Pathology Report [redacted] **CORRECTED**

Report Type ..... Pathology Report
Date of Event .... [redacted]
Sex ..... [redacted]
Authored by ..... [redacted]
Hosp/Group ..... [redacted]
Record Status .... CORRECTED

ADDENDA:
Addendum

MOLECULAR ANATOMIC PATHOLOGY TESTING:

Block 3K:

A. HRAS mutation IDENTIFIED (p.Q61K, c.181C>A).
B. Mutations in BRAF, NRAS61, KRAS12/13 NOT identified.
C. FISH test for RET/PTC rearrangement is pending.

NOTE:
DNA was extracted in the amount sufficient for testing.

BACKGROUND:
Mutations in either BRAF or RAS genes or RET/PTC rearrangements are found in more than 70% of papillary thyroid carcinomas (1). BRAF V600E (T1799A) mutation has been associated with more aggressive behavior of papillary carcinoma (2, 3). The association between BRAF V600E mutation and features of tumor aggressiveness have also been observed in papillary microcarcinomas (4). Mutations in the RAS genes or PAX8/PPARg rearrangement occur in ~70% of follicular thyroid carcinomas and with lower frequency in oncocytic (H?rthle cell) carcinomas (5). Regarding the specificity of these mutations for cancer, BRAF V600E mutation and RET/PTC and PAX8/PPARg rearrangements are overall specific for malignancy in the thyroid, although they have been reported with a very low frequency in benign thyroid lesions (6). RAS mutations occur in malignant and benign thyroid tumors, being found in ~40-50% of follicular and anaplastic carcinomas, 30-40% of follicular adenomas and 10-15% of papillary carcinomas (6).

1[redacted] et al. Correlation between genetic alterations and microscopic features, clinical manifestations, and prognostic characteristics of thyroid papillary carcinomas.[redacted]
2[redacted] et al. BRAF mutation predicts a poorer clinical prognosis for papillary thyroid cancer.[redacted]
3.[redacted] et al. BRAF V600E mutation and outcome of patients with papillary thyroid carcinoma: a[redacted] median follow-up study.[redacted]
4.[redacted] Analysis of differential BRAF(V600E) mutational status in high aggressive papillary thyroid microcarcinoma.[redacted]
5[redacted] et al. RAS point mutations and5PAX8-PPARg rearrangement in thyroid tumors: Evidence for distinct molecular pathways in thyroid follicular carcinoma[redacted]
6[redacted] Recent developments in the molecular biology of the thyroid.[redacted] Differential Diagnosis and Molecular Advances.[redacted]

MUTATIONAL ANALYSIS:
For paraffin-embedded surgical specimens, manual microdissection was performed to collect tumor tissue. Specimens with the minimum of 50% of tumor cells in a microdissection target are accepted for the analysis. Optical density readings were obtained. Real-time PCR was performed on the LightCycler platform to amplify BRAF codons 599-601, NRAS codon 61, HRAS codon 61, and KRAS codons 12/13 sequences. Post-PCR melting curve analysis was used to detect possible mutations. If required, the mutation type was confirmed by Sanger sequencing of the PCR product on ABI3130. DNA from samples positive for each of these mutations was used as positive controls. Amplification at 35 cycles or earlier was considered sufficient for the analysis. The limit of detection is approximately 10-20% of alleles with mutation present in the background of normal DNA.

[redacted]

[redacted]

My signature is attestation that I have personally reviewed the submitted material(s) and the above diagnosis reflects that evaluation.

Addendum

In-situ hybridization and immunohistochemistry have been performed on the invasive squamous cell carcinoma of the larynx in Part 3 and results are as follows:

STAIN	POSITIVE/NEGATIVE
HPV	negative
P16	negative
EGFR	3+

[redacted]

[redacted]

My signature is attestation that I have personally reviewed the submitted material(s) and the above diagnosis reflects that evaluation.

FINAL DIAGNOSIS:

PART 1: LYMPH NODES, NECK, LEVELS 2 - 4, SELECTIVE DISSECTION
SEVENTEEN LYMPH NODES, NO TUMOR PRESENT (0/17).

