represent areas of gain (blue arrows); the red bracket in the smooth signal indicates a relative loss (=2 copies) of a 2 Mb region within 11q24.3. (D) Chromosome analysis showing an unbalanced translocation between 11q and 22p, and resultant gain of a copy of 11q12.3qter attached to the short arm of one chromosome 22 (indicated by the blue bracket). (E) Chromosome 6p25 anomaly and IRF4 rearrangement shown by fluorescence in situ hybridisation.

of heterozygosity in case 1. The significance of these additional cytogenetic abnormalities is uncertain.

The diagnosis of BLL-11q is based on the presence of characteristic gain/loss patterns of 11q, together with BL/BLL morphology, GCB phenotype and lack of *MYC* rearrangement. The characteristic 11q aberration is key to making the diagnosis, but its presence alone is neither specific nor diagnostic since it may also be present in *MYC*+ BL or DLBCL.[16 20] The most sensitive modality for detecting this characteristic cytogenetic finding is DNA microarray. The 11q aberration can be visualised by chromosomal analysis, as seen in our two cases. However, chromosomal analysis relies on tumour cell viability and metaphase morphology, and the finding may not be characteristic for this aberration when resolution is low. Another potential diagnostic strategy is FISH for chromosome 11 abnormalities. Commercially available FISH probes for chromosome 11 regions may be used to detect gains within 11q and 11q terminal loss. Some groups have suggested a diagnostic strategy of *MYC* and chromosome 11 FISH, with reflex to DNA microarray if inconclusive.[21] Due to the variation of gain/loss spots among the cases,

depending on the probes used, the FISH method alone may miss some cases. On the other hand, some cases such as our case 3 may be falsely called positive. We recommend microarray or FISH testing on all *MYC*-negative HGBCLs with BL/BLL morphology. However, in low-resource regions, the tests could only be done on selected cases with high suspicion. Rymkiewicz *et al*[22] studied the flow cytometric immunophenotype in a series of 10 cases of BLL-11q, concluding that the immunophenotype was similar to that of BL except for a characteristic lower expression of CD38 and coexpression of CD16/CD56. If included in the analysis of CD10+ HGBCLs, this characteristic expression profile could be used to screen such cases and to determine the need for DNA microarray/FISH for detection of 11q abnormalities. The expression status of CD38 and CD16/56 is not known in our cases since these antibodies were not included in our FCM study.

Our case 3 was diagnosed in 2015 before the release of the 2016 WHO classification.[3] BLL morphology and mature B-cell phenotype were noticed, so a preliminary diagnosis of 'high-grade mature B-cell lymphoma, further classification pending cytogenetic results' was given. Chromosomal and FISH analysis was negative for *MYC*, *BCL2* and *BCL6* rearrangement, so BL or double-hit lymphoma was ruled out, and eventually, diagnosis of DLBCL was made. At that time, DNA microarray revealed the 11q aberration. When the new WHO classification was released in 2016,[3] the case was reanalysed, and it was found that the 11q aberration seen in this case was different from the cases reported as BLL-11q in literature; there was no distal loss. Interestingly, the tumour cells of this case showed strong and diffuse expression of MUM1, and there was a chromosomal rearrangement involving 6p25. FISH analysis confirmed *IRF4* rearrangement, which led to the diagnosis of LBL-IRF4. Gain of 11q22.3 through 11qter with no distal loss has been reported as one of the most frequent genomic aberrations associated with *IRF4* translocation-positive lymphomas.[23] Our case is concordant with this observation; however, our case is unusual in view of the presence of frequent mitotic figures and starry sky appearance, the additional cytogenetic abnormalities, including 14q and 17q structural rearrangements, and loss of heterozygosity of the entire chromosome X.

The diagnosis of LBL-IRF4 is based on high-grade morphological features, characteristic immunophenotype (B cells with diffuse and strong expression of MUM1 and BCL6) and the presence of *IRF4* rearrangements. The diagnosis of LBL-IRF4 should be suspected in high-grade FL or DLBCL cases with strong and diffuse expression of MUM1, especially in Waldeyer's ring and cervical regions, and should be confirmed by FISH analysis for *IRF4* rearrangement. Though frequent mitotic figures and starry sky appearance are not typical for LBL-IRF4, they can be seen in this entity, as shown in our case 3.

Historically, there has been no consensus on the diagnosis of *MYC*-negative HGBCLs with morphological features of BL. Given the poor interpathologist concordance on calling atypical BL or BLL, it is reasonable to use a single algorithm for BL and BLL when working up a paediatric case. The first step should be FISH analysis for *MYC* rearrangement. If *MYC* rearrangement is present, the diagnosis will be BL unless the morphology or phenotype is significantly atypical, then testing for *BCL2* and *BCL6* may be necessary to rule out double-hit/triple-hit lymphoma. If it is *MYC*-negative, based on our results and the published data,[7–12] four possibilities should be considered. The first consideration should always be BLL-11q, which seems under-recognised. The second possibility is '*MYC*-negative' BL. Approximately 10% of BLs lack *MYC* rearrangement by standard diagnostic methods but will show a molecular BL gene expression profile with gene expression analysis.[24] The third is LBL-IRF4. LBL-IRF4, like our case 3, can have a diffuse pattern with high proliferation index mimicking BL morphologically. The last is DLBCL. Some DLBCL cases can have a high proliferation index mimicking BL.

In summary, we identified three *MYC*-negativemature HGBCL cases with BL-like morphology within a recent 3-year period. Based on the 2016 WHO classification, two cases were consistent with BLL-11q, and one was consistent with LBL-IRF4. The detailed cytogenetic and histopathological findings of these cases have been described. Our findings highlight the importance of careful histopathological examination and proper cytogenetic testing for the accurate diagnosis of paediatric *MYC*-negative mature HGBCLs according to the new WHO classification. These cases were identified within a 3-year period in one children's hospital, suggesting these entities may be under-recognised.

Take home messages

- Burkitt-like lymphoma with 11q aberration (BLL-11q) and large B-cell lymphoma with *IRF4* rearrangement (LBL-IRF4) should be considered and properly tested in paediatric *MYC*-negative mature high-grade B-cell lymphomas (HGBCLs).
- Characteristic gain/loss patterns of 11q identified by cytogenetic studies (especially DNA microarray) is the key for the diagnosis of BLL-11q.
- BLL-11q is likely an under-recognised entity.
- Diffuse and strong expression of MUM1 and BCL6 in mature HGBCLs should raise concern for LBL-IRF4. The diagnosis should be confirmed by FISH study.

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ORCID iD
Weijie Li http://orcid.org/0000-0003-4922-5473

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