Leukemia (2008), 1–10

www.nature.com/leu

REVIEW

Translocations involving 8q24 in Burkitt lymphoma and other malignant lymphomas: a historical review of cytogenetics in the light of todays knowledge

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Burkitt lymphoma (BL) has a characteristic clinical presentation, morphology, immunophenotype and primary chromosomal aberration, that is, the translocation t(8;14)(q24;q32) or its variants. However, diagnostic dilemmas may arise in daily practice due to overlap of BL with subsets of other aggressive, mature B-cell lymphomas such as diffuse large B-cell lymphomas (DLBCL). Recently, two gene expression studies have described a distinct molecular profile for BL, but also showed the persistence of some cases intermediate between BL and DLBCL. An alternative approach to define BL is to consider (cyto)genetic data, in particular chromosomal abnormalities other than the t(8;14) or its variants. In this review the 'Mitelman Database of Chromosome Aberrations in Cancer,' harboring the majority of all published neoplasia-related karyotypes, was explored to define a cytogenetic profile of 'true' BL. This core subset of BL showed a very low complexity of chromosomal abnormalities with 40% of the cases having the IG–MYC fusion as the sole abnormality. In the remaining cases, additional recurrent but partially exclusive abnormalities included gains at chromosomes 1q, 7 and 12, and losses of 6q, 13q32–34 and 17p. Within the core subset, no differences were found between pediatric and adult patients. In addition, the genetic profile of the core subset was significantly different from BL with an 8q24 breakpoint not affecting one of the three immunoglobulin loci, BL with a translocation involving 18q21/BCL2, 3q27/BCL6 or 11q13/BCL1, additionally to a breakpoint at 8q24/MYC, and from other morphological types of lymphomas with an 8q24/MYC breakpoint. These groups showed a higher cytogenetic complexity than the core subset of BL. BL without a detectable 8q24/MYC breakpoint might be heterogeneous and deserves further studies. We suggest that, concordant with the WHO classification to be published in 2008, the diagnosis of BL should be restricted to cases with expression of CD10 and BCL6, absence or very weak expression of BCL2 protein, a homogeneously very high proliferation index and a proven IG-MYC translocation without evidence of a chromosomal translocation typical for other lymphoma entities. In addition, a high number of nonspecific cytogenetic abnormalities should suggest need for a critical review of the diagnosis of BL.
Leukemia advance online publication, 16 October 2008;
doi:10.1038/leu.2008.281
Keywords: Burkitt lymphoma; cytogenetics; MYC; translocation

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Dedication: This review is dedicated to the groundbreaking work of Professor Lore Zech on chromosome banding and t(8;14).
Received 21 May 2008; revised 6 August 2008; accepted 26 August 2008

Brief history of genetics in Burkitt lymphoma

Burkitt lymphoma (BL) was first described in 1958 by Denis P Burkitt as 'a sarcoma involving the jaws in African children'.[1] After the identification of the Epstein–Barr virus,[2] BL became a model for the exploration of chromosomal aberrations in cancer. From their cytogenetic analyses of BL cell lines, Kohn *et al*.[3] observed abnormalities of C-group chromosomes. In 1972, Manolov and Manolova[4] reported a marker band at the end of chromosome 14. Zech *et al*.[5] were the first to suggest that a translocation event occurred between the telomeric end of the long arm of chromosome 14 and the telomeric end of the long arm of chromosome 8. Variant translocations towards 2p and 22q were described in BL cell lines in 1979.[6,7] The molecular targets of these translocations were discovered to be MYC (at 8q24),[8,9] and genes coding for the immunoglobulin heavy chain (IGH at 14q32),[10] kappa light chain (IGK at 2p12)[11] and lambda light chain (IGL at 22q11).[12] All translocations juxtapose the MYC gene to one of the IG enhancers, which results in constitutive deregulation of MYC expression. Although it is considered to be the hallmark of BL, translocation of MYC is not specific, as it is also seen in other types of lymphomas.[13]

Current diagnostic dilemma

Although a correct diagnosis of classic BL can easily be made using a combination of clinical, histological, immunophenotypical and genetic criteria including the presence of a translocation involving MYC,[14] problems arise when considering other B-cell Non-Hodgkin's lymphomas (B-NHL) with one or more overlapping features with BL. Especially the distinction between BL and a small subset of diffuse large B-cell lymphoma (DLBCL) with multiple characteristics of BL (for example, morphological features, a MYC translocation and/or a very high proliferation index) appears to be problematic.[15] This distinction has important clinical implications for treatment and prognosis, as BL responds relatively poor to standard DLBCL therapy (CHOP-like regimen) but excellent to high-intensity chemotherapy.[16–18] As this distinction may be difficult using the current diagnostic tools,[19] several groups have focused on new parameters.[19–24] Recently, two comprehensive gene-expression studies on the subject have been published, showing some differences in outcome. In both studies, BL could be distinguished from other aggressive lymphomas by a distinctive gene expression pattern, that is, the combination of expression levels of multiple genes being able to predict a diagnosis of BL (predictor or index). Cases with a high index were called 'molecular BL' and cases with a low index 'non-molecular BL.' According to the Molecular Mechanisms in Malignant

Lymphomas (MMML) Network Project study,[25] the greater majority of mature aggressive B-cell lymphomas cases could be classified as either molecular BL or DLBCL, but an intermediate zone remained, as 48 of 219 (22%) of the cases could not be classified, as molecular BL or non-molecular BL. Moreover, 81% of all conventionally classified BL but also 11 of 165 (7%) morphologic DLBCL had a molecular profile of BL (Table 1a). No real intermediate group existed in the study of the Lymphoma/Leukemia Molecular Profiling Project (LLMPP);[26] however, 7 out of 20 (35%) morphologically difficult to classify DLBCL cases had a molecular profile of BL (Table 1b). Furthermore, in the MMML study bcl-2 expression and BCL2 translocations were observed in 9 and 1 out of the 44 molecular BL cases, respectively.[25] From the 53 molecular BL cases of the LLMPP study, 5 cases showed bcl2 protein expression and 3 had a BCL2 breakpoint in addition to the MYC breakpoint.[26] Both observations are in contrast with the criteria of the WHO classification from 2001.[14] These contradictions point to the possible utilization of additional criteria for a diagnostic definition of these intermediate lymphomas.

Role of genetics in the diagnosis of BL

Another approach to distinguish 'true' BL cases from mimickers might be the inclusion of genomic criteria in addition to the MYC-translocation. In 1982, Berger *et al*.[27] reported that approximately 40% of the BL/leukemia cases have a t(8;14) or variant translocation as the sole cytogenetic abnormality. The lack of complex genetic alterations in addition to a MYC translocation might therefore be suggestive for a 'true' BL.[28,29] The presence of an additional translocation as found in other types of B-NHL (for example, involving BCL2, BCL6 and/or CCND1) or a complex karyotype with multiple gains and/or losses would rather represent another type of B-NHL or a secondary transformed lymphoma.[30] This is supported by the publication of the MMML where array based comparative genomic hybridization (arrayCGH) was used to study genomic copy number imbalances in addition to fluorescent *in situ* hybridization (FISH) analysis for MYC, BCL2 and BCL6 translocations. In contrast to molecular BL, non-molecular BL were characterized by a less frequent IG-MYC translocation and by more additional genomic aberrations. 'Intermediate' cases were enriched for non-IG–MYC fusions, IG–MYC fusions in complex karyotypes and additional translocations of either BCL2 or BCL6.[25] A recent study of the LLMPP using CGH on BL revealed that the 'discrepant cases' (that is, molecular BL, but with aberrant morphology and/or immunophenotype) showed a more complex genetic make-up, including BCL2 breakpoints as detected by FISH.[31] These observations indicate that other genomic features than the MYC translocation status are important to distinguish 'true' BL from mimickers. The aim of this review is to define the cytogenetic profile of 'true' BL and to use this profile to distinguish BL from other B-cell lymphomas with an 8q24/MYC breakpoint.

Table 1 Summary of classification results based on gene-expression profiling

Pathological Dx	N	%	*Molecular Dx*	N	*% Group*	*% Total*
(a) Data from MMML (N = 219)[25]						
BL	36	16	BL	29	81	13
			DLBCL	3	8	1
			Intermediate	4	11	2
DLBCL	165	75	BL	11	7	5
			DLBCL	115	70	53
			Intermediate	39	23	18
Aggressive NOS	18	8	BL	4	22	2
			DLBCL	9	50	4
			Intermediate	5	28	2
(b) Data from LLMPP (N = 80)[a, 26]						
BL	45	56	BL	44	98	55
			DLBCL	1	2	1
			Intermediate			
DLBCL[b]	29	36	BL	8	28	10
			DLBCL	20	69	25
			Intermediate	1	3	1
Aggressive NOS	6	8	BL	1	16	1
			DLBCL	5	84	6
			Intermediate			

Abbreviations: BL, Burkitt lymphoma; DLBCL, diffuse large B-cell lymphomas.
[a]Total of 223 previously profiled DLBCL cases were excluded.
[b]Twenty nine morphologically 'difficult' DLBCL cases with an original diagnosis of atypical BL or Burkitt-like lymphoma but reclassified as DLBCL by the expert panel.

The Mitelman database as a comprehensive cytogenetic resource

To base our analyses on the largest available set of lymphomas we chose the 'Mitelman Database of Chromosome Aberrations in Cancer'[32] as our resource. The edition of the Mitelman database (September 2007) contained 6359 B-cell malignancies. Overall, 865 B-NHL with an 8q24 translocation were selected, including 538 BL. In addition, 108 cases of morphologic BL without a documented 8q24 translocation became part of the data set. Mature 'Burkitt' leukemias (ALL-L3 according to the FAB classification) were included, but not lymphomas and leukemias derived from precursor B-cells and plasma cell-related disorders.

Although we included series published between 1976[5] and 2007[33] and we realized that immunohistochemistry and FISH became gradually available after 1976, we relied on the diagnostic assignment of the cases as given through the database. We therefore defined a core subset of BL using a minimal set of criteria applied throughout the entire period. These criteria are (a) morphologic diagnosis of BL, (b) presence of a translocation between chromosome bands 8q24 (suggestive for the MYC locus) and 2p12, 14q32 or 22q11 (suggestive for involvement of one of the IG loci) and (c) no (additional) breakpoint at 18q21, 3q27 or 11q13 (expected targets are BCL2, BCL6 and CCND1, respectively). This core subset was extensively analyzed, and compared with other B-NHL with an 8q24 translocation as well as cases of morphologic BL that did not fulfill all these criteria.

All karyotyping data were converted to an 862 band-specific status map, with breakpoint and imbalance information, using karyotype parsing software developed as part of the Progenetix project (www.progenetix.net;[34] supplementary information). Statistical differences between genomic profiles of individual groups were determined with the CGHMultiArray software (http://webmath.tue.nl/mark/cgh/index.html) at a *P*-value <0.05.[35] The differences between groups were calculated with the Fisher's exact test for nominal values and the student's *t*-test for continuous variables. Although we are aware that the mentioned cytogenetic aberrations might involve other molecular targets, for readability purposes we have used molecular terms instead of cytogenetic terms for the following cytobands:

MYC for 8q24, BCL2 for 18q21, BCL6 for 3q27, CCND1 for 11q13, IGH for 14q32, IGK for 2p12 and IGL for 22q11.

