Understanding *MYC* driven aggressive B-cell lymphomas: pathogenesis and classification

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Abstract

*MYC* is a potent oncogene initially identified as the target of the t(8;14)(q24;q32) chromosome translocation in Burkitt lymphoma. *MYC* gene alterations have been identified in other mature B-cell neoplasms usually associated with an aggressive clinical behavior. Most of these tumors originate in cells that do not normally express MYC protein. The oncogenic events leading to MYC upregulation seem to overcome the inhibitory effect of physiological repressors such as BCL6 or BLIMP1. Aggressive lymphomas frequently carry additional oncogenic alterations that cooperate with MYC dysregulation, likely counteracting its pro-apoptotic function. The development of FISH probes and new reliable antibodies have facilitated the study of MYC gene alterations and protein expression in large series of patients providing new clinical and biological perspectives regarding MYC dysregulation in aggressive lymphomas. *MYC* gene alterations in large B-cell lymphomas are frequently associated with *BCL2* or *BCL6* translocations conferring a very aggressive behavior. On the other hand, MYC protein upregulation may occur in tumors without apparent gene alterations, and its association with BCL2 overexpression also confers a poor prognosis. In this review we integrate all this new information and discuss its perspectives, challenges and open questions for the diagnosis and management of the patients.
Introduction

*MYC* was initially identified as the target oncogene dysregulated by the t(8;14)(q24;q32) translocation in Burkitt lymphoma (BL). *MYC* rearrangements involving the heavy and light chain immunoglobulin loci (IG) as well as different non-IG genes were subsequently detected in other lymphoid neoplasms usually associated with very aggressive clinical behavior. The transforming oncogenic potential of *MYC* was initially demonstrated in cell lines and transgenic animal models. However, *MYC* dysregulation alone does not cause lymphoma, and the t(8;14) has been found at very low levels in blood and bone marrow of healthy individuals, indicating that this genetic alteration *per se* is not sufficient to trigger lymphomagenesis. BL and most lymphomas carrying *MYC* translocations are among the most proliferative tumors. Yet, *MYC* expression has been difficult to identify in the germinal center (GC), the lymphoid cell compartment with the highest proliferative fraction where most of these tumors originate. Understanding the possible role of *MYC* in normal lymphoid regulation and particularly the modulation of the germinal center reaction has been elusive.

Recent studies including basic immunology analyses, new animal models, next generation sequencing and clinicopathological observations are converging to provide a new perspective of the role of *MYC* in the lymphoid system and the pathogenesis of aggressive lymphomas. In this review we integrate all this new information and discuss new perspectives, challenges and open questions for the diagnosis and management of patients.
**MYC as a transcription factor**

MYC is a transcription factor forming heterodimers with the related protein MAX that bind to promoter regions of target genes and modulate their expression by the recruitment of specific co-activators and repressors.\(^1\text{-}^3\) Transcriptional activation of MYC is mediated by binding of the histone acetyltransferases CBP/p300, TIP60/GCN5 or the transcription factor P-TEFb, among others. Transcriptional repression is modulated by several mechanisms that include the interaction of these complexes with the transcription factor MIZ-1 preventing recruitment of the activating molecule p300 and facilitating binding of the gene silencing DNA-methyltransferase DNMT3a. Other transcription factors such as MAD may titrate out MYC from the complexes and then the MAX/MAD heterodimers recruit histone deacetylases (HDAC) that repress gene transcription.\(^3\) MYC may also repress gene expression by recruitment of HDAC to promoters containing E-boxes.\(^6\)