PART 2: LYMPH NODES, RIGHT NECK, LEVELS 2 - 4 INCLUDING LEVEL 2B, SELECTIVE DISSECTION

A. METASTATIC SQUAMOUS CELL CARCINOMA IN ONE OF FOURTEEN LYMPH NODES (2.2 CM) WITH SPINDLE CELL FEATURES (1/14).

B. EXTRACAPSULAR SPREAD IS PRESENT.

PART 3: LARYNX AND THYROID LOBE, RIGHT, TOTAL LARYNGECTOMY AND RIGHT THYROID LOBECTOMY (12 GRAMS)

A. INVASIVE SQUAMOUS CELL CARCINOMA, POORLY DIFFERENTIATED, KERATINIZING, WITH FOCAL SPINDLE CELL FEATURES, TRANSGLOTTIC, WITH SUPRA-AND SUB-GLOTTIC EXTENSION TO THE RIGHT TRUE AND FALSE FOCAL CORDS AND LEFT TRUE VOCAL CORD (5.5 CM).

B. ANGIOLYMPHATIC INVASION IS PRESENT.

C. PERINEURAL INVASION IS PRESENT.

D. THYROID AND CRICOID CARTILAGE, INVASION PRESENT.
E. MARGINS: THE RIGHT PYRIFORM SINUS SOFT TISSUE MARGIN AND RIGHT PRE-EPIGLOTTIC SOFT TISSUE MARGINS ARE POSITIVE FOR SQUAMOUS CELL CARCINOMA; OTHER MARGINS FREE (see also parts 5-9)
F. PATHOLOGIC STAGE: pT4aN1.
G. TRACHEOSTOMY WITH REACTIVE CHANGES, UNINVOLVED BY TUMOR.

H. RIGHT THYROID LOBE: PAPILLARY THYROID MICROCARCINOMAS, TWO FOCI, (0.9 AND 0.3 CM), CONFINED TO THYROID; NO ANGIOLYMPHATIC INVASION.
I. PATHOLOGIC STAGE: pT1aN0.
J. NON-NEOPLASTIC THYROID WITH KERATINIZING LYMPHOEPITHELIAL CYST (see comment).
K. NON-NEOPLASTIC THYROID WITH NODULAR HYPERPLASIA.

L. TWO CENTRAL LYMPH NODES, NO SQUAMOUS CELL CARCINOMA OR PAPILLARY THYROID CARCINOMA PRESENT (0/2).

PART 4: DEEP PYRIFORM SINUS, EXCISION
INVASIVE SQUAMOUS CELL CARCINOMA.
PART 5: RIGHT LATERAL MARGIN, EXCISION
NO TUMOR PRESENT.
PART 6: INFERIOR MARGIN, EXCISION
NO TUMOR PRESENT.
PART 7: LEFT PHARYNGEAL MARGIN, EXCISION
NO TUMOR PRESENT.
PART 8: SUPERIOR MARGIN, EXCISION
NO TUMOR PRESENT.
PART 9: NEW RIGHT LATERAL MARGIN, EXCISION
NO TUMOR PRESENT.

[redacted]

COMMENT:
The keratinizing lymphoepithelial cyst in the thyroid is lined by reactive, but bland, epithelium that is distinct from the patient' s solid high grade laryngeal squamous cell carcinoma and papillary thyroid carcinomas which are of the follicular variant.

[redacted]

My signature is attestation that I have personally reviewed the submitted material(s) and the final diagnosis reflects that evaluation.

GROSS DESCRIPTION:
The specimen is received in nine parts.
Part 1 is received fixed, labeled with the patient's name (initials xx) and "left neck levels 2, 3, 4." Received is an unoriented portion of fibroadipose tissue measuring 11.1 x 2.0 x 1.5 cm. The first third of the specimen contains up to eight potential soft, tan-pink lymph nodes ranging from 0.2 to 1.1 cm in greatest dimension. The middle third of the specimen contains up to two potential soft, tan-gray lymph nodes measuring 0.2 and 0.8 cm. The last third of the specimen contains no grossly identifiable lymph nodes.
Cassette code:
1A 1B multiple potential lymph nodes, first third
1C multiple potential lymph nodes, middle third