Case selection and subgroup definition

The data set included 865 B-NHL with a MYC translocation (Figure 1); 538 represented BL/leukemia and 327 cases another type of B-NHL (Supplementary Table 1). From the 538 BL cases, 525 (98%) had a translocation of MYC towards an IG locus (IG-MYC), the remaining 13 BL (2%) harbored a translocation of MYC towards a non-IG locus (non-IG-MYC). Out of the 327 other B-NHL with a MYC translocation, 256 (78%) harbored an IG-MYC translocation, while 71 cases (22%) harbored a non-IG-MYC translocation (significance of difference with BL $P<0.0001$). In addition, 108 morphologic BL cases without a confirmed MYC translocation were included.

In addition to the MYC translocation, 621 completely annotated translocation events were found in 380 cases. The most common translocations involved 18q21 (BCL2, $N=126$), 11q13 (CCND1, $N=35$) and 3q27 (BCL6, $N=31$), which are commonly seen in other types of lymphoma (for example, follicular lymphoma, mantle cell lymphoma and DLBCL). All cases with one or more of these translocations in addition to a MYC translocation were regarded as 'double-hit' (DH) lymphomas ($N=177$). In line with the new WHO classification of 2008, we used the expression DH throughout this manuscript and defined this as any kind of 8q24/MYC translocation plus any translocation involving 18q21/BCL2, 11q13/CCND1 or 3q27/BCL6. Nevertheless, we realize that the expression 'double-hit' is not uniformly used by authors and moreover might not fully cover the heterogeneity of this group. It might even be more appropriate to use the expression 'MYC plus' where behind 'plus' the specific second oncogene targeted could be mentioned (for example, 'MYC plus BCL2'). Fifty-three of the 177 DH lymphomas (29%) had a diagnosis of BL (44 IG-MYC and 9 non-IG-MYC cases) and 124 (71%) had a diagnosis of another type of B-NHL (76 IG-MYC and 48 non-IG-MYC cases).

Out of the total data set of 973 cases, age and gender were available for 808 (83%) and 932 (96%) cases, respectively, with gender being derived from karyotype information in a small subset of cases. When the cases were divided into five age cohorts (0–15, 16–30, 31–45, 46–60, 61–89 years), the percentage of morphological BL cases with IG-MYC decreased with age, while the percentage of DH BL cases and the other B-NHL cases (both IG-MYC and non-IG-MYC) increased with age. These differences were underscored by the age distributions of the single subgroups (Figure 2).

Definition of a core subset of BL—few cytogenetic differences between children and adults

Out of the 973 cases, 481 cases fulfilled our criteria for the core subset of BL (Figure 1; BL IG-MYC single hit): a morphological diagnosis of BL, a cytogenetic detection of an IG-MYC translocation and exclusion of a translocation of BCL2, BCL6 and/or CCND1. The distribution over the different age cohorts was very similar to that of population-based studies with almost half of patients being under the age of 15 years and a strong

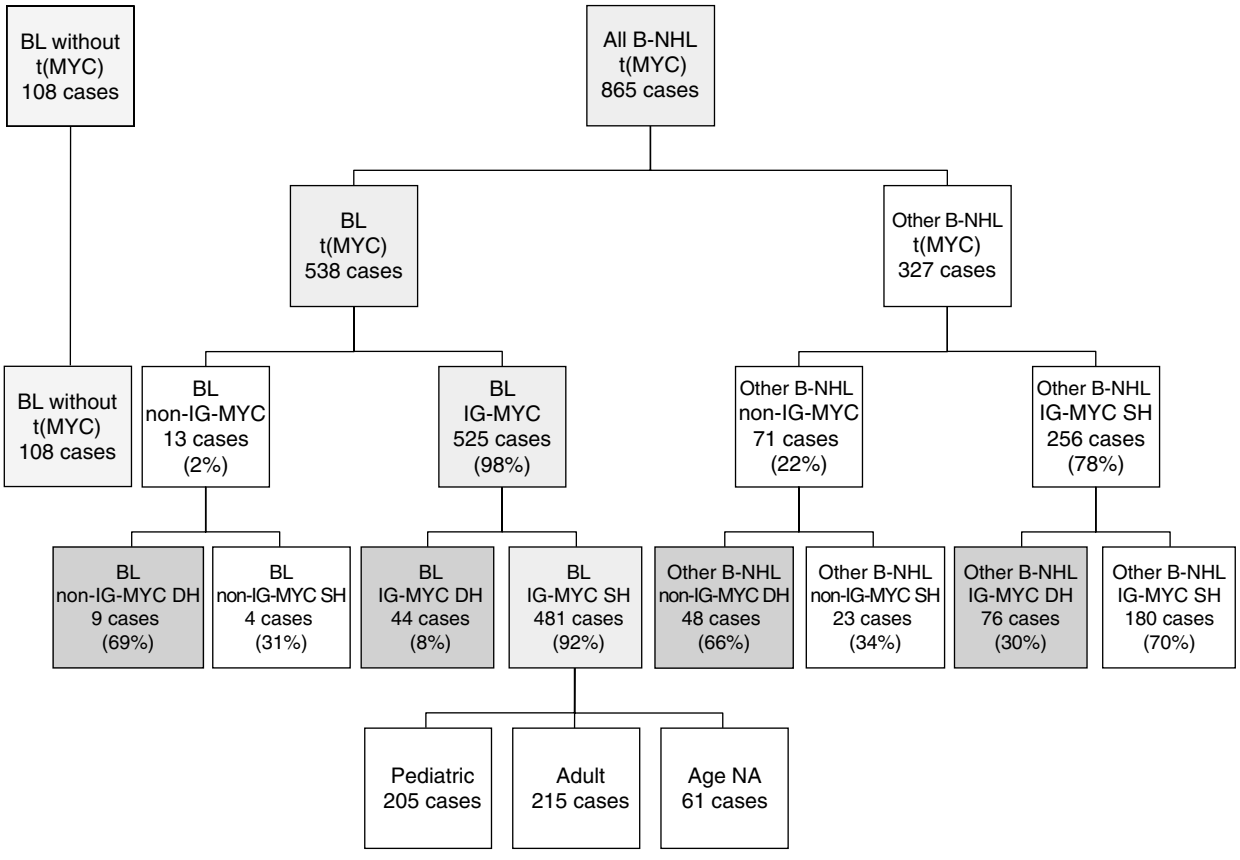

Figure 1 Flow chart of all cases from the Mitelman database.

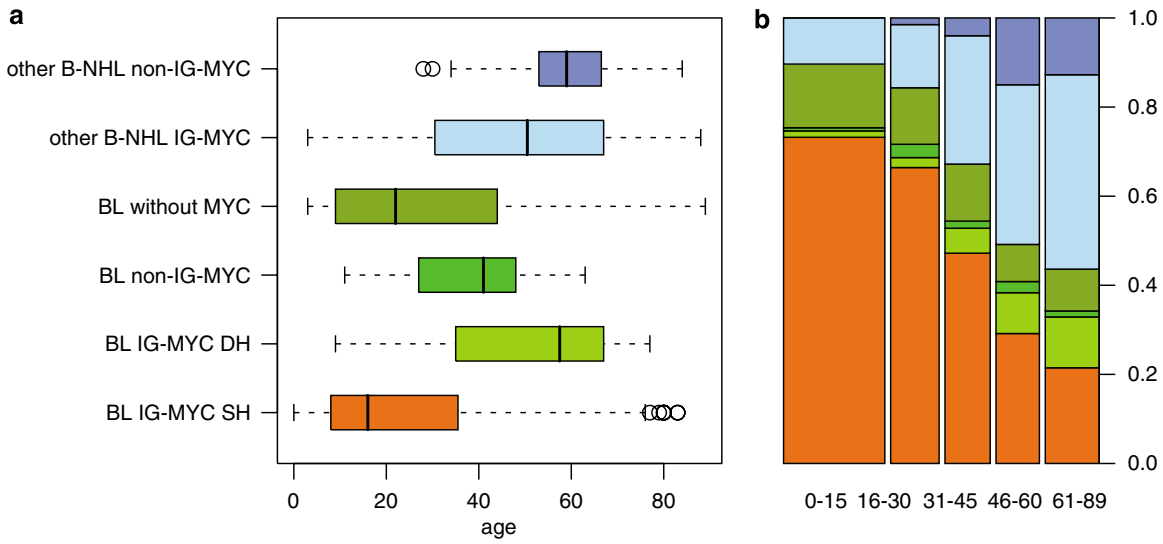

Figure 2 Age distributions of the different subgroups. (**a**) Box and Whisker plot with median and lower and upper quartiles. (**b**) Relative incidence of the different disorders in the different age cohorts. For colors, see panel **a**.

male preponderance.[36–38] As pediatric BL is thought to be prototypic for 'true' BL, we further limited the core subset to the 205 pediatric cases (age $\leqslant$15 years). Although even in this group the possibility of a misdiagnosis can not be excluded, the few potentially misdiagnosed cases should not have a strong impact on the overall cytogenetic profile. The mean age of this pediatric subset was 8 years and the majority of patients were male (74%). One hundred eighty-five patients (90%) had a classical t(8;14), 13 patients (6.5%) had a t(8;22) and 7 (3.5%) a t(2;8) variant translocation. In addition to this translocation, few other cytogenetic aberrations were found. Numerical imbalances and additional, non-recurrent translocations (not involving BCL2, BCL6 or CCND1) were detected in 122 and 36 of the 205 cases (60 and 18%, respectively). The most common aberrations (Figure 3) were singular events, with 87% of cases having 0–2 imbalances (0: 40%, 1: 33%, 2: 16% and $>$2 imbalances: 11%). As a surrogate for genomic complexity, we calculated the complexity score per case in accordance with the publication of the MMML.[25] In short, every non-continuous imbalance per chromosome was counted per case with an additional separation at chromosomal centromeres. Only imbalances were counted, whereas balanced translocations (copy number neutral) had no impact on the complexity score. Although the data cannot be directly compared to the complexity values from the MMML, as different techniques were used, our core BL subset also showed a very low complexity score as published for the molecular BL cases.[25] In addition, this complexity score linearly correlated with the number of aberrant chromosomes or cytobands (Supplementary Figure 1).

In the adult group ($>$15 years), the mean age was 35 years and again the majority of patients were male (75%). Compared with the pediatric group, more adult cases harbored a variant MYC translocation (22 versus 10%, $P=0.0003$). The most common numerical aberrations were almost identical except for a higher prevalence of gain of 8q in the adult group (Figure 3). Both the average number of imbalances (77% of adults having 0–2 numerical imbalances) and the genomic complexity score (Figure 4b) were very similar for children and adults. This supports recent studies,[25,26,39] suggesting that from a cytogenetic and gene expression point of view, BL in children and adults is the same disease. The differences between children and adults found by others in smaller cohorts[36,40,41] may represent the inclusion of BL mimickers. In light of these results and also the fact that the genetic profile of the patients with no data on age available were almost identical, we extended our core subset to BL IG-MYC single hit samples independent of patient age (Figure 3). The validity of our criteria is also supported by the absence of significant differences in complexity and the proportion of cases diagnosed as BL or another type of B-NHL, when we compared cases published before ($N=280$) and after 1994 ($N=201$) (Supplementary Figure 2).

