Bethesda System Cytology Categories and Molecular Alterations in Thyroid FNA Specimens

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I have nothing to disclose.

Overview

I. Practical Issues in Thyroid Cytology
II. Common Molecular Alterations in Thyroid Neoplasms
III. Performance Characteristics of Molecular Tests for Bethesda System Diagnoses
Thyroid nodules are common: increasing number of FNAs.

FNA: most accurate and cost-effective method for pre-operative evaluation of thyroid nodules.

Thyroid FNAs are performed by a variety of physicians.

Bethesda System for Reporting Thyroid Cytopathology (BSRTC).

Use and Role of Ancillary Testing.

Thyroid FNAs are considered to be accurate ...

However,

- Historically, thyroid FNA practice lacked standardization.
- Low Pre-test Probability: Only a few percent (~5-6%) of nodules are malignant.
- Majority of thyroid malignancies are low grade.
- 20-30% of cases: “indeterminate” diagnoses.
- Some false negative diagnoses (0-3%) are inevitable.
- Diagnostic resections (e.g. lobectomy) are often performed.
- Each year, ~25,000 thyroidectomies are performed for benign non-neoplastic diseases (although not all for diagnostic purpose).

Practical Issues

A. Indeterminate Diagnoses
B. Diagnostic Sorting
C. Ancillary Studies – Molecular Testing
Bethesda System for Reporting Thyroid Cytopathology (BSRTC) and Risk

<table>
<thead>
<tr>
<th>Category</th>
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<td>Malignant</td>
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Diagnostic Approach

Thyroid Cytology

- Minimize Unsatisfactory Cases
- Definitive Diagnosis
- No
- Atypia of Undetermined Significance / Follicular Lesion of US
- Follicular Neoplasm / Suspicious for FN
- Suspicious for Malignancy

- Benign
- Malignant

- Minimize False Negatives
- Minimize False Positives

- To cut or not to cut
- How much to cut
- Other management issues

Application of Molecular Testing to Thyroid Cytology

Bethesda Thyroid Committee V (2008)

- ... utilization of IHC or molecular techniques in this setting remains controversial.
- The specificity of BRAF, RET/PTC, and other markers for thyroid carcinoma is very promising, but limited validation precludes their widespread use.
- Standardized protocols with clinical validation may be required before nucleic acid-based ancillary studies can be widely utilized as an adjunct test in thyroid FNA samples.

Revised American Thyroid Association Guidelines (2009)

- Recent large prospective studies have confirmed the ability of genetic markers (BRAF, RAS, RET/PTC) and protein markers (galectin-3) to improve preoperative diagnostic accuracy for patients with indeterminate thyroid nodules.

- It is likely that some combination of molecular markers will be used in the future to optimize management of patients with indeterminate cytology on FNA specimens.

Cooper DS, et al. Thyroid;2009;19:1167-1214

Evaluation of Thyroid Nodules

- Cytology is diagnostic in 60-80% of cases
- Cytology is persistently non-diagnostic in 5-15% of cases
- Cytology indeterminate in 25-30% of cases

Mutational Analysis of Thyroid Nodules

- Testing for mutations:
  - BRAF V600E, K601E
  - NRAS codon 61
  - HRAS codon 61
  - KRAS codons 12/13
  - RET/PTC1, RET/PTC3
  - PAX8/PPARβ
- Testing for quality of FNA sample:
  - KRT7 expression
  - GAPDH expression

RNA/DNA Stabilization Reagent (Roche) stored -20°C
Mutations in Papillary Carcinoma

- **BRAF**
  - 45%
- **RET/PTC**
  - 15%
- **RAS**
  - 15%

~75% Papillary CA

- Normal
- **BRAF** V600E positive PTC: Tall Cell Variant
- **RET/PTC1** translocation: Papillary Carcinoma (Classic)

