

SAMPLE IMMUNOHISTOCHEMISTRY / MOLECULAR (FISH) / IN SITU HYBRIDIZATION REPORT

DIAGNOSIS:

ABC Hospital A06-20202 (Block A2)

Pelvic mass: Aggressive B cell lymphoma, most consistent with diffuse large B cell lymphoma, plasmablastic variant (so-called plasmablastic lymphoma); **positive** for a translocation involving the c-MYC gene by fluorescence in situ hybridization (FISH) using MetaSystems™ analysis (please see comments).

COMMENTS:

The identification of a translocation involving c-MYC suggests that this neoplasm may behave aggressively. Definitive subclassification of this neoplasm remains difficult. However, the reported immunohistochemistry studies (CD20-, CD79a-, CD45+, CD10+, CD138+) raise the concern for a plasmablastic lymphoma (diffuse large B cell lymphoma, plasmablastic variant). Additional in situ hybridization studies for EBV performed at PhenoPath Laboratories using the EBER-1 probe are positive, consistent with a plasmablastic lymphoma. Furthermore, the PAX-5 immunoreactivity confirms the B cell nature of this neoplasm. Although an atypical Burkitt lymphoma is a diagnostic consideration, particularly in light of the starry sky pattern histologically and high Ki-67-defined proliferative rate, the lack of CD20 and focal expression of bcl-2 argue against this possibility. Plasmablastic myeloma is also a diagnostic consideration, but the prominent EBV positivity in this case argues against this possibility; however, staging bone marrow evaluation may be helpful in excluding a plasmablastic myeloma. Careful clinical correlation is recommended.

REFERENCES:

1. Corbone, A., Gaidano, G., Ghoghini, A., Ferlito, A., Rinaldo, A., and Stein, H. Aids-related plasmablastic lymphomas of the oral cavity and jaws: a diagnostic dilemma. *Ann Otol Rhinol Laryngol* 108: 95-99, 1999
2. Delecluse, H., Anagnostopoulos, F., Dallenbach, F., Hummel, M., Marafioti, T., Schneider, U., Huhn, D., Schmidt-Westhausen, A., Reichart, P., Gross, U., and Stein, H. Plasmablastic lymphomas of the oral cavity: a new entity associated with the human immunodeficiency virus infection. *Blood* 89(4):1413-1420, 1997
3. ES Jaffe et al., WHO Classification of Tumours: Pathology & Genetics: Tumours of the Haematopoietic and Lymphoid Tissues, IARC Press (Lyon, France) 2001.
4. Kanungo A et al., *Mod Pathol* 19:25 2006.

SPECIMEN INFORMATION:

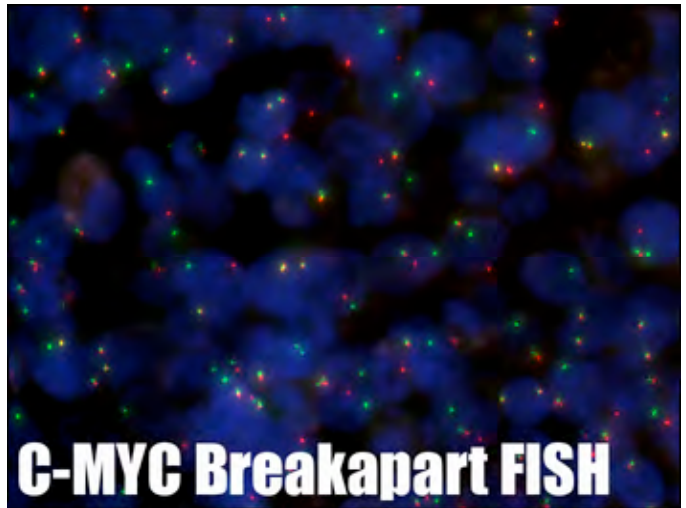
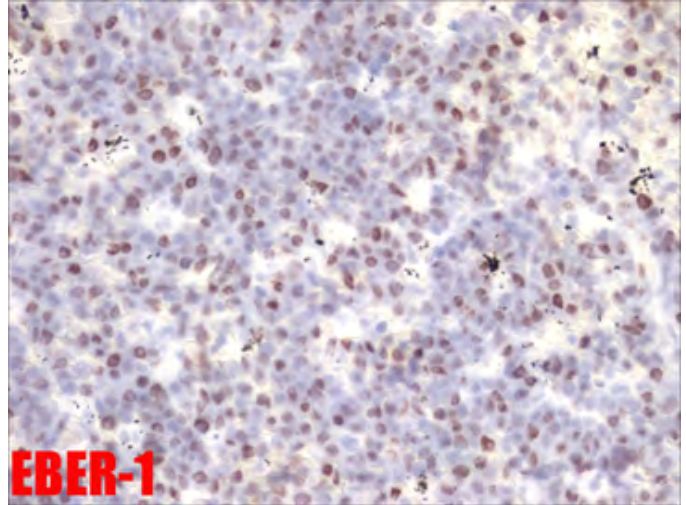
A1 = A06-20202, A2, 1 block

RECEIVED FOR THE FOLLOWING:

c-MYC FISH

CLINICAL HISTORY:

53 -year-old male with large pelvic mass involving the large and small bowel.



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 A2 (Surgery Date: 06/25/2006) - Pelvis (PP2006XXXXX A1)

Antibodies To	Clone	Result
PAX-5	24	Variably positive (in well-fixed foci)
bcl-6	PG-B6p	Focally positive (in well-fixed foci)
bcl-2	124	Variably positive (weak)
Ki-67 antigen (§)	MIB-1	100%

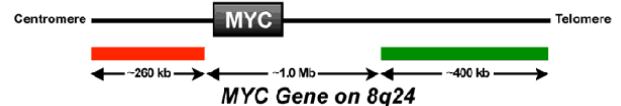
FLUORESCENCE IN SITU HYBRIDIZATION FINDINGS:

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 (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 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}.

Block A2 (Surgery Date: 06/25/2006) -Pelvis (PP2006XXXXX A1)

Dual Color Breakapart	Result (% of tiles positive)
c-MYC	Positive (15.9%)

Map of Dual Color c-MYC Breakapart Probes



IN SITU HYBRIDIZATION FINDINGS:

Deparaffinized sections of tissue are incubated with an end-labeled, digoxigeninated oligonucleotide DNA probe to the Epstein-Barr virus EBER1 mRNA as well as a positive internal control to U6 RNA. Localization is via a one-step procedure employing peroxidase-conjugated sheep anti-digoxigenin, using 3,3'-diaminobenzidine as chromogen. Positive and negative control slides for each test are reviewed and found to be satisfactory.

EBV-positive cells are identified by in situ hybridization using the EBER-1 probe (with appropriate internal U6 RNA control).

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.