The transcriptional program regulated by MYC includes 10-15% of all human genes. The main cell functions and pathways under control of MYC are cell proliferation and growth, DNA replication, protein biosynthesis and regulation of metabolism and energy. MYC promotes the transition from G0/1 to S phase activating directly and indirectly the expression of CCND2 and CDKs and downregulating cell cycle inhibitors.\(^1\text{-}^2\text{,}^7\text{-}^8\) The MYC transcriptional network also includes the direct regulation of a large number of micro-RNAs (miRs) with oncogenic or tumor suppressor function.\(^9\text{-}^10\) MYC upregulates the oncogenic miR 17-92-cluster but most miRs directly regulated by MYC are usually repressed.\(^9\) The miR 17-92-polycistron at 13q31 is commonly amplified in several subtypes of...
aggressive lymphomas\textsuperscript{11, 12} and its oncogenic function is mediated in part by the downregulation of PTEN, TP53 and E2F1 facilitating the activation of the PI3K/AKT pathway and inhibition of apoptosis, respectively.\textsuperscript{3} MYC represses several miRs with tumor suppressor function by the recruitment of HDAC.\textsuperscript{13} These miRs include miR15a/16-1, miR 26a, miR 29, and miR 34 that regulate crucial functions in the neoplastic development such as apoptosis (miR-15a/16-1 and miR-34 targeting BCL2 and TP53, respectively), proliferation (miR-29a targeting CDK6) or cell differentiation (miR-26a targeting EZH2).\textsuperscript{3, 14, 15} MYC itself is also negatively regulated by some miRs such as miR-34 and miR-494.\textsuperscript{14, 15} miR-494 is in turn repressed by EZH2, creating a positive autoregulatory loop (MYC/miR-26a/EZH2/miR 494) that sustains the persistent expression of MYC and EZH2 promoting the malignant phenotype of cells.\textsuperscript{14} The interactions of the networks regulated by MYC and its target miRs are complex and suggest fine-tuning of different processes that may be targeted by new therapies.\textsuperscript{13, 16}

Intriguingly, the gene profile transcriptionally regulated by MYC varies in different cell types with relatively little overlap.\textsuperscript{17} Two recent studies shed light on this puzzling observation showing that instead of activating a particular gene signature, MYC acts as an amplifier of the transcribed genes in a given cell by uploading to the promoters of active genes enhancing their transcription.\textsuperscript{18, 19} MYC does not bind to promoters of silent genes, and therefore acts as an activator of the preexisting transcription program. This function of MYC may be relevant to understand the increased aggressiveness of tumors associated with other oncogenic events carrying MYC alterations, and may offer perspectives for new therapies.\textsuperscript{18, 19}
A paradoxical role of MYC is the induction of apoptosis.\textsuperscript{1,3} The biological meaning of this function is not fully understood. It has been interpreted as a protective mechanism of cells to counteract the effects of oncogenic activation and avoid propagation of transformed cells. The mechanisms of MYC-mediated apoptosis may involve several pathways. Overexpression of MYC increases DNA replication possibly resulting in DNA damage that in turn triggers a TP53-mediated response leading to apoptosis. MYC expression also seems to downregulate, probably indirectly, antiapoptotic proteins such as BCL2 or BCLXL and upregulate pro-apoptotic elements such as BIM.\textsuperscript{20} This \textit{anti}-tumorigenic effect of \textit{MYC} may explain in part the need of other cooperative mechanisms for cell transformation and tumor progression.

The relevant oncogenic role of \textit{MYC} has stimulated the search for therapeutic strategies that may counteract its damaging functions. MYC protein itself has generally been considered “undruggable” and the potential approaches have been directed to reduce its expression, interfere with MAX dimerization or DNA binding or acting on downstream target genes. However, most of these strategies have been difficult to apply in \textit{in vivo} models.\textsuperscript{2,21,22} The recent discovery that MYC transcription depends on the regulatory function of BRD4 has offer new promising therapeutic opportunities.\textsuperscript{23,24} BRD4 is a member of the bromodomain and extran-terminal (BET) subfamily of proteins that bind to lysine acetylated histones and recruit elements required for transcription. Two small molecules, JQ1 and iBET, displace BRD4 from acetylated chromatin resulting in a downregulation of MYC and modulation of its transcriptional program, including the upregulation of MYC repressed miRs, with a marked anti-proliferative cell effect and tumor growth
These results have been confirmed in plasma cell myeloma (PCM) and BL cell lines with translocated \textit{IGH/MYC} and also in aggressive lymphomas with MYC overexpression not related to structural gene alterations suggesting that this strategy may be useful in a broad spectrum of MYC driven tumors.\textsuperscript{16,23-25} Although BRD4 binds to a high number of enhancers and promoters, its inhibition is particularly sensitive in very large and active enhancers called super-enhancers that regulate oncogenes such as \textit{MYC}. The addiction of PCM cells to MYC make the cells particularly sensitive to the BRD4 binding disruption on its super-enhancer.\textsuperscript{26}

