

SAMPLE IMMUNOHISTOCHEMISTRY / MOLECULAR (FISH) REPORT

DIAGNOSIS:

ABC Hospital A06-99999

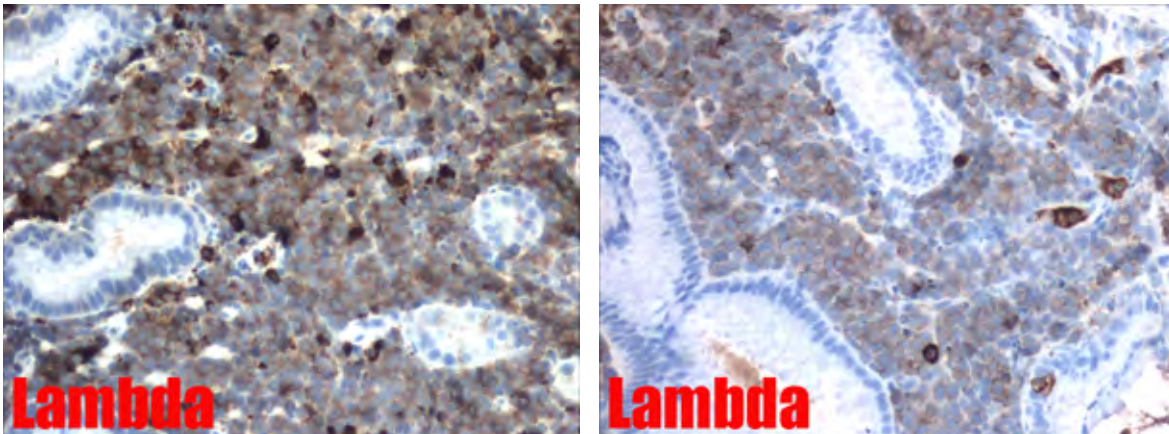
Gastric incisure biopsy: Aggressive B cell lymphoma, showing monoclonal lambda light chain expression, most consistent with atypical Burkitt lymphoma (please see comments).

ABC Hospital A05-88888

Gastric ulcer biopsy: Aggressive B cell lymphoma, showing monoclonal lambda light chain expression, most consistent with atypical Burkitt lymphoma; **positive** for a translocation involving c-MYC by FISH; **negative** for t(14;18) by FISH (please see comments).

COMMENTS:

Both the initial gastric biopsy (from 2005, A05-88888) and the current specimen (from 2006, A06-99999) show a similar involvement by an atypical lymphoid proliferation composed largely of medium- to large-sized cells and often associated with rather extensive necrosis. Some of the cells have a centroblast-like appearance with mild pleomorphism. The recent 2006 biopsy shows an immunophenotype similar to that previously reported in the 2005 biopsy (as per University One, Dr. Physician, their report UN-12345; slides not reviewed), in that the atypical infiltrate coexpresses CD20, CD10, and bcl-6, but not MUM-1 or bcl-2. The current biopsy also shows a very high proliferative rate which approaches 100% (as assessed by MIB-1), similar to that reported in the 2005 biopsy. In biopsies from both 2005 and 2006, there is weak lambda light chain restriction which provides further support that these neoplasms are likely related. Based upon the histologic and immunophenotypic findings, an aggressive B cell lymphoma is favored and raise the differential diagnosis which includes diffuse large B cell lymphoma and atypical Burkitt lymphoma. To address this question, particularly in light of the high proliferative index of this neoplasm and absence of bcl-2 positivity, additional fluorescence in situ hybridization studies looking for a translocation involving c-MYC and bcl-2 were performed. The identification of a c-MYC translocation in the absence of a t(14;18) is most in keeping with an atypical Burkitt lymphoma. Although the diagnosis of a diffuse large B cell lymphoma containing a c-MYC translocation was entertained, the histologic features (medium-large-sized cells, mild pleomorphism) and immunophenotypic findings (CD20+, CD10+, bcl-6+, bcl-2-, MIB-1 approaching 100%) are more consistent with an atypical Burkitt lymphoma, particularly as no other translocations {i.e. t(14;18)} characteristic of diffuse large B cell lymphoma could be demonstrated. Careful clinical correlation is recommended.