1D adipose tissue, last third
Part 2 is received fixed, labeled with the patient's name (initials xx) and "right neck levels 2, 3, 4 including 2B". Received is an unoriented portion of fibroadipose tissue measuring 12.0 x 2.5 x 1.4 cm. A 2.2 x 1.4 x 0.8 cm lymph node in the middle third of the specimen (designated level 2B) has a firm, tan-white cut surface with central necrotic material and pinpoint hemorrhages. Up to 6 other potential soft lymph nodes are identified in the middle third of the specimen ranging from 0.1 to 0.3 cm. The first, adjacent third of the specimen contains the most connective tissue and is presumed to represent level 3, having anatomically bent inferior to the middle third of the specimen. The first third of the specimen contains two soft tan lymph nodes measuring 1.3 and 2.2 cm. The opposite, last fatty third of the specimen is designated as level 4 and contains multiple potential lymph nodes ranging from 0.2 x 0.2 x 0.2 cm to 1.0 x 0.4 x 0.3 cm.
Cassette code:
2A enlarged lymph node, middle third (level 2B), serially sectioned and entirely submitted
2B multiple potential lymph nodes, middle third
2C up to two potential lymph nodes, first third
2D multiple potential lymph nodes, last third
Part 3 is received fixed, labeled with the patient's name (initials xx) and "total laryngectomy plus right thyroid lobe". Received is a laryngectomy specimen measuring 11.0 cm (superior to inferior) x 9.0 cm (medial to lateral) x 5.5 cm (anterior to posterior). The specimen contains a tracheostomy site with 5.5 x 4.0 cm ellipse of skin, 5.5 x 3.0 x 1.5 cm right thyroid lobe with 3.0 x 2.5 x 1.0 cm portion of isthmus, and seven tracheal rings.
An ulcerated, white, firm mass originates in the right glottis, measuring 5.5 cm (medial to lateral) x 4.0 cm (superior to inferior) x 3.0 cm (anterior to posterior). The mass has supra- and infra-glottic extension, and involves the right false vocal cord, right ventricle, right true vocal cord, left true vocal cord, pre-epiglottic soft tissue, thyroid cartilage, cricoid cartilage, anterior soft tissue and muscle, and right pyriform soft tissue margin. Grossly the hyoid bone is not involved. No lymph nodes are identified in the soft tissue.
The right thyroid lobe shows a dusky tan, firm, gelatinous mass measuring 1.4 x 1.2 x 2.0 cm (12 grams) in the mid zone, with multiple tan-white, firm nodules up to 0.3 cm immediately adjacent to and within the mass.
Digital photographs are taken.
Tissue from the lesion and normal tissue are banked for [redacted]
Ink code
Red tissue bank
Blue pre-epiglottic soft tissue margin
Orange soft tissue exposed after removal of the hyoid bone (not true margin)
Black all other margins
Cassette code:
3A bilateral posterior cricoid margins, shave
3B right pyriform sinus soft tissue margin, perpendicular
3C right pre-epiglottic soft tissue margin, perpendicular
3D anterior soft tissue margin and thyroid cartilage, perpendicular
3E mass involving thyroid cartilage with cricoid cartilage and thyroid gland
3F right false vocal cord, ventricle, and true vocal cord
3G left false vocal cord, ventricle, and true vocal cord
3H tracheal margin, shave
3I tracheostomy site with skin
3J 3N right thyroid nodule submitted from inferior to superior
Part 4 was received fresh for frozen intraoperative consultation, labeled with

the patient's name (initials xx) and "deep pyriform sinus". The specimen consists of two fragments of tan-white soft tissue measuring 0.4 x 0.2 x 0.2 cm and 0.1 x 0.1 x 0.1 cm. The specimen is entirely submitted for permanent section in cassette 4AFS.
Part 5 was received fresh for frozen intraoperative consultation, labeled with the patient's name (initials xx) and "right lateral margin". It consists of a tan-white soft tissue measuring 3.6 x 0.8 x 0.5 cm, with the clipped end inked in black. The specimen is entirely submitted for permanent section in cassette 5AFS.
Part 6 was received fresh for frozen intraoperative consultation, labeled with the patient's name (initials xx) and "inferior margin". It consists of a portion of soft tan-white tissue measuring 5.0 x 0.6 x 0.5 cm, with the clipped end inked in black. The specimen is entirely submitted for permanent section in cassette 6AFS.
Part 7 was received fresh for frozen intraoperative consultation, labeled with the patient's name (initials xx) and "left pharyngeal margin". Received is a portion of tan-white soft tissue measuring 3.5 x 0.4 x 0.4 cm, with the clipped end inked in black. The specimen is entirely submitted for permanent section in cassette 7AFS.
Part 8 was received fresh for frozen intraoperative consultation, labeled with the patient's name (initials xx) and "superior margin". Received is a portion of soft tan-white soft tissue measuring 5.5 x 0.6 x 0.5 cm, with the clipped end inked in black. The specimen is entirely submitted for permanent section in cassette 8AFS.
Part 9 was received fresh for frozen intraoperative consultation, labeled with the patient's name (initials xx) and "new right lateral margin, clip superior". Received is a portion of soft tan-white tissue measuring 3.7 x 0.6 x 0.5 cm, with the clipped end inked in black. The specimen is entirely submitted for permanent section in cassette 9AFS.
Dictated by: [redacted]

INTRAOPERATIVE CONSULTATION:
4AFS: RIGHT DEEP PYRIFORM SINUS, EXCISION (frozen section)
A. MALIGNANT.
B. INVASIVE SQUAMOUS CELL CARCINOMA ([redacted] M.D.).
5AFS: RIGHT LATERAL MARGIN, EXCISION (frozen section)
A. DEFER.
B. SMALL DETACHED CLUSTER OF ATYPICAL SQUAMOUS CELLS ([redacted]

6AFS: INFERIOR MARGIN, EXCISION (frozen section)
A. BENIGN.
B. NO TUMOR PRESENT ([redacted]).