\textbf{MYC regulation in germinal center cells}

Most aggressive lymphomas with \textit{MYC} alterations are related to follicular lymphoid cells but the role of MYC in GC formation and maintenance has been elusive until recently.\textsuperscript{27,28} \textit{MYC} is expressed in mature B-cells initiating GC formation and in a small subpopulation of B-cells of the light zone of the GC. However, MYC is absent in the highly proliferative cells of the GC dark zone. The sole expression of MYC in these selective subsets of B-cells explains the failure of previous studies using bulk GC cells to detect its expression. In the early steps of GC formation MYC is transiently upregulated in few B-cells before BCL6 is expressed (Fig 1). This expression seems to be induced by the initial interaction with antigens and T-cells and is essential for GC formation since its abrogation results in a complete absence of GCs. In subsequent steps, BCL6 is upregulated and directly represses \textit{MYC} by binding to its promoter. This switch between MYC
and BCL6 is associated with the formation of the dark zone of the CG and expansion of highly proliferating centroblasts. MYC is then re-expressed in a subset of activated cells of the light zone that have upregulated NF-κB and express IRF4 whereas BCL6 is downregulated. This MYC upregulation is again dependent on antigen and T-cell interactions. The light zone MYC-positive cells seem to correspond to a selected subpopulation of B-cells with high-affinity BCR that are prepared to re-enter the dark zone for a subsequent round of proliferation and further acquisition of IG somatic mutations perpetuating the GC reaction (Fig 1). MYC negative cells in the light zone will probably be the subset primed to exit the GC as memory cells or early plasmablasts. BLIMP1 induction in these latter cells will promote the plasma cell differentiation program and will repress MYC expression by binding to its promoter (Fig 1).  

The absence of MYC in cells of the GC dark zone is intriguing and raises the question of the mechanisms sustaining the high proliferative program of these cells. Recent gene expression profiling studies of isolated cells from the GC dark and light zones have identified different transcriptional programs. TCF3 (E2A), a potent transcription factor highly expressed in the DZ, upregulates genes required for GC function including \textit{CCND3} and \textit{E2F2} promoting cell proliferation. Interestingly, TCF3 induces its own negative inhibitor ID3 that is also a target of MYC. These elements may create an autoregulatory loop controlling the transition of cells between the dark and light zones. ID3 expression promoted by TCF3 may contribute to the attenuation of the TCF3 program allowing the cell to move from the dark to the light zone (Fig 2A). The expression of MYC in light zone cells would sustain this effect by the induction of ID3.
MYC dysregulation in aggressive B-cell lymphomas

MYC gene alterations were initially identified in lymphoid neoplasms by cytogenetic and molecular genetic studies that recognized 8q24 translocations, and MYC gene rearrangements, amplifications or mutations. These studies were, however, difficult to apply on a routine basis in large series of patients. The development of MYC fluorescence in-situ hybridization (FISH) probes and - more recently - a monoclonal antibody that specifically recognizes MYC protein in routinely processed tissues has simplified the analysis of these alterations in routine practice. These two technical advances have facilitated the study of MYC in large cohorts of patients expanding our view and perspective of these alterations in different subtypes of aggressive lymphomas (Table 1). Intriguingly, most of these tumors originate in cells that do not express MYC protein. The oncogenic events leading to the upregulation of MYC seem to overcome the inhibitory effect of physiological repressors such as BCL6 in GC cells or BLIMP1 in terminally differentiated B-cells (Table 2). In addition, these aggressive lymphomas appear to have acquired additional oncogenic alterations that seem to cooperate with MYC dysregulation by counteracting especially its pro-apoptotic function (Table 2).

