Pathology From 1/1/2004 through 12/29/2014

08/22/2014 **Interpretive Lab Test** **Final**

Accession Number: BL14K30690 Report Status: Final
Type: Interpretive Lab Test
Specimen Type: Paraffin embedded brain biopsy / BS14-N41488-B2
Procedure Date: 08/22/2014
Ordering Provider: ENNIO A. CHIOCCA M.D.

CASE: BL-14-K30690
PATIENT: STEVEN KEATING

Pathologist: Lynette M Sholl, M.D.

CLINICAL DATA:

Clinical History: None given.
Clinical Diagnosis: Astrocytoma

DNA was isolated from a brain tumor biopsy, BS14-41488-B2. DNA methylation patterns in the CpG island of the MGMT gene (Genbank accession number AL355531 nt46931-47011) was determined by chemical (bisulfite) modification of unmethylated, but not methylated, cytosines to uracil and subsequent PCR using primers specific for either methylated or the modified unmethylated DNA (Esteller et al. Cancer Res. 1999;59:793-797.) The PCR products were analyzed in duplicate parallel runs by capillary gel electrophoresis. The sensitivity of the assay based on DNA dilutions studies is at least 1:1000.

RESULT:
The analyzed region of the MGMT promoter is partially METHYLATED (1 of 2 aliquots).

INTERPRETATION:
MGMT (O6-methylguanine DNA methyltransferase) is a DNA repair gene. Methylation of the promotor leads to gene silencing and loss of MGMT expression. A recent study that tested the methylation status of the same region of the MGMT promoter in glioblastomas found that MGMT promoter methylation was an independent favorable prognostic factor and was associated with a survival benefit in patients treated with temozolamide and radiotherapy. (Hegi M, Diserans A, Gorlia T et al. MGMT Gene Silencing and Benefit from Temozolomide in Glioblastoma. N Engl J Med 2005;352:997-1003.)

These tests were developed and their performance characteristics determined by the Molecular Diagnostics Laboratory, Brigham and Women's Hospital. They have not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary.

Final Diagnosis by Lynette M Sholl M.D., Electronically signed on Wednesday September 03, 2014 at 04:53:20PM

Pathology from 1/1/2004 through 12/29/2014 (cont)

08/19/2014 **Surgical Pathology** **Amend/Addenda**

Accession Number: BS14N41488 Report Status: Amend/Addenda
Type: Surgical Pathology
Specimen Type: LEFT FRONTAL TUMOR
Procedure Date: 08/19/2014
Ordering Provider: ENNIO A. CHIOCCA M.D.

CASE: BS-14-N41488
PATIENT: STEVEN KEATING

********** Addended Report **********
Resident: David Meredith, M.D., Ph.D.
Pathologist: Umberto De Girolami, M.D.

PATHOLOGIC DIAGNOSIS:

A-D. SPECIMEN DESIGNATED "LEFT FRONTAL TUMOR" (FSA, SMA):

NEWLY DIAGNOSED TUMOR (Surgery #1)

DIFFUSE ASTROCYTOMA, W.H.O. Grade 2 (ICD-0 9400/3)

IDH1(R132H) MUTATION POSITIVE (by IHC)
TP53 PROTEIN POSITIVE (by IHC, suggestive of mutation)
BRAF(V600E) NOT DETECTED (by IHC)

NOTE:
There is evidence that the tumor may lie at the higher end of the grade 2 spectrum with focal regions having slightly higher than average cellularity and atypia. Mitotic activity was detected in block B1, but this region was very small (only a few high power fields) and mitoses were not detected in other regions of the tumor. The proliferation rate in the region with mitotic activity and the vast majority of the tumor was low (not exceeding 4%). Therefore while grading as WHO Grade 3 was considered it was not felt to be warranted at this time given the overall findings in a well sampled tumor.

Classification of the tumor as MIXED GLIOMA, WHO GRADE 2 would also be appropriate given that reliable criteria for distinction of diffuse astrocytoma and mixed glioma have not been established and the clinical significance of distinguishing between these two entities is not clear.

