Role of MYC in pediatric and adult B-cell lymphoma patients

Aukema, Sietse Marten

9

Summary, general discussion
& future perspectives

CHAPTER 9: SUMMARY OF INDIVIDUAL CHAPTERS

In chapters**1** and **2** I briefly introduced the basic diagnostic and clinical aspects of mature B cell lymphomas (chapter **1**) and the classical cytogenetic features of follicular lymphoma, Burkitt lymphoma and diffuse large B cell lymphoma (DLBCL), as well as of rare lymphomas that have intermediate features of both these lymphomas (chapter **2**).

In chapter **3** we analyzed by conventional cytogenetics (karyotyping) paired Burkitt lymphoma samples taken at initial diagnosis and at relapse(s) in order to investigate if and what chromosomal aberrations occur in patients suffering from relapse. At initial diagnosis Burkitt lymphoma karyotypes showed a low complexity comparable to Burkitt lymphomas not showing later relapse, while the karyotypes from the corresponding relapses showed an (sometimes dramatic) increase in karyotype complexity with a high frequency of recurrent secondary aberrations. This increase in karyotype complexity was likely a consequence of clonal heterogeneity already present at initial diagnosis and subsequent clonal selection and evolution (and not chemotherapy induced) as (i) the secondary aberrations observed at relapse have been described to occur (although at a much lower frequency) also in primary Burkitt lymphoma samples and (ii) the observed clonal evolution pattern in relapsing Burkitt lymphoma patients in our study paralleled that seen in Burkitt lymphoma cell lines. This, for Burkitt lymphoma unusual, high karyotype complexity and/or particular chromosomal changes involved herein likely contribute to the very poor prognosis of Burkitt lymphoma at relapse.

Chapter **4:** In this chapter we analyzed the clinico-pathologic characteristics of a subset of mostly pediatric / adolescent Burkitt lymphomas with (aberrant) BCL2 expression. We observed variable BCL2 expression in 23% of lymphomas that were (conventionally) diagnosed as Burkitt lymphoma on basis of histology, immunohistochemistry and FISH. BCL2 protein expression varied both quantitatively (percentage of tumor cells) and qualitatively (intensity of staining), but was except for two cases never homogeneously strong. All cases had a *MYC* break but no *BCL2* or *BCL6* break upon FISH analysis. Using 'digital multiplex gene expression profiling' with a modified Burkitt-lymphoma classifier, 13 of 17 BCL2 expressing cases showed a mBL profile and 4 an intermediate BL profile, but none showed a non-BL profile. Interestingly, no clinical differences were seen between the two subgroups and in a homogeneously treated group of pediatric patients no differences in outcome were observed. This indicates that in contrast to DLBCL,

double expression of MYC and BCL2 in Burkitt lymphoma has no clinical implications, at least not in pediatric / adolescent patients, mostly tested in this series.

Chapter **5:** This chapter focused on mutational analysis in Burkitt lymphoma. By whole-genome sequencing we identified in an explorative cohort of 17 pediatric Burkitt lymphomas, additional to the already identified mutations affecting such as *ID3*, *TCF3 and CCND3* recurrent *PCBP1* mutations in 3 cases (18%). We subsequently confirmed this by Sanger sequencing in 3/28 (11%) cases of an independent mixed pediatric/adult validation cohort. The *PCBP1* mutations were identified in both pediatric and adult Burkitt lymphoma patients and predominantly affected the KH III domain of the protein either resulting in complete domain loss or amino acid changes. The mutations could hereby lead to a reduced or even loss-of-function of the protein and interfere with the normal functions including nuclear trafficking and pre-mRNA splicing. Remarkably all six Burkitt lymphoma cases with a *PCBP1* mutation were IRF4/MUM1 positive by immunohistochemistry in contrast to only 12/32 (39%) of the *PCBP1*-wild type cases.

Chapter **6**: Here we explored the cytogenetic and clinico-pathologic aspects of double-hit lymphomas (DHL). By reviewing the published cytogenetic literature and exploration and analysis of 689 *MYC*+ lymphomas included in the Mitelman database we identified both previously known (*BCL2*+/*MYC*+, *BCL6*+/*MYC*+ and *BCL2*+/*BCL6*+/*MYC*+) as well novel DHL combinations (*9p/MYC*+, *BCL3*+/*MYC*+, *CCND1*+/*MYC*+) and established a revised definition and concept of DHL with DHL defined as a *MYC* breakpoint in combination with another recurrent chromosomal breakpoint. In addition we reviewed and outlined the individual oncogenes involved in DHL as well as the timing of their rearrangement in oncogenesis as well as their possible functional synergy in DHL. Finally, we made an in-depth review of the pathologic as well as clinical characteristics highlighting their frequent presentation with BM and especially CNS involvement in 10-50% of the cases.

Chapter **7**: In this chapter we analyzed the clinico-pathologic and molecular characteristics of 80 *MYC*-rearranged non-Burkitt lymphomas (as defined by gene expression profiling, non-mBL) including 33 *MYC* single-hit non-Burkitt lymphomas (*MYC* SHL non-BL) and 47 double-hit lymphomas (DHL). We compared *MYC* SHL non-BL with DHL and made as well subset analyses of the *BCL2*+/*MYC*+, *BCL6*+/*MYC*+ and *BCL2*+/*BCL6*+/*MYC*+ DHL / triple hit lymphoma. We showed that *MYC* SHL non-BL have, as assessed by array-CGH, a chromosomal

complexity comparable to *MYC* DHL and have a comparable poor outcome as well. This suggests that in *MYC* SHL non-BL cases chromosomal translocations other than *BCL2* and/or *BCL6* (e.g. *PAX5*) and other chromosomal aberrations (e.g. genomic gains and losses) are involved. In the subset analysis of DHL *BCL2*+/*MYC*+ more often showed a GCB-like gene expression profile while that in *BCL6*+/*MYC*+ was more often ABC-like or unclassifiable. The *BCL2*+/*BCL6*+/*MYC*+ showed more similarities with *BCL2*+/*MYC*+ than *BCL6*+/*MYC*+ DHL. Most importantly, this strongly underlines the importance to distinguish Burkitt lymphoma that is always *MYC* single-hit from *MYC*-SHL non-Burkitt lymphomas, and indicates that in case of doubt between Burkitt lymphoma and non-Burkitt lymphoma array-CGH (or similar method) and/or gene expression profiling should be performed.