Burkitt lymphoma

Burkitt lymphoma is composed of highly proliferating mature B-cells expressing a germinal center phenotype. It frequently presents in extranodal sites in children and young adults. Epidemiological studies have recognized three
variants including endemic, sporadic and HIV associated variants. The genetic hallmark of BL is the MYC translocation usually with the IGH locus but also with immunoglobulin light chain genes. These translocations are usually the sole chromosomal aberration or are associated with few additional alterations. In addition to MYC translocations, BL harbors also MYC and TP53 mutations in around 60% and 40% of the cases, respectively.\textsuperscript{35,36} Similar MYC mutations have been identified in DLBCL and seem to be introduced via the germinal center somatic mutational machinery.\textsuperscript{37} Most of these mutations target functional domains that enhance the oncogenic potential of MYC by different mechanisms including increased protein stability and transcriptional function or by impairing the induction of the proapoptotic element BIM.\textsuperscript{37,38}

Gene expression profiling studies have identified different signatures of BL and diffuse large B-cell lymphomas (DLBCL).\textsuperscript{39,40} The gene expression profile of BL is similar to that of cells of the GC dark zone whereas the expression profile of GCB and ABC DLBCL is more similar to that of light zone cells, suggesting that these lymphomas have an origin in different GC compartments.\textsuperscript{30} The relationship of BL to dark zone cells seems paradoxical since these cells do not normally express MYC.

Recent genomic sequencing studies have identified novel recurrent somatic mutations in BL.\textsuperscript{31,41,42} The most remarkable findings are frequent activating mutations in TCF3 and inactivating mutations in its inhibitor ID3. The inactivation of ID3 likely impedes TCF3 in its inhibitory effect resulting in a constitutive activation of this pathway (Fig 2A). ID3 mutations (38-68%) are more frequent than those of TCF3 (11%). They are detected in about 70% of sporadic and HIV-associated BL
but in only 40% endemic tumors. These mutations are essential for the survival of BL cells and, therefore, constitute a necessary cooperating mechanism of MYC in the pathogenesis of BL.⁴¹ TCF3 and ID3 are expressed in the GC cells of the dark, but not the light, zone suggesting that mutations of these genes may retain the tumor cells in their differentiation compartment of the GC. The activation of TFC3 promotes the survival of BL cells thus intensifying BCR signaling through the phosphoinositide-3-kinase (PI3K) pathway and their proliferation by upregulating the expression of CCND3 (Fig 2A).³¹ Reinforcing the relevance of this mechanism, activating mutations of CCND3 have been detected in 38% of sporadic BL and occasional endemic tumors.³¹:⁴¹ In a recent mouse model, PI3K activation cooperating with MYC induces a lymphoma that resembles human BL, including the acquisition of CCND3 mutations.⁴ ID3 and TCF3 mutations have not been identified in DLBCL reinforcing the idea that they are a cooperating mechanism of MYC to develop and maintain the identity of BL.

**Diffuse large B-cell lymphoma (DLBCL) with MYC translocations**

Approximately 5-14% of DLBCL have been reported to carry MYC translocations.⁴³:⁴⁴ MYC amplification, although not systematically studied, has been reported in 2% of DLBCL in a recent study.⁴⁵ An amplification of the translocated allele, a phenomenon named complicon, has been observed in some cases.⁴⁶ Low copy number gains of MYC are more common in DLBCL (19-38%) and may be associated with higher levels of mRNA expression.⁴⁷ The presence of an underlying MYC translocation or amplification cannot be reliably predicted
based on morphological and immunophenotypic features, although these cases
tend to be positive for CD10 and BCL6 and may be negative for BCL2 (Fig
3A).45,48,49

In keeping with this, and in accordance with the concept of MYC
translocations arising in the GC microenvironment, most MYC rearranged DLBCL
show a GC-type gene expression profile and/or a GC phenotype.40,49 MYC
rearranged DLBCL may either arise de novo, or may represent a high grade
transformation of an antecedent low-grade lymphoma, most commonly follicular
lymphoma (FL). In the latter case, the MYC gene rearrangement is frequently
accompanied by a t(14;18)(q32;q21) chromosome translocation/BCL2
rearrangement. In larger series, around 40% of patients whose tumors carry a dual
MYC/BCL2 translocation were reported to have a history of, or are diagnosed with,
concurrent FL.50 On the other hand, 60-80% MYC rearrangements in supposedly
“bona fide” de novo DLBCL are accompanied by either BCL2 or BCL6
rearrangements, thus indicating that this “double hit” scenario can also occur
without clinical indication of a preceding low grade disease (Fig 2A).44,49,51

