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Experience with provisional WHO-entities large B-cell lymphoma with *IRF4*-rearrangement and Burkitt-like lymphoma with 11q aberration in paediatric patients of the NHL-BFM group

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Summary

Large B-cell lymphoma with *IRF4* rearrangement, and Burkitt-like lymphoma with 11q aberration are two provisional lymphoma entities in the 2017 revision of the WHO classification of lymphoid neoplasms. Despite being more frequent in young patients, knowledge regarding their true incidence and clinical features in unselected cohorts of paediatric and adolescent patients is limited. We screened for both entities among paediatric patients (<18 years of age) in the German NHL-BFM (Non-Hodgkin lymphoma Berlin-Frankfurt-Münster) group. Among follicular lymphomas and diffuse large B-cell lymphomas (DLBCL), 7/34 cases (21%) showed an *IRF4* break-apart pattern by fluorescence *in situ* hybridisation (FISH) and are associated with stages I and II disease ($P = 0{\cdot}043$). Among lymphomas morphologically resembling Burkitt lymphoma, DLBCL and high-grade B-cell lymphoma, unclassifiable, 13/102 cases (13%) lacked a *MYC* break-apart pattern but were positive for 11q proximal gain and telomeric loss by FISH. *MYC*-negative Burkitt-like lymphomas with the typical 11q gain-loss pattern by FISH were older ($P = 0{\cdot}004$), showed less male predominance ($P = 0{\cdot}003$), lower stage ($P = 0{\cdot}040$), lower serum LDH level ($P = 0{\cdot}01$) and less abdominal involvement ($P = 0{\cdot}008$) compared to high grade B-cell lymphomas without 11q gain-loss pattern. Both entities showed excellent outcome with overall survival of 100% when managed according to NHL-BFM strategies and may provide candidates for future therapy de-escalation in clinical trials.

Keywords: non-Hodgkin lymphoma, paediatric haematology, paediatric oncology, chromosome 11q, B cells.

Large B-cell lymphoma with *IRF4* rearrangement (LBCL-*IRF4*) and *MYC* translocation-negative Burkitt-like lymphoma with 11q aberration (mnBLL,11q) are two provisional lymphoma entities in the 2017 revision of the World Health Organisation (WHO) Classification of Lymphoid Neoplasms.[1] First described by our group in 2011[2] and 2014[3], both entities are mature B non-Hodgkin lymphomas (B-NHL) and are mostly found in children and adolescents; they are hence highly relevant to paediatric haematologists and oncologists. After their initial description, only a few patient series of these provisional entities have been published.[4,5] The prevalence of LBCL-*IRF4* and mnBLL,11q among paediatric B-NHL has also not been studied systematically. Moreover, the clinical features of these two lymphomas are only known from small case series and isolated case reports. In this study we report an extensive series of LBCL-*IRF4* and mnBLL,11q, allowing us to estimate the frequency and the clinical relevance of these subtypes in

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patients treated in Germany according to NHL-BFM (Non-Hodgkin lymphoma Berlin-Frankfurt-Münster) protocols.

Material and methods

Patient cohort

To identify potential cases of LBCL-*IRF4*, the pathology records and formalin-fixed paraffin-embedded (FFPE) tumour tissue of all patients <18 years of age with follicular lymphoma (FL) grade 3A and 3B, FL with areas of diffuse large B-cell lymphoma (transformed FL) and *de novo* diffuse large B-cell lymphoma (DLBCL) from 2012 to 2017 were retrieved from the Kiel Lymph Node Registry. These cases are designated as 'morphological FL/DLBCL' in this manuscript.

To identify potential cases of mnBLL,11q, the pathology records and paraffin blocks of all patients <18 years of age with Burkitt lymphoma (BL), *de novo* DLBCL or high-grade B-cell lymphoma (HGBCL), unclassifiable, with features intermediate between DLBCL and BL according to the 2008 revision of the WHO classification, diagnosed from 2014 to 2017, were retrieved from the Kiel Lymph Node Registry. These cases are designated as 'morphological BL/DLBCL/HGBCL' in this report.

The patient demographics and clinical data of both cohorts were compared to a control cohort in the NHL-BFM database with similar diagnoses to evaluate them for potential selection bias.

Two patients in the BL/DLBCL/HGBCL cohort overlapped with our recent publication on the mutational landscape of mnBLL,11q,[6] and one patient in the FL/DLBCL cohort overlapped with our previous publication on paediatric DLBCL.[7] The two cohorts do not otherwise overlap with our previous publications on the initial description of LBCL-*IRF4* and mnBLL,11q.[2,3]

A flowchart describing the case selection process can be found in Figures S1 and S2.

Fluorescence in situ *hybridisation (FISH) studies*

For cases of morphological FL/DLBCL, the results of *IRF4* break-apart fluorescence *in situ* hybridisation (FISH) and, for a subset of *IRF4* break-apart pattern positive cases, *IRF4*-IGH fusion FISH at the time of diagnosis were obtained from the files of the Kiel Lymph Node Registry. For cases without information on *IRF4* rearrangement at diagnosis, FISH – using commercially available *IRF4* break-apart probes (ZYTOVISION GmbH, Bremerhaven, Germany) – was retrospectively performed on freshly cut paraffin sections of the FFPE tumour blocks or tissue microarrays (TMAs), composed of two cores of 0·6 mm diameter from each specimen. The hybridisation results were visualised on Zeiss Axioscope fluorescence microscope (Zeiss, Jena, Germany).