The overall size of the resection is very large.
The tumor infiltrates adjacent brain parenchyma.

W.H.O. Histologic Grading Criteria
Cellularity: moderate
Atypia: moderate
Mitoses: present (but small focal region only)
Vascular Proliferation: not present
Necrosis: not present

Immunohistochemistry performed at BWH demonstrates the following staining profile in lesional cells (block B1):

OLIG2 positive (50% of cells, c/w astrocytoma)
GFAP positive (weak, variable)
IDH1(R132H) positive (possibly heterogeneous ~70% of cells)
TP53 positive (50% of cells)

Pathology from 1/1/2004 through 12/29/2014 (cont)

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Synaptophysin       negative, no abnormal neurons
NeuN                     negative, no abnormal neurons
SMI31               negative, no abnormal neurons
BRAF(V600E)              negative
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The formally quantified MIB-1 proliferation index is 2% (computer aided, block B1; 4/231 cells counted).

Analysis for MGMT promoter methylation status will be performed (block B2) and the results reported separately by the BWH Center for Advanced Molecular Diagnostics.

Array comparative genomic hybridization (Oncocopy) will be performed (block C1) and results reported separately by the Cytogenetics laboratory.

Somatic mutation profiling (Oncopanel) will be performed (patient consented to Oncopanel study 11-104).

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Tumor Tissue Adequacy:              Large {>1.0 cm in multiple blocks}
Primary Advanced Study Block:       C1, 2.5 cm (t60 n00), scrolls ok
Secondary Advanced Study Blocks:    B1, 2.5 cm (t60 n00), scrolls ok
Clinical trial block:               C1
Tissue Microarray Block:            C1
MGMT block:                         B2
Tissue submitted to tissue bank:    Yes
Clinical frozen tissue:       Yes
Consent Status for Tissue Research:     Full 11-104, 10-417
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The case was reviewed at the Neuropathology Staff Conference.

PATHOLOGY CLINICAL NOTES: 26 year old male with non-enhancing left frontal mass discovered in 2007 via research fMRI. Followed with serial scans until 2010. Now presents with seizure-like symptoms and increased size of the mass compared to last imaging in 2010.

CLINICAL DATA:
History: Not given.
Operation: Not given.
Operative Findings: Not given.
Clinical Diagnosis: Left frontal tumor.

TISSUE SUBMITTED:
A/1. Left frontal tumor.
B/2. Left frontal tumor.
C/3. Left frontal tumor.
D/4. Left frontal tumor.

O.R. CONSULTATION:
SPECIMEN LABELED "#1. LEFT FRONTAL TUMOR" (FSA, SMA):
Glioma without definite anaplastic features; further classification and final grading awaits permanent sections.

OR Consultation by: Umberto De Girolami, M.D.
Resident: David Meredith, M.D., Ph.D.

The senior physician certifies that he/she personally conducted a gross and/or microscopic examination of the described specimen(s) and rendered or confirmed the rapid diagnos(es) related thereto.

Pathology from 1/1/2004 through 12/29/2014 (cont)

GROSS DESCRIPTION:
The specimen is received fresh, in four parts, each labeled with the patient's name and unit number.

Part A, labeled "#1 Left frontal tumor" consists of an irregular, tan-pink, gelatinous soft tissue fragment (4.0 x 1.6 x 0.9 cm). A representative section is frozen as FSA and smeared as SMA. Representative sections are submitted for research and for Oncopanel.

Micro A1: FSA remnant, 1 frag, ESS.
Micro A2: Multi frags, ESS.

Part B, labeled "#2 Left frontal tumor" consists of an irregular, tan-white to tan-gray soft tissue fragment (4.5 x 2.5 x 2.0 cm). A representative section is given to the tissue bank, clinically frozen and given for research. The remainder is entirely submitted.

Micro B1-B2: Multi frags toto, ESS.