In contrast to BL, MYC rearrangements in DLBCL are usually seen in the
context of complex karyotypic alterations,40,52 and MYC is more frequently
translocated to immunoglobulin (IG) light chain loci (IGL) or to non-IG genes such
as BCL6, BCL11A, PAX5 or ICAROS49.53 MYC rearrangements in DLBCL have
predominantly been demonstrated in patients >60 years of age with higher clinical
stage, higher international prognostic index (IPI) values, and frequent presentation
in extranodal sites, but these features are not consistent in all studies.44,51,54
A MYC rearrangement predicted an inferior outcome in DLBCL in most studies, but it is not yet entirely clear if this is due to the MYC rearrangement itself, or rather due to the fact that 58%-83% of MYC translocated DLBCL harbor dual or even triple translocations also targeting BCL2 and/or BCL6. Amplifications, but not low copy number alterations, also, have been associated with shorter overall survival.

MYC protein expression is seen in the majority of DLBCL, but the number of positive cells strongly varies from case to case. MYC protein is highly expressed (>70% of cells) in the nuclei of DLBCL with MYC rearrangements or amplification. However, only one third of DLBCL with substantial (>30-40% cells) MYC protein expression do carry MYC gene alterations. This suggests that mechanisms other than gene rearrangements are responsible for elevated protein expression in a considerable proportion of DLBCL. Protein overexpression of MYC has been associated with inferior prognosis in some studies, but as is the case with MYC translocations, MYC overexpression in DLBCL may not be predictive of an inferior prognosis on its own, since there is good evidence that it is the dual deregulation of both MYC and BCL2 expression that is strongly correlated with shorter survival. Immunohistochemical expression scores using MYC, BCL2, and possibly BCL6, are able to identify patients with poor prognosis even within IPI subgroups.
B cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma (BCLU)

B cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma (BCLU) is a provisional category in the WHO 2008 lymphoma classification. BCLU is primarily defined by morphological features (Fig. 3B). Interestingly, MYC rearrangements can be detected in 30%-50% of these tumors. As the name implies, BCLU represents an “intermediate” category with the tumors displaying features of both DLBCL and BL. The term “intermediate” lymphomas was derived from gene expression profiling studies, in which tumors were classified as “molecularly intermediate”, if their gene expression profile was neither fully consistent with BL, nor with DLBCL. BCLU is a disease of older patients presenting with nodal or extranodal disease usually in an advanced clinical stage. Both by morphology as well as by immunohistochemistry, some cases in this category bear more resemblance to BL, while others are more reminiscent of DLBCL (Table 3).

In contrast to BL, BCLU generally harbors many more aberrations in addition to 8q24 (MYC) translocations. The molecular structure of MYC translocations varies between BL and BCLU. In the former, MYC-IGH translocations prevail, while MYC is more frequently translocated to IGL or to non-IG genes in the latter. Although the exact frequency is not known, the MYC rearrangement is accompanied by translocations involving either BCL2, BCL6, or both in roughly 20%. These particular tumors are referred to as “double hit” (DH) or “triple hit” (TH) lymphomas. Gene expression profiling analyses have shown a profile intermediate between BL and DLBCL in some of the DH/TH lymphomas, while others display a
Burkitt gene expression profile.\textsuperscript{40,62} DH/TH lymphomas usually are aggressive neoplasms presenting with high clinical stage, high LDH, frequent extranodal manifestations and bone marrow and CNS infiltration. A satisfactory therapeutic approach is lacking, and the average survival time is short, usually less than one year.\textsuperscript{50,63-65} Although the outcome of the patients in most, if not all, reports has been described as poor, cases with an antecedent or simultaneously occurring FL may fare still worse.\textsuperscript{65}

“Double hit” (triple hit) lymphoma

The DH/TH genetic constitution is not restricted to DLBCL and BCLU. It has also been observed in follicular lymphoma,\textsuperscript{66} and in B-cell lymphoblastic leukemia/lymphoma (TdT\textsuperscript{+}).\textsuperscript{65,67,68} Most DH lymphomas harbor concomitant \textit{MYC} and \textit{BCL2} gene translocations, but a minority of them has \textit{MYC/BCL6} rearrangements. In Mitelman’s database of cytogenetic alterations in cancer, 62\% of DH/TH cases harbor \textit{MYC/BCL2} translocations, a triple hit constellation involving \textit{MYC}, \textit{BCL2} and \textit{BCL6} is encountered in 16\%, and \textit{MYC/BCL6} rearranged cases account for only 8\%.\textsuperscript{69,70} More recently, \textit{MYC/BCL6} rearranged lymphomas have been reported to be more often CD10 negative but IRF4/MUM1 positive, and cytogenetically less complex than their \textit{MYC/BCL2} counterparts.\textsuperscript{71} In general, in most studies carried out, patients with DH/TH have been reported to run a dismal clinical course.\textsuperscript{50,54,63-64}
Plasmablastic lymphoma and plasma cell myeloma