For cases with morphological BL/DLBCL/HGBCL, the results of *MYC* break-apart FISH, *MYC*-IGH fusion FISH and 11q status by gain-loss FISH or OncoScan Assay (Thermo Fisher Scientific, Waltham, MA, USA) were obtained from the files of the Kiel Lymph Node Registry. For cases without information on *MYC* breakpoint or 11q status at diagnosis, they were retrospectively analysed using commercially available *MYC* break-apart probes (Abbott, Wiesbaden, Germany) on freshly cut paraffin sections or TMAs as described above. 11q gain/loss triple-colour FISH probes (ZYTOVISION GmbH) were then applied to cases that were negative for *MYC* break-apart FISH and *MYC*-IGH fusion. The hybridisation results were visualised on Zeiss Axioscope fluorescence microscope (Zeiss, Jena, Germany). Cases showing proximal gain in the 11q23 region and telomeric loss in 11q24·1-ter in at least 20% of lymphoma cells, or those with only telomeric loss, were interpreted as 'positive for 11q typical gain-loss pattern', as scored according to previous studies.[8]

Immunohistochemical studies

The MUM1 expression status of the *IRF4* break-apart positive FL/DLBCL cases was retrieved from the pathology records of the Kiel Lymph Node Registry. For cases without information on MUM1 at the time of diagnosis, MUM1 immunohistochemistry (IHC) was performed retrospectively (clone MUM1P, Dako, dilution 1:100) on the FFPE tumour blocks by automated IHC stainer (Leica BOND, Leica Biosystems, Germany), according to the manufacturer's instructions.

Clinical data and statistical analysis

The clinical data of each patient was retrieved from the NHL-BFM Study Centre in Münster, including gender, age, Murphy/St. Jude staging, serum lactate dehydrogenase (LDH) level at diagnosis, presence/absence of B-symptoms, central nervous system (CNS) or bone marrow involvement, NHL-BFM risk group status, event-free survival and cumulative incidence of disease relapse. Statistical analysis was performed using IBM SPSS (IBM Corporation, Armonk, NY, USA) and SAS-PC 9·4 software (SAS Institute, Cary, NC, USA). Kaplan-Meier survival analysis was performed on the event-free survival data and compared using the log rank test. For each statistical test, a *P*-value of less than 0·05 was reported as statistically significant.

Ethical approval

This research was approved by the Institutional Review Board/Ethics Committee of the medical faculty of the University of Kiel (review number D447/10) and conducted in accordance with the Declaration of Helsinki. All patients were registered in the clinical trials and/or registry of the BFM-NHL group and informed consent was obtained by the parents.

Results

Prevalence of IRF4 *break-apart in FL/DLBCL*

From 2012 to 2017, 34 patients with morphological FL/DLBCL were identified (four FL grade 3A/3B, three transformed FL and 27 DLBCL). Of these, 2/4 cases of FL grade 3A/3B (50%), 2/3 cases of transformed FL (67%) and 3/27

Table I. Prevalence of *IRF4* break-apart in patients with morphological FL/DLBCL.

Histology	*IRF4* break-apart positive
FL grade 3A/3B	2/4 (50%)
FL grade 3A/3B with DLBCL	2/3 (67%)
DLBCL	3/27 (11%)
Total	7/34 (21%)

FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma.

cases of *de novo* DLBCL (11%) were positive for *IRF4* break-apart FISH, either at the time of diagnosis or by retrospective FISH analysis, yielding a total of 7/34 (21%) patients with positive *IRF4* chromosomal breakpoint in the entire FL/DLBCL cohort Table I. Four of these cases were also positive for *IRF4*-IGH fusion. The clinical parameters of these patients and the characteristics of their lymphomas are described in Tables II and III, respectively. Figure 1 shows the morphology and the FISH finding of a representative case.

Prevalence of 11q aberration in morphological BL/DLBCL/HGCBL

From 2014 to 2017, 102 patients with morphological BL/DLBCL/HGBCL were identified (71 BL, 11 HGBCL unclassifiable and 20 *de novo* DLBCL). Seventeen cases of *de novo* DLBCL overlapped with the FL/DLBCL cohort. Thirty-one out of 102 BL/DLBCL/HGBCL cases (30%) were negative for *MYC* chromosomal breakpoint, either at the time of diagnosis or by retrospective FISH analysis (26 cases negative for both *MYC* break-apart pattern and *MYC*-IGH fusion, five cases negative for *MYC* break-apart pattern only). In these patients, 6/6 cases of morphological BL, 5/6 cases of HGBCL, unclassifiable, and 2/19 DLBCL cases were positive for the typical 11q gain-loss pattern on FISH analysis, yielding a total of 13/102 patients (13%) with typical 11q gain-loss pattern in the entire BL/DLBCL/HGBCL cohort Table IV. When the DLBCL cases were excluded, 11/82 cases (13%) of BL/HGBCL were positive for the typical 11q gain-loss pattern. A further 11/12 cases (92%) of BL/HGBCL cases lacking a *MYC* breakpoint were positive for 11q typical gain-loss pattern (nine cases negative for both *MYC* break-apart pattern and *MYC*-IGH fusion, two cases negative for *MYC* break-apart pattern only).

The clinical parameters of these patients and the characteristics of their lymphomas are described in Tables V and VI, respectively. Figure 2 shows the morphology of a representative case and FISH results.

Table II. Clinical features of the seven patients with *IRF4* break-apart positive large B-cell lymphoma.

Case no.	Gender	Age	Tumour localisation	Stage (St. Jude/Murphy)	NHL-BFM risk group
IRF4-1	F	13·1	Small intestine	II	B-NHL R2
IRF4-2	F	14·3	Tonsil	II	B-NHL R2
IRF4-3	M	12·0	Tonsil	I	B-NHL: Watch and wait
IRF4-4	M	7·0	Cervical lymph nodes and tonsil	II	B-NHL R2
IRF4-5	M	11·3	Nasopharynx	II	B-NHL R2
IRF4-6	F	5·7	Cervical and pre-tracheal lymph nodes	II	B-NHL R2
IRF4-7	M	17·6	Tonsil	I	B-NHL R1

M, male; F, female.

Table III. Morphology, immunophenotype and molecular findings of the seven patients with *IRF4* break-apart positive large B-cell lymphoma.

