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Simple karyotype and bcl-6 expression predict a diagnosis of Burkitt lymphoma and better survival in *IG-MYC* rearranged high-grade B-cell lymphomas

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Rearrangement of *MYC* with immunoglobulin genes is a hallmark of Burkitt lymphoma. However, this rearrangement is not entirely specific and is often accompanied by varying numbers of additional cytogenetic abnormalities. This study aimed to assess the impact of karyotypic complexity, in correlation with comprehensive immunophenotypic analyses on the diagnosis and clinical outcomes of 34 cases of MYC-IG rearranged lymphomas that included Burkitt lymphoma (twenty-two cases), diffuse large B-cell lymphoma (three cases), unclassifiable B-cell lymphoma with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma (six cases), and plasmablastic lymphoma (three cases). Additional cytogenetic abnormalities were observed in 26 of 34 cases (76%), including four cases (12%) that harbored dual translocations involving *BCL-2* or *BCl-6*. Burkitt lymphoma cases had a significantly lower number of additional abnormalities (mean of 1.7), compared with unclassified B-cell lymphoma (3.3), diffuse large B-cell lymphoma (21.7), and plasmablastic lymphoma (6.7). Cases with simple karyotype ($\leqslant$2 additional abnormalities) were more likely to have a diagnosis of Burkitt lymphoma (89 *versus* 33% in patients with $>$2 additional abnormalities, $P<0.01$) and express bcl-6 (95 *versus* 47%, $P<0.01$). In addition, Burkitt lymphoma, bcl-6 expression, and simple karyotype were individual predictors of better overall survival. However, in multivariate analyses, only bcl-6 expression remained an independent predictor, although survival could be further stratified by karyotypic complexity in bcl-6(+) patients. We conclude that simple karyotype and bcl-6 expression suggest a diagnosis of Burkitt lymphoma and may portend better overall survival. These results may be very useful in the diagnosis and stratification of *MYC-IG* rearranged high-grade B-cell lymphomas.
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Rearrangement of *MYC* (v-myc myelocytomatosis viral oncogene homolog) with immunoglobulin (IG) heavy chain [t(8;14)(q24;q32)] or light chain [t(2;8)(p12;q24) or t(8;22)(q24;q11.2)] genes is a hallmark of Burkitt lymphoma.[1] However, these rearrangements are not entirely specific as they are also seen occasionally in other high-grade B-cell lymphomas,[2–7] including those in the newly proposed entity 'B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma' (hereafter referred to as unclassifiable B-cell lymphoma) in the 2008 World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissue.[8] Furthermore, *MYC* rearrangements are often accompanied by varying numbers of additional cytogenetic abnormalities. These chromosomal abnormalities likely contribute to disease biology and response to therapy.

While the impact of additional cytogenetic abnormalities detected by conventional karyotype

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on clinical outcomes in Burkitt or Burkitt-like lymphomas has been the subject of several studies,[9–11] there is very little information regarding their diagnostic value in the classification of high-grade B-cell lymphomas. Although prompt diagnosis of Burkitt lymphoma and other high-grade B-cell lymphomas carrying *MYC* rearrangements is clinically important because they are aggressive and may require more intensive therapy,[12] the diagnosis sometimes can be challenging.[2–4]

Two major gene expression profiling studies recently sharpened the molecular distinction between Burkitt lymphoma and diffuse large B-cell lymphoma. Despite this, there remain borderline cases with a molecular signature intermediate between these entities.[13,14] In the 2008 WHO classification, the newly established unclassifiable B-cell lymphoma category reflects this diagnostic challenge. This category is heterogenous and is not considered a distinct disease entity, but is useful in allowing the classification of cases not meeting criteria for classical Burkitt lymphoma or diffuse large B-cell lymphoma. It is also the first time that karyotypic abnormalities in addition to *MYC* rearrangement are integrated in the classification. The classification states that cases of Burkitt lymphoma often have a *MYC*-simple karyotype, defined as no or only few additional karyotypic changes or less than six abnormalities detected by (array) comparative genomic hybridization (CGH), whereas cases of unclassifiable B-cell lymphoma often have a *MYC*-complex karyotype.[8] However, the statement '*only few*' is not numerically defined and array CGH is not used in routine clinical practice.

The purpose of this study was to assess the impact of additional karyotypic cytogenetic abnormalities detected by conventional karyotype, in correlation with comprehensive immunophenotypic analyses, on the diagnosis and clinical outcomes of 34 cases of *MYC-IG* rearranged high-grade B-cell lymphomas that included Burkitt lymphoma, unclassifiable B-cell lymphoma, diffuse large B-cell lymphoma, and plasmablastic lymphoma.

Materials and methods

Case Identification

Search of an institutional database for high grade B-cell lymphomas with conventional karyotypes showing *IG-MYC* rearrangement from 2000 to 2009 identified 34 cases that had flow cytometric immunophenotyping and sufficient material to evaluate morphology and perform additional immunohistochemical studies. Clinical data were obtained by review of electronic medical records. The study was approved by the Institutional Review Board at the University of Texas Southwestern Medical Center at Dallas.

Histology, Immunohistochemistry, and *In situ* Hybiridization

Routine hematoxylin and eosin-stained sections were prepared from formalin-fixed and/or B5-fixed paraffin blocks. Bone marrow trephine biopsy specimens were fixed in B-5 or Zenker solution, washed, decalcified, and processed. Bone marrow aspirate smears were prepared and stained with Wright-Giemsa stain. Immunohistochemical analysis was performed on a tissue microarray in 17 cases (duplicate 0.8 mm cores) and individual whole sections in 17 cases by previously described methods[15] using a broad panel of antibodies, including CD10 [56C6], Bcl-6 [GL19E/A8] (Ventana, Tucson, AZ, USA), CD20 [L26] (Signet Laboratories, Dedham, MA, USA), Bcl-2 [124], and Ki-67 [30-9] (all from Dako, Capinteria, CA, USA), CD44 [156-3C11] (Lab Vision, Fremont, CA, USA), IRF-4 [MUM-1] (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and TCL-1 [4042] (Cell Signaling Technology, Danvers, MA, USA). A case was scored as positive if immunostaining was obtained in more than 20% of the neoplastic cells. *In situ* hybridization analysis for EBV encoded small RNA (EBER) was performed using a Novocastra ISH kit according to the manufacturer's instructions.

Flow Cytometry

All cases were immunophenotyped using 4-color FACSCalibur flow cytometry instruments with CELLQuest software (Becton Dickinson, San Jose, CA, USA) and analyzed using cluster analysis with Paint-a-Gate Software (Becton Dickinson) as previously described.[16] The four 4-color combinations (FITC/PE/PerCP/APC) used to characterize the B-cell populations were CD10/CD19/CD20/CD38, kappa/lambda/20/38, lambda/kappa/CD5/CD19, and FMC-7/CD23/CD5/CD19. The level of CD38-APC expression (clone HB7 from BD Biosciences) was quantified as mean fluorescence intensity (MFI).

Karyotype and Fluorescence *in situ* Hybridization (FISH)

Conventional chromosome analysis was performed by the standard cytogenetics protocols for neoplastic studies. Karyotype description conforms to the International System for Human Cytogenetic Nomenclature (ISCN) 2005.[17] FISH analysis was performed on either cell suspensions or paraffin blocks using a commercially available tricolor dual fusion FISH probe set for *MYC–IGH* rearrangement [t(8;14)] with chromosome 8 centromeric probe (CEP8) as internal control (Vysis). If t(8;22) or t(2;8) is present, the probe set that detects the *MYC* loci will show an extra signal on chromosome 22 or 2. The *IgH* rearrangement probe is a dual color, break apart probe (Abbott, Abbott Park, IL, USA) that detects

chromosome breaks of the immunoglobulin heavy chain locus at 14q32. The *IGH–BCL*-2 rearrangement/t(14;18) probe is a dual color, dual fusion probe that detects the juxtaposition of the *IgH* locus at 14q32 to the *BCL2* sequence at 18q21.