Chapter **8**: In this chapter we studied MYC expression in 25 cases of transformed follicular lymphomas (tFL) in relation to *MYC*-rearrangements and the *MYC* partner and compared these features with those of the original untransformed FL. We showed that MYC is frequently but not always up-regulated in tFL. In most-high-expressors *MYC*-rearrangements were detected (9/22 cases, 41%), the highest expression being found in the 4 cases with IG-*MYC* juxtaposition. Interestingly, although the numbers are small, it seems that non-IGH partners (including IGL, IGK, *PAX5* and *BCL6*) are frequent in these lymphomas.

CHAPTER 9: GENERAL DISCUSSION AND PERSPECTIVES

I. Technical approaches for diagnosing aggressive *MYC*-R lymphomas: *Should all DLBCL and DLBCL-BL be screened for MYC-R?* At present *MYC*-FISH is not routinely performed on all newly diagnosed DLBCL, also not in most (tertiary) centers. Many laboratories apply FISH first on selected cases with a (presumed) higher likelihood of having a *MYC*-R, e.g. those with a GCB-like 'cell-of-origin' (COO) defined with immunohistochemistry by the Hans-algorythm[1] and/or gene expression profiling,[2-4] high MYC by IHC,[5,6] DLBCL with immunoblastic morphology[7,8] or lymphomas with DLBCL-BL intermediate morphology.[9-11] Ki67 is not a reliable marker to screen for *MYC*-R.[2-4,12,13] In fact the same is true for immunohistochemically staining for MYC protein (MYC IHC) as the percentage of positive staining cells is highly variable in *MYC*-rearranged DLBCL, much more heterogeneous than in Burkitt lymphoma, in which usually >80% of the tumor cells are positive.[14] This means that a very low threshold level of as low as 20% positive cells should be used to select cases with a *MYC* breakpoint.[15] Moreover, estimating the percentage of MYC positive cells the subject of inter-observer variability.[15,16]

Importantly, MYC IHC cannot replace *MYC* FISH as *MYC*-R positive cases with high MYC expression still have worse outcome compared to *MYC*-not rearranged cases with a comparable high MYC expression. This also suggests that the poor outcome cannot be explained by MYC expression alone.[17,18] It therefore remains recommended to screen all DLBCL, irrespective of immunophenotype or morphological variant, as well as all DLBCL-BL intermediates for *MYC*-rearrangements. Finally, it should be emphasized that conventional cytogenetics is still the only available routine diagnostic technique providing information about *MYC* breakpoints and the *MYC* partner (IG- and non-IG), in addition giving a genome wide overview on other numerical and structural aberrations, e.g. 18q21/*BCL2*, 3q27/*BCL6* and/or 19q13/*BCL3* and many others.[19] Moreover, if necessary, FISH can be applied (e.g. to detect cryptic rearrangements) on these chromosomal preparations and interphase nuclei as well. See Table 1 for an overview of different diagnostic tools to assess MYC expression and aberrations.

In general it is advised to screen all DLBCL cases with a *MYC* break-apart (BAP) FISH test. If desired this could be complemented with dual color dual fusion assay (DCDF) as *MYC* rearrangement due to insertions of IGH (and IGK or IGL) in *MYC* or vice versa might be missed in up to 10% of cases[4] using a *MYC* BAP only. As shown in chapters 6 and 7, non-molecular-BL lymphomas with a *MYC*

breakpoint frequently have a non-*IG* partner and also have frequently an IGK or IGL in case of an IG partner. As IGK-*MYC* and IGL-*MYC* dual color dual fusion probes are not commercially available (only IGK and IGL break apart) this hinders a further differentiation in lymphomas that have a *MYC* break but no IGH-*MYC* co-localization (see below and Fig 2). It should be realized that using IGK and IGL BAP probes as surrogate for IGK-*MYC* and *MYC*-IGL DCDF probes[20] is inaccurate and may lead to false positive results (Fig. 1).[7,21]