Case no.	Tumour morphology	CD20	CD10	BCL6	MUM1	Hans Classifier	BCL2	c-MYC	Ki-67	*IRF4* break-apart FISH	*IRF4*-IGH fusion FISH
IRF4-1	FL grade 3A with DLBCL	++	++	na	++	GCB type*	++	na	90%	Positive	Positive
IRF4-2	FL grade 3B	++	++	++	++	Not applicable	++	na	80%	Positive	Positive
IRF4-3	FL grade 3A	++	na	++	++	Not applicable	++	na	60%	Positive	Positive
IRF4-4	FL grade 3B with DLBCL	++	++	na	++	GCB type	++	+	80%	Positive	Positive
IRF4-5	DLBCL	++	–	++	++	Non-GCB type	++	na	>80%	Positive	na
IRF4-6	DLBCL	++	–	+	++	Non-GCB type	++	na	80%	Positive	na
IRF4-7	DLBCL	++	–	++	++	Non-GCB type	++	na	na	Positive	na

FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma; ++, strongly positive; +, weakly positive; –, negative; na, not available; GCB, germinal centre B-cell.

*Case is also GCB type by Nanostring.[7]

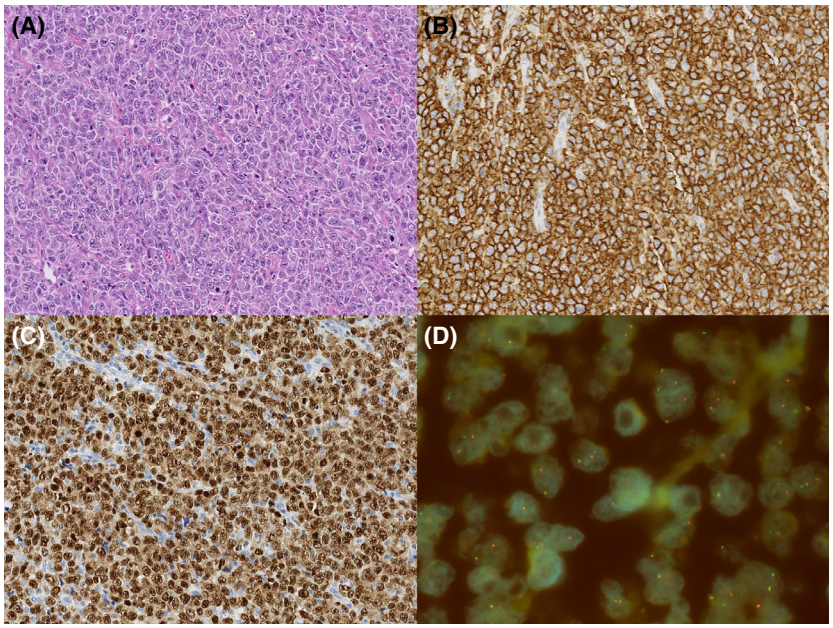

Fig 1. Morphology of *IRF4* break-apart positive large B-cell lymphoma. (A) The tumour showed large cell morphology on H&E staining. (B) The lymphoma cells were diffusely positive for CD20 immunohistochemistry. (C) The tumour cells also strongly expressed IRF4/MUM1. (D) FISH study using *IRF4* break-apart probes. The lymphoma cells displayed one fusion signal and one break-apart signal, confirming the presence of *IRF4* chromosomal breakpoint. [Colour figure can be viewed at wileyonlinelibrary.com]

Table IV. Prevalence of *11q* and *MYC* chromosomal changes in patients with morphological BL/DLBCL/HGBCL.

Tumour histology	*MYC* breakpoint-negative, 11q typical gain-loss pattern positive	*MYC* breakpoint negative, 11q typical gain-loss pattern negative
BL	6/71 (8%)	0/71 (0%)
HGBCL	5/11 (45%)	1/11 (10%)
DLBCL	2/20 (10%)	17/20 (85%)
Total	13/102 (13%)	18/102 (17%)

BL, Burkitt lymphoma; DLBCL, diffuse large B-cell lymphoma; HGBCL, high-grade B-cell lymphoma, unclassifiable, with features intermediate between BL and DLBCL, according to the 2008 WHO classification.

MUM1 expression status in IRF4 *breakpoint positive FL/DLBCL*

All seven cases of *IRF4* breakpoint-positive FL/DLBCL cases were positive for MUM1 expression by IHC, either at diagnosis or by retrospective analysis Table III.

Clinical features of patients with IRF4 *breakpoint-positive FL/DLBCL*

Clinical features were available for at least 24 patients with FL/DLBCL Table VII. Patients with *IRF4* breakpoint-positive lymphoma had a significantly lower tumour stage compared to *IRF4* breakpoint-negative tumours ($P = 0{\cdot}043$). There was no significant difference in gender, age, presence/absence of B symptoms and NHL-BFM risk groups of patients with *IRF4* breakpoint-positive lymphoma *versus IRF4* breakpoint-negative cases. There was no significant difference in tumour localisation between *IRF4* breakpoint-positive and *IRF4* breakpoint-negative lymphomas Table VIII.

Clinical features of patients with 11q typical gain-loss pattern positive BL/DLBCL/HGBCL

Clinical features were available for at least 85 patients with BL/DLBCL/HGBCL Table IX. The patients with *MYC* breakpoint-negative and 11q gain-loss positive tumours are older than those of the other two groups (median age 13·9 years vs. 9·1 years vs. 7·5 years, $P = 0{\cdot}004$). They also showed less male predominance ($P = 0{\cdot}003$), lower serum LDH level ($P = 0{\cdot}01$), lower tumour stage ($P = 0{\cdot}04$) and lower NHL risk group ($P = 0{\cdot}010$). Ten cases are negative for both *MYC* break-apart pattern and *MYC*-IGH fusion, and three cases are negative for *MYC* break-apart pattern only, Table VI. Otherwise, there was no significant difference in serum LDH level, B-symptoms and CNS involvement among these three groups of tumours.