Classification of Lymphomas

Classification of lymphomas were based on the criteria outlined in the 2008 WHO classification by integration of morphology, immunophenotype, and cytogenetic/genetic results on *IG–MYC* rearrangement,[1,8,18,19] and detailed as follows:

(1) Burkitt lymphoma: typical cohesive growth of monotonous medium-sized lymphoma cells, with prototypic immunophenotype of CD10(+)/bcl-6(+)/bcl-2(−) and high Ki-67 (>95%), and *IG–MYC* rearrangement. One deviation from this profile, such as slight atypical cytomorphological features, weak expression of bcl-2, or lack of bcl-6, was considered acceptable.
(2) Unclassifiable B-cell lymphoma: morphological features/nuclear pleomorphism intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma with a typical Burkitt lymphoma immunophenotype; or morphologically more typical of Burkitt lymphoma but with an atypical immunophenotype, such as strong bcl-2 expression or with an atypical genetic feature, such as dual translocations involving *IG–BCL2* rearrangement. Atypical cytomorphological features included increased nuclear irregularity, slight nuclear pleomorphism, and/or prominent single nucleoli.
(3) Diffuse large B-cell lymphoma: proliferation of large lymphoma cells with nuclear size clearly exceeding a macrophage nucleus and often with greater nuclear pleomorphism regardless of immunophenotype and genetic features.
(4) Plasmablastic lymphoma: proliferation of large neoplastic cells with immunoblast or plasmablast cytomorphology and with an immunophenotype of terminally differentiated B-cells, and frequent expression of EBER.

The number of additional cytogenetic abnormalities was not used as a major determining factor in the classification, as there is no specific guideline in the 2008 classification of lymphomas. All cases were classified by joint review of two hematopathologists (AS and WC).

Statistical Analysis

Descriptive analyses were performed using frequencies, means, medians, and ranges. Dichotomous variables were compared between two groups using the χ^2-test or Fisher's exact test. The continuous variables were compared by Student's *t*-test. The difference in the number of additional cytogenetic abnormalities among four diagnostic categories was analyzed by one-way ANOVA followed by Tukey's multiple comparison method. Survival curves were calculated for overall survival (OS) according to Kaplan–Meier method and compared using the two-sided log-rank test. Proportional hazardous survival analysis was performed for OS with different analyzed parameters (diagnosis, additional cytogenetic abnormalities, CD10, bcl-2, bcl-6, MUM-1, stage, age, gender) as covariates. OS was the time from diagnosis to the date of last follow-up or death from any cause. *P*-value less than 0.05 was considered significant. All data were analyzed with SAS 9.1 (Insightful Corp. Seattle, WA, USA) or Prism (Version 5.0a; GraphPad Software, La Jolla, CA, USA).

Results

Clinical Characteristics

Clinical data and other major clinicopathological findings of 34 cases of *IG–MYC* rearranged B-cell lymphomas are presented in Table 1. There were 28 males and 6 females, aged from 2 to 66 years (median 35). Seventeen patients were immunocompromised due either to human immunodeficiency virus infection (15 patients, 44%) or to post-transplant immunosuppression (two patients, 6%). All Burkitt lymphomas were either sporadic or associated with immunosuppression. Twenty-four patients presented with extranodal disease (71%), and the remaining presented with nodal disease alone (29%). Therapeutic regimens were variable, depending on the age, original diagnosis, and available treatment protocol.

Cytogenetic (Karyotype and FISH) Characteristics

Of 34 cases with *IG–MYC* rearrangement, 27 (79%) had rearrangement with the immunoglobulin (IG) heavy chain gene (*IgH*) [t(8;14)(q24.1;q32)], three (9%) with the IG kappa light chain gene [t(2;8)(p12;q24.1)] and four (12%) with the IG lambda light chain gene [t(8;22)(q24;q11.2)]. FISH confirmed *IGH–MYC*/t(8;14) in 11 cases, *MYC* rearrangement in two cases, *IGH* rearrangement in two cases, and *IGH/BCL2*/t(14;18) in two cases. Case 25 had a cryptic t(8;14) that was confirmed by FISH. Table 2 lists the details of karyotypic and relevant FISH findings in each case.

Four patients (12%), aged between 50–64 years (accounting for 44% (4/9) of those older than 40 years), harbored an additional *IGH* rearrangement with either *BCL-2* (t(14;18)(q32;q21) in cases 21, 23, and 33], or *BCL-6* [t(3;14)(q27;q32) in case 30). Twenty-six cases (76%) had additional cytogenetic abnormalities besides *MYC* rearrangement, five (15%) had one additional abnormality, six (18%) had two, five (15%) had three, and 10 (29%) had ⩾4 additional abnormalities. Representative cases with

Table 1 Clinicopathological features of *MYC-IG* rearranged lymphomas

Case	*Age/sex*	*Site*	*Diagnosis*	*HIV*	*ACA*	*Ki-67*[a]	*CD10 IHC/FC*	*Bcl-2*	*Bcl-6*	*TCL-1*	*CD44*	*CD38 MFI*	*MUM1*	*EBER/ISH*	*Treatment*	*Outcome f/u (mo)*
1	35/M	BM	PBL	+	0	70	ND/−	−	−	−	ND	7931	+	+	Steroids	D/0.73
2	13/M	LN	BL	−	0	>95	+/+	−	+	+	−	620	−	−	ANHL01P1	A/19
3	21/M	Abdomen	BL	[b]	0	>95	+/+	−	+	+	−	645	−	+	HyperCVAD	A/28
4	31/M	LN	BL	+	0	>95	+/+	−	+	−	+	655	−	NA	HyperCVAD	A/96
5	44/M	Testis	BL	+	0	>95	+/+	−	+	+	−	3183	+	+	NA	A/19
6	39/M	BM	BL	+	0	>95	ND/+	−	+	ND	ND	600	−	ND	NA	A/0.1
7	6/M	Abdomen	BL	−	0	>95	+/+	−	+	+	−	1100	+	+	ANHL01P1	A/12
8	35/M	BM	BL	+	0	>95	+	−	+	ND	ND	4500	−	−	HyperCVAD	A/1.5
9	5/M	Omentum	BL	−	1	75	+/+	−	+	+	+	1202	+	−	ANHL01P1	A/37
10	31/M	LN	INT	+	1	>95	+/+	−	+	+	−	1203	−	−	HyperCVAD/ICE	D/17
11	57/M	LN	BL	+	1	>95	+/+	−	+	+	−	2435	−	+	CODOX-M	A/26
12	29/M	LN	BL	+	1	>95	+/+	−	+	+	−	2017	−	+	CODOX-M	A/7
13	62/M	BM	BL	−	1	NI	ND/+	−	+	ND	ND	2198	−	ND	CODOX-M	A/16
14	2/M	Face	BL	−	2	>95	+/+	−	+	+	−	1988	+	−	NA	A/85
15	11/M	Terminal Ileum	BL	[b]	2	>95	+/+	weak+	+	+	−	1001	+	+	NA	A/0.1
16	8/M	Tonsil	BL	−	2	>95	+/+	−	+	+	p+	1829	+	−	ANHL01P1	A/39
17	3/M	Omentum	BL	−	2	>95	+/+	−	+	+	−	1372	+	−	POG 9317	A/89
18	35/F	Ileocecum	BL	+	2	>95	+/+	−	+	+	−	1274	−	+	NA	A/5
19	19/M	BM	BL	−	2	>95	ND/+	−	+	ND	ND	2091	−	ND	HyperCVAD/CODOX-M/SCT	D/13
20	2/F	BM	BL	−	3	NI	ND/+	ND	+	ND	ND	1200	ND	ND	NA	D/1
21	64/M	BM	INT	−	3	NI	ND/+	+	−	ND	ND	3070	−	ND	HyperCVAD-R	A/7
22	32/M	BM	BL	+	3	>95	ND/+	−	−	ND	ND	1061	−	ND	NA	A/9
23	65/M	BM	INT	−	3	>95	ND/+	−	−	ND	ND	271	−	ND	CODOX-M	D/3
24	35/F	Paraspine	INT	−	3	90	+/+	+	+	+	−	2694	+	−	HyperCVAD	A/10
25	56/F	LN	INT	−	4	>95	+/ND	weak+	+	ND	ND	4457	ND	+	HyperCVAD	A/0.8
26	9/M	Ileum/cecum	BL	−	4	>95	+/+	−	+	+	−	1228	+	−	POG9219	A/100
27	40/M	Anus	PBL	+	5	70	+/−	−	−	+	−	2627	+	+	NA	A/7
28	66/M	BM	BL	−	5	>95	ND/+	−	+	ND	ND	1001	ND	−	HyperCVAD	A/3
29	36/M	BM	BL	+	6	>95	ND/+	−	−	ND	ND	1198	−	ND	HyperCVAD	D/7
30	50/M	BM	INT	+	6	NI	ND/+	−	−	ND	ND	2059	+	ND	CODOX-M	D/5
31	51/F	Peritoneaum	DLBCL	−	9	75	+/+	−	+	+	−	642	−	−	CHOP-R/RICE/DHAP/SCT	D/7
32	35/F	BM	PBL	+	15	NI	ND/−	+	−	−	ND	4558	+	+	CHOP	D/3
33	66/M	LN	DLBCL	−	24	90	+/+	+	+	−	ND	1047	+	−	CHOP-R	A/18
34	57/M	LN	DLBCL	−	32	>95	+/+	−	−	ND	ND	2339	−	ND	CHOP-R	A/4