The most common aberrations in this core subset (occurring in $>$4% of the cases) were copy number gains involving 1q, 7 and 12, and losses involving 6q, 13q32–34 and 17p (Figure 5). To investigate co-occurrence of the different cytogenetic imbalances, we performed cluster analyses on all 696 cases with imbalances (Figure 4c; Supplementary Figure 3). As a compromise between cross-talk between neighboring intervals and resolution, we used a reduced cluster matrix (86 regions). Figure 4c shows that the core BL clusters together. Focusing on core BL cases (Supplementary Figure 4), it seemed that gain of 1q is associated with lack of other recurrent abnormalities, with a mostly random scattering of abnormalities in the other cases.

The cytogenetic profile of the BL core subset is different from the cytogenetic profile of other B-NHL cases with a MYC translocation

A MYC translocation is not specific for BL,[12] and is also seen in a subset of morphological DLBCL,[42–44] follicular lymphoma[45] or mantle cell lymphoma.[46] We found 327 of such cases in our data set, with the majority having a diagnosis of DLBCL ($N=135$; 41%), B-cell lymphoma-NOS ($N=79$; 24%) and follicular lymphoma ($N=52$; 16%) (Supplementary Table 1). Apart from the DLBCL cases, these cases are commonly thought to represent transformed disease and therefore may harbor a non-IG-MYC breakpoint or additional translocations (for example, involving BCL2 or CCND1), as well as a higher number of numerical aberrations.[47–50] Indeed, the complexity score was

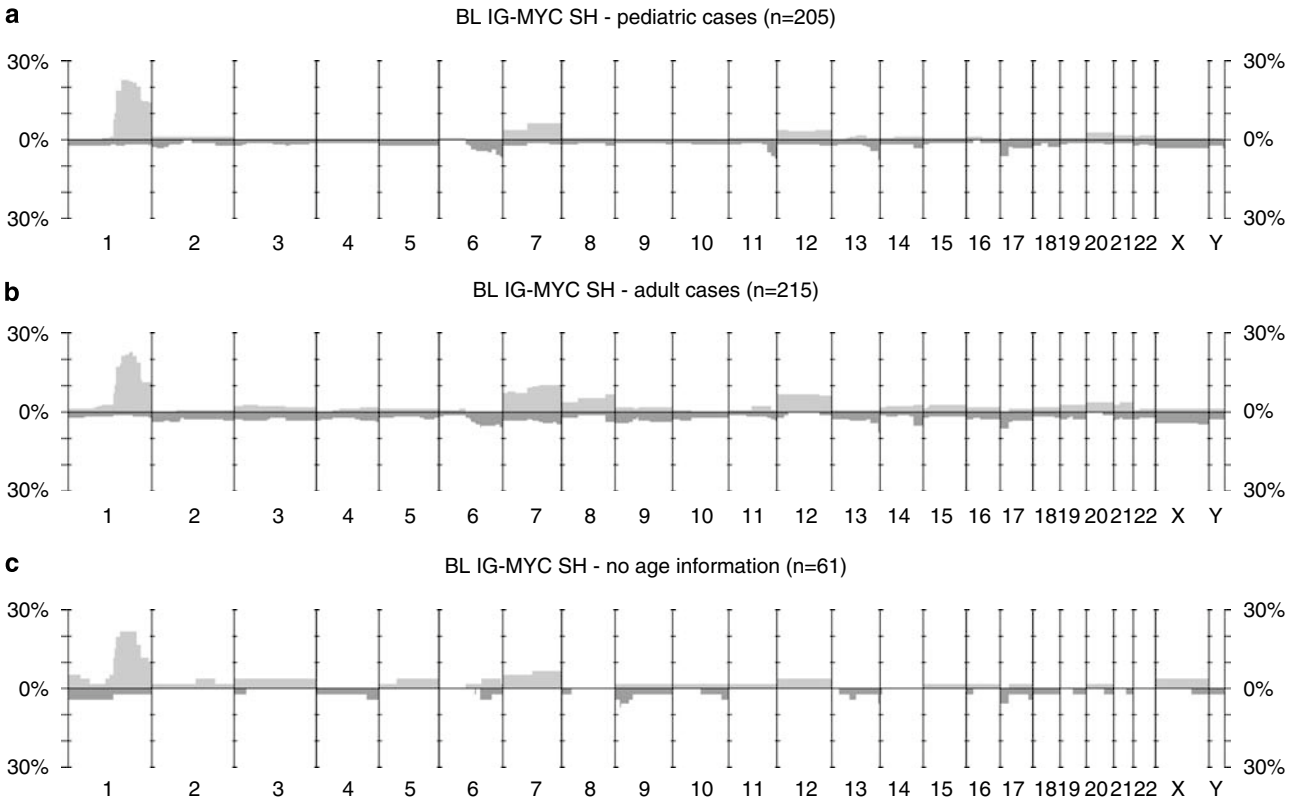

Figure 3 Histogram of genetic aberrations in core Burkitt lymphoma (BL). Histograms for numerical imbalances in pediatric (**a**), adult (**b**) and patients of unknown age (**c**) from the core subset of BL at a virtual 862 band resolution. Green/light grey: gain; red/dark grey: loss.

much higher in the other B-NHL cases (Figure 4a; significance of difference $P<0.0001$) and gains of 7, 11p, 11q11–22, 12, 18 and X and loss of 4q, 6q13–27, 9, 10p, 15, 17p13 and 17q11–23 were more common compared with the BL core subset (Figure 5). In addition, patients were older (mean 50 versus 23.5 years, $P<0.0001$; Figure 2), and the male/female ratio was lower (Supplementary Table 3). These differences persisted when limiting the group to cases with a morphological diagnosis of DLBCL (data not shown).

Morphologic BL without a reported MYC translocation show genetic differences from the core subset

Although in the WHO classification a MYC translocation is a prerequisite for the diagnosis of BL, cases with typical phenotype (morphology and immunohistochemistry) and a very high proliferation index but lacking a MYC translocation often are diagnosed as BL. The precise classification and treatment of these cases is under ongoing discussion. Some authors suggest that such cases should by definition be classified (and treated) as DLBCL,[41] but others claim that such cases behave more aggressively than DLBCL and would benefit from more aggressive therapy.[20] We compared this group ($N=108$) with the core subset. The genomic complexity was higher (mean 4.5 versus 1.7 $P<0.0001$; Figure 4a). Specifically, gain of 6p, 7p, 11p and 18 and loss of 9, 14q32, 16 and 22 were more common (Figure 5). In addition, this group showed a higher mean age of 29 years (Figure 2, $P=0.014$), and the male/female ratio was lower than that in the core subset (Supplementary Table 2). We hypothesize that these 8q24 translocation negative BL cases might represent three subgroups: (a) cases in which the original karyotyping missed a MYC translocation, (b) cases that harbored a different mechanism by which MYC can be overexpressed or (c) cases that were misdiagnosed as BL. In case of (a) or (b), molecular techniques (for example, FISH) could have provided additional information. The most important conclusion could therefore be that this group should prospectively be reduced by applying strict immunohistochemical criteria in combination with proper additional molecular techniques, to detect hidden MYC translocations and to exclude additional breakpoints involving BCL2, BCL6 or CCND1. The final classification (and treatment) of the remaining cases without any indication of a MYC translocation as either BL, DLBCL or a separate entity should be subject of further research.

BL with a non-IG-MYC translocation (BL non-IG-MYC) is a rare entity and is different from the core subset

The activation of MYC by juxtaposition towards one of the IG loci is considered the canonical starting point of BL and therefore a condition *sine qua non* for the diagnosis. Other mechanisms such as amplification or deregulation by juxtaposition of MYC with other genes might also lead to MYC overexpression and as a result a BL-like morphology.[51,52] Non-IG-MYC translocation may occur as a secondary event (for example, in follicular lymphoma), resulting in a transformed lymphoma with a BL-like morphology.[53] Although the numbers were small, we compared the genetic profile of the 13 BL with a non-IG-MYC breakpoint with that of the core subset. The genomic complexity of the 13 BL non-IG-MYC cases was much