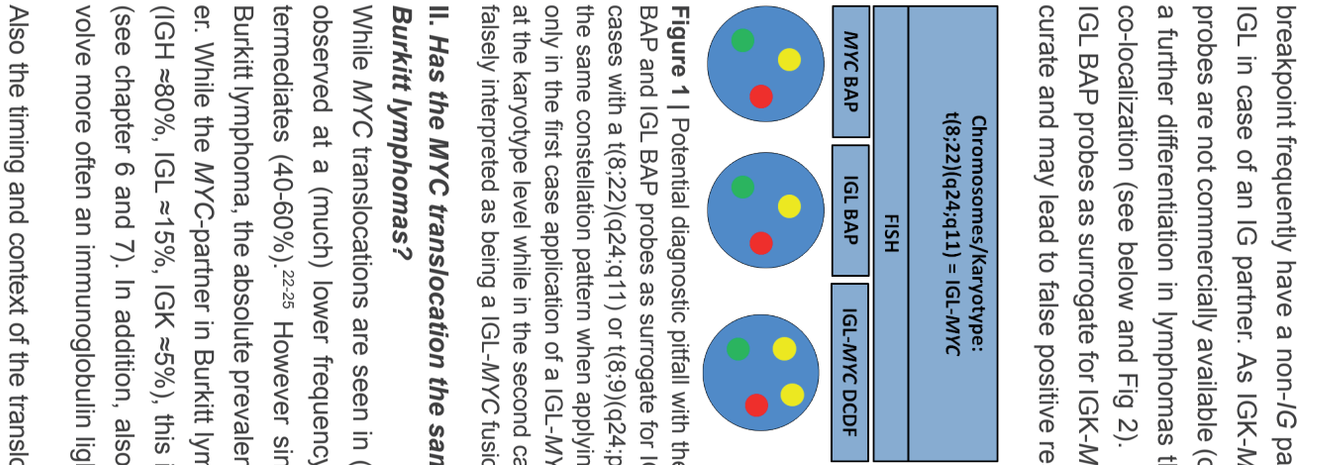

Figure 1 | Potential diagnostic pitfall with the application and interpretation of using *MYC* BAP and IGL BAP probes as surrogate for IGL-*MYC* dual-color-dual-fusion probes. Both cases with a t(8;22)(q24;q11) or t(8;9)(q24;p13) & t(3;22)(q27;q11) in the karyotype have the same constellation pattern when applying *MYC* and *IGL* BAP probes only. However, only in the first case application of a IGL-*MYC* DCDF probe will show the 'true' situation at the karyotype level while in the second case *PAX5-MYC* and IGL-*BCL6* fusions are falsely interpreted as being a IGL-*MYC* fusion.

II. *Has the MYC translocation the same role in Burkitt and MYC-R non-Burkitt lymphomas?*

While *MYC* translocations are seen in (virtually) all Burkitt lymphomas, they are observed at a (much) lower frequency in DLBCL (5-15%) and DLBCL-BL intermediates (40-60%).[22-25] However since DLBCL is much more frequent than Burkitt lymphoma, the absolute prevalence of *MYC* breakpoints in DLBCL is higher. While the *MYC*-partner in Burkitt lymphoma is by definition one of the IG loci (IGH ≈80%, IGL ≈15%, IGK ≈5%), this is more often a non-IG partner in non-BL (see chapter 6 and 7). In addition, also when having an IG-partner this may involve more often an immunoglobulin light chain locus (IGL, IGK) in non-BL.[22,26-30]

Also the timing and context of the translocation differ: while the IG-*MYC* translocation in Burkitt lymphoma is considered to be a primary, disease initiating event occurring in the context of a low genomic complexity (and in ≈40% of the cases being the only cytogenetic abnormality[27,31-33]), it is a secondary event occurring in the context of other imbalances and translocations in non-Burkitt lymphomas (Fig. 2).[14,34-37]

Tabel 1 | key diagnostic tools for the pathologist to analyze MYC expression and chromosomal aberrations

Technology	Karyotyping	M-FISH	FISH	Array-CGH / SNP-array /Oncoscan	IHC	Quantitative real-time-PCR	Nanostring / DMGE
material input	Fresh material, cell suspension (PB, LN, BM, Ascites, PE)	Fresh material (good quality), cell suspension (PB, LN, BM, Ascites, PE)	Fresh material (cell suspension), FFPE	DNA from fresh material (SNP-array, array-CGH), FFPE (low input) (Oncoscan)	FFPE	mRNA from fresh material, FFPE	mRNA from fresh material, FFPE
analysis	Genome wide	Genome wide	Targeted	Genome wide	Targeted	Targeted (multiplex)	Targeted (panels)
(un)balanced translocations	Yes (might miss cryptic rearrangements)	Yes	Yes (only for the applied assay)	No	No	No	In principle possible
gains/losses	Yes	Yes	Yes (only for the applied assay)	Yes	No	No	In principle possible
overexpression	No	No	No	No	Yes (protein)	Yes (mRNA)	Yes (mRNA)
costs, handling	High, labor intensive, specialized laboratory and staff needed.	High, labor intensive, specialized laboratory and staff needed.	Relatively short turnaround time. Applicable in many laboratories.	High costs	Applicable in all laboratories. Low-costs, quick turnaround time. Interobserver variability in assessment of MYC staining	Low cost, easy handling, quick turn-around-time. Mutations and translocations may interfere with detection of MYC in commercially available assays[132]	Relatively low costs.
Detection of subclonal aberrations	Yes	Yes	Yes	No (>30%)	Yes	No	No

Abbreviations: PB, peripheral blood ; LN, lymph node ; BM, bone marrow ; PE, pleural effusion ; FFPE, formalix fixed paraffin embedded ; DMGE, digital multiplex gene expression