On tumour localisation, *MYC* breakpoint-negative 11q gain-loss positive tumours were less likely to have abdominal involvement ($P = 0{\cdot}008$), Table X. There is no significant difference in nodal involvement when the *MYC* breakpoint-negative 11q gain-loss positive cases were compared with *MYC* breakpoint-positive cases (10/13 cases vs. 41/68 cases, $P = 0{\cdot}353$ by Fisher's exact test).

Table V. Clinical features of the 13 patients with *MYC* breakpoint-negative 11q gain-loss FISH positive Burkitt-like B-cell lymphoma.

Case No.	Gender	Age	Tumour localisation	Stage (St. Jude/ Murphy)	NHL-BFM risk group
11q-01	M	8·3	Cervical lymph nodes	na	B-NHL R2
11q-02	M	16·7	Cervical lymph nodes	II	B-NHL R2
11q-03	M	10·8	Bone and soft tissue (multiple sites)	IV	(Non-BFM therapy)
11q-04	M	14·8	Cervical lymph nodes and pharynx	II	B-NHL R2
11q-05	F	9·7	Cervical lymph nodes	II	B-NHL R2
11q-06	M	7·6	Ileum	II	B-NHL R1
11q-07	M	14·2	Cervical lymph nodes	I	B-NHL R2
11q-08	F	17·7	Tonsil	I	B-NHL R2
11q-09	F	10·2	Cervical lymph nodes	III	B-NHL R2
11q-10	M	13·9	Abdominal lymph nodes, caecum, ascites	II	B-NHL R2
11q-11	M	16·8	Abdominal lymph nodes	III	B-NHL R2
11q-12	M	13·4	Abdominal, retroperitoneal and pelvic lymph nodes, liver, pancreas	III	B-NHL R2
11q-13	F	15·0	Cervical, clavicular and abdominal lymph nodes, pleural effusion, ascites	III	B-NHL R3

M, male; F, female; na, not available.

Of note, when the analysis was limited to cases with BL morphology (71 patients), patients with *MYC* breakpoint-negative 11q gain-loss positive lymphomas were still significantly older than *MYC* breakpoint-positive cases ($P < 0{\cdot}001$). In view of a limited number of *MYC* breakpoint-negative 11q gain-loss positive tumours with BL morphology, analysis regarding the clinical presentation are of limited value. However, there was no significant difference in the frequency of abdominal involvement between these two groups. (Table SI).

Clinical outcome in FL/DLBCL according to IRF4 *rearrangement*

The two-year event-free survival (EFS) of patients with *IRF4* breakpoint-positive FL/DLBCL was 100%, compared to 88·5% in *IRF4* breakpoint-negative tumours (Fig 3). No significant difference was observed in the cumulative incidence of relapse between these two groups of patients (data not shown).

Clinical outcome of patients with BL/DLBCL/HGBCL according to 11q aberration

The two-year EFS of patients with *MYC* breakpoint-negative 11q gain-loss positive BL/DLBCL/HGBCL was 100%, compared to 93·8% in *MYC* breakpoint-negative 11q gain-loss negative tumours, and 92·5% in *MYC* breakpoint-positive tumours Fig 4. No significant difference was observed in the cumulative incidence of relapse among these three groups of patients (data not shown).

When the analysis was limited to cases with pure BL morphology, the two-year EFS of patients with *MYC* breakpoint-negative 11q gain-loss positive lymphomas was 100%, compared to 95·2% in *MYC* breakpoint-positive BL (Fig 5).

Analysis on potential cohort bias

The FL/DLBCL cohort showed similar distribution of gender, age, stage, serum LDH level, B-symptoms and B-NHL risk group compared to a control cohort of patients with similar diagnoses in the NHL-BFM database. The BL/DLBCL/HGBCL cohort has fewer patients with Burkitt leukaemia compared to the control cohort (11/134 patients vs. 27/82 patients), otherwise the cohort showed no significant difference in gender, age, serum LDH level, B-symptoms and B-NHL risk group compared to the control.

Discussion

Large B-cell lymphoma with *IRF4*-rearrangement and Burkitt-like lymphomas with 11q aberrations were introduced as provisional entities in the 2017 revision of the WHO classification of lymphoid neoplasms.[1] Despite their molecular profile being recently studied in detail,[5-6,9,10] there is very limited data on the incidence and clinical presentation of these two entities.[3,9] As both seem to occur predominantly in children and adolescents, we evaluated the incidence and the clinical characteristics of these lymphomas in a large cohort of patients <18 years of age and representative of lymphoma patients in central Europe. To the best of our knowledge, our study presents one of the larger series of paediatric B-NHL with *IRF4* rearrangement and 11q aberration in the literature. All patients are of European origin and treated according to NHL-BFM protocols. Therefore, our data represent the clinical experience of the NHL-BFM group in managing these lymphomas.

As reported previously, LBCL-*IRF4* may present as FL grade 3, DLBCL or transformed FL.[2] Although we cannot exclude that our analyses missed a few cases with *IRF4* rearrangements cryptic to our FISH approach, we estimate that – based on our cohort – at least 20% of all FL grade 3 and

Table VI. Morphology, immunophenotype and molecular findings of the 13 patients with *MYC* breakpoint-negative, 11q gain-loss FISH positive B-cell lymphoma.