Abbreviations: A, alive; ACA, additional cytogenetic abnormalities; BL, Burkitt lymphoma; CHOP, cyclophosphamide, hydroxydaunomycin, oncovin, prednisone; CHOP-R, CHOP+rituximab; BM, bone marrow; BL, Burkitt lymphoma, CODOX-M, cyclophosphamide, doxorubicin, high-dose methotrexate; D, dead; DLBCL, diffuse large B-cell lymphoma; DHAP, cytosine arabinoside, cisplatin and dexamethasone; HyperCVD, fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone, alternating with cycles of high-dose methotrexate and cytarabine; ESHAP, etoposide, cytarabine, cisplatinum and methylprednisolone; ICE, ifosfamide-carboplatin-etoposide; INT, lymphoma unclassifiable, with features intermediate between BL and DLBCL; LN, lymph node; NA, treatment information not available; ND, not done; NI, not interpretable; PBL, plasmablastic lymphoma.

[a]All stains were performed by immunohistochemistry (IHC) unless specified. Mean fluorescence intensity (MFI) of CD38 was assessed by flow cytometry (FC), EBV viral infection by *in situ* hybridization (ISH) for EBV-encoded small RNA (EBER), CD10 by both IHC and FC.

[b]Post cardiac and renal transplant for cases 3 and 15, respectively.

Table 2 Karyotypic results in high grade B-cell lymphomas with *MYC-IG* rearrangement

Case	*ACA*	*Cytogenetics*
1	0	46,XY, **t(8;14)(q24.1;q32)**[a] [20]
2	0	46,XY,**t(8;14)**(q24.1;q32) [20]
3	0	46,XY, **t(8;14)(q24.1;q32)**[a] [20]
4	0	46,XY,**t(8;14)**(q24.1;q32) [5]
5	0	46,XY,**t(8;22)**(q24.1;q11.2) [20]
6	0	46,XY,**t(8;14)**(q24.1;q32) [16]
7	0	46,XY,**t(8;14)**(q24.1;q32) [19]
8	0	46,XY,**t(8;14)**(q24.1;q32)[a][20]
9	1	46,XY,der(6)t(1;6)(q12;q13),**t(8;14)(q24.1;q32)**[a] [20]
10	1	47,XY,**t(8;14)**(q24.1;q32),+12 [18]
11	1	47,XY,t**(8;14)(q24.1;q32)**[a],+12 [20]
12	1	47,XY,**t(8;14)(q24.1;q32)**[a],+20 [12]/46,XY,**t(8;14)**(q24.1;q32),add(17)(p11.2) [4]
13	1	46,XY,del(4)(q27q31),**t(8;14)(q24.1;q32)**[a] [10]
14	2	46,XY,dup(1)(q?12q?24),i(7)(q10),**t(8;14)**(q24.1;q32) [8]
15	2	46,XY,der(2)del(2)(q?13q21)del(2)(q23q31),**t(8;22)**(q24.1;q11.2),del(17)(p11.2)
16	2	47,XY,**t(8;14)(q24.1;q32)**[a],add(18)(q21),+19,add(18) [4]
17	2	46XY, **t(8;14)**(q24.1;q32), add(13)(q34), del(17)(p11.2) [1]
18	2	47,+X,idic(X)(p11.2)x2,**t(8;14)**(q24.1;q32) [20]
19	2	47,XY,+del(7)(p15p22),**t(8;14)**(q24.1;q32) [10]/47,idem,ins(1;1)(q25;q31.2q21) [3]
20	3	47,XX,**t(8;14)(q24.1;q32)**[a],+12 [15]/46,idem,-X [3]/47,idem,-X,+mar [2]
21	3	46,XY,del(6)(q15q21),**t(8;22)**(q24.1;q11.2),**t(14;18)**(q32;q21),add(22)(q13) [7]
22	3	47,XY,+8,**der(8)t(8;14)**(q24.1;q32),t(8;14)(q24.1;q32),dup(11)(q13q23),add(13)(q22) [19]
23	3	46,XY,dup(1)(q42q21),**t(8;22)**(q24;q11.2)[a],-12,**t(14;18)(q32;q21)**[c],t(15;21) (p11.2;q11.2),+der(?)t(?;12)(?;q13) [21]/46,idem,-dup(1) [3]/47,idem, +der(8)t(8;22)(q24;q11.2) [4]
24[d]	3	48,XX,+der(X)t(X;1)(q26;q21),**t(2;8)**(p12;q24.1),+**der(8)t(2;8)**(p12;q24.1)[a],der(11)t(11;14)(p15;q32)[b], add(14)(q32) [10]
25[e]	4	48,XX,dup(11)(q13q23),+12,ins(14;8)(q32;q24.1q24.1),+20.ish ins(14;8)(MYC+,IGH+;MYC+,IGH-)[17]/45∼48,idem, add(9)(q34)[cp3]
26	4	46,XY,**t(8;14)**(q24.1;q32) [2]/46,idem,t(2;21)(p21;q22) [15]/45,idem,-Y,t(4;17)(q21;p11.2),del(6)(q21q23) [4]
27	5	47,XY,add(6)(p23),+7,add(8)(p23),**t(8;14)**(q24.1;q32),der(13)t(13;15)(p12;q13), der(21)t(1;21)(q12;q22) [13]
28	5	46,XY,del(2)(p21p23),**t(8;14)(q24.1;q32)**[a] [3]/47,idem,-del(2)(p21p23),+7[1]/47,idem,-del(2)(p21p23),+der(7)del(7)(p13p15)inv(7)(p22q11.2)[15]
29	6	47,XY,**t(2;8)**(p12;q24.1),add(3)(q27),t(12;22)(p13;q11.2),+add(12)(q24.1) add(14)(q32),der(14)t(1;14)(q12;p11.2)add(14)(q32), add(15)(q24) [15 cells]/ 47,idem,add(2)(q31),-add(3)(q27),+add(3)(q27) [4]
30	6	47,XY,der(3)t(3;14)(q27;q32),**der(8)t(8;14)**(q24.1;q32)**t(3;14)(q27;q32)**[b],del(10)(q23q23),**der(14)t(8;14)**(q24.1;q32),+der(17)t(1;17)(q12;p13) [3]/48,idem,+X [9 cells]/50,idem,+X,+7,+add(14)(q32) [2]/50,idem,+X,+add(14)(q32),+20 [5]
31[f]	9	43,XX,del(1)(q42q44),**der(2)t(2;8)(p13;q24)**[a] ,der(3)t(2;3)(p13;q27),-4,add(5)(q33), add(8)(q24),add(9)(q22), -10, add(10)(q24),-15,add(17)(p13) [13]
32	15	48∼49,XX,del(1)(p34.1p36.3),add(2)(p11.2),add(2)(q31),der(8)t(8;8)(p23;q11.2),**der(8)t(8;14)**(q24.1;q32),add(9)(p22), der(12)t(1;12)(q21;p13)ins(12;?)(p13;?)add(12)(q24.1),add(13)(p11.2),add(14)(q32),der(14)add(14)(p11.2)t(8;14)(q24.1;q32),15,add(16)(q22),add(17)(p11.2), add(21)(q22),+3∼4 mar [cp4 cells]
33	24	51,X,Y,+X,der(1)add(1)(p36.3)del(1)(q42q44),der(1)del(1)(p32p36.1) ins(1;?)(q21;?),add(2)(p11.2),der(2)t(2;7)(p21;q11.2),del(3)(p13p25), add(5)(q31),+der(5)t(5;14)(p14;q24)t(14;18)(q32;q21),+der(6)t(6;18)(q27;q21)**t(14;18)**(q32;q21)[c],+7,add(8)(q24.1),ins(8;?)(q22;?),+add(10)(q22),+11,-13, der(13)t(13;14)(q32;q32),-14,**der(14)t(8;14)**(q24;q32)[a],der(16)t(7;16)(q11.2;p13.3),del(17)(p11.2p13), der(18)t(7;18)(q11.2;p11.2),+der(?)(?::14q32->14q32::?),+mar [8]
34	32	80∼85<4n>-X,-Y,del(1)(p32p36.1),-2,ins(2;?)(q31;?)x2,-4,-4,-5,add(5)(p15), add(6)(q13),add(6)(q23),ins(6;?)(q23;?),add(7)(q11.2),**t(8;14)**(q24;q32)[a] x2,add(9)(q22),add(9)(q22),-10,-10,-11,-12,-13,-15,+16,add(16)(p11.2),add(16)(p13.3),-18,-18,-20,-21,-22,-22,+7∼13mar[cp5]/ 75∼87,idem,add(19)(p13.3)[cp10]