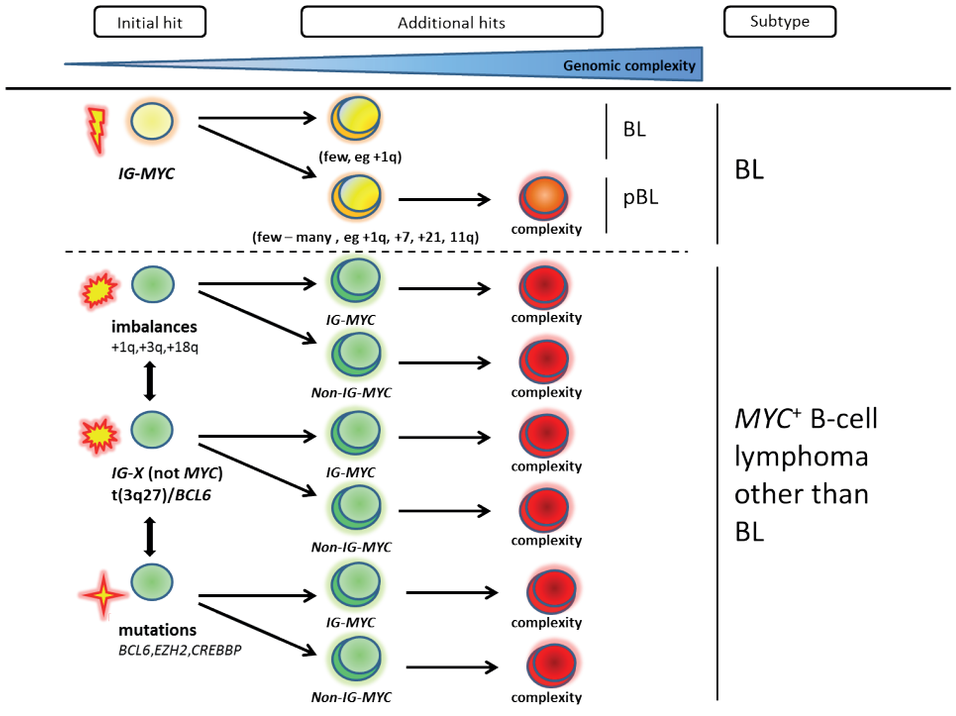

Figure 2 | Genetic model for *MYC* induced lymphomagenesis in Burkitt lymphoma (BL) and *MYC*-positive lymphomas other than BL. In BL, the IG-*MYC* translocation is an initial event and is accompanied by no or few secondary genomic aberrations resulting in an overall low genomic complexity. On the other hand, in other *MYC* positive lymphomas the IG-*MYC* or often non-IG-*MYC* translocation is a secondary event often involved in disease progression or high-grade transformation. The initial hits in these lymphomas consist of IG-translocations involving oncogenes other than *MYC* (IG-X), genomic imbalances, mutations or more likely a combination of these. So here, the *MYC* translocation occurs in the context of a much more complex genomic background as is the case in BL and results in a much higher genomic complexity. pBL indicates progressive Bukitt lymphoma. Figure from Aukema SM & Siebert R *MYC*+ lymphomas other than Burkitt lymphoma, Hematology Education: the education program for the annual congress of the European Hematology Association 2012;6(1):65-74. With permission.

In addition, the mutational context might be important for the role of the *MYC* translocation with in Burkitt lymphoma (but not or very rarely in non-BL) frequent *ID3*/*TCF3* and *CCND3* mutations and a strong dependence on PI3K signaling.[7,38-44] In contrast, in DLBCL a large number of heterogeneous mutations can be observed, also in *MYC*-rearranged cases.[45-51]

Although adult Burkitt lymphoma may have overall an inferior prognosis compared to pediatric Burkitt lymphoma, also in adults very high survival rates of 90-

100% can be achieved in limited disease stage (and CNS negative) patients.[52,53] This is in striking contrast to *MYC*-rearranged non-BL where also 'single-hit lymphomas' (so with *MYC* rearrangement but, as in Burkitt lymphoma, without *BCL2* and *BCL6* rearrangements) have a poor outcome.[7] Thus, while being both 'single-hit lymphoma' they have a clinically strikingly different behavior.

What could explain the dramatic difference in clinical outcome between adult MYC-R (single-hit) non-Burkitt lymphoma (DLBCL, DLBCL/BL intermediate) and Burkitt lymphoma?

Several biological differences between Burkitt lymphoma and single-hit non-BL could contribute to this (Table-2). (i) In SHL non-Burkitt lymphoma, BCL2 expression can be observed in 40~80% of the cases, which is considerably higher than in Burkitt lymphoma where (weak) BCL2 expression can be seen in up to 20-25% of cases.[7,21,54-56] In addition, while (nearly) all Burkitt lymphoma have a GCB-like or unclassifiable cell of origin gene expression profile, *MYC* SHL non-BL only have a 'favorable' GCB-like (or unclassifiable) cell-of-origin in 50-70% of cases.[7,21,56]

Although many prognostic markers may have prognostic impact only in individual lymphoma subtypes, karyotype/genomic complexity has been shown to be an adverse prognostic across many lymphoma entities.[23] Indeed, genomic complexity (as assessed by array-CGH) is significantly higher but also more diverse in non-Burkitt SHL compared to Burkitt lymphoma.[7] Thus, *MYC* SHL non-BL may harbor additional chromosomal translocations including *PAX5* and IGH breaks, often with unidentified (oncogene) partners.[7] For a comparison of biological characteristics between Burkitt lymphoma and *MYC* SHL non-BL, see Table 2.

III. Clinical approaches for pediatric and adult Burkitt lymphoma

At initial diagnosis pediatric Burkitt lymphoma and also pediatric DLBCL have an excellent prognosis when treated with appropriate high intensity and short duration poly-chemotherapy.[33,57-64] On the other hand, the prognosis in adult Burkitt lymphoma is considerably worse compared to pediatric Burkitt lymphoma. Several biological and clinical factors, alone or combined, could contribute to this[65] which will be point-by-point discussed below: **(i)** biologically differences between adult and pediatric Burkitt lymphoma, **(ii)** inproper inclusion of non-BL lymphomas, in particular SH DLBCL and 'double-hit' lymphomas in adults, and **(iii)** increased treatment toxicity and decreased treatment intensity in adults.