Case No.	Tumour morphology	CD20	CD10	BCL6	MUM1	Hans Classifier	BCL2	c-MYC	Ki-67	11q gain-loss FISH	*MYC* break-apart FISH	*MYC*-IGH fusion FISH
11q-01	HGBCL	++	++	na	na	GCB type	−	na	90%	11q23 gain and 11q24 loss	Negative	Negative
11q-02	BL	++	++	++	-	GCB type	−	na	95%	11q23 gain and 11q24 loss	Negative	Negative
11q-03	HGBCL	++	++	na	na	GCB type	−	na	100%	Trisomy 11 (CEP11 and 11q23) with heterozygous 11q24 deletion	Negative	Negative
11q-04	HGBCL	++	++	na	na	GCB type	−	na	100%	11q23 gain and 11q24 loss	Negative	na
11q-05	HGBCL	++	++	na	na	GCB type	−	na	100%	11q23 gain and 11q24 loss	Negative	Negative
11q-06	HGBCL	++	++	na	na	GCB type	++	na	100%	11q23 gain and 11q24 loss	Negative	na
11q-07	DLBCL	++	++	na	na	GCB type	+	na	90%	11q23 gain and 11q24 loss	Negative	Negative
11q-08	DLBCL	++	++	na	na	GCB type	++	na	80%	11q23 gain and 11q24 loss	Negative	Negative
11q-09	BL	++	++	++	na	GCB type	−	na	90%	11q23 gain and 11q24 loss	Negative	Negative
11q-10	BL	++	++	++	na	GCB type	−	na	95%	11q24 loss only	Negative	Negative
11q-11	BL	++	++	++	na	GCB type	−	++	100%	11q24 loss only (by OncoScan-Array)	Negative	na
11q-12	BL	++	++	na	na	GCB type	−	na	100%	11q23 gain and 11q24 loss	Negative	Negative
11q-13	BL	++	++	na	na	GCB type	−	na	100%	11q23 gain and 11q24 loss	Negative	Negative

BL, Burkitt lymphoma; DLBCL, diffuse large B-cell lymphoma; HGBCL, high-grade B-cell lymphoma, unclassifiable, with features intermediate between BL and DLBCL according to the 2008 WHO classification; ++, strongly positive; +, weakly positive; −, negative; na, not available; GCB, germinal centre B-cell.

DLBCL by morphology harbour an *IRF4* chromosomal translocation in this age group in central Europe. Assuming that FL/DLBCL comprise about 10% of all lymphomas in children,[11] the overall incidence is certainly very low and probably in the range of 1–2%, even among lymphomas in children and adolescents.

According to the literature, many LBCL-*IRF4* tumours occur in the Waldeyer ring (e.g., tonsils). In our cohort, 5/7 patients have lymphoma involvement of the tonsils or nasopharynx, confirming that the Waldeyer Ring is indeed a predilection site of involvement. Since these lymphomas frequently present as localised disease (stage 1 or 2), it is not surprising that the outcome is excellent when treated according to NHL-BFM therapy. Hence, LBCL-*IRF4* may present a subgroup of mature B-NHL for therapy de-escalation in future clinical trials. Despite patients with LBCL-*IRF4* being treated similarly with *IRF4* rearrangement-negative B-NHL in current protocols, we recommend testing all cases of MUM1-positive FL or DLBCL in children and adolescents for chromosomal breaks in *IRF4* in order to gather more experience with this new entity.

To estimate the frequency of mnBLL,11q, we screened *de novo* DLBCL and lymphomas with BL or HGBCL morphology for this new WHO entity. This genetic aberration has also been reported in typical BL with *MYC* translocations and DLBCL [9]. Though DLBCL mostly lack the typical terminal deletion, the 11q gain-loss pattern might not be restricted to mnBLL,11q, similar in the way that *MYC* translocations are not restricted to BL. Since we intended to identify the new entity rather than 11q aberrations *per se*, we restricted the analysis of 11q to mature B-NHL with BL/DLBCL/HGBCL morphology in children and adolescents who were negative for *MYC* breakpoint by FISH. In this subgroup of morphologically and genetically predefined lymphomas, a substantial number of cases fulfilled the criteria of the new WHO entity Burkitt-like lymphoma with 11q aberration. Our data suggest that, in the age group of children and adolescents presenting with mature aggressive B-NHL, the ratio

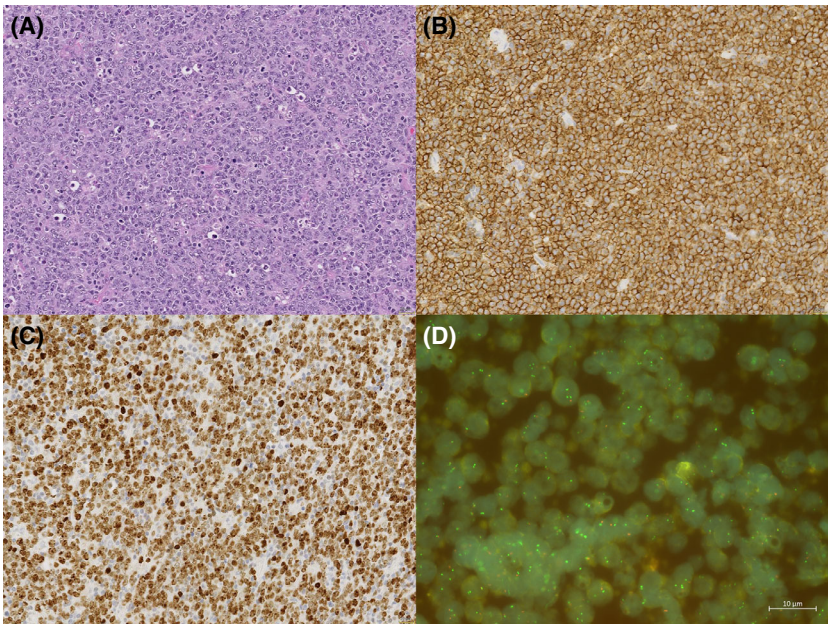

Fig 2. Morphology of high-grade B-cell lymphoma with positive 11q gain-loss FISH. (A) The tumour showed high-grade lymphoma morphology on H&E staining. (B) CD20 immunohistochemistry confirms B-cell phenotype of the tumour. (C) Ki-67 immunohistochemistry. Over 90% of the cells expressed Ki-67. (D) FISH using 11q gain/loss triple-colour probes showed two to three green signals in the 11q23·3 region but only one orange signal at the 11q24·3 region, confirming the presence of typical 11q gain-loss pattern. [Colour figure can be viewed at wileyonlinelibrary.com]

Table VII. Clinical features of patients with morphological FL/DLBCL with and without *IRF4* breakpoint.

