Correlation of *MYC* Rearrangement with MYC Immunohistochemistry in Aggressive B-cell Lymphomas

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Abstract

Background
Diffuse large B-cell lymphoma (DLBCL) and related entities are the most common type of non-Hodgkin lymphomas. While *MYC* rearrangement is a molecular hallmark of Burkitt lymphoma, it can be seen in other B cell lymphomas. In aggressive B-cell lymphomas (ABL), it is suggested to be associated with tumor progression and poor prognosis. In this study, we focus on aggressive B-cell lymphomas and correlation of *MYC* translocation by fluorescence in-situ hybridization (FISH) and C-MYC expression by immunohistochemistry (IHC).
Design
We analyzed a cohort of 760 consult cases that were diagnosed as ABL including Burkitt lymphoma, B-cell lymphoma unclassifiable (including double hit lymphomas), and DLBCL and selected cases where *MYC* FISH studies and C-MYC IHC were performed.
Results
In a cohort of 760 consult cases, there was *MYC* FISH and corresponding IHC data available on 117 cases (Table 1). In this series, the highest levels of C-MYC expression correlated with *MYC* translocation. However, even with high cut-offs (>90%) of C-MYC expression by IHC some cases lacked evidence of translocation. In addition, rare cases with low C-MYC expression had evidence of translocations.
Conclusion
Our series confirms that likelihood of *MYC* rearrangement increases with high immunohistochemical expression of C-MYC. However, C-MYC may be overexpressed on translocation negative cases, and rare cases may lack high expression of C-MYC but still have evidence of translocation.

Background

Diffuse large B cell lymphoma (DLBCL) is the most commonly diagnosed subtype of lymphoma worldwide. The current World Health Organization (WHO) classification includes several subtypes which are based on a combination of clinical, immunohistochemical, and genetic differences. Many large studies have shown variation in immunohistochemical and genetic features of aggressive B cell lymphomas. The presence of a variety of immunohistochemical, genetic and clinical features have an impact on prognosis of these patients. However, in most cases, the specific diagnosis (e.g. lymphoma type) and clinical stage are still the most important features in prognosis.

Additional insight into prognosis and pathobiology of DLBCL will continue by using additional methods to subclassify cases. In this study, we focus on aggressive B-cell lymphomas and correlation of *MYC* translocation by fluorescence in-situ hybridization (FISH) and C-MYC expression by immunohistochemistry (IHC).

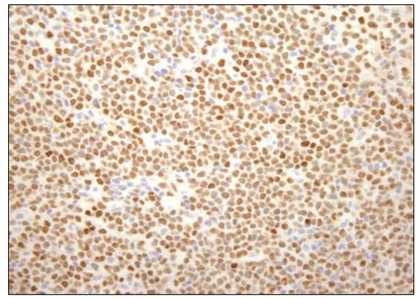
Materials & Methods

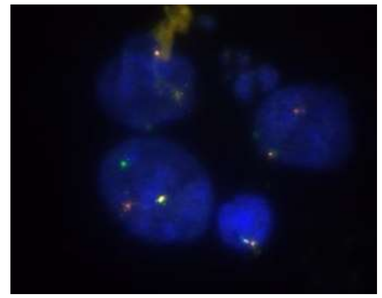
All cases were reviewed and diagnosed by DPO. The diagnoses were made in accordance with the 2008 WHO classification for hematopoietic and lymphoid tumors using a combination of immunohistochemical, genetic and other studies, as appropriate, to establish the diagnosis. The tissues were evaluated using both standard hematoxylin and eosin (H&E) staining and immunohistochemistry. Immunohistochemical stains were performed on a variety of platforms from Ventana (Tucson, AZ), Leica BioSystems (Buffalo Grove, IL), and Dako (Carpenteria, CA) using standard methodologies.

Most cases were evaluated using an extensive panel of immunohistochemical stains including CD20, CD3, CD5, CD10, cyclin D1, BCL2, BCL6, Ki67, and CD30. A subset of cases was further evaluated with immunohistochemical stains MUM1, GCET1, LMO2, FOXP1, and MYC. Fluorescence in situ hybridization (FISH) studies were performed in a subset of cases using standard methods (Abbott Molecular; Des Plaines, IL). This included lymphoma-associated translocations of MYC, IGH/MYC, IGH/BCL2 and BCL6 in most circumstances. Approximately midway through the study, FISH testing was predicated on the presence of MYC immunohistochemical expression of >50%, except in cases with other indications of high-grade features.

Correlation of MYC immunohistochemistry with *MYC* FISH and Ki67

MYC IHC (n = 117)	POSITIVE MYC FISH (n=39)	Ki67 RESULTS IN MYC POSITIVE
100%	10/11 (91%)	90% - 2; 100% - 8
90%	16/23 (70%)	70% - 3; 80% - 1; 90% - 4; 100% - 8
80%	4/16 (22%)	90% - 2; 100% - 2
70%	5/19 (26%)	40% - 1; 60% - 2; 90% - 2
60%	2/15 (13%)	30% - 1; 80% - 1
50%	0/11 (0%)	
<50%	2/22 (9%)*	100% - 2





Left: IHC for MYC at near 100%; Right: positive MYC FISH

Results

We evaluated the correlation of MYC immunohistochemical expression in 117 cases with the presence or absence of MYC translocation (either with IGH partner, non-IGH partner or both in most cases).This included cases of Burkitt lymphoma, DHL, and DLBCL. In most cases, a cut-off for testing MYC FISH was at 50% or greater expression of MYC. There was a general trend that using higher cutoffs correlated more strongly with the presence of a translocation. At 50-59%, no cases were identified with a MYC translocation, with positivity increasing to 91% of cases with 100% MYC expression showing MYC translocation.

Further we found two cases with relatively low MYC expression (5%, 35%), which harbored MYC translocations. When evaluating Ki67 expression in MYC translocation positive cases, we found that 7/39 (18%) had 70% or lower expression of Ki67.

MYC expression and correlation with *MYC* FISH results

MYC CUT OFF	NUMBER	% POSITIVE BY FISH
100%	10/11	91%
90%+	26/44	59%
80%+	30/60	50%
70%+	35/79	44%
60%+	37/84	44%
50%+	37/95	39%
>0%	39/117	33%

Discussion

- Cases with unusually low MYC expression may indicate an aberrant MYC protein, and may be an indication to test for MYC translocations
- 18% of cases with Ki67 of 70% of less (30-70%) had MYC translocations
- Ki67 does not appear to be an accurate screen for predicting which cases should undergo FISH studies for MYC translations
- We propose that 50% MYC expression as a cut off would allow maximal detection without over-utilizing FISH studies