Table 2 | basic clinico-pathologic and genetic aspects of Burkitt lymphoma and *MYC* SHL non-BL

	Burkitt lymphoma (% of cases affected)	*MYC* SHL non-BL (% affected). All data from[7]
Age at diagnosis	Bi-modal age distribution: peaks at 5-10 years (pediatric) [27,73,133-135] and 35-45 (adult)[25,71,126]	57 years
M:F	Pediatric age group: male predominance: 2-10:1 [27,58,73,133-136] Adults: no/slight male predominance 1-3:1 [25,57,71,126,129]	1:1
Prognosis	(very) favorable	poor
MYC-IG-partner	100[2]	71
Key other chr. aberrations	+1q, +7q/+7, 11q, 17p-, + 21	+1q , +3q27, -6q, +7p/q, 9p-, 17p-,+18q21
BCL2 expression (% of cases)	0-20[30,55,106,133-137]	77
ID3 Mutations (% of cases)	≈35-70[40,42-44]	12
CD10 (% of cases)	95-100[30,55,106,133-140]	66
BCL6 (% of cases)	95-100[30,106,133-137,139,140]	72
MUM1/IRF4 (% of cases)	20-40[135,136,139-142]	54
Cell-of-origin (GEP) (% of cases)	GCB (91), unclassified (9)[106]	GCB (52%), unclassified (19), ABC (29)
Genomic complexity	MYC simple : 29/34 (85) [106] MYC complex : 5/34 (15)	MYC simple : 7/21 (33) MYC complex : 14/21 (66)

(i) Are there any biologically differences between adult and pediatric Burkitt lymphoma?

The topic of possible biological differences between adult and pediatric Burkitt lymphoma has been subject of many studies. Overall most studies (using conventional cytogenetics, comparative genomic hybridization and SNP-arrays) have not found any gross differences between both.[27,58,66-68] This is in contrast to pediatric and adult DLBCL where striking differences are observed in, amongst others, chromosomal translocations, COO and genomic complexity.[69-71] Only one recent study found several gains and losses enriched in or even specific for adult versus pediatric Burkitt lymphoma.[72] Moreover, these authors found *ID3* mutations in all adult Burkitt lymphoma, a mutation frequency different to those found in other studies.[40,42,44] Finally there may be other differences related to lymphomagenesis: for instance pediatric Burkitt lymphomas more frequently present in the gastro-intestinal tract[73] and have a lower rate of EBV infection compared to adults.[74]

(ii) Inproper inclusion of non-BL lymphomas, e.g. 'double-hit' lymphomas

The criteria for the diagnosis of Burkitt lymphoma and the inclusion in clinical studies have been subject to changes over time. In addition, many studies did not have adequate (molecular) cytogenetic workup and, due to the relative rarity of adult Burkitt lymphoma, many studies were conducted over a long time and were retrospective in nature. So studies focusing on adult 'Burkitt lymphoma' might in fact contain also aggressive non-Burkitt lymphomas[75-78] which in the light of today's knowledge represent very poor outcome double-hit or *MYC* SHL non-(molecular)-BL (see above) lymphomas with a high genomic complexity.[27,67] Indeed, among the 203 *BCL2*+/*MYC*+ DHL identified in the Mitelman database 30 (15%) were classified as Burkitt lymphoma/leukemia (chapter 5).[22]

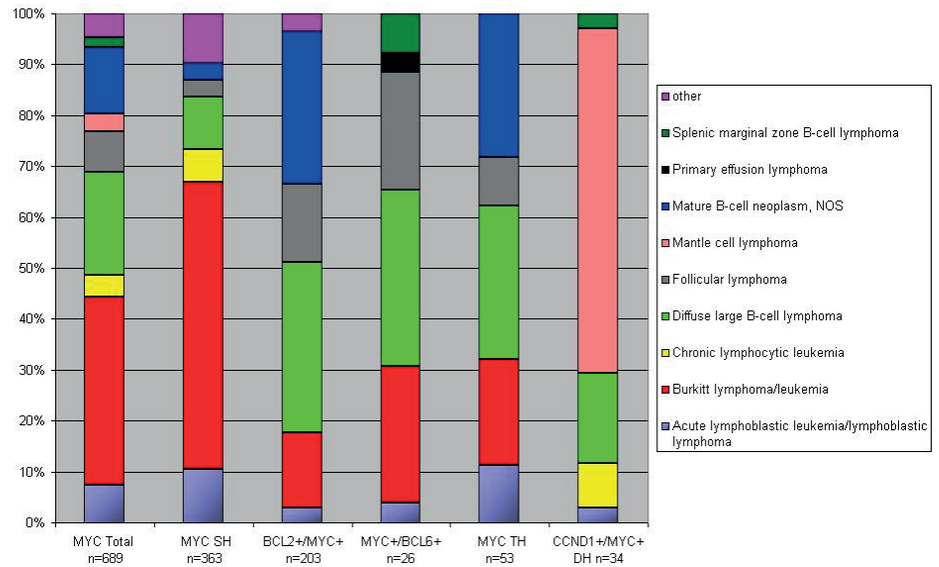

Figure 3 | Distribution of morphologies according to breakpoints. 30/203 (15%) of *BCL2+/MYC+* DHL have diagnosis of Burkitt lymphoma/leukemia

For the establishment of a solid diagnosis of Burkitt lymphoma, and to exclude lymphomas that mimic Burkitt lymphoma such as double-hit lymphomas in adult patients, it is recommended to perform:

(i) IHC for CD10, CD20, CD79a, CD3, BCL2, MUM1/IRF4, Ki67/MIB1 and TdT, and (ii) Conventional cytogenetics (bone marrow, peripheral blood, and also lymph nodal samples) and / or a FISH panel (for the detecting of cryptic rearrangements) with *MYC* BAP, IGH-*MYC* (and if available also IGK-*MYC* and IGL-*MYC*),

BCL2 BAP and *BCL6* BAP, and in case of doubt also
(iii) Dedicated gene expression profiling with Burkitt (and COO) classifier.[55,79,80]
Finally in some cases
(iv) Array-CGH or Oncoscan analysis to detect chromosomal imbalances[7] or mutational analyses for *ID3*/*TCF3*, *CCND3* and other genes could be performed.[7,40,43,81]

On the other hand, in pediatric Burkitt lymphoma studies and trials DLBCL are frequently included. However, this does not seem to impact survival as pediatric DLBCL only very rarely or never comprises DHL (due to the virtual absence of *BCL2* translocations and only very rare *BCL6* translocations)[69] and has, in contrast to the situation in adults, a prognosis very similar to Burkitt lymphoma.[58,61-64,82] with the exception that differences in relapse patterns are seen with DLBCL showing later relapses than Burkitt lymphoma.[59,62]

(iii/iv) increased treatment toxicity and decreased treatment intensity.
For (older) adults treatment toxicity and decreased treatment intensity are a major problem leading to a high percentage of treatment related morbidity and mortality.[65,83] In addition, the group of adult Burkitt lymphoma includes all patients age >16 or >18 and consequently the mean or median age may widely vary across the conducted studies. Although the cut-off between 'younger' and 'older/elderly' adults may vary between studies, a common finding is that adults over >55 years do (significantly) worse compared to the 'younger' adults,[53,76-78] partly due to a lower likelihood of achieving complete remission.[77,78] Interestingly, also already within the pediatric population age might be a factor with children having lower cumulative progression and higher survival rates compared to adolescents[60] but data on this are not uniform.[82,84]

(Management of) Burkitt lymphoma patients at disease progression or relapse
In Burkitt lymphoma virtually all relapses occur within one year after diagnosis[33,57,59,85,86] with (very) late relapses mostly representing new de novo tumors rather than 'true' relapses.[87-90] Relapses are both from a biological and clinical perspective challenging as these patients have a very poor survival rate of only 10-30%.[91] The clinical challenges include that (as Burkitt lymphoma follows a linear clonal evolution pattern) tumors often are already exposed to and resistant to multiple lines and classes of chemotherapy. We recently showed that Burkitt lymphoma at relapse has a high genomic complexity with, among others, frequent deletions of 13q and 17p. Currently we are analyzing diagnosis – relapse

pairs by whole exome sequencing and this might lead to the identification of (targetable) relapse specific mutations, a situation comparable to the situation in pediatric ALL.[92-94]

As relapses are associated with such a detrimental outcome a challenge would be to predict the cases that will later show progression or relapse. Karyotype complexity at initial diagnosis (being comparably low to Burkitt lymphoma in general) does not seem to predict later relapse.[33] What remains possible is that, in analogy to the prognostic impact of very small *TP53*^{mut} subclones in CLL and adult B/T-ALL, also in Burkitt lymphoma very small but undetectable subclones at initial diagnosis determine the later clinical behavior.[95-97] Indeed, also in Burkitt lymphoma several observations points to an important role for *TP53* mutations and clonal heterogeneity: (i) site-specific *TP53* mutations have been identified in biopsies taken from different sites in the same patient at the same time,[98] (ii) relapse or progression acquired mutations in *TP53* have been described,[98-100] and (iii) Burkitt lymphoma cell lines show a much higher *TP53* mutations frequency compared to primary samples.[100] These findings suggest that *TP53* mutations are a late(r) event associated with disease progression, but may have been already present in a subclone of the tumor at presentation.

Additionally suboptimal dosed and administered chemotherapy allowing the development and/or outgrowth of therapy resistant subclones may play a very important role. Indeed, in a recent study all but one of the cases showing later relapse had prolonged mean intercycle times [53] suggesting that it is of pivotal importance to not only give the complete dose of the cytotoxic drugs (with no reduction in the number of cycles) but also adhere to the scheduled time frame and not allow longer intervals. Indeed, as (i) the secondary aberrations seen in Burkitt lymphoma can, albeit at a much lower frequency, also be observed at initial diagnosis and (ii) the clonal evolution pattern seen in patients parallels that seen in Burkitt lymphoma cell lines, this suggest the presence of subclonal aberrations already present at initial diagnosis and / or their very rapid expansion after survival of few cells surviving chemotherapy. Most importantly these data strongly argue against chemotherapy induced abnormalities.

At present the best treatment of relapse may be in preventing it at initial diagnosis.[53,78,101] If relapse or disease progression occurs, re-biopsy for present and future therapeutic purposes (especially in case of easy accessible sites as PB and/or BM involvement) should be considered in the framework of large multi-center

studies as the identification of novel and alternative therapeutic targets other than chemotherapy for those individual patients is needed. This need is especially high in poor-resource countries including Southern-Sahara Africa were the progression and relapse rate far exceeds that of the Western world and reducing relapse rate here would be of tremendous impact.[102-104]

IV Clinical – biology based - approaches for DHL and other *MYC*+ non-BL lymphomas