7AFS: LEFT PHARYNGEAL MARGIN, EXCISION (frozen section)
A. BENIGN.
B. NO TUMOR PRESENT ([redacted]
8AFS: SUPERIOR MARGIN, EXCISION (frozen section)
A. BENIGN.
B. NO TUMOR PRESENT ([redacted]
9AFS: NEW RIGHT LATERAL MARGIN, CLIP SUPERIOR, EXCISION (frozen section)
A. BENIGN.
B. NO TUMOR PRESENT ([redacted]).

MICROSCOPIC:

Microscopic examination substantiates the above diagnosis.

The following statement applies to all immunohistochemistry, Insitu Hybridization Assays (ISH & FISH), Molecular Anatomic Pathology, and Immunofluorescent Testing:
The testing was developed and its performance characteristics determined by the [redacted] Department of Pathology, as required by the CLIA [redacted] regulations. The testing has not been cleared or approved for the specific use by the U.S. Food and Drug Administration, but the FDA has determined such approval is not necessary for clinical use. Tissue fixation ranges from a minimum of [redacted] to a maximum of [redacted] hours.
This laboratory is certified under the Clinical Laboratory Improvement Amendments of [redacted] ("CLIA") as qualified to perform high-complexity clinical testing. Pursuant to the requirements of CLIA, ASR's used in this laboratory have been established and verified for accuracy and precision. Additional information about this type of test is available upon request.
CASE SYNOPSIS:
SYNOPTIC DATA - LARYNX RESECTIONS
TYPE OF LARYNGECTOMY: Total
TUMOR LATERALITY: Bilateral
ATTACHED STRUCTURES: Neck dissection, Pyriform sinus, Tracheotomy, Thyroid, Skin
TUMOR LOCATION/SEGMENT: Transglottic
TUMOR SIZE: Maximum dimension: 5.5 cm
HISTOLOGIC TYPE OF TUMOR: Squamous cell carcinoma
HISTOLOGIC GRADE: Poorly differentiated/Undifferentiated
STRUCTURES INVOLVED BY TUMOR: True cord, False cord, Ventricle, Pyriform sinus, Subglottis (greater than 1 cm subglottic extension), Thyroid cartilage, Pre-epiglottic space, Extralaryngeal soft tissue
LYMPH NODES: Lymph nodes positive, Right: 1
Total number of right sided lymph nodes examined: 16
Lymph nodes positive, Left: 0
Total number of left sided lymph nodes examined: 17
EXTRACAPSULAR SPREAD OF LYMPH NODE METASTASES
No
INTRA-PERINEURAL INVASION: Present
VASCULAR INVASION: Yes
SURGICAL MARGIN INVOLVEMENT: Positive (invasive tumor)
T STAGE, PATHOLOGIC: Glottis, pT4a
N STAGE, PATHOLOGIC: pN1
M STAGE, PATHOLOGIC: pMX

SYNOPTIC DATA - PRIMARY THYROID TUMORS
SPECIMEN TYPE: Lobectomy
TUMOR SITE: Right Lobe
TUMOR FOCALITY: Multifocal
TUMOR SIZE (largest nodule): Greatest Dimension: 2.0 cm
HISTOLOGIC TYPE**: Papillary carcinoma
PRIMARY TUMOR (pT): pT1a
REGIONAL LYMPH NODES (pN): pN0
Number of regional lymph nodes examined: 1
Number of regional lymph nodes involved: 0
DISTANT METASTASIS (pM): Not applicable
EXTRATHYROIDAL EXTENSION: Not identified
MARGINS: Margins uninvolved by carcinoma
LYMPH-VASCULAR INVASION: Not identified