Abreviation: ACA, additional cytogenetic abnormalities.

[a]Fluorescence *in situ* hybridization (FISH) confirmed t(8;14)/*MYC-IgH* in 11 cases or *MYC* rearrangement in two cases.

[b]FISH confirmed *IgH* rearrangement in cases 24 and 30.

[c]FISH confirmed *IGH/BCL2* rearrangement [(14;18) translocation] in cases 23 and 33.

[d]By FSIH: evidence of a *MYC* rearrangement, an extra copy of the 5' end of the *MYC* gene and an extra copy of the *IGH* (14q32).

[e]cryptic (8;14) rearrangement confirmed by FISH

[f]By FISH: evidence of two abnormal clones with a *MYC* gene rearrangement, one of which also has extra copies of the *MYC* (8q24) and *IGH* (14q32) genes.

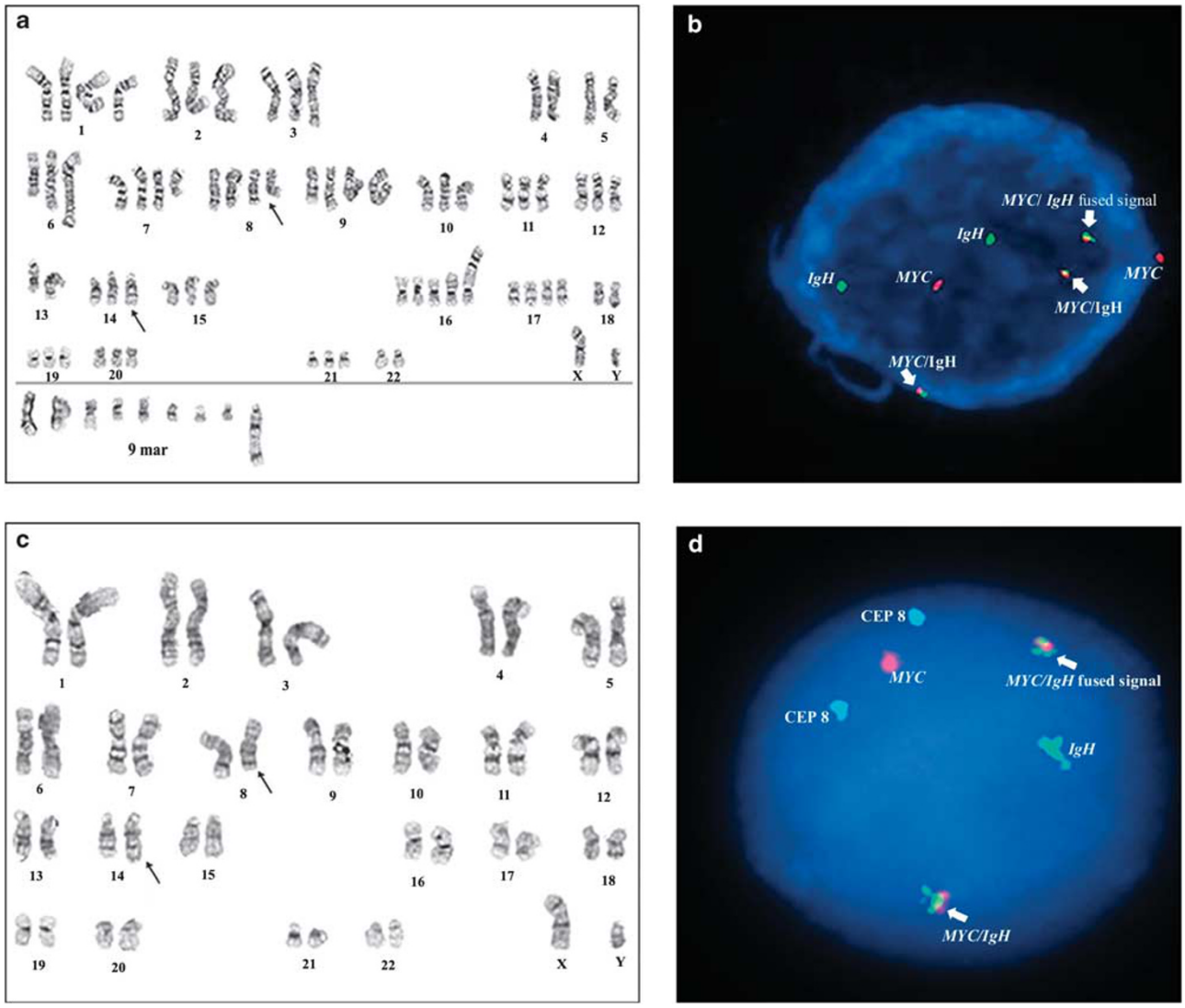

Figure 1 Representative karyotypic and fluorescence *in-situ* hybridization (FISH) findings in cases 34 (panels **a** + **b**) and 2 (panels **c** + **d**). (**a**) shows a karyotype with a t(8;14) and having 32 additional cytogenetic abnormalities. (**b**) shows the FISH findings for one of three abnormal clones found (three fused signals for the *MYC/IgH*, two red signals for the normal *MYC* alleles and two green signals for the normal *IgH* alleles). (**c**) shows a karyotype with an t(8;14) and having no additional cytogenetic abnormalities, while (**d**) shows a classical *MYC/IgH* rearragement FISH pattern: two fused signals for the *MYC/IgH* rearrangement, 1 red signal for the normal *IgH* allele, 1green signal for the normal *MYC* allele and two aqua signals for the two centromeric probes for chromosome 8.