Part C, labeled "#3 Left frontal tumor" consists of multiple tan-pink soft tissue fragments (4.0 x 3.0 x 2.0 cm in aggregate). A representative section is given for research and clinically frozen. The remainder is entirely submitted.

Micro C1-C4: Multi frags toto, ESS.

Part D, labeled "#4 Left frontal tumor" consists of multiple irregular, tan-white soft tissue fragments (2.8 x 1.7 x 1.2 cm in aggregate), which are submitted in toto.

Micro D1-D2: Multi frags, ESS.

CASE NUMBER: 41488

Dictated by: Taft, Kristin

By his/her signature below, the senior physician certifies that he/she personally conducted a microscopic examination ("gross only" exam if so stated) of the described specimen(s) and rendered or confirmed the diagnosis(es) related thereto.

Final Diagnosis by Keith L Ligon M.D., Ph.D., Electronically signed on Saturday September 06, 2014 at 07:35:53PM

ADDENDUM:

Results of Oncocopy (array CGH) were reviewed and found to support the histopathologic diagnosis of a low grade glioma without unfavorable features.

INTEGRATIVE DIAGNOSIS (including histopathology, IHC, and array CGH results):

DIFFUSE ASTROCYTOMA
GRADE 2
IDH1(R132H) MUTATION POSITIVE

This concludes all planned clinical testing on the case.

Pathology from 1/1/2004 through 12/29/2014 (cont)

Addendum #1 by Keith L Ligon M.D., Ph.D., Electronically signed on Saturday September 06, 2014 at 07:39:30PM

Pathology from 1/1/2004 through 12/29/2014 (cont)

08/19/2014 **Cytogenetics** **Final**

Accession Number: CG14R05315 Report Status: Final
Type: Cytogenetics
Specimen Type: cg-FFPE
Procedure Date: 08/19/2014
Ordering Provider: ENNIO A. CHIOCCA M.D.

CASE: CG-14-R05315
PATIENT: STEVEN KEATING
Cytogeneticist: Ligon Ph.D., Azra Hadi

SOLID TUMOR ARRAY CGH ANALYSIS

RESULT:
arr[hg19]
3q26.31q28(171,681,429-188,500,069)x3,7p22.3q26.2(42,976-155,143,600)x3,(8)x3

INTERPRETATION:
Several copy number changes were identified following array comparative genomic hybridization (aCGH) of this formalin-fixed, paraffin-embedded (FFPE) primary brain tumor specimen. The following genomic imbalances were noted:

(1) a 16.8 Mb single copy gain of 3q, which includes PIK3CA and SOX2,

(2) polysomy 7

(3) polysomy 8

The findings are CONSISTENT the histopathologic diagnosis of a DIFFUSE ASTROCYTOMA WHO GRADE 2 or other low grade IDH-mutated astrocytoma/mixed glioma.

Gains involving Chr 7 and 8 are common in diffuse astrocytoma. In isolation however, they are not specific or diagnostic of low grade glioma or any other tumor type.

Aberrations commonly correlated with less favorable outcomes in diffuse astrocytoma (PTEN/10q loss, CDKN2A/9q loss, etc) are NOT DETECTED.

The findings are NOT CONSISTENT with an oligodendroglioma as there is no evidence for 1p/19q co-deletion.

See Table 1 (below) for a list of selected genes/regions that were evaluated specifically for this interpretation.

COMMENTS:
Array - based comparative genomic hybridization (aCGH) was performed using the stock 1x1M Agilent SurePrint G3 Human CGH Microarray chip to identify tumor - specific genomic copy number changes. Genomic DNA isolated from the FFPE specimen submitted was hybridized with genomic DNA isolated from a reference DNA sample representing a pool of karyotypically normal individuals (Promega, Madison, WI). The array platform contains 963,029 probes spaced across the human genome with a 2.1 kb overall median probe spacing and a 1.8 kb probe

spacing in RefSeq genes. A genomic imbalance is reported when a minimum of eight (8) consecutive probes, which correspond to approximately 14-16 kb, show an average log2 ratio above +0.18 or below -0.30. Amplifications are reported when an average log2 ratio for a given interval is equal to, or greater than, +2.0.