Nikiforov YE Nat Rev Endocrinol 2011: 7: 569
NRAS positive: FVPTC

BRAF K601E positive Follicular Variant PTC

Mutations in Follicular Carcinoma

Nikiforov YE Nat Rev Endocrinol 2011: 7: 569
<table>
<thead>
<tr>
<th>Clinical Prevalence</th>
<th>BRAF V600E</th>
<th>BRAF K601E</th>
<th>RAS</th>
<th>RET/PTC</th>
<th>PAX8/PPARγ</th>
<th>CTNNB1</th>
<th>TP53</th>
<th>AKT1</th>
<th>RET</th>
<th>NTRK1</th>
<th>PI3K</th>
<th>PTEN</th>
<th>AKT1</th>
<th>BRAF V600E positive PTC</th>
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<tbody>
<tr>
<td>~80%</td>
<td>Classic</td>
<td>FVPTC</td>
<td>Rare FC</td>
<td>Solid</td>
<td>Solid</td>
<td>Classic</td>
<td>FC (FA)</td>
<td>PD Ca</td>
<td>ACa</td>
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<td>BRAF V600E positive PTC</td>
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<tr>
<td>~15%</td>
<td>TCV</td>
<td>FVPTC</td>
<td>Classic</td>
<td>Solid</td>
<td>Solid</td>
<td>Classic</td>
<td>FC (FA)</td>
<td>PD Ca</td>
<td>ACa</td>
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<tr>
<td>&lt;1%</td>
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<td>Rare FC</td>
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<td>§ (RET-negative)</td>
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<td>♯ (sporadic)</td>
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Clinical Prevalence

<table>
<thead>
<tr>
<th>Clinical Prevalence</th>
<th>~80%</th>
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<th>&lt;1%</th>
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</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>-</td>
<td>45</td>
<td>15</td>
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<tr>
<td>RAS</td>
<td>20</td>
<td>40-50</td>
<td>30-54</td>
<td>50</td>
</tr>
<tr>
<td>RET/PTC</td>
<td>10-20</td>
<td>-</td>
<td>~0</td>
<td>-</td>
</tr>
<tr>
<td>PAX8/PPARγ</td>
<td>35</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NTRK1</td>
<td>~5%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CTNNB1</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>70</td>
</tr>
<tr>
<td>TP53</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20-30</td>
</tr>
<tr>
<td>AKT1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5-10</td>
</tr>
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<td>-</td>
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<td>~10</td>
<td>-</td>
<td>-</td>
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<td>20</td>
<td>70</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RAS</td>
<td>50</td>
<td>68</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RET/PTC</td>
<td>30</td>
<td>54</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<tr>
<td>TP53</td>
<td>6</td>
<td>6</td>
<td>-</td>
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</tr>
<tr>
<td>CTNNB1</td>
<td>~66</td>
<td>~66</td>
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BRAF V600E positive PTC

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Ricarte Filho JC Cancer Res 2009; 69: 4885
Nikiforov YE Nat Rev Endocrinol 2011; 7: 569
Theoharis C Curr Opin Oncol 2012; 24: 35
Moura MM J Clin Endocrinol Metab 2011; 96: E183
BSRTC Diagnoses

Indeterminate Diagnoses:

Underlying Issues

Sampling/Technical/Visualization
- Unsat – poor sampling, obscuring elements
- AUS/FLUS – partial sampling of neoplasm, focal nuclear atypia
- AUS/FLUS – heterogeneity in pattern

Lesional Characteristics
- Follicular Neoplasm/Suspicious for FN
- Suspicious for Malignancy – criteria incompletely fulfilled
Bethesda System for Reporting Thyroid Cytopathology (BSRTC) and Risk

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Unsatisfactory/Non-diagnostic

- Definition: Specimen does not fulfill adequacy criteria.
  - Fewer than 6 groups of well-preserved, well-stained follicular cell groups (10 cells each).
  - Poorly prepared, poorly stained, or obscured follicular cells.
  - Cyst fluid.
- Recommended Management: Repeat FNA.
- Key Issues:
  - Minimize number of cases placed into this category.
  - Are there lesional cells that may be tested by other methods?

Unsatisfactory/Non-diagnostic
Molecular Testing on Unsatisfactory Samples

- 11 studies from Italy, Korea, and USA.
- Molecular Tests:
  - **BRAF, RAS, RET/PTC, TRK1**
- Positive Findings:
  - Range: 0-26.4%
  - Overall: 31/278 (11.2%)
- Follow-up:
  - 23/24 BRAF + cases with malignant outcome [23 PTC; 1 FP BRAF (NH)]
  - 3/5 RAS + cases with malignant outcome (2 PTC, 1 HCC, 2 FA)
  - 1/1 RET/PTC + cases with malignant outcome (1 PTC)

Unsatisfactory/Non-diagnostic

- Optimize specimen sampling and processing for better visualization of lesional cells.
- Molecular testing on Unsatisfactory/Non-diagnostic samples may be worthwhile, depending on the adequacy threshold.