Plasmablastic lymphoma (PBL) is an aggressive neoplasm composed of a diffuse proliferation of large B cells usually with immunoblastic morphology and the phenotype of a terminally differentiated B-cell characterized by the loss of mature B-cell markers and expression of plasma cell-related antigens (Fig 3C).\(^6^0\) Epstein-Barr Virus (EBV) infection with latency I is common but not found in all cases. These tumors usually present in extranodal sites, and frequently in mucosae of the head and neck region in patients with different immunodeficiency states, mainly HIV infection.\(^7^2\)-\(^7^4\) MYC translocations are encountered in 41-49% of PBL, virtually all of them with an \(IG\) gene as partner and usually in the context of multiple chromosomal aberrations, and seem to confer an inferior prognosis.\(^7^3\)-\(^7^6\)

MYC activation also seems to play a role in the progression of plasma cell neoplasms particularly from monoclonal gammopathy of undetermined significance (MGUS) to plasma cell myeloma (PCM). This progression is associated with increased levels of MYC expression in the absence of structural alterations of the gene.\(^7^7\) MYC rearrangements have been found in 0-15% of unselected PCM but in 45% of advanced tumors, particularly in those with extramedullary involvement, and in 65% of PCM cell lines, suggesting that MYC structural alterations are associated with progression of the tumors.\(^7^3\);\(^7^8\);\(^7^9\) Contrary to PBL, MYC in PCM is frequently rearranged to non-\(IGH\) loci.\(^7^9\) The functional relevance of MYC in PCM has been highlighted by the addiction of these cells to MYC for survival.\(^8^0\);\(^8^1\)

Some patients with PBL and MYC rearrangements have overlapping features with PCM,\(^8^2\) but the clinical context, immunodeficiency status and EBV infection will help to distinguish these two entities. Secondary PBL transformed from CLL or
FL also frequently harbor MYC translocations.\cite{83,84} All these observations suggest that MYC aberrations do also play a role in the pathogenesis of aggressive lymphoid neoplasms with terminal B-cell differentiation. This finding is remarkable since most aggressive B-cell lymphomas with MYC rearrangements have a GC phenotype.

The terminal B-cell differentiation program is triggered by BLIMP1, a transcription factor highly expressed in PBL.\cite{85,86} BLIMP1 represses genes that maintain the mature B-cell identity such as PAX5 and promotes the expression of genes involved in plasma cell differentiation such as XBP1. BLIMP1 also represses MYC and other genes controlling cell proliferation and cell growth. The frequent presence of MYC translocations in these tumors may be required to overcome the repressing effect of BLIMP1 on MYC (Fig 2B). PCM and probably also related neoplasias have an active unfolded protein response (UPR), a protective antiapoptotic mechanism triggered in the endoplasmic reticulum, that ensures the proper handling of the high protein load produced in these cells.\cite{87} This protective mechanism may help to bypass the pro-apoptotic effect of MYC. Interestingly, MYC oncogenic activation also seems to promote the UPR in transformed cells as a mechanism to escape from its apoptotic effects.\cite{88}

**ALK+ large B-cell lymphoma**

ALK-positive large B-cell lymphoma is an aggressive tumor composed of immunoblasts with a plasmablastic phenotype and expression of ALK protein due to activating gene rearrangements with different partner chromosomes.\cite{89} These
tumors lack mature B-cell markers and express BLIMP1 and XBP1. Contrary to other PBL, these tumors do not carry MYC translocations but express high levels of MYC protein. The mechanism activating MYC in these tumors is not clear, but may be a consequence of STAT3 activation. STAT3 is a downstream effector of ALK, and is phosphorylated in ALK+ DLBCL. STAT3 also induces the expression of BLIMP1 promoting plasma cell differentiation (Fig 2B). Similar to PBL, the activation of MYC by STAT3 may be a mechanism to overcome the repressing effects of BLIMP1.