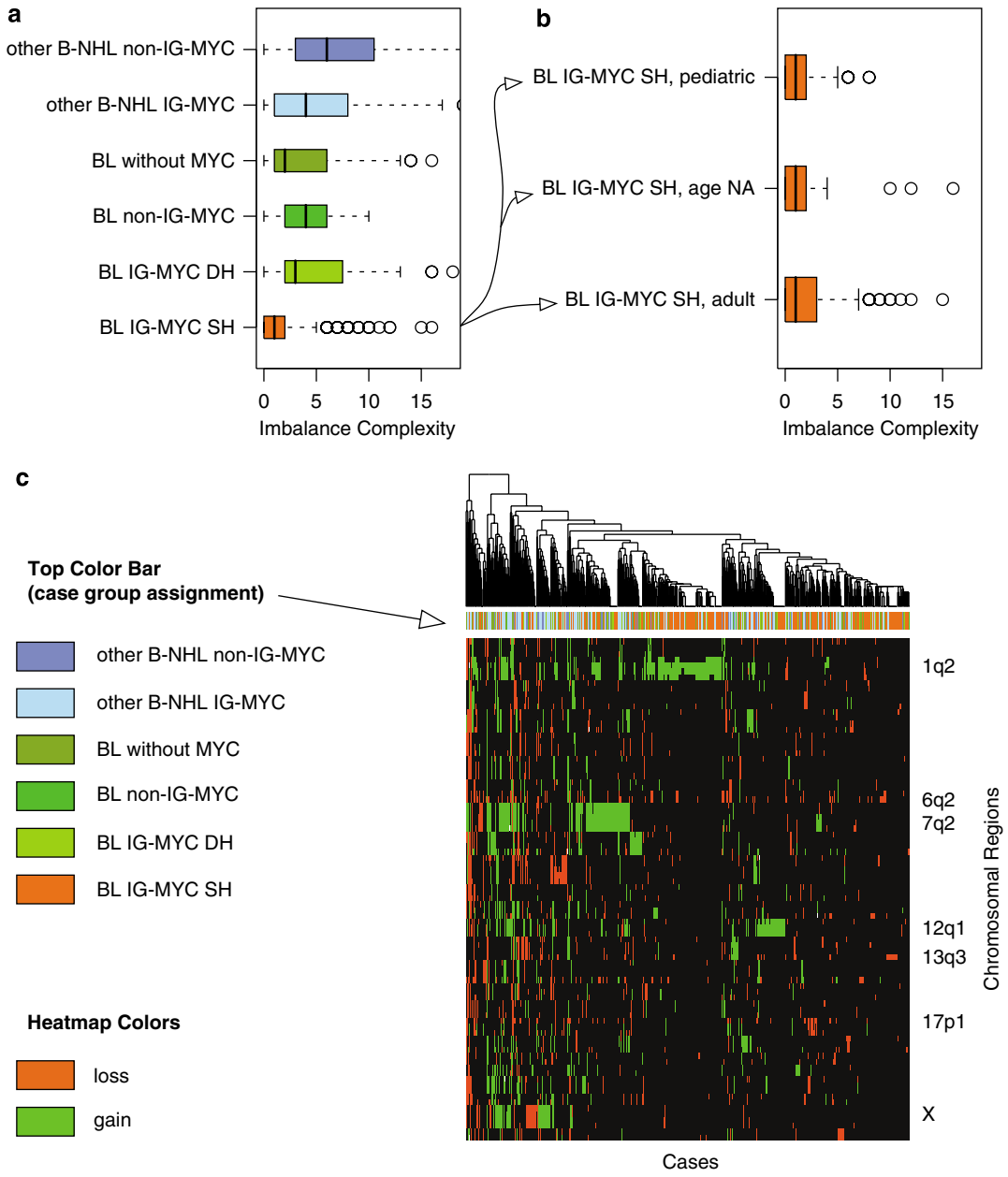

Figure 4 Complexity score and cluster analysis of the different subgroups. (**a**) Shows the imbalance complexity scores for the main subgroups analyzed and (**b**) for the three age subgroups of the core subset. Box and Whisker plots with median values, lower and higher quartiles. (**c**) Shows distribution of genomic gains and losses, as determined from the karyotypes of the 696 cases with imbalances, out of the total 973 cases. For visualization and ordering, the resolution of the genomic status matrix was reduced to 86 intervals (for example, '1q2'), and cases were clustered according to similarity of their imbalance profiles. The color bar on the top represents the assignment of cases to one of the six subgroups. Chromosomal regions run from 1pter (top) to Yqter (bottom), with some regions highlighted due to frequent involvement in BL IG-MYC single hit (and X for guidance). Green: gain; red: loss.

higher (mean 4.6 versus 1.7 $P=0.0003$; Figure 4a) and gain of 7p and 8q22–23 and loss of 8q24, 13q32–34 and X were more common (Figure 5). In addition, the age distribution was skewed towards a higher age (Figure 2) and there was a female preponderance (Supplementary Table 2).

Our data show that a non-IG-MYC translocation is extremely rare in cases morphologically diagnosed as BL. This corroborates the publication of Bertrand *et al*.[53] on the largest series ($N=17$) of such B-NHL, of which only one case had a BL morphology. These authors suggested that the non-IG-MYC is a secondary event. In line with this conclusion, 9 out of our 13 cases (69%) harbored two translocations involving MYC and also BCL2, BCL6 or CCND1 (to be discussed below). Therefore, we suggest that BL non-IG-MYC cases should not be considered as 'true' BL.

Double-hit BL (BL IG-MYC DH) should not be considered as 'true' BL

In 44 out of the 525 BL cases with an IG-MYC breakpoint, other recurrent translocations were found in addition to the MYC-translocation. We found 31 breakpoints at BCL2 (70% of all

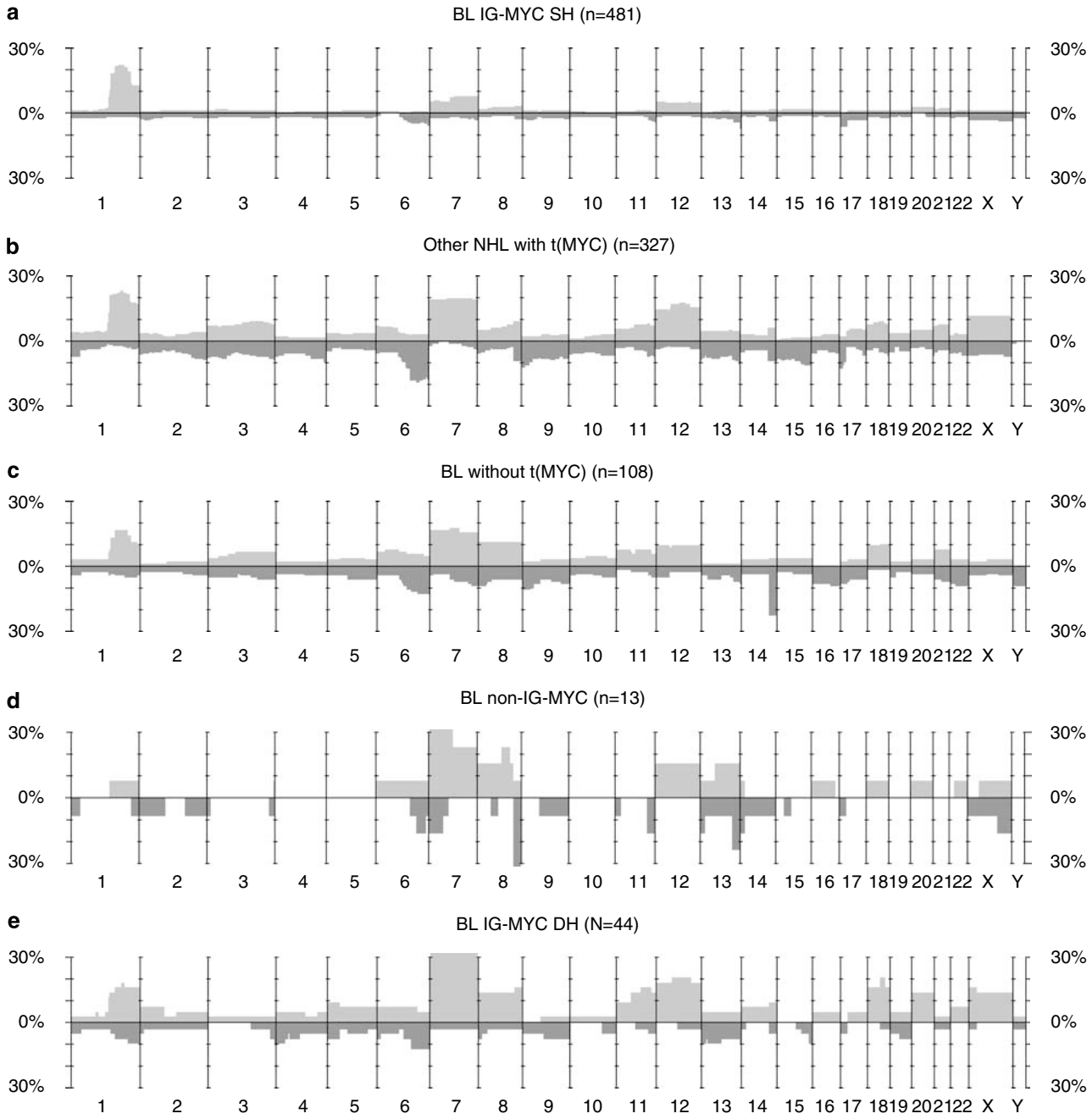

Figure 5 Genetic aberrations in all subgroups. Histogram of genetic aberrations of all different subgroups at a virtual 862 band resolution (**a**–**e**). Green/light grey: gain; red/dark grey: loss.

cases), 15 at BCL6 (34%) and 5 at CCND1 (11%), 6 cases (14%) having triple hits for MYC, BCL2 and BCL6. As described in the previous paragraph, the IG-MYC translocation in such cases is thought to be the second hit. Various authors have already suggested that such DH BL cases should be considered a separate entity from 'true' BL.[30,54,55]

When comparing the 44 BL IG-MYC DH cases with the core subset, they appeared to be two separate entities. DH BL patients were much older (mean 51 years, $P<0.0001$, Figure 2), very much similar to the other B-NHL group. Also the genomic complexity of the DH cases was much higher than that in the core subset (mean 5.4 versus 1.7, $P<0.0001$; Figure 4a). Gains of 7, 8, 11, 12, 18, 20 and X and loss of 3q27–29, 6q and 15q26 were significantly more common in the DH subgroup (Figure 5). The overlap between BL IG-MYC DH and other B-NHL cases was supported by the fact that DHs were also very frequent in the group of other B-NHL (124/327, 38%). These observations support the available clinical data on this type of lymphoma. The patients have a very aggressive disease that is refractory to current chemotherapeutic treatment. Overall survival is very short, even if treated with high-intensity chemotherapy as used for BL.[56]

Another intriguing observation is that a variant MYC translocation towards 2p or 22q occurred more often in DH lymphomas (55%), compared with the core subset (16%). According to Au *et al.*,[57] this supports the hypothesis that the

IG-MYC translocation is a secondary event, as one IGH locus is already occupied by the primary translocation (that is, t(11;14) or t(14;18)) and the other IGH allele should remain functional for B-cell receptor signaling.

The LLMPP study[25] suggested that at least some DH lymphomas may have a molecular profile that is very similar to that of 'true' BL. A larger series of such lymphomas has to be studied independently from the designed BL predictors to know whether these lymphomas really are similar to 'true' BL or not. For now, there are strong indications that DH BL should be considered a separate disease from 'true' BL. In the new WHO classification of 2008, it is advised that these cases are labeled as a separate group 'B-cell lymphoma with features intermediate between DLBCL and BL'.

Summary and conclusions

In view of the therapeutic and prognostic consequences, the diagnosis of BL should be made with the highest level of certainty. So far this is done with conventional tools, including clinical aspects.[14] With these techniques, the distinction is still hard to make in a subset of cases, especially in the 'gray-zone' between BL and DLBCL, and additional criteria might be of use. In this review, we studied the cytogenetic profile of 'true' BL using the unique, comprehensive collection of cytogenetic data in the 'Mitelman database.'[32] We defined a core subset of BL, that is, lymphomas that were registered as such on a morphological basis, contained an IG-MYC translocation and did not harbor chromosomal translocations of the BCL2, BCL6 or CCND1 loci. As a proof of principle, we compared the cytogenetic profile of this core subset with those of other B-NHL with a MYC breakpoint, with BL cases without a MYC breakpoint, with BL cases with a non-IG-MYC breakpoint and with DH BL cases. The validity of the used criteria for BL was underscored by the absence of any significant increase in the genomic complexity score with age as well as the similarity for cases published before and after the introduction of the REAL classification in 1994. Interestingly, lack of differences in genomic complexity between pediatric and adult cases was independently found by the MMML[39] and LLMPP.[31]

We demonstrated that these core BL cases are obviously different from other B-NHL cases with a MYC translocation, as the latter harbor more and also other genetic aberrations than 'true' BL. In 'true' BL gains of 1q, 7 and 12 are found as common recurring events, while (the even less frequent) losses mostly involve 6q, 17p as well as 13q32–q34. Besides differences in cytogenetic complexity and pattern, the available epidemiological data (age and gender distribution) also differed between the two groups. Obviously, morphological criteria remain essential to distinguish BL from other lymphomas. Without availability of gene expression-profiling data, cases with an IG–MYC fusion without evidence of a DH should not be considered as BL, unless indicated by histological appearance. This is supported by our analysis of all 661 IG-MYC single hit lymphoma cases, in which 21% of the 475 cases with a complexity score of 0–2 had an original diagnosis other than BL (Figure 6; Supplementary Figure 5). On the other hand, we are aware of the fact that this study cannot give any guideline to definitely identify the rare individual lymphomas that are morphologically different from BL but nevertheless should be considered as molecular BL based on advanced molecular profiling.