IGH–MYC/t(8;14) with 32 additional abnormalities (case 34) and with no additional abnormalities (case 2) are illustrated in Figure 1.

Immunophenotypic Characteristics by Flow Cytometry

All but the three cases of plasmablastic lymphoma expressed CD10, CD19, and CD20, and all were negative for CD3 and CD5. CD38 was positive in all cases by flow cytometry with a mean MFI of 2009 (median 1372, range 271–7931), significantly higher than diffuse large B-cell lymphoma without *MYC* rearrangement (mean MFI of 880 in our previous study[20]). Twenty-six cases (76%) had a MFI higher than 1141, which is considered an indicator for *MYC* rearrangement with a moderately high sensitivity (75%) and specificity (81%).[20] A representative flow cytometry plot from case 13 is presented in Figure 2.

Classification of *IG–MYC* Rearranged Lymphomas

Of 34 cases with *IG–MYC* rearrangement, 22 cases (65%) were classified as Burkitt lymphoma, six (18%) as unclassifiable B-cell lymphoma, three (9%) as diffuse large B-cell lymphoma, and three as plasmablastic lymphoma (9%) (Table 1).

Of the 22 Burkitt lymphoma cases, 17 (77%) had a typical morphology of monotonous medium-sized lymphoma cells (Figure 3a), the typical Burkitt lymphoma immunophenotype [CD10(+)/bcl-6(+)/

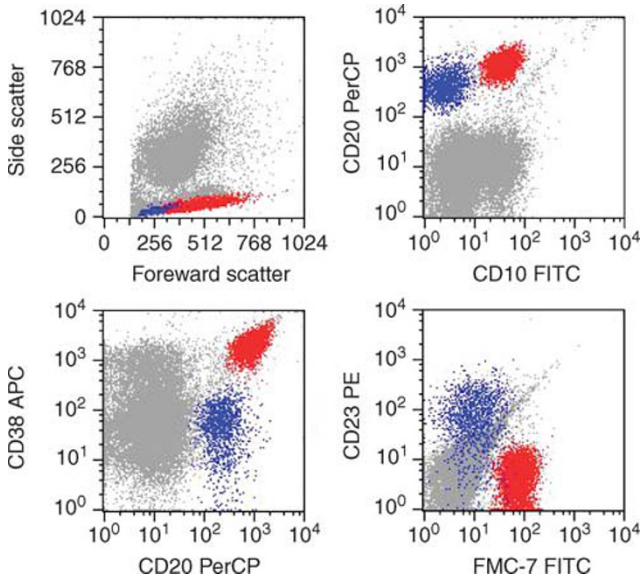

Figure 2 Four-color flow cytometric analysis on case 13 shows that neoplastic B cells (in red) are medium to large in size and express CD10, CD20, bright CD38 (MFI of 2198), FMC-7, and largely lack CD23. Normal B cells are painted in blue.

bcl-2(−)], and high Ki-67 (>95%). Five cases (23%) had one atypical feature, slight nuclear pleomorphism in case 4, a moderately high Ki-67 (75%) in case 9, weak bcl-2 expression in case 15, and lack of bcl-6 expression in cases 22 and 29.

Of six cases classified as unclassifiable B-cell lymphoma, three had dual translocations. Cases 21 and 23 had slight nuclear pleomorphism, had both t(8;22) and t(14;18), and lacked bcl-6. Case 21 also expressed bcl-2. Case 30 had classical Burkitt lymphoma morphology, but had both t(8;14) and t(3;14) and lacked bcl-6. Cases 10, 24, and 25 had great nuclear pleomorphism intermediate between Burkitt lymphoma and diffuse large B-cell lymphoma, beyond what is acceptable for Burkitt lymphoma with atypical morphology (Figure 3b). Cases 10 and 25 had a typical Burkitt lymphoma immunophenotype and case 24 expressed bcl-2 and had a Ki-67 of 90%.

All three cases classified as diffuse large B-cell lymphoma had proliferation of large lymphoid cells with greater nuclear pleomorphism and prominent nucleoli (Figure 3c) with Ki-67 of 75 to >95%. Case 33 had dual translocations [t(8;14) and t(14;18)] and expressed bcl-2. Case 34 lacked bcl-2 and bcl-6.

All three cases classified as plasmablastic lymphoma had proliferations of large immunoblasts or large lymphoid cells with plasmablastic features, such as central to eccentrically located prominent nucleoli (Figure 3d), and had an immunophenotype of terminally differentiated B cells, largely lacking B-cell lineage markers (CD19, CD20). All cases lacked bcl-6. CD10 and bcl-2 were expressed in only one case. Ki-67 staining was 70–75%. EBER was positive in all cases.

Expressions of TCL-1, CD44, and MUM-1 in *IG-MYC* Rearranged Lymphomas

Previous studies have shown that an immunophenotype of TCL-1(+)/CD44(−) could predict for *MYC* rearrangement.[5] Expression of TCL-1 and CD44 was assessed in 21 and 18 cases with available material, respectively. Of these, 17 of 21 (81%) expressed TCL-1 (Figure 3e), whereas 15 of 18 (83%) lacked CD44 (Figure 3f). Of the 18 cases in which both markers were assessed, 15 (83%) had an immunophenotype of TCL-1(+)/CD44(−). MUM-1 was expressed in 36% of Burkitt lymphoma (8 of 22) and 50% of non-Burkitt lymphoma cases (6 of 12) ($P=0.487$).

Segregation of *IG-MYC* Rearranged Lymphomas by Cytogenetic Complexity

We found that the mean number of additional cytogenetic abnormalities was significantly different among four different diagnostic groups (Table 3), ranging from 1.7 for Burkitt lymphoma, to 3.3 for unclassifiable B-cell lymphoma, 6.8 for plasmablastic lymphoma and 21.7 for diffuse large B-cell lymphoma ($P<0.001$). In addition, the mean number of additional abnormalities in Burkitt lymphoma was significantly lower than in unclassifiable ($P<0.05$) or all non-Burkitt cases (mean 8.8, $P=0.0164$).