**Summary and Perspectives**

The recent elucidation of the function of MYC in the development of the GC has provided a framework to also better understand its role in lymphomagenesis. The availability of new FISH probes and antibodies have facilitated the study of MYC alterations in aggressive lymphomas. MYC translocations are a diagnostic feature of BL, and in this disease, are frequently associated with a simple karyotype and somatic mutations activating the TCF3/ID3 pathway. In contrast, MYC gene alterations seem to represent secondary events associated with complex karyotypes in large B-cell lymphomas (LBCL) and are frequently associated with BCL2 or BCL6 translocations that confer a remarkable aggressiveness to the tumors. A number of LBCL have MYC protein upregulation independent of gene alterations. The concomitant overexpression of BCL2 protein in these tumors is associated with poor prognosis. Although most studies concur on the prognostic value of these “double” genetic or immunohistochemical “hits” it is not completely clear if both have a similar significance. Further studies are
needed to clarify how these new findings should be incorporated in the clinic. Immunohistochemical studies are easier to perform than genetic analyses. The difficulties, however, to reproduce quantitative scores for some markers may preclude their routine application suggesting that a screening approach using immunohistochemistry combined with FISH studies may be a helpful strategy.

Although BL and DLBCL are distinctive lymphoma entities, molecular and pathological studies have recognized a subgroup of very aggressive tumors with intermediate features that are difficult to classify in these well-defined categories. These intermediate lymphomas (BCLU) are a diagnostic challenge and their clinical and biological significance is not completely clear. All these tumors are difficult to control with current therapeutic strategies. The integration of the new genetic and molecular diagnostic tools with novel treatment regimens and drugs may help to overcome the dismal prognosis of these malignancies.

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Table 1: Aggressive lymphomas with MYC genetic and protein alterations

**MYC Genetic alterations**
- Burkitt lymphoma (BL)
- Diffuse large B-cell lymphoma (DLBCL)
- B-cell lymphoma unclassifiable intermediate between DLBCL/BL (BCLU)
- Plasmablastic lymphoma
- Transformed lymphoma (rare)

**MYC protein overexpression without evidence of genetic aberrations**
- Diffuse large B-cell lymphoma
- ALK+ large B-cell lymphoma
Table 2. MYC activation and cooperating mechanisms in aggressive B-cell lymphomas

<table>
<thead>
<tr>
<th>Lymphoma</th>
<th>Normal cell counterpart</th>
<th>MYC inhibitory physiological mechanism</th>
<th>MYC oncogenic activation</th>
<th>MYC cooperating mechanism*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burkitt</td>
<td>DZ germinal center cell</td>
<td>BCL6</td>
<td>MYC-Translocation</td>
<td>TCF3/ID3/CCND3 mutations</td>
</tr>
<tr>
<td>GCB- DLBCL</td>
<td>LZ germinal center cell</td>
<td>BCL6</td>
<td>MYC-Translocation/?</td>
<td>BCL2 Translocation</td>
</tr>
<tr>
<td>ABC- DLBCL</td>
<td>LZ germinal center cell</td>
<td>BCL6</td>
<td>BCR/MYD88 activation</td>
<td>BCL2 overexpression (18q amplifications)</td>
</tr>
<tr>
<td>PBL</td>
<td>Plasmablast</td>
<td>BLIMP1</td>
<td>MYC-Translocation</td>
<td>ER-stress response?</td>
</tr>
<tr>
<td>ALK+ LBCL</td>
<td>Plasmablast</td>
<td>BLIMP1</td>
<td>ALK-STAT3 activation ?</td>
<td>ER-stress response?</td>
</tr>
</tbody>
</table>

GCB: Germinal center B cell; ABC: Activated B cell; DLBCL: Diffuse large B-cell lymphoma; PBL: Plasmablastic lymphoma; LBCL: Large B cell lymphoma; DZ: Dark zone; LZ: Light zone

*TP53 and MYC mutations present in all types of lymphomas may help the tumor cells to escape the apoptotic effect of MYC\textsuperscript{20,38}
Table 3. Morphological, immunological and genetic features differentiating BL, BCLU, and DLBCL with MYC rearrangements

