Validation and Application of Tissue FISH for Diffuse Large B-Cell Lymphoma Testing; the Fox Chase Experience

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Diffuse large B-cell lymphoma (DLBCL) is the most common histologic subtype of non-Hodgkin lymphoma. *BCL2*, *BCL6*, and *MYC* are the most common oncogenes involved in DLBCL, and translocations of these genes are associated with an aggressive clinical course. We validated a fluorescence in situ hybridization (FISH) assay for the analysis of DLBCL. For the validation of each probe, 5-µ sections from 20 normal lymph node samples were examined to establish statistically significant cutoff values, which ranged from ≥ 6% to ≥ 12% (95% CI) of analyzed cells for the individual probes. Between 12/2021 and 03/2023, clinical testing was performed on 49 DLBCL samples, 45 (92%) of which had an abnormality of one or more of the three oncogenes tested. *BCL2*, *BCL6*, and *MYC* rearrangements were detected in 15 (31%), 16 (33%), and 9 (18%) specimens, respectively. One-hit rearrangements were identified in 24 cases, with rearrangements of *BLC6* and *BCL2* most commonly observed (11 and 10 cases, respectively). Four cases had rearrangements of *MYC* and either *BCL2* or *BCL6*. Such high-grade B-cell lymphomas, typically referred to as "double-hit" lymphomas, are known to be aggressive. Another case had rearrangements of both *BCL2* and *BCL6*. Two cases had rearrangements of *MYC, BCL2*, and *BCL6* – "triple-hit" lymphomas – an entity that reportedly shares features with Burkitt lymphoma. Interestingly, 6 other lymphomas had single signals for all three oncogenes, suggesting the existence of a hypodiploid or near-hypodiploid cell population in these cases. Our findings suggest that FISH testing is an important adjunct in the workup of patients with DLBCL, as these results may affect clinical management. Given that these 3 oncogenes encode proteins associated with cell proliferation or pro-survival pathways, their translocation-mediated up regulation may be associated with a poor prognosis, particularly in the "multi-hit" lymphomas. Thus, these patients would likely benefit from intensified chemo-therapeutic approaches, including novel immunotherapies.

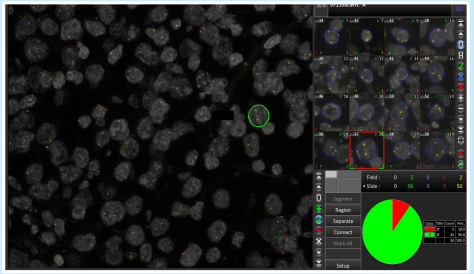

Figure 1. Screenshot of MetaCyte mode for Tissue FISH in Neon, using the Metafer Scanning and Imaging Platform. The panel on the left shows one of several manually selected fields of view (FOVs) of the FFPE tissue specimen. From all of the FOVs, a gallery of 50 individual nuclei (25/reader) is created for the purpose of scoring, a subset of which is shown in the upper right panel. Below the gallery, the table outlines the breakdown of nuclei with each FISH signal pattern.

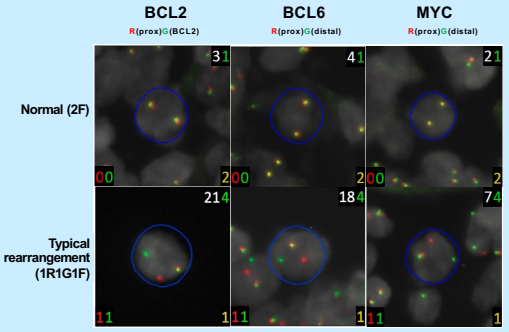

Figure 2. Examples of normal (2F) and typical rearrangement (1R1G1F) signal patterns.

	1 Probe	2 Probes	3 Probes	Total Cases
1 rearrangement	MYC (3) BCL2 (10) BCL6 (11)			24
2 rearrangements		BCL6/MYC (2) BCL2/MYC (2) BCL2/BCL6 (1)		5
3 rearrangements			BCL2/BCL6/MYC (2)	2
3+ fusion signals	MYC (10) BCL2 (2) BCL6 (3)	BCL2/BCL6 (2)	BCL2/BCL6/MYC (4)	21
1 fusion signal	MYC (1)	BCL2/MYC (2) BCL2/BCL6 (3)	BCL2/BCL6/MYC (6)	12

Table 1. Summary of the BCL2, BCL6, and MYC alterations observed by FISH in 49 FFPE lymphoma tissue specimens. The number of cases is listed in parentheses next to each probe(s).

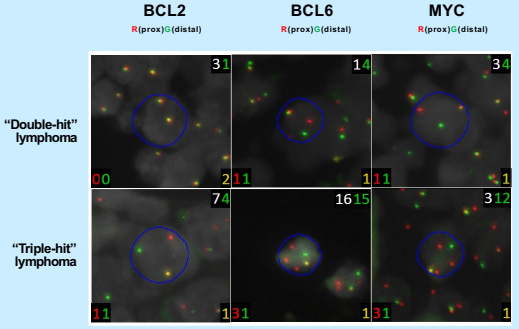

Figure 3. Examples of "multi-hit" lymphomas. The upper panels are from a "double-hit" lymphoma where rearrangements were observed with the *BCL6* (1R1G1F) and *MYC* (1R1G1F) break apart probes; *BCL2* exhibited the normal signal pattern (2F). The lower panels are from a "triple-hit" lymphoma where rearrangements were observed in all three break apart probes: *BCL2* (1R1G1F), *BCL6* (1~3R1G1F), and *MYC* (1~3R1G1F).

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