This assay cannot exclude: (1) chromosome imbalances when the proportion of tumor cells in the original sample is less than 50%; (2) chromosome imbalances smaller than the resolution of the chip, or (3) tumor heterogeneity, particularly if an abnormal clone is not sufficiently represented in the original sample. This assay is not designed to identify balanced chromosomal rearrangements (e.g., balanced reciprocal translocations, inversions or insertions), ploidy changes, uniparental disomy or DNA methylation. The composition of this array is based on the UCSC hg19 (GRCh37), Feb. 2009 (http://genome.ucsc.edu/cgi-bin/hgGateway). This test was developed and its performance determined by the BWH Cytogenetics Laboratory as required by the CLIA '88 regulations. This test is used for clinical purposes.

INDICATION FOR TEST:
Astrocytoma
BS14-N41488-C1

TABLE 1:

Gene/Region	Chromosome Band	Copy Number Change	Nucleotides (GRCh37//hg19)
MYCL1	1p34.2	No change detected	
CDKN2C	1p33	No change detected	
PIK3C2B	1q32.1	No change detected	
MDM4	1q32.1	No change detected	
AKT3	1q44	No change detected	
MYCN	2p24.3	No change detected	
PIK3CA	3q26.32	16.8 Mb single copy gain	chr3:171,681,429-188,500,069
SOX2	3q26.33	16.8 Mb single copy gain	chr3:171,681,429-188,500,069
FGFR3	4p16.3	No change detected	
PDGFRA	4q12	No change detected	
MYB	6q23.3	No change detected	
PARK2	6q26	No change detected	
EGFR	7p11.2	Single copy gain/polysomy	chr7:42,976-155,143,600
EGFRvIII	7p11.2	Not detected	
CDK6	7q21.2	Single copy gain/polysomy	chr7:42,976-155,143,600
MET	7q31.2	Single copy gain/polysomy	chr7:42,976-155,143,600
BRAF	7q34	Single copy gain/polysomy	chr7:42,976-155,143,600
FGFR1	8p11.23-p11.22	Single copy gain/polysomy	chr8:161,472-145,978,744
MYC	8q24.21	Single copy gain/polysomy	chr8:161,472-145,978,744
CDKN2A	9p21.3	No change detected	
PTEN	10q23.31	No change detected	
FGFR2	10q26.13	No change detected	
CCND2	12p13.32	No change detected	
CDK4	12q14.1	No change detected	
MDM2	12q15	No change detected	
RB1	13q14.2	No change detected	
TP53	17p13.1	No change detected	
NF1	17q11.2	No change detected	
INI1	22q11.23	No change detected	

Pathology from 1/1/2004 through 12/29/2014 (cont)

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NF2  22q12.2   No change detected
1p-  N/A  Whole arm loss not detected
4p-  N/A  Whole arm loss not detected
Monosomy 6     N/A  Not detected
6q-  N/A  Whole arm loss not detected
Polysomy 7     N/A  Detected  chr7:42,976-155,143,600
7p-  N/A  Whole arm loss not detected
Monosomy 10    N/A  Not detected
10q- N/A  Whole arm loss not detected
11p- N/A  Whole arm loss not detected
Monosomy 14    N/A  Not detected
idic(17p11.2)  N/A  Not detected
18q- N/A  Whole arm loss not detected
19q- N/A  Whole arm loss not detected
Monosomy 22    N/A  Not detected

Other:
 Polysomy 8    N/A           Detected
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REPORT by Azra Hadi Ligon Ph.D., on Wednesday September 03, 2014 at 11:30:18AM
Final Diagnosis by Keith L Ligon M.D., Ph.D., Electronically signed on Saturday September 06, 2014 at 06:30:36PM