Both the 2005 biopsy (see left image above) and the 2006 biopsy (see right image above) show convincing weak lambda light chain restriction.

REFERENCES:

Allen M. Gown, MD, Chief Pathologist • Lynn Goldstein, MD, Pathologist • Patricia L. Kandalaf, MD, Pathologist
Steven J. Kussick, MD, PhD, Pathologist • Harry Hwang, MD, Pathologist

- Haralambieva E, Boerma EJ, van Imhoff GW, Rosati S, Schuurung E, Muller-Hermelink HK, Kluin PM, Ott G. Clinical, immunophenotypic, and genetic analysis of adult lymphomas with morphologic features of Burkitt lymphoma. *Am J Surg Pathol.* 2005 Aug;29(8):1086-94.
- McClure RF, Remstein ED, Macon WR, Dewald GW, Habermann TM, Hoering A, Kurtin PJ. Adult B-cell lymphomas with burkitt-like morphology are phenotypically and genotypically heterogeneous with aggressive clinical behavior. *Am J Surg Pathol.* 2005 Dec;29(12):1652-60.

SPECIMEN INFORMATION:

A1 = A06-99999, 1 block/1 H&E
 B1 = A05-88888, 1 block/1 H&E

RECEIVED FOR THE FOLLOWING:

Confirm same tumor

IMMUNOHISTOCHEMICAL FINDINGS:

Tissue sections (along with appropriate positive control) are incubated with the following antibody. Localization is via a biotin-free, polymer-based immunoperoxidase technique according to an optimized protocol. The controls are reviewed for appropriate positive and negative reactivity and found to be satisfactory.

Block A06-99999 (Surgery Date 06/28/2006) -Gastric ulcer (PP2006XXXX A1)

Target population: Tumor

Antibodies To	Clone	Result
CD20	L26	Uniformly positive
CD3	PS1	Negative
CD10, CALLA	56C6	Uniformly positive
bcl-6	PG-B6p	Uniformly positive
bcl-2	124	Negative
MUM1/IRF4	MUM1p	Negative
Kappa light chains	polyclonal	Negative (lambda restricted)
Lambda light chains	polyclonal	Small subset of cells positive (lambda restricted)
Ki-67 antigen (S)	MIB-1	>95%

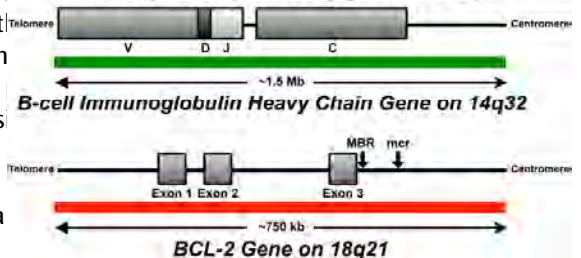
Block A05-88888 (Surgery Date: 05/05/2005) -Gastric ulcer (PP2006XXXX B1)

Antibodies To	Clone	Result
bcl-2	124	Negative
Kappa light chains	polyclonal	Negative (lambda restricted)
Lambda light chains	polyclonal	Small subset of cells positive (lambda restricted)

FLUORESCENCE IN SITU HYBRIDIZATION FINDINGS:

Deparaffinized tissue sections, following digestion/pretreatment along with appropriate positive/negative controls, are incubated with a Vysis detection system an analyte specific reagent containing 2 separate probes (SpectrumGreen™), and the second to the bcl-2 gene on 18q21 (SpectrumC t(14;18) FISH is performed using the MetaSystems™ Metafer scanning system. With signals, MetaSystems™ can detect gene fusions in areas of paraffin tissue section focus/tile sampling methodology, tiles with signals that are in close proximity (<=2 percentage of tiles containing positive signals is calculated. The threshold for positive neoplastic cases which do not contain the translocation of interest. A positive case fusion genes detected is 3 standard deviations above the mean of this negative t(14;18)(q32;q21) FISH assay is 15.9% {3 standard deviations above the mean of a