Furthermore, we conclude that both DH BL cases as well as the extremely rare BL cases with a non-IG-MYC breakpoint (which in fact mainly represent DH lymphomas) should be separated from 'true' BL, as both their genomic and epidemiological profile are much more similar to other types of B-NHL. This is supported by the MMML data on gene expression and by clinical data showing that such patients have a very aggressive disease that is hardly curable with conventional or high-intensity chemotherapeutic regimens. The awareness on such DH cases is needed especially in older patients, as almost one-third of all cases diagnosed as BL in the database above the age of 60 years appeared to be a DH BL.

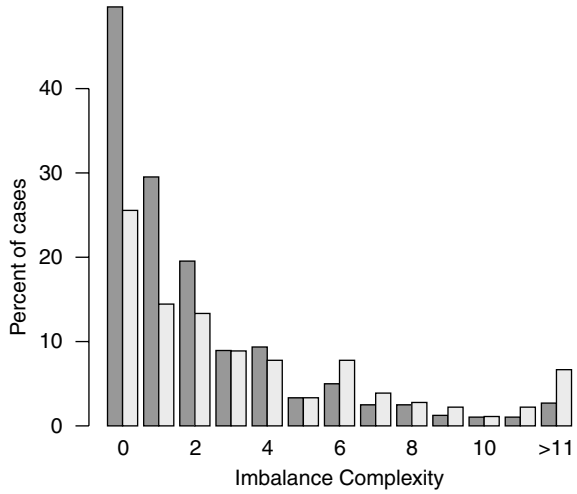

Figure 6 Morphology versus imbalance complexity in IG-MYC single hit lymphomas. All lymphoma cases with an IG-MYC breakpoint but no 18q21, 3q27 or 11q13 breakpoint were analyzed for imbalance complexity and original histological diagnosis. A histological diagnosis of BL (red/dark grey bars) is associated with a lower complexity and other diagnoses (blue/light grey bars) are gradually increasing in cases with a higher complexity.

There are practical consequences from this review. While lacking the molecular resolution of FISH methods, cytogenetic analysis has the great advantage of giving a 'bird's eye' overview of the many relevant genetic abnormalities in BL, that is, the partner of the MYC locus, the presence or absence of additional translocations and presence or absence and number of other structural abnormalities. Therefore, the authors strongly advise to submit material of any lymphoma suspicious for BL or mimicking diseases for conventional cytogenetic analysis by metaphase karyotyping. In addition, a simple MYC segregation FISH test should be carried out in all cases. More elaborate FISH tests for MYC, IG loci and also BCL2, BCL6 and eventually also CCND1, should be carried out in all BL with some abnormality in morphology or immunohistochemistry for CD5, CD10, BCL2, BCL6 or Ki-67. The use of fewer tests should be strictly restricted to pediatric patients <15 year. Another point to consider is that, although a FISH split signal assay with two probes flanking the MYC gene is the most sensitive and fast assay for detection of MYC breakpoints, it is not able to distinguish IG-MYC from non-IG–MYC fusions and does not cover all MYC breakpoints, that is, far 5′ and 3′ breakpoints as well as small insertions are not detected.[58,59] Furthermore, the steady increase in the age of lymphomas that mimic BL strongly emphasizes that there is no distinct age at which a pathologist can safely make a diagnosis of BL without any ancillary cytogenetic or molecular studies.

Acknowledgements

RS′ research on BL is supported by the Deutsche Krebshilfe and the Kinder-Krebs-Initiative Buchholz, Holm-Seppensen.

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)

TRANSLOCATIONS INVOLVING 8Q24 IN BURKITT LYMPHOMA AND OTHER MALIGNANT LYMPHOMAS – A HISTORICAL REVIEW OF CYTOGENETICS IN THE LIGHT OF TODAYS KNOWLEDGE

EG Boerma, R Siebert, PhM Kluin, M Baudis

Supplementary Data

TABLES

Supplementary Table 1: Morphological Diagnosis of all 973 Included Cases

ICD-O 3 code	Standardized Diagnosis	Cases
3229	Burkitt lymphoma, NOS	646
3226 2/3	Diffuse large B-cell lymphoma, NOS	135
3197	Malignant lymphoma, B-cell NOS	78
3230	Follicular lymphoma, NOS	52
3274 1/3	B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma	29
3224 1/3	Mantle cell lymphoma	18
3229 2/3	Splenic marginal zone lymphoma, NOS	4
3233	Marginal zone lymphoma, NOS	4
3313 1/3	Hairy cell leukemia	4
3223 2/3	Malignant lymphoma, lymphoplasmacytic	1
3226	Primary effusion lymphoma	1
3226 1/3	Mediastinal large B-cell lymphoma	1
All cases		**973**

Supplementary Table 2: Gender distribution

Subgroup	Male	Female	Ratio
BL IG-MYC SH	322	120	2.7
BL IG-MYC SH - pediatric	152	53	2.9
BL IG-MYC SH - adult	153	62	2.5
BL IG-MYC DH	28	16	1.8
BL nonIG-MYC	5	8	0.6
BL without MYC	66	42	1.6
Other B-NHL	217	108	2

Gender distribution in the different subsets of the 973 B-NHL. The core subset of BL IG-MYC SH cases shows an exceptional high male:female ratio (2.7), with only slight age related variation. The numbers are based on cases with available gender (and age for pediatric/adult) information.

Supplementary Table 3: "Non-Ig" Burkitt Lymphomas

Mitelman ID	Karyotype	Age	Gender	PMID
FM-11886-11-1	50, XX, add(3)(q27), +del(7)(q21), t(7;8)(q33;q24), +12, +13, t(14;18)(q32;q21), +18	59	female	17230227
FM-11436-1-1	46, XX, t(3;8)(q27;q24), der(14)t(13;14)(q14;p11) / 46, idem, der(8)t(3;8)t(3;8)(q29;q22-24)	11	female	16772123
FM-10797-4-1	46, XY, t(1;6)(q32;p21), t(3;13)(q27;p12), +t(6;21)(q16;p11), +der(8)t(8;14)(q22;p11), -13, t(14;19)(q34;q13), +1-4mar	28	male	15642390
FM-10797-5-1	47-48, X, del(X)(q22), t(3;3)(p23;q27), del(7)(p?), add(8)(q24), +del(8)(q24), +1-2mar	61	female	15642390
FM-10797-6-1	46, XX, t(1;8)(p34;q24)	27	female	15642390
FM-10797-6-2	48, XX, t(1;8), add(3)(p26), +6, +7, del(9)(q?)	27	female	15642390
FM-10623-12-1	45, X, -X, der(2)del(2)(p11)del(2)(q24), -8, add(8)(q24), der(11)t(8;11)(q13;p15), del(15)(q1?3q1?5), +der(16)t(?8;16)(p11;q2?3)	14	female	15125609
FM-10095-1-1	46, XY, add(1)(p36), add(6)(q21), add(7)(p13), t(8;9)(q24;p13), add(13)(q32), t(14;18)(q32;q21)	48	male	12708908
FM-9750-14-1	50, XY, +der(X)t(X;1)(p11;q2?1), add(4)(p1?), +add(7)(q22), ins(8;?)(q22-24;?), add(11)(q23), +12, t(14;18)(q32;q21), +20	42	male	12147652
FM-6229-1-1	46, XX, t(2;3)(p12;q27), del(8)(q24), t(14;18)(q32;q21)	41	female	8616793
FM-6482-7-1	46, XY, der(1)t(1;8)(q42;?q21), t(2;8)(q37;q24), del(6)(q23q25), del(11)(q23), der(13)t(8;13)(?q24;q32-34), t(14;18)(q32;q21), del(17)(p11)	47	male	8580796
FM-1137-6-1	46, XX, +7, t(8;11)(q24;q13), der(13;22)(q10;q10), t(14;18)(q32;q21), add(17)(p?)	63	female	6592036
FM-3061-11-1	46, XY, t(1;14)(q11;q32), del(8)(q24), -14	17	male	2751244
FM-10016-17-1	46, XY, add(2)(q32), del(3)(q27), der(3)add(3)(p21)add(3)(q27), add(4)(q31), add(5)(q11), add(7)(q31), der(8)t(3;8)(p24;q24), -14, del(15)(q23), -16, add(17)(q24), der(18)t(8;18)(q24;q22), add(19)(p13), der(19)t(1;19)(q11;p13), +2mar	NA	male	12529293
FM-10086-4-1	45, X, -X, del(1)(p13p22), -2, der(8)t(2;8)(q13;q24), del(9)(p12), t(11;14)(q13;q32), t(12;18)(q13;q23), -13, add(15)(p15), add(21)(p13), +2mar	50	female	12481006
FM-10126-991488-1	46, XY, der(1)t(1;8)(p36;q24), der(10)t(3;10)(q21;q26), t(14;18)(q32;q21)	NA	male	12742158
FM-10375-5-1	46, XY, add(2)(q32), del(3)(q27), der(3)add(3)(p21)?add(3)(q2?7), add(4)(q3?1), add(5)(q11), add(7)(q31) or del(7)(q31q35), der(8)t(3;8)(p24;q24), -14, del(15)(q23), -16, add(17)(q24), der(18)t(8;18)(q24;q22), add(19)(p13), der(19)t(1;19)(q11;p13), +2mar	NA	male	14562288
FM-10378-30-1	65-71, XX, -X, der(1)t(1;8)(p36;?q24)x2, der(1)t(1;8)(q41;q22), -2, der(3)t(3;8)(p24;?q23), +7, der(8)del(8)(p21)del(8)(q23), i(8)(q10), -9, +11, +12, +13, t(14;18)(q32;q21), der(16), del(17)(p11), +der(22), der(22)t(2;22)(?;q13)x2	NA	female	12930384
FM-10378-52-1	47, XY, der(1)t(1;8)(?;q24), -3, t(5;6)(p15;q23), +7, der(8)t(1;8)(p36;q24), der(12)t(5;12)(?;q24), der(13)t(1;8;13)dup(13), t(14;18)(q32;q21), del(15)(q24), der(18)t(3;18)(?q26;q23)x2	NA	male	12930384
FM-11012-1-1	78, XXY, +1, t(2;3)(q21;q27)x2, +3, +5, +6, -8, -10, +13, t(14;18)(q32;q21), +17, der(17)t(8;17)(q2?4;p11)t(4;8)(?;q2?4)x2, +18, +20, +22, dmin	40	male	15852472
FM-11012-2-3	49, XY, +Y, hsr(1)(q3?1), hsr(2)(p?), t(3;22)(q27;q11), +7, der(8)t(8;18)(q24;q?)hsr(8)(q24), +12, der(14)t(14;16)(q32;q?), der(17)t(16;17)(?;p11)t(7;16)	28	male	15852472
FM-11144-7-1	47, XY, t(1;6)(p35;p25), +12 / 48, idem, +9 / 47, idem, t(8;9)(q24;q11) / 47, idem, t(9;15)(q11;p11) / 47, idem, t(9;15)(q11;q26) / 47, idem, t(9;19)(q11;p13)	57	male	15510210
FM-11189-15-1	47, XX, t(3;14)(q27;q32), dup(6)(p21p25), +der(8)t(8;20)(q24;q11), add(10)(p15) / 47, idem, t(X;5)(q13;q33)	75	female	16156859
FM-11298-31-1	46, X?, t(5;8)(q11;q24)	NA	NA	16179374
FM-11428-18-1	47, XY, t(8;18)(q24;p11), +12	69	male	16519699