Should SHL non-BL be treated different than DHL/THL? Few studies have compared the clinical (and biological) features of *MYC* SHL vs *MYC* DHL. This comparison is complicated by the definition of DHL, e.g. considering only *BCL2*+/*MYC*+ and *BCL2*+/*BCL6*+/*MYC*+ D/THL as DHL or also *BCL6*+/*MYC*+. In addition, *MYC* SHL may harbor (also among adults) morphological DLBCL representing mBL at the molecular level.[27,105,106]

From a biological perspective also in *MYC* SHL non-Burkitt lymphomas the *MYC* translocation is very likely a secondary event and these lymphomas have a genomic complexity comparably with DHL (significantly higher than Burkitt lymphoma) suggesting that aberrations other than *BCL2* and/or *BCL6* translocations (e.g. other translocations, gains/losses, mutations) play an important role in these lymphomas.[7,14,56] Results regarding prognostic impact on OS are conflicting with most[7,14,21,56] but not all[107] studies showing *MYC* SHL non-BL also to have a negative prognostic impact. Due to very small numbers a direct comparison between the DHL subsets (*BCL2*+/*MYC*+ and *BCL6*+/*MYC*+) is difficult, but it is suggested by most[7,28,108-110] but not all[21,111] studies that patients with *BCL6*+/*MYC*+ DHL have a similar poor to an even poorer outcome compared to those with *BCL2*+/*MYC*+ DHL. In addition to small numbers this comparison is further complicated by the fact that *BCL6* may have, in contrast to *BCL2*, a wide variety of translocation partners including *MYC*[112] and it could be argued if these cases with a t(3,8)(q27;q24) represent genuine DHL (only one of both oncogenes, e.g. *MYC*, may be activated by *BCL6*).[113]

Should the MYC-partner be assessed and guide therapy? Both in research as well in routine diagnostics the assessment of *MYC* partners other than IGH (IGK-*MYC*, IGL-*MYC*) remains difficult. As previously discussed, at present the only methods to detect these partners are conventional cytogenetics, FISH with non-commercially available probes and theoretically also WGS (Lopez, Aukema et al., in preparation). For Burkitt lymphoma there are at present no indications

that the IG-*MYC* partner, IGH or variant, has any gross biological and clinical implications.[23] However, the ability to detect an IG-*MYC* partner has differential diagnostic (BL vs non-BL) and also clinical implications, since the absence of any IG partner (IGH, IGK and IGL) of *MYC* excludes a diagnosis of Burkitt lymphoma and supports a different diagnosis such as a non-BL with a *MYC* breakpoint.

In adult non-Burkitt lymphoma several studies have shown that an IG-*MYC* translocation results in (statistically significant) higher levels of MYC activation at both mRNA[7,114] as well as protein level[21]. Regarding prognostic impact the results are not completely in line with each other, some studies suggesting a poorer outcome only when an IG-*MYC* partner is present[21,115], while others showed no prognostic difference.[7] This possible correlation between outcome and the *MYC* partner might be explained by the observation that translocation with an IG partner results in higher MYC expression compared to a non-IG partner.[7,21,114] Thus it seems that for unknown reasons and opposite to what might be expected from Burkitt lymphomas that have a very high MYC expression, a high MYC expression in DLBCL is associated with poorer outcome.[116,117]

V. Future perspectives on therapy: The clinical management of patients with *MYC*-R positive non-Burkitt lymphomas remains a true challenge. Several clinical trials focusing on optimizing the therapy for MYC positive lymphomas are currently being conducted or have been recently completed (Table 3). Most of them are (modifications on) intermediate- (R-EPOCH) or high-intensity treatment regimens (R-HyperCVAD) trying to overcome the treatment resistance in these cases by using more intensified treatment regimens than the standard R-CHOP. Interpretation of results coming from these trials may be difficult as the studies focus on various entities (e.g. DLBCL, intermediates/Burkitt-like, plasmablastic lymphomas) and, moreover, assessment of MYC-positivity is either not specified or includes both *MYC*-translocation positive cases or also MYC-IHC positive cases. Some studies already suggest that intensified front-line/induction chemotherapy with e.g. R-EPOCH and R-Hyper-CVAD, R-M/C, R-CODOX-M/R-IVAC results in a longer PFS compared to R-CHOP[108,118] but also show that further intensification (e.g. high intensity R-Hyper-CVAD, R-M/C, R-CODOX-M/R-IVAC) may not necessarily result in a better outcome compared to intermediate intensity R-EPOCH.

As high (which means above threshold as set in individual studies) levels of MYC and BCL2 proteins or both are expressed in respectively ≈30-60%, ≈50-80% and ≈20-50% of DLBCL blocking either one or both proteins appears an attractive

therapeutic approach. A potential disadvantage of MYC inhibition may lay therein, that the concomitant reduction in proliferation may also result in a reduced sensitivity to chemotherapy.[83] A selective BCL2- inhibitor ABT-199/Venetoclax has, either alone or in synergy with other drugs, been shown to have high *in vitro* and *in vivo* (including mouse models and in patients) anti-tumor activity in various lymphoma entities with overexpression of BCL2.[125-129] This may be a worthwhile approach in especially these lymphomas since some papers suggest that independent of the cell of origin (COO), double expression of BCL2 and MYC in DLBCL is associated with a very poor outcome.[123] Other therapeutic options may include PI3-kinase pathway or BET-domain protein inhibitors.[130,131]