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Low risk indeterminate category

Definition: Specimens that contain
- Cells with architectural and/or nuclear atypia
- Not sufficient for FN/SFN, SMC, or PMC
- Atypia is more than what can be ascribed to benign changes.

Recommended Management: Repeat FNA.

Key Issues:
- What is the yield of molecular testing?
- Is molecular testing useful?

Atypia of Undetermined Significance/
Follicular Lesion of Undetermined Significance

AUS/FLUS

Key Mutations
- **BRAF** mutation
- **RAS** mutation
- **RET/PTC**
- **PAX8/PPAR**

Atypia of Undetermined Significance/
Follicular Lesion of Undetermined Significance

Key Mutations
- **BRAF** mutation
- **RAS** mutation
- **RET/PTC**
- **PAX8/PPAR**
Molecular Testing on AUS/FLUS

- 11 studies from Italy, Korea, and USA.
- Molecular Tests: 
  - BRAF, RAS, RET/PTC, TRK1, PAX8/PPARγ
- Positive Findings: 
  - Range: 0-31.9%
  - Overall: 119/915 (13.0%) 
- Follow-up: 
  - 75/76 BRAF+ cases with malignant outcome [75 PTC; 1 FP BRAF (NH)]
  - 22/29 RAS+ cases with malignant outcome (outcome 18 PTC, 4 FC, 7 FA),
  - 1/1 RET/PTC+ cases with malignant outcome (1 PTC)
  - 1/1 PAX8/PPARγ+ cases with malignant outcome (1 PTC)

BRAF Mutations

- Prevalence: 40-45% of papillary carcinomas
- 20-30% of poorly differentiated/anaplastic carcinomas

BRAF V600E

- Papillary Carcinoma: most common genetic alteration in PTC (45%)
  - Older age at presentation
  - Classic papillary carcinoma, tall cell variant
  - Extrathyroidal extension
  - Higher tumor stage at presentation, LN metastasis
  - Higher rate of tumor recurrence and tumor-related mortality
  - Propensity for dedifferentiation
  - Among primary thyroid neoplasms – highly specific for PTC, PD Ca, AC
  - Present in other non-thyroid malignancies: melanoma, colon, lung
- Poorly Differentiated Carcinoma
- Anaplastic Ca
- NOT in Follicular Ca (with rare exceptions)
- However, not all BRAF positive PTCs are aggressive.
AUS/FLUS and \textit{BRAF}

\begin{itemize}
  \item 119 \textit{BRAF} positive cases
    \begin{itemize}
      \item 11 AUS/FLUS
        \begin{itemize}
          \item Atypia: Architectural (3), Cytologic (6), Arch + Cyto (2)
        \end{itemize}
      \item \textit{BRAF} subtype
        \begin{itemize}
          \item V600E (6)
          \item K601E (5)
        \end{itemize}
      \end{itemize}
    \end{itemize}

\begin{itemize}
  \item Outcome Malignant 11/11
    \begin{itemize}
      \item FV PTC (5) – 4 K601E, 1 V600E
      \item Classic PTC (4) – 4 V600E
      \item TCV PTC (1) – 1 V600E
      \item Solid PTC (1) – 1 K601E
    \end{itemize}
\end{itemize}

A subset of AUS/FLUS cases (13%) are shown to have mutations by current molecular methods.