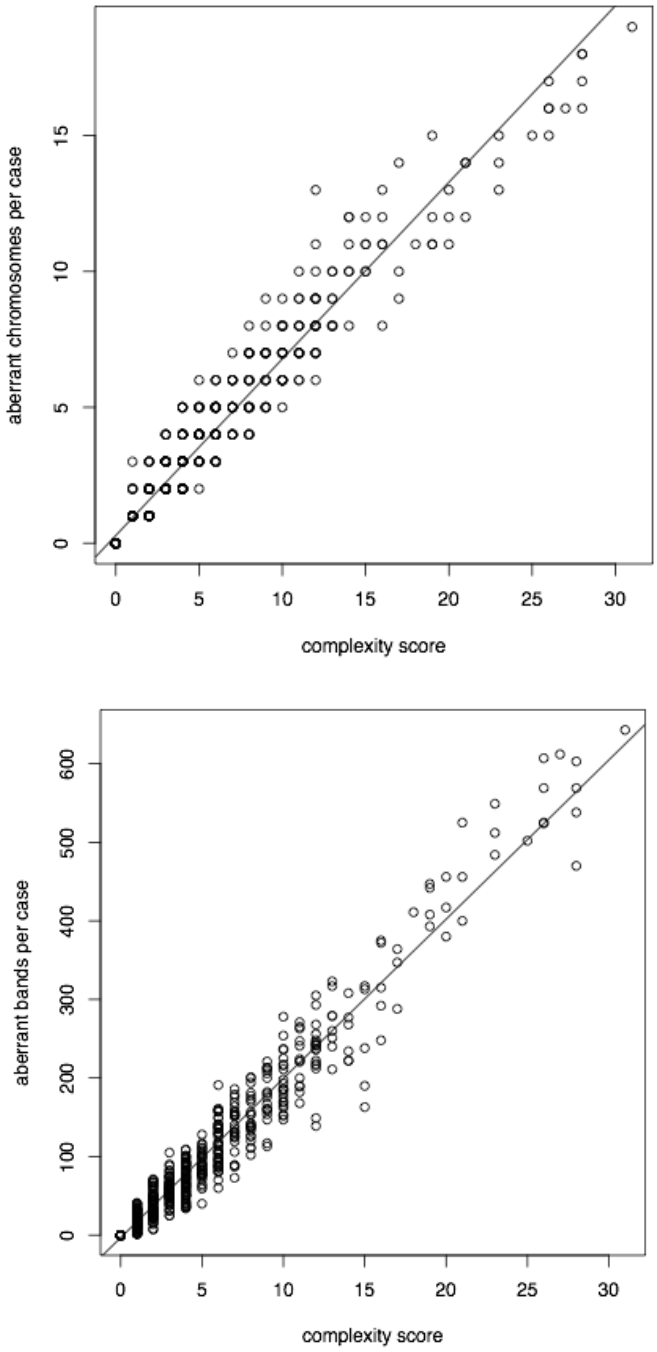
Mitelman ID	Karyotype	Age	Gender	PMID
FM-11711-23-1	46-51, XY, +Y, der(2)t(2;21)(q21;q11), +3, +del(5)(q?q?), der(8)t(8;12)(q24;?), +11, -13, del(14)(q22q32), +der(14)t(13;14)(q?;q?)inv(14)(p?q?), +15, der(18)t(2;18)(?;q21), +19, -20	NA	male	16840733
FM-11711-7-1	46, XY, del(13)(q13q22) / 46, XY, del(7)(q11), der(8)t(8;10)(q24;q?)t(10;13)(q?;q?), der(10)t(10;13)(q11;q?)t(7;13)(q11;q?), der(13)t(13;16)(q11;p11), der(16)t(10;16)(?;p11)ins(10;13)(?;q?)t(10;13)(?;q?)	NA	male	16840733
FM-11729-1-1	48, XX, t(3;8)(q27;q24), del(6)(q21q23), +12, +18	53	female	17175379
FM-11886-1-1	55, XY, +X, t(3;14;18)(q27;q32;q21), del(3)(q26q27), +der(3)t(1;3)(q21;q27), +5, +6, t(8;9)(q24;p13), +11, +12, +13, +16, der(17)t(1;17)(q21;p13), +20	45	male	17230227
FM-11886-13-1	49, XX, +X, t(1;8;13)(q31;q24;q22), del(6)(q22q27), +7, t(14;18)(q32;q21), add(17)(p12), +21	64	female	17230227
FM-11886-17-1	46-52, XY, inv(2)(p16q13), t(4;8)(q21;q24), der(5)(q32), del(6)(q21), del(9)(q11q32), +11, -13, -15, +der(16)(p11), i(17)(q10), -18, +21, +mar	36	male	17230227
FM-11886-2-1	50, X, +X, -Y, +add(6)(q12), +add(7)(q12), +add(8)q24), t(8;9)(q24;p13), t(14;18)(q32;q21), +20 / 51, idem, idem, +add(2)(q12)	56	male	17230227
FM-11886-3-1	49, XX, t(1;4)(q31;p16), t(8;9)(q24;p13), +13, +add(14)(q32), t(14;18)(q32;q21), del(15)(q22), +21	69	female	17230227
FM-11886-5-1	48, XX, t(2;22)(p12;p11), add(3)(q23), add(4)(q31), +i(6)(p10), t(7;8)(p12;q24), add(19)(q13), +20	80	female	17230227
FM-11886-6-1	48, XY, del(2)(q34), t(3;8)(q27;q24), +7, +der(8)t(3;8), t(14;18)(q32;q21)	53	male	17230227
FM-11886-7-1	49, XX, t(3;8)(q27;q24), +7, +der(8)t(3;8), del(10)(q24q25), +12, t(14;18)(q32;q21)	56	female	17230227
FM-11924-3-1	45, XY, t(8;14)(q24;q23q31), ins(13;8)(q14;q24q24), der(17)t(17;22)(p1?2;q1?2), -22 / 45, idem, del(2)(q?)	70	male	17452258
FM-11960-2-1	49-50, XX, +add(3)(p13), add(5)(p15), +add(7)(q21), der(8)t(8;15)(q24;q14), t(9;14)(p13;q32), +10, +add(12)(p13), +der(12)add(12)(p13)add(12)(q24), -15, add(17)(q25), +18	NA	female	17556073
FM-1258-2-1	45, XY, -8, t(8;10)(q12;p14), t(8;11)(q24;q13), add(14)(q32)	84	male	6513578
FM-2024-25-1	45, X, t(X;8)(p12;q24), del(1)(p12p31), add(6)(q?), add(7)(q?), der(9)t(9;13)(p21;q21), t(11;14)(q13;q32), -13	61	female	3312844
FM-2703-27-1	45, X, -Y, add(1)(p2?), der(1)t(1;1)(p36;q12), +dup(1)(p31p36), -2, dup(3)(q13-21q26), -6, der(8)t(4;8)(q21;q24), +der(8)t(8;9)(p23;p21), dup(12)(q13q21), -13, add(14)(q32), -15, -19, -19, -22, +3mar	NA	male	3416308
FM-3053-1-1	77, XXX, +X, +1, +1, add(1)(p13)x3, add(3)(p12), +add(3)(p21), -4, -6, t(6;7)(p21;q36), +der(7), t(8;14)(q24;q11)x2, -9, +11, +12, +13, der(13)t(1;13)(p13;q32)x2, +14, -15, +der(16)t(16;17)(p11;q11), -17, +18, +20, +21, -22	47	female	2535034
FM-3135-33-1	48, XY, add(1)(q?21), t(8;9)(q24;p13), t(14;18)(q32;q21), der(15)t(1;15)(q21;p11)t(1;11)(q42;q13), +2r	67	male	2506953
FM-3135-34-1	47-48, X, -Y, +X, add(2)(q11), del(2)(p11), -4, del(6)(q2?3q2?5), del(7)(p15), add(8)(p?21), t(8;9)(q24;p13), t(12;19)(p11;q13), t(14;18)(q32;q21), add(18)(q23), +der(18)t(14;18), add(22)(q13), +mar / 91, XX, -Y, -Y, +X, +X, del(1)(p22p36), add(2)x2, del(3)(p?13p?25)x2, -4, -4, del(6)x2, del(7)x2, t(8;9), add(8)x2, der(9)t(8;9), -12, t(14;18)x2, -17, +der(18)x2, -19, add(22), +2mar	57	male	2506953
FM-3556-1-1	47, X, der(Y)t(Y;1)(q12;q21), t(2;8)(q14;q24), +3, t(14;19)(q32;q13)	60	male	2208056
FM-3913-23-1	44, Y, add(X)(q26), t(1;11)(p36;q13), add(3)(q29), -4, -22 / 44, idem, der(8)t(4;8)(q12;q24), -5, -21	65	male	1913607
FM-4345-8-1	47, X?, +X, +3, ?t(4;8)(q33;q24), ?t(6;8)(q25;q24), i(12)(q10), -18	NA	female	1560313
FM-4382-14-1	46, XX, t(6;8)(p21;q24), t(14;18)(q32;q21) / 47, idem, +8	NA	female	1638478
FM-4395-9-1	46, XY, t(8;11)(q24;q13) / 46, XY, t(11;14)(q13;q32)	59	male	1381952
FM-4487-1-1	47, XX, t(1;5;10)(p34;q13;q22), del(7)(q22q32), +der(8)t(8;15)(p23;q13) / 47, idem, t(5;14)(q31;q11) / 47, idem, dic(6;8)(q25;q24) / 47, idem, t(11;17)(q13;p13) / 47, idem, der(7)del(7)(q22q32)t(1;7)(q25;q36) / 48, idem, +r	75	female	1394107