Clinical features at disease presentation	*IRF4* break-point-positive	*IRF4* break-point-negative	P-value
Gender			
Male	4/7 (57%)	16/25 (64%)	$P = 0{\cdot}740$ (χ^2 test)
Female	3/7 (43%)	9/25 (36%)	
Age			
<10 years	2/7 (29%)	10/24 (42%)	
10–14 years	4/7 (57%)	7/24 (29%)	
≥15 years	1/7 (14%)	7/24 (29%)	
Median age (1st to 3rd quartile)	12·0 (7·0–14·3)	12·0 (8·7–15·9)	$P = 0{\cdot}832$ (Wilcoxon)
Stage (St. Jude/Murphy)			
Stage I	2/7 (29%)	8/22 (36%)	**$P = 0{\cdot}043$** (χ^2 test)
Stage II	5/7 (71%)	4/22 (18%)	
Stage III	0/7 (0%)	7/22 (32%)	
Stage IV	0/7 (0%)	3/22 (14%)	
Serum LDH (U/l)			
<500	7/7 (100%)	20/25 (79%)	
≥500	0/7 (0%)	5/25 (21%)	
Median serum LDH (U/l) (1st to 3rd quartile)	251 (198–307)	308·5 (226–424)	$P = 0{\cdot}228$ (Wilcoxon)
B-symptoms present	0/7 (100%)	4/25 (16%)	$P = 0{\cdot}258$ (χ^2 test)
NHL-BFM risk group			
B-NHL: Watch and wait	1/7 (14%)	1/17 (6%)	$P = 0{\cdot}770$ (χ^2 test)
B-NHL R1	1/7 (14%)	3/17 (18%)	
B-NHL R2	5/7 (71%)	10/17 (59%)	
B-NHL R3	0/7 (0%)	2/17 (12%)	
B-NHL R4	0/7 (0%)	1/17 (6%)	

As some clinical data were missing for several patients, the total number of cases ranges from 24 to 32 in each category.
Bold underline represents items that have *P*-value less than 0·05 (i.e. statistically significant).
FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma.

of patients with mnBLL,11q to *MYC*-rearrangement-positive BL may be close to 1:10. Since this entity has been reported to occur frequently as post-transplant lymphoproliferative disorders (PTLD),[12] the incidence will certainly be heavily dependent on the patient cohort analysed. PTLD were not included in our study, but the specimens analysed by us are fairly representative for mature aggressive B-NHL in children and adolescents in central Europe.

As we selected the cohort based on archived tumour biopsy available in the Kiel lymph node registry (although

Table VIII. Tumour localisation of *IRF4*-positive lymphomas morphological FL/DLBCL.

Tumour localisation	*IRF4* break-apart-positive	*IRF4* break-apart-negative	Fisher's exact test (*P*-value)
CNS involvement	0/7 (100%)	0/23 (100%)	*P* = 1·000
Head and neck involvement (including cervical lymph nodes)	5/6 (83%)	17/22 (77%)	*P* = 1·000
Axillary and clavicular lymph nodes involvement	0/6 (0%)	4/22 (18%)	*P* = 0·549
Thoracic involvement (including pleural and pericardial effusion)	1/6 (17%)	2/22 (9%)	*P* = 0·530
Any abdominal involvement (including ascites, abdominal and retroperitoneal lymph nodes, intestinal and solid organ tumours)	0/6 (0%)	8/23 (35%)	*P* = 0·148
Inguinal lymph nodes involvement	0/6 (0%)	2/22 (9%)	*P* = 1·000
Bone marrow involvement	0/7 (0%)	2/17 (12%)	*P* = 1·000

As some tumour localisation data were missing for several patients, the total number of patients range from 22 to 29 in each category. FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma.

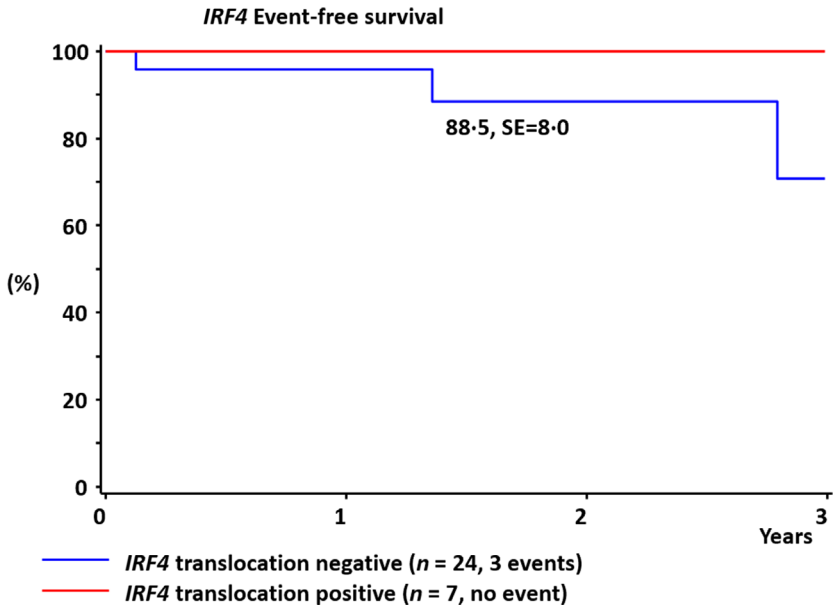

Fig 3. Two-year event-free survival of patients with morphological FL/DLBCL with positive *IRF4* break-apart by FISH. A statistical test was not performed as there were too few events to draw any meaningful conclusion. [Colour figure can be viewed at wileyonlinelibrary.com]