Tabel-3 | Key phase II and III studies registered at clinical trials.gov (accesses 2016.06.01) with search term 'MYC' and condition 'lymphoma'

study	Sponsor	Phase	Official title	lymphoma	Assessment of MYC / *MYC*	Age (yrs)	drugs	Study arms	alloca-tion	out-come	Status
NCT 01092182	National Cancer Institute (NCI)	II	Phase II Study of Dose-Adjusted EPOCH+/-Rituximab in Adults With Untreated Burkitt Lymphoma, c-MYC Positive Diffuse Large B-Cell Lymphoma and Plasmablastic Lymphoma	BL, DLBCL,c-MYC Positive, PBL	c-MYC Positive, method not specified	≥18 - 100	EPOCH-R (21 days for 6 cycles), EPOCH-RR 21 days for 3 cycles	(A) BL Low Risk: EPOCH-RR, (B) BL High Risk EPOCH-R, (C) DLBCL high risk EPOCH-R	Non-randomized	PFS, EFS and OS	recruiting
NCT 02213913	University of Chicago	I/II	Prospective, Multi-center Phase I/II Trial of Lenalidomide and Dose-Adjusted EPOCH-R in MYC-Associated B-Cell Lymphomas	CD20+ B-cell lymphoma other than BL (various entities, e.g. DLBCL, FL, MCL, SMZL, HL, CLL, WM, HCL)	DHL: C-myc: break with FISH or karyotype (not ICN) or IHC >40% plus either: Breaks in BCL-2 (FISH, karyotype) or IHC >70%	≥18	lenalidomide, DA-EPOCH-R	lenalidomide, DA-EPOCH-R	NA	PFS 1,2 & 3 years, MTD	recruiting

study	Sponsor	Phase	Official title	lymphoma	Assessment of MYC / *MYC*	Age (yrs)	drugs	Study arms	alloca-tion	out-come	Status
NCT 01854372	The University of Texas Health Science Center at San Antonio	II	Rituximab-Hyper-CVAD (R-HCVAD) Alternating With Rituximab-Methotrexate-Cytarabine-(R-MC) in Newly Diagnosed Patients With Diffuse Large B-Cell Lymphoma With MYC-Rearrangement. A Phase 2, Multi-Center, Open Label Study (CTRC# 11-53)	DLBCL, intermediate (Burkitt-like) lymphoma	C-myc rearrangement by FISH or conventional cytogenetics	≥18 – 70	R-HCVAD and R-MC	R-HCVAD and R-MC	NA	PFS	Withdrawn prior to enrollment
NCT 02674750	Curis, Inc.	II (open-label)	Open-Label, Phase 2 Study to Evaluate the Efficacy and Safety of CUDC-907 With and Without Rituximab in Patients With Relapsed/Refractory MYC-Altered Diffuse Large B-Cell Lymphoma	Relapsed/ Refractory-DLBCL	FISH (central testing) or IHC (≥40%)	≥18	CUDC-907, Rituximab	CUDC-907 or CUDC-907 + R	Non-randomized	ORR	recruiting
NCT 02815397	Rush University Medical Center	II	DA-EPOCH-RM: A Phase II Study Evaluating the Efficacy and Safety of Metformin in Combination With Standard Induction Therapy (DA-EPOCH-R) for Previously Untreated C-myc+ Diffuse Large B-Cell Lymphoma	DLBCL (+subtyping)	c-myc breaks by karyotype/ FISH and/or IHC ≥ 40%; this includes double hits (with bcl-2 breaks found using cytogenetics/FISH) and/or double expressors (bcl-2 ≥ 70% by IHC); ICN not considered positivity for c-myc	≥ 18	Metformin given in addition to standard of care treatment	Single arm, open label	NA	18 month progression-free survival	recruiting
NCT 02481310	Northwestern University	Phase I & II	A Phase I-II Trial of DA-EPOCH-R Plus Ixazomib as Frontline Therapy for Patients With MYC-aberrant Lymphoid Malignancies: The DACIPHOR Regimen	BL, DLBCL/ BL, DLBCL, MYC gene mutation, PBL	FISH (no central testing required), in addition testing for BCL2/6 by FISH	≥18	DA-EPOCH-R Plus Ixazomib	DA-EPOCH-R Plus Ixazomib	NA	Impact of COO by Hans on response rate, PFS, OS (DLBCL), consolidation SCT	recruiting

study	Sponsor	Phase	Official title	lymphoma	Assessment of MYC / *MYC*	Age (yrs)	drugs	Study arms	alloca-tion	out-come	Status
NCT 01914718	Fudan Univer-sity	II	Dose-adjusted EP-OCH-R in Patients With Diffuse Large B-cell Lymphoma: a Phase II Study	DLBCL	c-MYC Posi-tive, method not specified	≥18 – 70	DA-EP-OCH-R	DA-EP-OCH-R	NA	PFS	termi-nated

Yrs, years ; EPOCH; Etoposide, Prednisolone, Oncovin/Vincristine, Cyclophosphamide, Hydroxydaunorubicin/doxorubicin ; HCVAD , Hyper-CVAD hyperfractionated- Cyclophosphamide, Vincristine, Doxorubicin, Dexamethasone, Methlotrexate, Cytarabine ; DA, dose adjusted. R, Rituximab ; SCT, stem cell transplantation BL, Burkitt lymphoma ; DLBCL, diffuse large B-cell lymphoma, PBL, plasmablastic lymphoma ; FL, follicular lymphoma ; MCL, mantle cell lymphoma, SMZL; splenic marginal zone lymphoma ; HL, Hodgkin lymphoma ; CLL, chronic lymphocytic leukemia ; WM, Waldenström macroglobulinaemia ; HCL, hairy cell leukemia ; COO, cell of origin

PFS, progression free survival, EFS, event-free survival, OS, overall survival ; ORR, overall response rate ; MTD, maximum tolerable dose ICN, increased copy number ; FISH, fluorescent in situ hybridization ; IHC, immunohistochemistry ; NA, not available.

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