Map of Dual Color, Dual Fusion Probes for t(14;18) FISH (IgH/BCL-2)



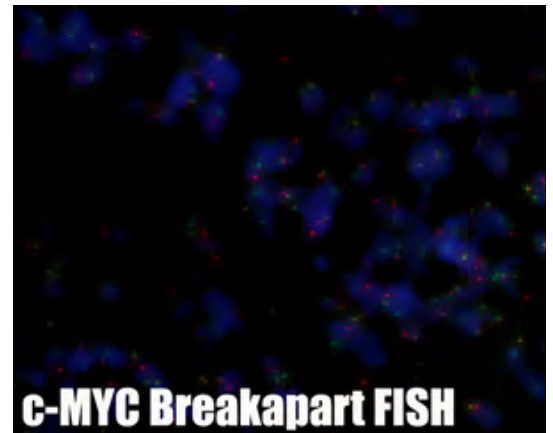
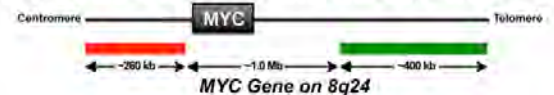
t(14;18) negative cases is 7.8%}. The sensitivity and specificity of the t(14;18)(q32;q21) FISH assay were 93.5% and 100%, respectively (n=123, including 62 follicular lymphomas).

Block A05-88888 (Surgery Date: 05/05/2005) - Gastric ulcer (PP2005XXXXX B1)

Dual Color/Dual Fusion Probe Set	Result (% of tiles positive)
t(14;18)(q32;q21) involving bcl-2	Negative (6.8%)

Deparaffinized tissue sections, following digestion/pretreatment along with appropriate positive/negative controls, are incubated with a Vysis detection system, an analyte specific reagent containing 2 separate probes (see probe map below) for the c-MYC gene on chromosome 8q24 (a 5' SpectrumOrange probe and a 3' SpectrumGreen probe), which span the break point region common in Burkitt's lymphoma. Quantitative morphometric analysis is performed using the MetaSystems™ Metafer scanning system. With its ability to analyze 3D distances between FISH signals, MetaSystems can detect breakapart signals in areas of paraffin tissue sections selected by the pathologist. Using an extended focus/tile sampling methodology, tiles with distant unpaired signals (≥ 10 pixels in distance) are considered positive and the percentage of tiles containing positive signals is calculated. The threshold for positivity is established from a group of immunophenotypically characterized cases which do not contain the translocation of interest. A positive case is defined as a case in which the mean number of positive tiles detected is 3 standard deviations above the mean of this negative control group. The threshold established for the c-MYC FISH assay is 2.2% {3 standard deviations above the mean of a negative control group of cases which do not contain c-MYC translocations; mean of this negative control group is 0.7%; n=39}.

Map of Dual Color c-MYC Breakapart Probes



Fluorescence in situ hybridization studies using breakapart probes show numerous unpaired red and green signals, indicating a translocation involving the c-MYC gene.

Block A05-88888 (Surgery Date: 05/05/2005) - Gastric ulcer (PP2005XXXXX B1)

Dual Color Breakapart	Result (% of tiles positive)
c-MYC	Positive (17.2%)

ELECTRONICALLY SIGNED

Steven J. Kussick, M.D., Ph.D.
Pathologist

In compliance with CMS regulations, the pathologist's signature on this report indicates that the case has been personally reviewed, and the diagnosis made or confirmed by the Pathologist.

NOTE: Some of the tests reported here may have been developed and performance characteristics determined by PhenoPath Laboratories. They have not been cleared or approved by the U.S. Food and Drug Administration (FDA). However, the FDA has determined that such clearance or approval is not necessary. Pursuant to the requirements of CLIA, this laboratory has established and verified the accuracy and precision of all tests, and additional information about these tests is available upon request. PhenoPath Laboratories is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical laboratory testing.