The 34 cases were separated into two groups, those with simple karyotypes (group I, additional cytogenetic abnormalities $\leqslant 2$, $n=19$) and those with complex karyotypes (group II, additional abnormalities >2, $n=15$), and their clinicopathological features were compared (Table 4). Patients in group I were younger (median age = 29) than those in group II (median age = 50), which likely reflects the fact that most pediatric Burkitt lymphoma cases (6 of 8, 75%) had simple karyotypes. There was no difference in gender or immune status between the two groups. Group I cases were more likely to be classified as Burkitt lymphoma (17 of 19, 89%) compared with those in group II (5 of 15, 33%, $P=0.011$). Similarly, bcl-6 was more often expressed in group I (18 of 19, 96%) than in group II (7 of 15, 47%, $P=0.0042$), indicating a significant inverse correlation between bcl-6 and karyotypic complexity. In fact, group I cases were more likely to have an immunophenotype of $CD10^+/bcl\text{-}6^+/bcl\text{-}2^-$ (17 of 19, 89%) than those in group II (3 of 14, 21%, $P=0.0002$). In addition, there was a trend towards a low stage disease (stage I+II) in group I (7 of 17, 41%) compared with group II (3 of 15, 20%; $P=0.265$). Other parameters, including Ki-67, EBV status, CD38 MFI, MUM-1, and CD44, showed no significant difference between the groups.

Characteristics Associated with Overall Survival

The median follow-up duration in this cohort was 9 months (range 0.1–100). At the last follow-up, 25 patients were alive. In univariate analyses,

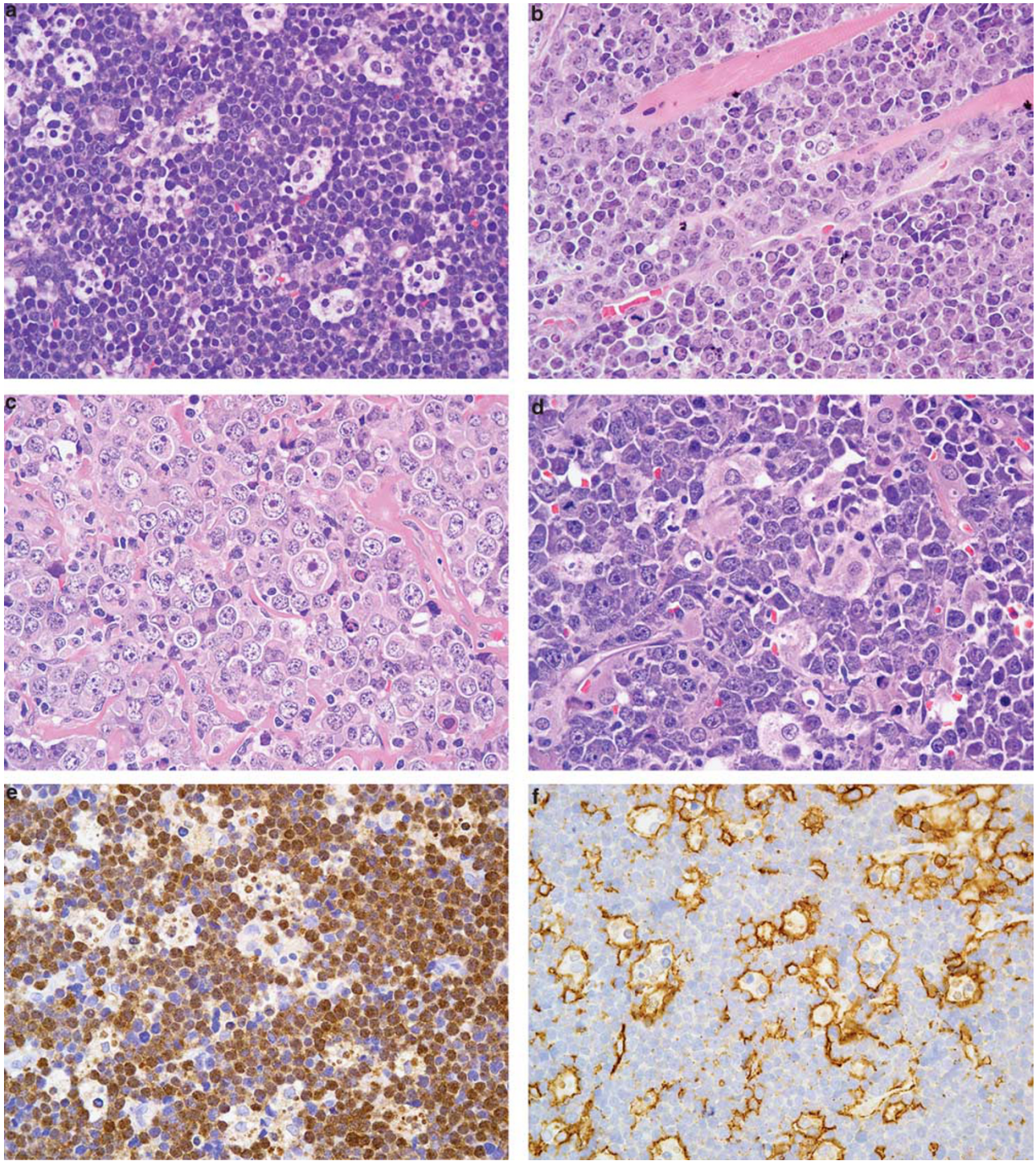

Figure 3 Representative lymphoma of each type (H&E stains for **a–d**, × 500; immunohistochemistry for E and F, × 500). (**a**) Burkitt lymphoma morphology (case 14): diffuse infiltrate of medium-sized monomorphous cells with round nuclei, multiple small nucleoli and a moderate amount of cytoplasm. (**b**) Morphology of lymphoma unclassifiable with features intermediate between Burkitt lymphoma and diffuse large B-cell lymphoma (case 25): resembling Burkitt lymphoma except there is greater pleomorphism in nuclear size and shape and more prominent nucleoli. (**c**) Diffuse large B cell lymphoma morphology (case 34): diffuse infiltrate of large cells with significant pleomorphism and prominent nucleoli. (**d**) Plasmablastic lymphoma morphology (case 27): diffuse infiltrate of large cells with immunoblastic features including single prominent nucleolus. (**e** and **f**): expression of TCL-1 and lack of CD44 in Burkitt lymphoma, respectively (case 14).

overall survival was better for patients with Burkitt lymphoma (median undefined) *versus* those with diffuse large B cell lymphoma or unclassifiable B-cell lymphomas (median 7 months) or plasmablastic lymphoma (median 3 months) ($P=0.0209$; Figure 4a), for patients whose lymphoma expressed bcl-6 (median undefined) *versus* those who were bcl-6(-) (median 7 months) ($P=0.0019$; Figure 4b),

Table 3 The number of additional cytogenetic abnormalities among the 4 *MYC-IG* rearranged diagnostic lymphoma categories

	Mean ± s.e.	*Median, range*	P-*value*
BL ($n=22$)	1.7 ± 0.36	1.5, 0–6	
Non-BL ($n=12$)	8.8 ± 2.9	4.5, 0–32	<0.05, BL *versus* non-BL
INT ($n=6$)	3.3 ± 0.67	3, 1–6	<0.05, BL *versus* INT
DLBCL ($n=3$)	21.7 ± 6.7	24, 9–32	<0.05, INT *versus* DLBCL
PBL ($n=3$)	6.67 ± 4.4	5, 0–15	<0.05, INT *versus* PBL

Abbreviations: BL, Burkitt lymphoma (BL); DLBCL, diffuse large B-cell lymphoma; INT, B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL; PBL, plasmablastic lymphoma; s.e., standard error.

Table 4 Clinicopathological features of *MYC-IG* rearranged lymphomas according to the number of additional cytogenetic abnormalities: ⩽2 (group I) and >2 (group II).