ADDITIONAL PATHOLOGIC FINDINGS: Thyroiditis
Other: Keratinizing lymphoepithelial cyst, and nodular hyperplasia
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PATIENT HISTORY:
CHIEF COMPLAINT/ PRE-OP/ POST-OP DIAGNOSIS: Laryngeal cancer.
PROCEDURE: Total laryngectomy.
SPECIFIC CLINICAL QUESTION: Not provided.
OUTSIDE TISSUE DIAGNOSIS: Not provided.
PRIOR MALIGNANCY: Not provided.
CHEMORADIATION THERAPY: Not provided.
ORGAN TRANSPLANT: Not provided.
IMMUNOSUPPRESSION: Not provided.
OTHER DISEASES: Not provided.
[redacted]
HISTO TISSUE SUMMARY/SLIDES REVIEWED:
Part 1: Left Neck Level 2-4
Taken: [redacted]
Stain/cnt Block
H&E x 1 A
H&E x 1 B
H&E x 1 C
H&E x 1 D
Part 2: Right Neck Level 2-4 including Level 2B
Taken: [redacted]
Stain/cnt Block
H&E x 1 A
H&E x 1 B
H&E x 1 C
H&E x 1 D
Part 3: Total Laryngectomy and Right Thyroid Lobe
Taken: [redacted]
Stain/cnt Block
H&E x 1 A
H&E x 1 B
H&E x 1 C
Decal x 1 D
H&E x 1 D
H&E x 1 E
H&E Recut x 1 F
IEGFR x 1 F
IBNKNC x 1 F
IBNKNC x 1 F
IBNKNC x 1 F
IBNKNC x 1 F
IBNKNC x 1 F
IBNKNC x 1 F
H&E x 1 F
HPV x 1 F
IISH x 1 F
P16 x 1 F
V-EGFR x 1 F
H&E x 1 G
H&E x 1 H
H&E x 1 I
H&E x 1 J

MAP H&E Recut x 1 K
MAP H&E Recut x 1 K
MAPBNK x 1 K
MAPBNK x 1 K
MAPBNK x 1 K
MAPBNK x 1 K
MAPBNK x 1 K
MAPBNK x 1 K
MAPBNK x 1 K
MAPBNK x 1 K
MAPBNK x 1 K
MAPBNK x 1 K
H&E x 1 K
ISEQADDON x 1 K
ITHY-PE x 1 K
iret x 1 K
H&E x 1 L
H&E x 1 M
RHHE Lev ___ x 1 N
RHHE Lev ___ x 1 N
RHHE Lev ___ x 1 N
RHHE Lev ___ x 1 N
RHHE Lev ___ x 1 N
H&E x 1 N
Part 4: Deep Piriform Sinus
Taken: [redacted]
Stain/cnt Block
H&E x 1 AFS
Part 5: Right Lateral Margin
Taken: [redacted]
Stain/cnt Block
H&E x 1 AFS
Part 6: Inferior Margin
Taken: [redacted]
Stain/cnt Block
H&E x 1 AFS
Part 7: Left Pharyngeal Margin
Taken: [redacted]
Stain/cnt Block
H&E x 1 AFS
Part 8: Superior Margin
Taken: [redacted]
Stain/cnt Block
H&E x 1 AFS
Part 9: New Right Lateral Margin
Taken: [redacted]
Stain/cnt Block
H&E x 1 AFS
[redacted]
SPECIAL Procedures:
In Situ Procedure
Interpretation
PROBE: LSI EGFR/CEP7 Dual-Color Probe ([redacted]
Cytogenetic Location: 7p12 / 7p11.1-q11.1
3F: EGFR FISH STUDIES PERFORMED ON THE SQUAMOUS CELL CARCINOMA ARE NEGATIVE.
Number of cells analyzed: 60

Ratio EGFR/CEP7: 1.10
High Polysomy: 0%
SNR (signal to nucleus ratio): 2.6
Low Polysomy: 13(21.7%)
Trisomy: 13(21.7%)
Disomy: 34(56.7%)
3K: RET/PTC: The targeted area of the tissue showed 0(0%) of the cells with the rearrangement pattern and 60(100%) of the cells with the normal pattern. The targeted area is considered NOT rearranged for the RET/PTC region. The RET/PTC1 Probe did not contain the rearrangement pattern.

[redacted]

My signature is attestation that I have personally reviewed the submitted material(s) and the above diagnosis reflects that evaluation.
Results
EGFR FISH analysis was manually performed and quantitatively assessed by analysis of a minimum of 60 cells using the EGFR SpectrumOrange and the CEP7 SpectrumGreen probes.
EGFR FISH positive:
High Polysomy: > four gene copies in > 40% of cells
Gene Amplification: Ratio gene/chromosome more than two or > 15 gene copies in > 10% of cells
EGFR FISH negative:
Disomy: < two gene copies in more than 90% of the cells
Trisomy: three gene copies in more than 10% of cells
Low Polysomy: > four gene copies in more than 10% but less than 40% of cells
References:

[redacted]