Mitelman ID	Karyotype	Age	Gender	PMID
FM-4846-34-1	46, XX, t(11;14)(q13;q32) / 47, idem, +7 / 46, idem, der(8)t(8;12)(q24;q22), t(8;12)	69	female	8499640
FM-5067-1-1	47, XY, der(1)i(1)(q10)del(1)(q32), +dup(1)(p11p34), del(6)(q?), t(8;11)(q24;q11), t(14;18)(q32;q21)	30	male	8221615
FM-5645-4-1	47, XY, +5, t(5;8)(p15;q24), add(15)(p11)	72	male	7524766
FM-5681-23-1	46, XY, t(8;11)(q24;q32), t(11;14)(q13;q32), dup(11)(q1?q1?)	NA	male	8204891
FM-603-10-1	51, XY, der(6)t(1;6)(p22;q13-15), der(8)t(8;16)(q24;p11), +11, +12, der(13)t(2;13)(q21;q14), del(15)(q22), +16, +20, +mar	53	male	7372340
FM-6061-5-1	46, XY, del(3)(p21), der(8)t(8;11)(q24;q13), t(8;11)(p12;q13), dup(11)(q13q25), t(11;14)(q13;q32)	59	male	7656198
FM-7062-13-1	46, XY, t(8;13)(q24;q14)	NA	male	9290957
FM-7091-1-1	50, XY, t(1;5)(p36;q31), +8, +der(18)t(18;22)(q21;q11), t(18;22), +20, +21 / 50, idem, der(2)t(1;2)(q21;q37) / 50, idem, der(3)t(1;3)(q21;p26) / 50, idem, der(4)t(1;4)(q21;q35) / 50, idem, der(5)t(1;5)(q21;p15) / 50, idem, der(8)t(1;8)(q21;q24) / 50, idem, der(9)t(1;9)(q21;p24) / 50, idem, der(17)t(1;17)(q21;q25)	51	male	9309114
FM-7434-478-1	46, XY, add(4)(q35), t(4;8)(q21;q24), del(9)(q12q32), t(11;14)(q13;q32), add(15)(q25-26)	NA	male	9444933
FM-7530-11-1	43-46, XY, t(7;8)(p11-13;q24), add(20)(p11-12)	NA	male	9593268
FM-7530-9-1	43-46, XY, t(1;8;11)(q25;q24;q21) / 42-46, XY, add(14)(q32)	NA	male	9593268
FM-7941-37-1	45, X, -Y, -6, add(8)(q24), der(15)?t(8;15)(q24;q22), idic(17)(p11), +mar	65	male	10326592
FM-8274-15-1	49, XY, +X, t(1;6)(p36;q22), t(8;14)(q24;q13), -13, +19, +21, +22	34	male	10389925
FM-8321-33-1	44-45, X, -X, t(1;8)(q22;q24), del(6)(q15q23), -9, t(11;14)(q13;q32), -13, -21, +3mar / 44-45, idem, der(1)	63	female	10602418
FM-8576-8-1	42-46, XY, t(8;14)(q24;q23)	NA	male	10718214
FM-8597-32-1	46-48, XY, +del(X)(q26), +2, der(8)?t(4;8)(p11;q24), t(14;18)(q32;q21)	NA	male	10862046
FM-871-14-1	46, X, ?t(X;8)(q22;q24)	72	female	6850608
FM-871-73-1	82, XXX, -X, del(1)(p22), ?del(2)(q32), t(2;4)(q32;q35), -3, t(3;6)(p25;q21), -4, -5, t(5;6)(q15;q27), del(6)(q21), -8, ?t(8;15)(q24;q24), -9, -10, -11, -12, del(12)(p11), -14, +t(14;18)(q32;q21), -16, -17, -18, -19, -22, +3mar	65	female	6850608
FM-9035-1-1	43-44, X, -Y, add(3)(p11), t(8;9)(q24;q13), der(11)t(11;14)(q13;q32), der(14)t(9;14)(q13;q32)ins(14;?)(q32;?), der(17)t(3;17)(q13;p11)	58	male	11064480
FM-9035-4-1	45, XY, dic(8;9)(q24;p24), -9, t(11;14)(q13;q32)	56	male	11064480
FM-9084-14-1	48, XY, del(3)(q13q24), +7, ins(9;8)(p12;q12q24), +12, t(14;18)(q32;q21)	NA	male	11243387
FM-9084-16-1	46, X, t(X;14)(p12;q32), t(1;5;6)(p35;p15;p21), t(8;13)(q24;q34), -13, der(14)t(14;18)(q32;q21), der(17)t(13;17)(q14;p11)	NA	female	11243387
FM-9225-27-1	45, X, -X, t(1;4;3)(q23;q23;p25), del(6)(q16q27), del(8)(p13), der(8)t(8;9)(q24;p21), t(11;14)(q13;q32), del(13)(q?) / 43, idem, der(7)t(7;11)(p15;q14), t(11;14), -9, -13	66	female	11579465
FM-9393-11-1	50, XY, +X, der(1)t(1;2)(q32;q23), +der(3)t(3;8)(q27;q24), t(3;14)(q27;q32), del(6)(q21q27), +9, +dup(18)(p11p11), der(22)t(1;22)(q32;q11)	NA	male	11850073
FM-9442-2173-1	46, XX, t(1;14)(q21;q32), t(8;9)(q24;q13)	44	female	11753646
FM-9541-11-1	55, XY, del(3)(q26q27), +der(3)t(1;3)(q21;q27), t(3;14;18)(q27;q32;q21), +5, +6, t(8;9)(q24;p12), +11, +12, +13, +16, der(17)t(1;17)(q21;p13), +20	NA	male	11920179
FM-9541-12-1	50, XX, add(3)(q27), +del(7)(q21), t(7;8)(q33;q24), +12, +13, t(14;18)(q32;q21), +18	NA	female	11920179
FM-9737-11-1	47, XY, del(2)(q21q31), t(3;22)(q27;q11), del(6)(q13q15), der(8)t(2;8)(q21;q24), +11, der(20)t(1;20)(q21;q13)	NA	male	12378526
FM-9737-16-1	46, Y, t(X;9)(q23;p24), t(1;8)(q21;q24), add(5)(p15), add(7)(p22), t(11;14)(q13;q32), add(12)(p11), i(17)(q10), -18, +mar	NA	male	12378526
FM-9737-24-1	92-94, XXYY, der(1)t(1;1)(q21;q?)x2, der(1)t(1;8)(q21;q24)x2, t(3;22)(q27;q11)x2, -6, add(8)(q24), +add(8), der(8)t(1;8)(q?;q24)x3, dup(12)(q12q22)x2, +13, -15, i(17)(q10), +r, +mar	NA	male	12378526

Mitelman ID	Karyotype	Age	Gender	PMID
FM-9749-1-1	74, X, -X, -X, der(1)t(X;1)(q13;p13), der(1)t(1;8)(p11;q24)t(8;17)(q22;q25), der(3)t(2;3)(q31;q27), del(6)(q21q25), der(7)t(7;10)(p22;q22), der(8)t(2;8)(q31;q24), der(9)ins(9;2)(q34;q35q31), der(10)t(10;22)(p11;q11), der(12)ins(12;1)(p11;q32q11), i(12)(p10), der(17)t(3;17)(q13;p11), der(19)t(19;21)(p13;q11)t(21;21)(q22;q11)t(21;22)(q22;q13), der(21)t(12;21)(p11;p11-13), +2mar	60	female	11721971
FM-9882-1-1	45, XY, dic(8;9)(q24;p24), -9, t(11;14)(q13;q32)	55	male	12153165
FM-9882-4-1	42-45, XY, add(2)(p25), del(6)(q?21), ins(8;?)(q24;?), del(9)(p22), t(11;14)(q13;q32), del(12)(q15), -14, -15, add(16)(q24)	53	male	12153165
FM-9882-5-1	43-44, XY, add(3)(p11), t(8;9)(q24;q13), der(11)t(11;14)(q13;q32), der(14)t(9;14)(q13;q32)ins(14;?)(q32;?), -15, der(17)t(3;17)(q13;p11), -21, +1-2mar	61	male	12153165