	Group I (n = *19*)	*Group II* (n = *15*)	P-*value*
Additional cytogenetic abnormalities (range, mean)	0–2, 0.89	3–32, 8.3	**0.0053**
Age range, median (year)	2–62, 29	2–66, 50	**0.0085**
Gender (male/female)	18/1	10/5	0.663
Stage (I+II)/(III+IV)	7/10	3/12	0.265
Immunosuppression	11/19 (58%)	6/15 (40%)	0.491
Classified as Burkitt lymphoma	17/19 (89%)	5/15 (33%)	**0.0011**
CD10+ (FC+IHC)	18/19 (95%)	14/15 (93%)	1.0
CD10+/Bcl-6+/Bcl-2-	17/19 (89%)	3/14 (21%)	**0.0002**
CD38 MFI (FC), mean, range	2046 (619–7931)	1963 (271–4457)	0.89
Bcl-2+	1/19 (5.3%)	5/15 (33%)	0.066
Bcl-6+	18/19 (95%)	7/15 (47%)	**0.0042**
Ki-67>95	16/18 (89%)	7/10 (70%)	0.32
MUM-1+	8/19 (89%)	6/13 (46%)	1.0
TCL-1+	13/15 (87%)	5/7 (71%)	0.56
CD44+	3/14 (21%)	0/4 (0%)	0.62
EBV EBER+	9/16 (56%)	3/8 (38%)	0.87

All stains were performed by immunohistochemistry (IHC) unless specified. Mean fluorescence intensity (MFI) of CD38 was assessed by flow cytometry (FC), EBV viral infection by *in situ* hybridization (ISH) for EBV encoded small RNA (EBER), CD10 by both IHC and FC.

and for patients in group I (median undefined) *versus* those in group II (median 7 months) ($P=0.0104$; Figure 4c). However, in multivariate analysis, only bcl-6 expression remained as an independent significant predictor of better survival ($P=0.0042$). Although cytogenetic complexity was not an independent prognostic indicator for the entire cohort, it did predict survival within the bcl-6(+) subgroup ($n=25$). Of these patients, those in group I with simple karyotype had a better overall survival than those in group II with complex karyotype ($P=0.043$) (Figure 4d).

Clinical stage and age were not a significant prognostic indicator, likely due to the small number of cases in this study. Other immunohistochemical markers had no significant impact on survival.

Discussion

To the best of our knowledge, this is the first study to explore the impact of conventional cytogenetic abnormalities in correlation with comprehensive immunophenotypic analyses on the classification and prognosis of *MYC–IG* rearranged high-grade B-cell lymphomas using the criteria outlined in the 2008 WHO classification. Our results indicated that simple karyotype (additional cytogenetic abnormalities ⩽2) predicts a diagnosis of Burkitt lymphoma, bcl-6 expression, and better overall survival. Furthermore, these *MYC–IG* rearranged high-grade B-cell lymphomas frequently expressed bright CD38 by flow cytometry. As this result could be available within hours of a primary diagnostic procedure, it can be used to initiate conventional and/or molecular cytogenetic testing for *MYC*, as well as *BCL-2* and *BCL-6* rearrangements, especially in older patients.

High-grade B-cell lymphomas with *MYC–IG* rearrangement encompass heterogeneous diagnostic categories including Burkitt lymphoma, diffuse large B-cell lymphoma, unclassifiable B-cell lymphoma, and plasmablastic lymphoma. Distinguishing Burkitt lymphoma from these morphological mimics remains a challenge, which is at least partially related to the subjective evaluation of cytomorphological features[3,4,21] and the evolving definition of these lymphomas in light of recent gene-expression profiling studies.[13,14] The latter is reflected in the 2008 WHO classification system that includes the newly established unclassifiable B-cell lymphoma and an evolving definition of Burkitt lymphoma. The previous atypical/Burkitt-like and plasmacytoid morphological variants are no longer designated. Cases with features slightly deviating from the typical medium-sized monomorphous

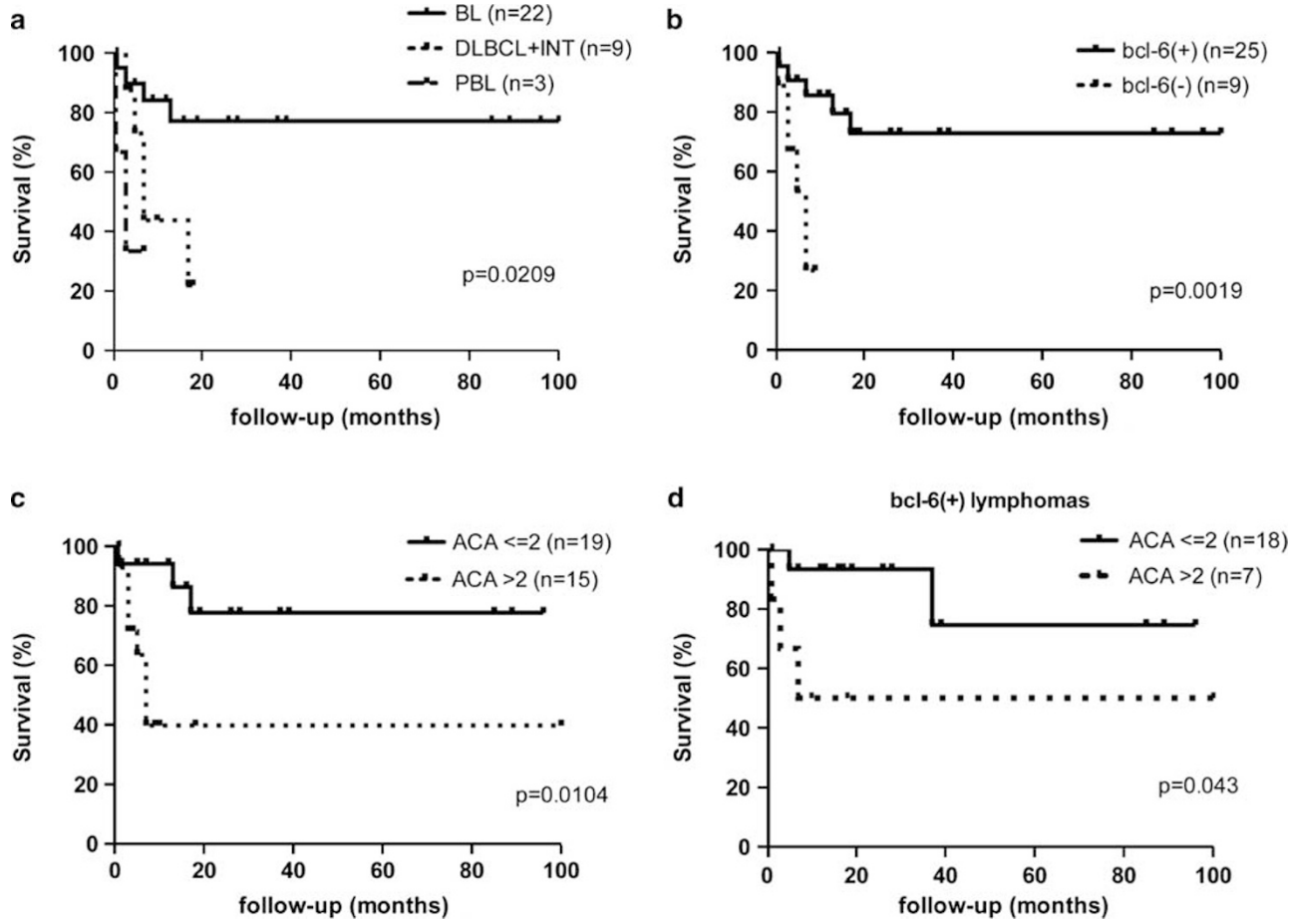

Figure 4 Lymphoma-specific survival was estimated by Kaplan–Meier method. (**a**) Analysis by lymphoma type, Burkitt lymphoma (BL), diffuse large B-cell lymphoma (DLBCL), lymphoma unclassifiable with features intermediate between Burkitt lymphoma and diffuse large B-cell lymphoma (INT), and plasmablastic lymphoma (PBL). (**b**) Analysis by bcl-6 positivity in all lymphomas. (**c**) Analysis by the number of additional cytogenetic abnormalities (ACA) in all lymphomas. (**d**) Analysis by additional cytogenetic abnormalities (ACA) only in bcl-6(+) lymphomas.