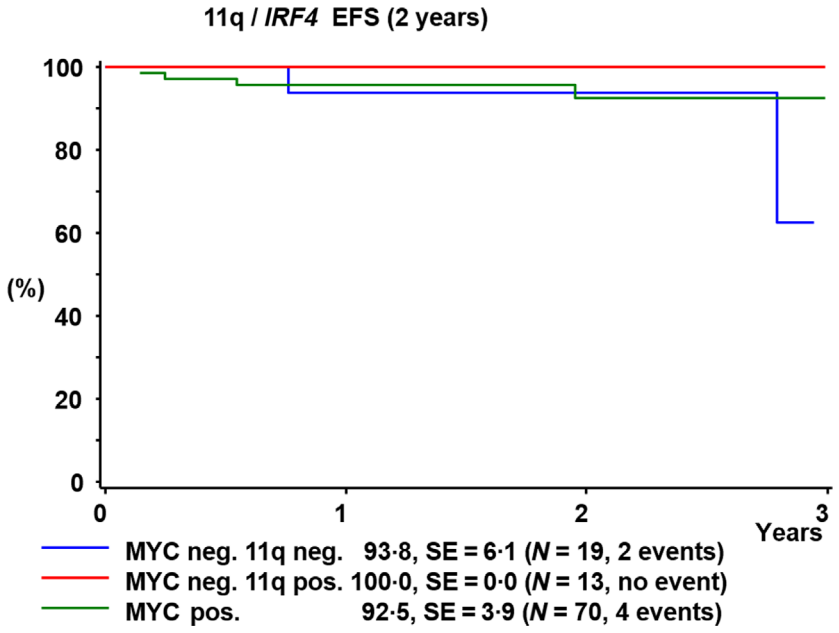

Fig 4. Event-free survival of patients with morphological BL/DLBCL/HGBCL with positive typical 11q gain-loss pattern by FISH. A statistical test was not performed as there were too few events to draw any meaningful conclusion. [Colour figure can be viewed at wileyonlinelibrary.com]

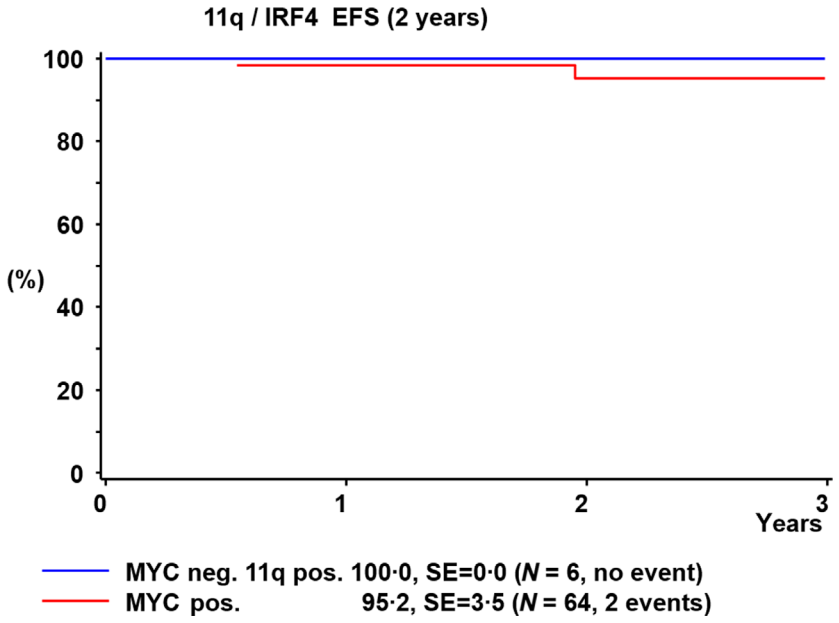

Fig 5. Event-free survival of patients with morphological BL with positive typical 11q gain-loss pattern by FISH, excluding cases with morphological DLBCL and HGBCL. A statistical test was not performed as there were too few events to draw any meaningful conclusion. [Colour figure can be viewed at wileyonlinelibrary.com]

Table IX. Clinical features of patients with morphological BL/DLBCL/HGBCL according to *MYC* and *11q* status.

Clinical features at disease presentation	*MYC* break-point- negative and 11q typical gain-loss pattern positive	*MYC* break-point- negative and 11q typical gain-loss pattern negative	*MYC* break-point-positive	*P*-value
Gender				
Male	9/13 (69%)	11/19 (58%)	63/70 (90%)	**P = 0·003** (χ^2 test)
Female	4/13 (31%)	8/19 (42%)	7/70 (10%))	
Age				
<10 years	3/13 (23%)	11/19 (58%)	46/70 (66%)	
10–14 years	6/13 (46%)	3/19 (16%)	17/70 (24%)	
≥15 years	4/13 (31%)	5/19 (26%)	7/70 (10%)	
Median age (1st quartile to 3rd quartile)	13·9 (10·2–15·0)	9·1 (5·7–15·8)	7·5 (5·3–11·5)	**P = 0·004** (Wilcoxon)
Stage (St. Jude/Murphy)				
Stage I	2/12 (17%)	4/18 (22%)	2/57 (4%)	**P = 0·040** (χ^2 test)
Stage II	6/12 (50%)	4/18 (22%)	14/57 (25%)	
Stage III	3/12 (25%)	8/18 (45%)	31/57 (54%)	
Stage IV	1/12 (8%)	2/18 (11%)	10/57 (17%)	
Serum LDH level (U/l)				
<500	11/13 (85%)	14/19 (74%)	42/70 (60%)	
≥500	2/13 (15%)	5/19 (26%)	28/70 (40%)	
Median serum LDH level (1st quartile to 3rd quartile)	271 (205–320)	317 (249–518)	429 (288–782)	**P = 0·01** (Wilcoxon)
B symptoms present	0/13 (0%)	6/19 (32%)	22/70 (31%)	*P* = 0·06
NHL-BFM risk group				
Watch and wait	0/12 (0%)	1/15 (7%)	0/58 (0%)	**P = 0·010**
B-NHL R1	1/12 (8%)	4/15 (27%)	1/58 (2%)	
B-NHL R2	10/12 (83%)	7/15 (46%)	30/58 (52%)	
B-NHL R3	1/12 (8%)	2/15 (13%)	14/58 (24%)	
B-NHL R4	0/12 (0%)	1/15 (7%)	8/58 (13%)	
B-NHL R4 with positive CNS disease	0/12 (0%)	0/15 (0%)	5/58 (9%)	

As some clinical data were missing for several patients, the total number of patients ranges from 85 to 102 in each category.
Bold underline represents items that have *P*-value less than 0·05 (i.e. statistically significant).