<table>
<thead>
<tr>
<th>Feature</th>
<th>BL</th>
<th>BCLU</th>
<th>DLBCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Architecture</td>
<td>Cohesive</td>
<td>Often cohesive</td>
<td>Variable</td>
</tr>
<tr>
<td>Starry sky pattern</td>
<td>As a rule</td>
<td>Very often</td>
<td>Sometimes</td>
</tr>
<tr>
<td>Cell size</td>
<td>Medium</td>
<td>Medium/large</td>
<td>Large</td>
</tr>
<tr>
<td>Chromatin distribution</td>
<td>Coarse</td>
<td>Variable</td>
<td>Fine</td>
</tr>
<tr>
<td>Proliferation (Ki67)</td>
<td>&gt;95%</td>
<td>Variable, often &lt;95%</td>
<td>Variable, more often &lt;95%</td>
</tr>
<tr>
<td>CD10</td>
<td>Almost always</td>
<td>Frequent</td>
<td>30%</td>
</tr>
<tr>
<td>BCL2 protein expression</td>
<td>Negative/weak</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>MYC Translocation</td>
<td>&gt;90% (IGH)</td>
<td>35-50% (IGL and non-IG)</td>
<td>5-14% (IGL und non-IG)</td>
</tr>
<tr>
<td>BCL2/BCL6 translocation in addition to MYC</td>
<td>No</td>
<td>Frequent</td>
<td>50-70%</td>
</tr>
<tr>
<td>Complex karyotype</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Figures

Figure 1. **MYC expression and regulation in the formation of the normal germinal center reaction.** MYC is initially expressed in B-cells after interaction with antigens and T-cells and is essential for germinal center (GC) formation. The subsequent upregulation of BCL6 represses MYC and initiates the formation of the GC dark zone (DZ). MYC is re-expressed in a subset of cells of the light zone (LZ) after NF-kB upregulation that will re-entry into the DZ for subsequent rounds of IG somatic mutations. MYC negative cells in the LZ exit the GC as memory cells or early plasmablasts. BLIMP1 induction will promote plasma cell differentiation and repress MYC.

Figure 2. **Oncogenic mechanisms of MYC in aggressive mature B-cell lymphomas.** (A) In Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL) MYC is activated by gene translocations or amplifications. Activation of the TCF3/ID3 pathway cooperates with MYC in BL whereas $BCL2$ and/or $BCL6$ translocations are the cooperating mechanisms in DLBCL. (B) In plasmablastic lymphomas (PBL) MYC is activated by translocations whereas in ALK+ large B-cell lymphomas (LBCL) MYC is upregulated indirectly by the oncogenic effect of ALK and STAT3 activation. In both tumors, MYC activation overcomes the suppressor effect of BLIMP1. The activation of the unfolded protein response (UPR) may be a survival mechanism to counterbalance the pro-apoptotic function of MYC. Stars represent somatic mutations, green and red boxes indicate activating and suppressing mechanisms, respectively.

Figure 3. **Aggressive lymphomas with MYC translocations.** (A) *MYC* rearranged diffuse large B cell lymphoma. Note large blastic cells with broad cytoplasm and large nuclei, finely dispersed nuclear chromatin, and single prominent nucleoli (H&E x400). (B) B-cell lymphoma, unclassifiable with features intermediate between DLBCL and BL. In comparison with BL, the tumor cells are slightly larger and harbour more irregular nuclei, sometimes with single nucleoli. Some histiocytes are interspersed, but there is no clear-cut starry sky pattern (H&E x400). (C) Plasmablastic lymphoma *MYC* rearranged. The cells are small to intermediate in size. A Giemsa stain highlights the plasmablastic features of the tumor cells. CD20 was negative in this tumor (Giemsa, x1000).
Figure 1
Figure 2A
**Figure 2B**

**ALK** ➞ **STAT3** ➞ **MYC** ➞ **Survival**

**ALK** ➞ **BLIMP1** ➞ **Plasma Cell Differentiation**

**ALK+ LBCL/PBL**
Understanding *MYC* driven aggressive B-cell lymphomas: pathogenesis and classification

German Ott, Andreas Rosenwald and Elias Campo