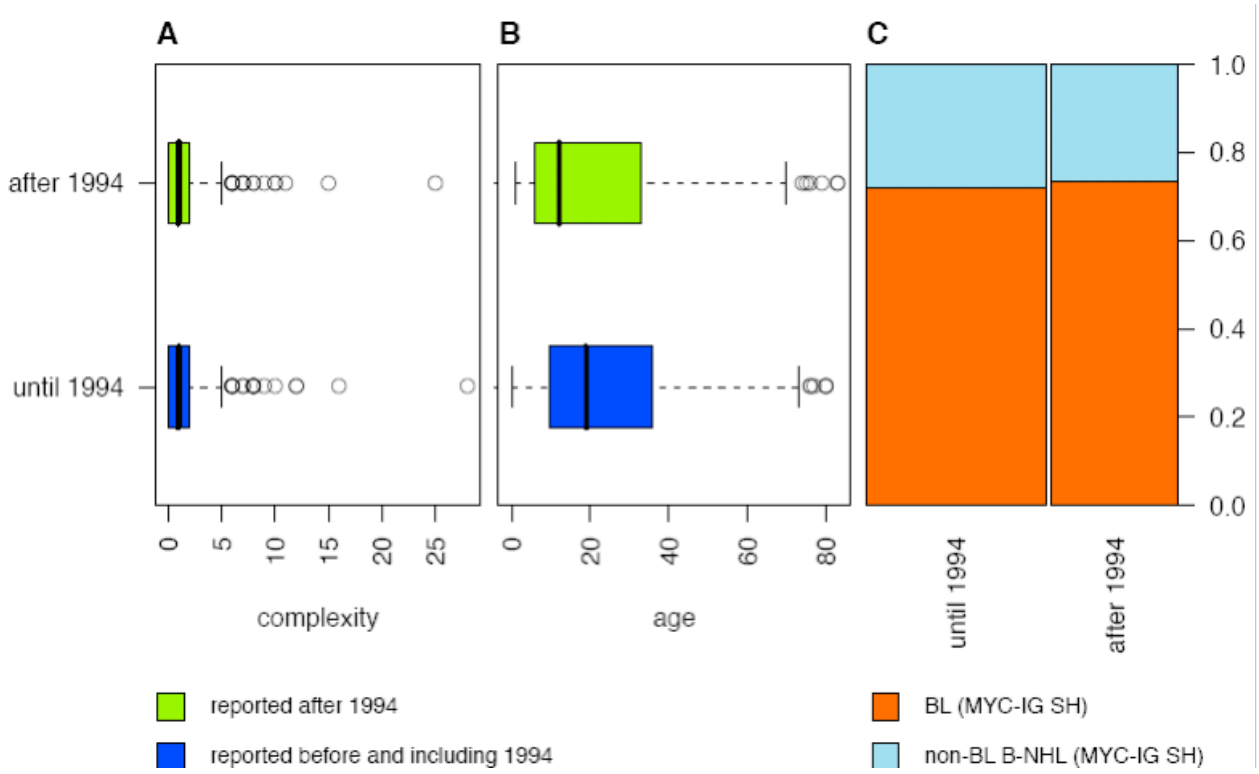
FIGURES

Supplementary Figure 1: Complexity score validation



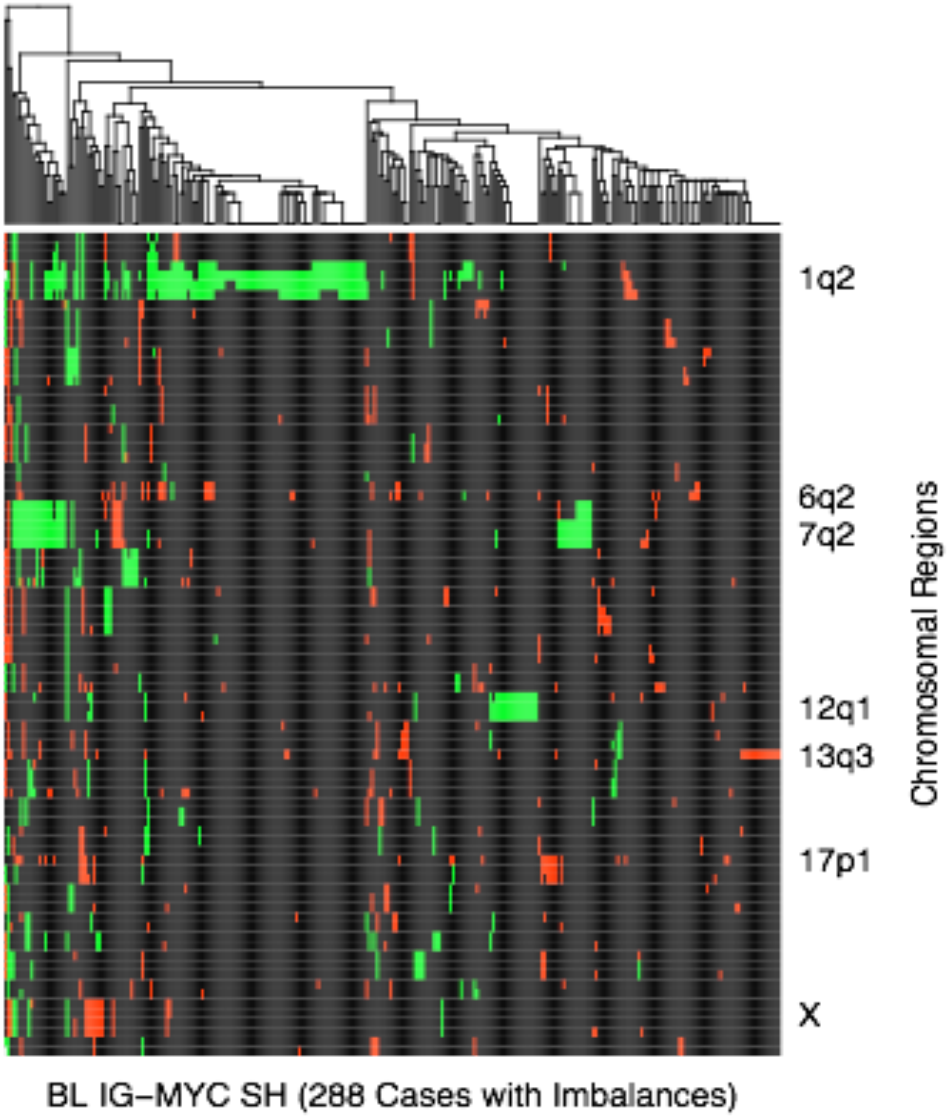
These figures show the distribution of the genomic complexity score (see main text) in comparison to the number of chromosomes (top) or bands (out of 862; bottom) with imbalances per case. Overall, linear relations between the different scores can be observed for cytogenetic data.

Supplementary Figure 2: No essential differences in karyotypic complexity between core BL-SH cases published before and after 1994.



Panel A shows the complexity for IG-MYC BL-SH cases published before (N=280) and after 1994 (N=201). The year 1994 was chosen because of the publication of the REAL classification. Panel B shows the age distribution for both series with a comparable overall age distribution but a higher number of young children in the later interval (indicated by the down-shifted median). Panel C shows that within the total group of lymphomas with an IG-MYC breakpoint and without a breakpoint at 18q21, 3q27 or 11q13, no quantitative diagnostic shift had occurred between both intervals.

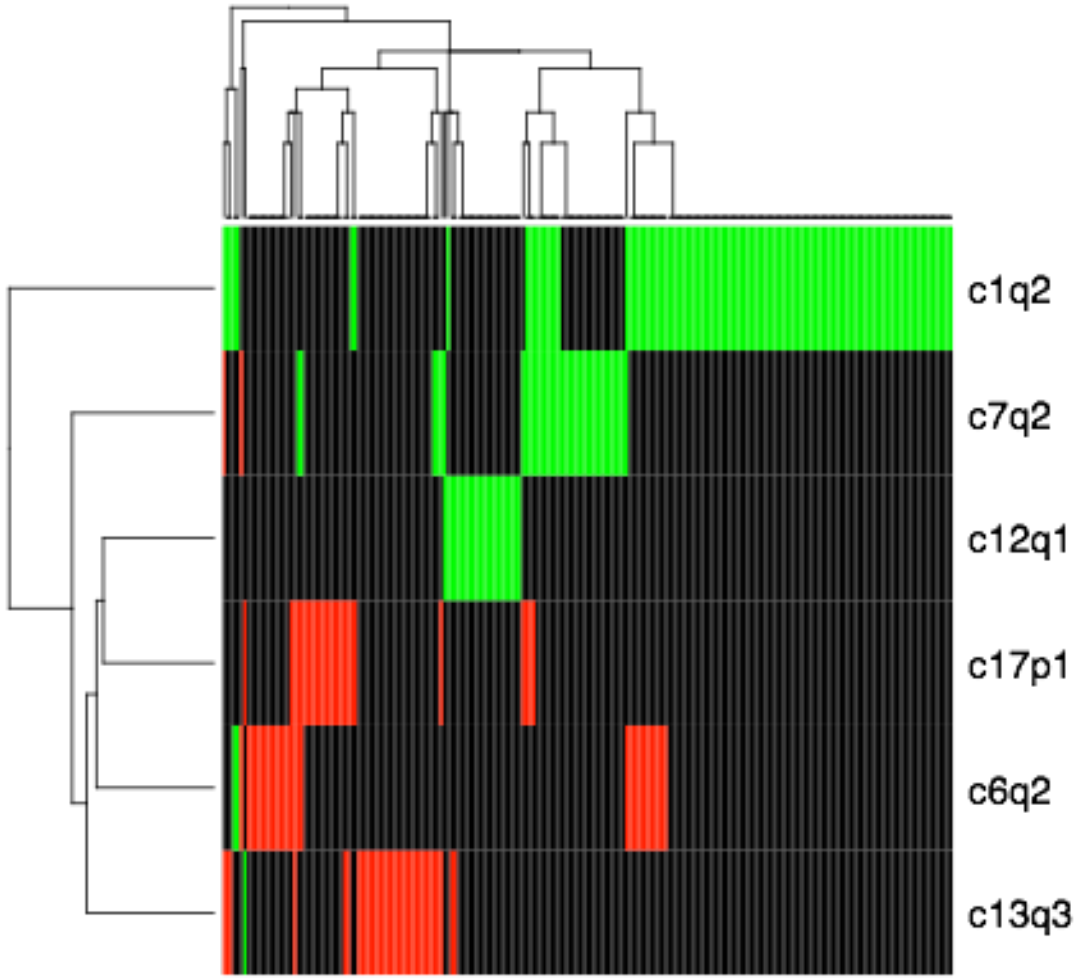
Supplementary Figure 3: Imbalance pattern in BL IG-MYC SH cases



For an ordered visualization of case specific gains and losses, genomic imbalances were clustered for similarity of their aberration pattern (conf. figure 5). Here, 288 core subset cases (BL IG-MYC SH) with imbalances are shown. The structure of the heatmap is defined through the recurring, mostly singular events (note gains on 1q) and a scattering of events with low recurrence.

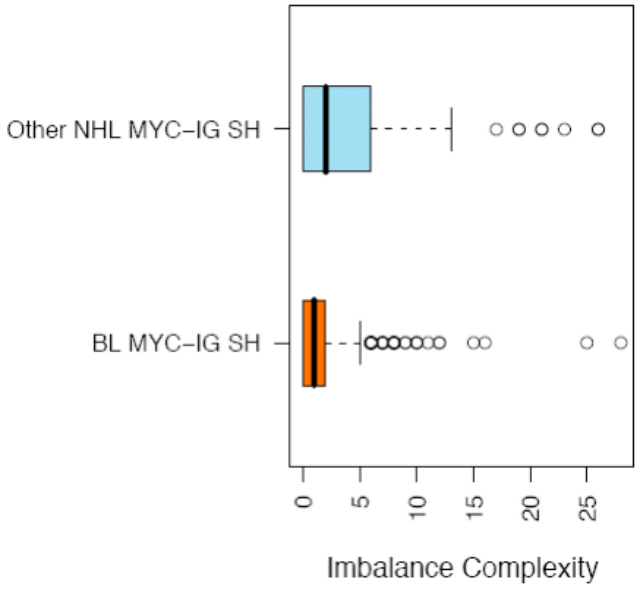
Chromosomal regions run from 1pter (top) to Yqter (bottom), with some regions highlighted due to frequent involvement in BL IG-MYC SH (and X for guidance). Green = gain, red = loss.

Supplementary Figure 4: Relation of recurring imbalances in BL IG-MYC SH cases



To better determine possible relationships of the most frequent imbalances, only cases from the BL IG-MYC SH subset which had at least one unambiguous occurrence of one of the six most frequent imbalances were selected, resulting in 205 out of 481 cases. Only few overlapping occurrences could be observed, to the point of near exclusiveness.

Supplementary Figure 5: Imbalance complexity in all SH lymphomas with an IG-MYC breakpoint.



To determine whether BL and other lymphomas that harbour a IG-MYC breakpoint but no breakpoints at 3q27, 11q13 or 18q21 are different, the imbalance complexity was analyzed. BL MYC-IG SH cases have an apparent lower complexity in their genomic imbalances.

TECHNICAL NOTES

Conversion of karyotype data

Karyotype data was converted from the ISCN conform annotation acquired from the Mitelman database, as described previously (Baudis, Biotechniques 2006). By convention, the overall genomic dosage per genomic interval was used as endpoint. A combination of chromosomal aberrations resulting in a loss and a gain of the same segment. As example, a karyotype containing "+i(7)(q10)" would result in a gain status for 7q (4 copies), and a balanced status for 7p. Genomic status data was assigned to a virtual high-resolution karyotype consisting of 862 bands mapped to the genome "Golden Path". For some downstream analyses, the data was re-mapped to lower resolution matrices, with intervals containing both copy number gains and losses being assigned a "NA" value.

In contrast to CGH methods, karyotype annotations may contain ambiguous elements and therefore not always be completely resolved. Examples would be marker chromosomes ("mar"), incomplete karyotypes ("inc") or structural changes in which not all components are defined ("add", "t(1;?)(q21;?"). In a small number of instances, the karyotype parser may fail an annotation due to the lack of training for this particular instance. However, the technical parser limitations are considered small compared to the overall fidelity of banding analysis.

For the purpose of this study, abnormalities used for group assignment (translocations involving the *MYC*, *BCL6*, *CCND1*, *BCL2*) were verified by manual karyotype inspection.

Reference:

Baudis, M. Online database and bioinformatics toolbox to support data mining in cancer cytogenetics. BioTechniques (2006) vol. 40 (3) pp. 269-70, 272