Table X. Tumour localisation of morphological BL/DLBCL/HGBCL according to *MYC* and 11q status.

Tumour localisation	*MYC* breakpoint-negative and 11q typical gain-loss pattern positive	*MYC* breakpoint-negative and 11q typical gain-loss pattern negative	*MYC* breakpoint-positive	χ^2 test (*P*-value)
CNS involvement	0/13 (0%)	0/15 (0%)	6/68 (9%)	$P = 0{\cdot}268$
Head and neck involvement (including cervical lymph nodes and thyroid)	8/13 (62%)	9/16 (56%)	27/68 (40%)	$P = 0{\cdot}221$
Axillary and clavicular lymph nodes	1/13 (8%)	4/15 (27%)	4/66 (6%)	$\mathbf{P = 0{\cdot}048}$
Thoracic involvement (including pleural and pericardial effusion)	1/13 (8%)	0/16 (0%)	6/67 (9%)	$P = 09{\cdot}464$
Any abdominal involvement (including ascites, abdominal and retroperitoneal lymph nodes, intestinal and solid organ tumours)	5/13 (39%)	8/17 (47%)	52/69 (75%)	$\mathbf{P = 0{\cdot}008}$
Inguinal lymph nodes	0/13 (0%)	2/15 (13%)	4/65 (6%)	$P = 0{\cdot}353$
Any nodal involvement (including superficial and deep nodes)	10/13 (77%)	9/16 (56%)	41/68 (60%)	$P = 0{\cdot}464$
Bone marrow involvement	0/13 (0%)	2/16 (13%)	15/66 (23%)	$P = 0{\cdot}122$

As some tumour localisation data were missing for several patients, the total number of patients ranges from 93 to 99 in each category.

patients with Burkitt leukaemia may be diagnosed purely by bone marrow aspiration without lymph node biopsies), our analysis was restricted to nodal BL excluding Burkitt leukaemia. This may explain the bias against Burkitt leukaemia in our cohort. Nevertheless, recently published profiles of this entity in flow cytometry analysis[10] will certainly foster the identification of mnBLL,11q in leukaemic patients and provide an even more complete picture of the incidence of the disease.

The differences in the clinical presentation of *MYC* breakpoint-positive BL and mnBLL,11q found in our study support the concept that both diseases share similar histopathological and immunophenotypical features,[3,10] but differ strikingly in molecular genetic features.[5,6] Given the fact that mnBLL,11q molecularly differ from typical BL, and that according to the data in this study Burkitt-like lymphomas show excellent outcome under current NHL-BFM treatment, mnBLL,11q may also be considered as a subgroup of lymphomas for dose-reduction. However, currently there is no evidence at all that the good outcome is caused by lymphoma biology or by the presentation at low stages and risk group. In order to understand whether identification of mnBLL,11q and its distinction from typical BL is clinically relevant, larger cohorts of this disease and a clinical comparison with BL of the same stage/risk group will be required.

One limitation of our method is that we defined *IRF4* rearrangement as lymphomas that are positive for *IRF4* break-apart FISH. This strategy would miss approximately 10% of LBCL-*IRF4*[13] as they exhibit *IRF4*-IGH fusion but are negative for *IRF4* break-apart using commercially available probes. Hence the prevalence of *IRF4*-rearrangement in paediatric FL/DLBCL may be even higher than the 21% reported here, if *IRF4*-IGH fusion analysis is incorporated. Likewise, using *MYC* break-apart probes alone would miss approximately 5–10% of *MYC* rearrangements and cryptic *MYC* insertions in HGBCLs.[14,15] The proportion of DLBCL/HGBCL in the '*MYC* breakpoint negative 11q gain-loss negative' group may hence be lower if *MYC*-IG fusion probes were incorporated.

Despite presenting a large series of these two provisional entities in this study, our cohort is still too small to answer the above questions. Joint efforts are required to generate cohorts large enough to test for the clinical relevance, independent of the stage. Given the fact that the relative frequencies of LBCL-*IRF4* and mnBLL,11q are rather high in children and adolescents with B-NHL according to our data, together with the fact that commercial FISH probes for *IRF4* and 11q are now widely available, we believe that the diagnosis of these two entities will be easier and the unanswered questions regarding the clinical relevance can be resolved in the foreseeable future.

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Author contributions

R.K.H.A-Y. and W.K. designed the research study and wrote the manuscript; I.O. and W.K. provided histology review and IHC analysis; L.A.P., W.W. and B.B. provided clinical data; R.S. and W.K. provided molecular analysis; R.K.H.A-Y. and M.Z. provided statistical analysis, all authors approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table SI. Tumour localisation of morphological BL according to *MYC* and 11q status. As some tumour localisation data was missing for several patients, the total number of patients in each category ranges from 66 to 70.

Fig S1. Case selection process of identifying potential cases of large B-cell lymphoma with *IRF4* rearrangement.

Fig S2. Case selection process of identifying potential cases of Burkitt-like lymphoma with 11q aberration. BA^+: Break-apart pattern positive; BA^-: Break-apart pattern negative.

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