cytomorphology are classified simply as Burkitt lymphoma if they otherwise fit immunophenotypically and genetically because these 'atypical' Burkitt lymphomas have a molecular signature similar to classic BL.[14] In addition, rare cases that are otherwise characteristic of Burkitt lymphoma but lack *MYC–IG* rearrangement are acceptable for Burkitt lymphoma as these cases bear a Burkitt-like molecular signature,[13,14] and may upregulate *MYC* expression by alternative mechanisms possibly involving the modulation of microRNA.[22]

Although the new diagnostic criteria are clearly outlined, the practical application is not always straightforward. For example, cases with features slightly deviating from the typical medium-sized monomorphous cytomorphology may be acceptable for Burkitt lymphoma,[14] whereas cases with greater cytomorphological variations would be classified as unclassifiable or diffuse large B-cell lymphoma. Unfortunately, the borderline between slight and great cytomorphological variations remains imprecise and subjective.[3,4,21] Therefore, the use of additional diagnostic modalities may be helpful.

Indeed, karyotypic abnormalities in addition to *MYC* rearrangement are integrated in the 2008 classification system for the first time. Cases of Burkitt lymphoma often have a *MYC*-simple karyotype, whereas cases of unclassifiable B-cell lymphoma often have a *MYC*-complex karyotype.[8,14] Our studies support this statement. We found that Burkitt lymphoma cases had low number of additional cytogenetic abnormalities (mean of 1.7), compared with unclassifiable (3.3) and diffuse large B-cell lymphomas (21.7), which is also in agreement with the recent observations that approximately 80% of Burkitt lymphoma cases have a simple karyotype with a low cytogenetic imbalance complexity score of 0–2.[23] Furthermore, using the cut-off of two additional cytogenetic abnormalities, we found that there were a number of significant differences between the patients with simple (group I) *versus* complex (group II) karyotypes. Notably, group I cases were more likely to have a diagnosis of Burkitt lymphoma and express bcl-6. These results indicate that the karyotypic complexity might supplement morphological evaluation to separate

Burkitt lymphoma from other high grade B-cell lymphomas and that the appropriate threshold to define simple karyotype may be two additional abnormalities. Our study reiterates the importance of submission of material for karyotypic analysis given its diagnostic importance and the advantage of having a 'bird's eye' overview of the many relevant genetic abnormalities.

Our results also support that unclassifiable B-cell lymphoma cases are rich in karyotypic complexity in addition to dual translocations involving *BCL-2* or *BCL-6*.[4,7,24] Three of six unclassifiable cases in this study harbored such dual translocations. The fourth such case was a diffuse large B-cell lymphoma. All of these so-called 'double-hit' lymphoma cases were older adults (>40 years old) and accounted for 44% of those in patients over 40. Given the unfavorable clinical outcome of these lymphomas,[6,24] conventional and/or molecular cytogenetic testing for *BCL-2* and *BCL-6* rearrangements in *MYC*-rearranged lymphomas may be diagnostically and clinically helpful, especially in those patients older than 40.

IG-MYC rearrangement has been reported in up to 10% of diffuse large B-cell lymphomas.[25–27] In this study, all *IG-MYC* rearranged diffuse large B-cell lymphomas had complex karyotypes, as has been previously reported.[14] Three plasmablastic lymphomas in this cohort carried *IG–MYC* rearrangement with a variable karyotypic complexity; such rearrangement was found in about one-third of such lymphoma in our recent study.[28]

Recognition of *MYC* rearrangement is important because it portends a more aggressive disease and may prompt more timely and intensive therapy.[2,12] However, *MYC*-rearranged lymphomas may be underrecognized because karyotype and/or FISH analysis are not performed in all cases. Therefore, recognition of the features suggestive of *MYC* rearrangement may be important to prompt additional testing. Previous studies have suggested that high proliferation fraction (Ki-67>90%), typical Burkitt lymphoma immunophenotype ($CD10^+$/bcl-6^+/bcl-2^-), and a $CD38^+$/$CD44^-$/TCL-1^+ pattern may predict the presence of *MYC* rearrangement.[2–5] In this study, 87% of cases had a high Ki-67 (>95%), and 83% of cases expressed TCL-1 and lacked CD44, which is in general consistent with these published results. However, the immunophenotype of $CD10^+$/bcl-6^+/bcl-2^- is mainly present in group I cases (89%) and at very low rate in group II cases (21%), implying that such immunophenotype may not be a reliable predictor in *MYC*-complex lymphomas. Consistent with our previous study, the majority of cases in this cohort expressed bright CD38 with an MFI higher than 1141, the threshold that is considered an indicator for *MYC* rearrangement with a moderately high sensitivity and specificity.[20] Importantly, such bright CD38 expression is consistently present in group II *MYC*-complex lymphomas (87%), indicating that bright CD38 expression may be a better predictor of *MYC* rearrangement in this group.

While this is a retrospective study and patients were treated variably, survival analysis did indicate that Burkitt lymphoma, bcl-6 expression, and simple karyotype appear to be predictors of better clinical outcomes. These results are in keeping with the previously reported poor overall survival of patients with *MYC*-rearranged diffuse large B-cell lymphoma,[14,25] and unclassifiable cases. The latter likely represent atypical Burkitt lymphoma classified by 2001 WHO classification system, and 'double-hit' lymphomas.[4,6,24] It is interesting to note that all three cases of *MYC*-rearranged diffuse large B-cell lymphoma in this study were treated with R-CHOP (rituximab, cyclophosphamide, hydroxydaunomycin, oncovin, prednisone), which may not be as effective in these patients compared with those without *MYC*-rearrangement.[13,25] In multivariate analysis, only bcl-6 expression remained an independent predictor of better overall survival. Nonetheless, within the subgroup of bcl-6 positive patients, survival could be further stratified by karyotypic complexity, as patients with positive bcl-6 and simple karyotype had the best clinical outcomes. Although unclassifiable and *MYC*-rearranged diffuse large B-cell lymphomas may benefit from intensified chemotherapeutic regimens used for BL,[6,25,29] future prospective studies in a large number of patients are needed to investigate the optimal therapy for these aggressive lymphomas.

In this study, we focused on the impact of karyotypic abnormalities on the classification of these challenging *MYC*-rearranged lymphomas. This study design is different from recent studies that targeted high-proliferative gray-zone lymphomas with variable features similar to those of Burkitt lymphoma, in which there was no karyotypic analysis and many cases lacked *MYC* rearrangement.[2–4] Although the 2008 criteria include up to 10% of molecularly defined Burkitt lymphoma without demonstrable *MYC* rearrangement, it is unknown how this would affect our daily practice. It is probably prudent to assess for a *MYC* rearrangement in all cases having a high index of suspicion for such abnormalities.

Notably, we analyzed pediatric and adult cases together for classification and karyotypic complexity. Although pediatric and adult Burkitt lymphoma cases have some differences in morphology and immunophenotype,[3,4] from a genomic complexity and gene expression point of view, Burkitt lymphoma in children and adults is the same disease.[13,14,23,30,31] It should also be noted that our study focused on *MYC-IG* rearrangement. Future studies should include non-*IG* partner genes to comprehensively assess the spectrum of *MYC*-rearranged lymphomas.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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