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 ($P = 0.043$). 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 ($P = 0.004$), showed less male predominance ($P = 0.003$), lower stage ($P = 0.040$), lower serum LDH level ($P = 0.01$) and less abdominal involvement ($P = 0.008$) 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.
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,

and one patient in the FL/DLBCL cohort overlapped with our previous publication on paediatric DLBCL.

The two cohorts do not otherwise overlap with our previous publications on the initial description of LBCL-IRF4 and mnBLL,11q.

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). 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.

**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
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/HGBCL

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, 6/8 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 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.

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 | IRF4 break-apart FISH | IRF4-IGH fusion |
|----------|-------------------|------|------|------|------|-----------------|------|-------|----------------------|----------------|
| 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    | 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
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 \((P = 0.043)\). 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, \(P = 0.004\)). They also showed less male predominance \((P = 0.003)\), lower serum LDH level \((P = 0.01)\), lower tumour stage \((P = 0.04)\) and lower NHL risk group \((P = 0.010)\). Ten cases are negative for both MYC breakpoint pattern and MYC-IGH fusion, and three cases are negative for MYC breakpoint 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 \((P = 0.008)\), 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 \((10/13 \text{ cases vs. } 41/68 \text{ cases}, P = 0.353 \text{ by Fisher's exact test})\).

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.

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 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 ($P < 0.001$). 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,\(^2,4,6,9,10\) there is very limited data on the incidence and clinical presentation of these two entities.\(^{5,6}\) 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

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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, 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. 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.

| Case No. | Tumour morphology | CD20 | CD10 | BCL6 | MUM1 | Hans Classifier | BCL2 | c-MYC | Ki-67 | 11q gain-loss FISH | MYC breakapart 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      | ++   | na    | 100% | 11q24 loss only (by OncoScan-Array) | Negative          | Negative          |
| 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.
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), 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                                  | 9/13 (69%)                                      | 11/19 (58%)                                    | 63/70 (90%)             | P = 0.003 (χ² test) |
| Male                                    | 4/13 (31%)                                      | 8/19 (42%)                                     | 7/70 (10%)              |         |
| Female                                  |                                                 |                                                |                         |         |
| Age                                     | 3/13 (23%)                                      | 11/19 (58%)                                    | 46/70 (66%)             |         |
| <10 years                                |                                                 |                                                |                         |         |
| 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 (χ² 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                       |                                                |                                                |                         |         |
| Watch and wait                           | 0/13 (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).
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\textsuperscript{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,\textsuperscript{5,10} but differ strikingly in molecular genetic features.\textsuperscript{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\textsuperscript{13} as they exhibit IRF4-IGH fusion but are negative for IRF4 break-apart using commercially available probes. Hence the prevalence of MYC-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.\textsuperscript{14,15} The proportion of DLBCL/HGBCL in the ‘MYC breakpoint negative 11q gain-loss negative’ group may hence be lower if MYC-IGH 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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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 | \( \chi^2 \) test (P-value) |
|-----------------------------------------------------|---------------------------------------------------------------------|---------------------------------------------------------------------|-------------------------|-----------------------------|
| CNS involvement                                     | 0/13 (0%)                                                          | 0/15 (0%)                                                          | 6/68 (9%)               | 0.268                       |
| Head and neck involvement (including cervical lymph nodes and thyroid) | 8/13 (62%)                                                        | 9/16 (56%)                                                        | 27/68 (40%)            | 0.221                       |
| Axillary and clavicular lymph nodes                 | 1/13 (8%)                                                          | 4/15 (27%)                                                        | 4/66 (6%)               | \( 0.048 \)                |
| Thoracic involvement (including pleural and pericardial effusion) | 1/13 (8%)                                                          | 0/16 (0%)                                                          | 6/67 (9%)               | \( 0.0946 \)               |
| Any abdominal involvement (including ascites, abdominal and retroperitoneal lymph nodes, intestinal and solid organ tumours) | 5/13 (39%)                                                        | 8/17 (47%)                                                        | 52/69 (75%)            | \( 0.008 \)                |
| Inguinal lymph nodes                                | 0/13 (0%)                                                          | 2/15 (13%)                                                        | 4/65 (6%)               | 0.353                       |
| Any nodal involvement (including superficial and deep nodes) | 10/13 (77%)                                                        | 9/16 (56%)                                                        | 41/68 (60%)            | 0.464                       |
| Bone marrow involvement                             | 0/13 (0%)                                                          | 2/16 (13%)                                                        | 15/66 (23%)            | 0.122                       |

As some tumour localisation data were missing for several patients, the total number of patients ranges from 93 to 99 in each category.
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 S1. 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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