- if molecular was positive, the probability of malignancy was 88%
  - Benign outcome cases were Follicular Adenomas.
- if molecular was negative, the probability of malignancy was 5.9%

Opinions are split
- Authors of studies with low yield view that molecular testing are not useful.
- Others view that valuable information is provided for a minority but a significant number of AUS/FLUS cases.
- 10% of our BRAF positive cases were AUS/FLUS.
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Follicular Neoplasm/Susp for FN

- **Medium risk** indeterminate category
- Definition: Cellular specimen with microfollicles and small amount of colloid.
- Recommended Management: Lobectomy
- Key Issue: Is there a marker that would help distinguish benign from malignant follicular processes?
Follicular Neoplasm/Susp for FN

- Key Mutations
  - \textit{BRAF} K601E mutation
  - \textit{RAS} mutation
  - \textit{PAX8/PPAR\gamma}

\textit{RAS}

- Follicular Adenoma (30%)
- Follicular Carcinoma (40%)
- Follicular variant of papillary carcinoma (45%)
  - Tumor encapsulation
  - Lack of lymph node metastasis
  - Bilateral disease is more common
  - Progression from FA to FC to Dedifferentiation

- \textit{RAS} in Hyperplastic nodules
  - Undercalled FA?
  - Benign non-neoplastic nodule with mutation?

Molecular Testing on FN/SFN

- 6 studies from Italy, Korea, and USA.
  Zatelli (2009)
  Prosil (2010)
  Kim SK (2011)
  Nikiforov (2011)
  Marchetti (2012)
  Kang (2012)

- Molecular Tests:
  - \textit{BRAF, RAS, RET/PTC, PAX8/PPAR\gamma}

- Positive Findings:
  - Range: 0-17.8%
  - Overall: \textit{48/398 (12.1\%)}

- Molecular results correlate:
  - 11/11 \textit{BRAF} + cases with malignant outcome (all PTC)
  - 29/34 \textit{RAS} + cases with malignant outcome (26 PTC, 3 FC, 5 FA)
  - No benign hyperplastic \textit{BRAF} + cases.
Molecular Panel on FN/SFN Dx

- **RAS mutations:** NRAS > HRAS > KRAS
- **PAX8/PPARγ**
- **BRAF V601E mutation**
- **FVPTC >> CL PTC >> Follicular Carcinoma**

<table>
<thead>
<tr>
<th>Cancer Risk</th>
<th>Management</th>
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<tbody>
<tr>
<td>Positive</td>
<td>87% Total Thyroidectomy</td>
</tr>
<tr>
<td>Negative</td>
<td>14% Lobectomy</td>
</tr>
</tbody>
</table>

Benign outcome cases were RAS+ FA.


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### Typical Colloid Poor FN/SFN vs Colloid Rich FN/SFN

<table>
<thead>
<tr>
<th>Parameter for Comparison</th>
<th>Typical Colloid-Poor FN/SFN</th>
<th>Colloid-Rich FN/SFN</th>
<th>Typical vs Colloid-Rich P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probability of Malignancy</td>
<td>104/360 (28.9%)</td>
<td>24/71 (33.8%)</td>
<td>P = 0.49</td>
</tr>
<tr>
<td>Probability of Benign Hyperplasia</td>
<td>155/360 (41.1%)</td>
<td>42/71 (57.7%)</td>
<td>P = 0.03</td>
</tr>
<tr>
<td>Probability of FA</td>
<td>99/360 (27.5%)</td>
<td>6/71 (8.5%)</td>
<td>P &lt; 0.001</td>
</tr>
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### Bethesda System for Reporting Thyroid Cytopathology (BSRTC) and Risk

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</table>
Suspicious for Malignant Cells

- **High risk** indeterminate category
- Definition: Some features of malignancy raise a strong suspicion for malignancy (but not sufficient for a definitive diagnosis).
- Recommended Management: Lobectomy or Total Thyroidectomy
- Key Issue: Can a positive molecular result push the diagnosis to “positive”?

Suspicious for Malignant Cells (Susp for PTC)

Molecular Testing on Suspicious for Malignant Cells

- 11 studies from Italy, Korea, and USA.
- **Molecular Tests:**
  - *BRAF, RAS, RET/PTC, PAX8/PPARγ*
- **Positive Findings:**
  - Range: 17.6 - 85.2%
  - Overall: 279/478 (58.4%)
- **Molecular results:**
  - 200/200 *BRAF* + cases with malignant outcome (all PTC)
  - 7/10 *RAS* + with malignant outcome (6 PTC, 1 FC, 1 FA, 2 “benign”)
  - 4/4 *RET/PTC* + cases with malignant outcome (all PTC)
Suspicious for Malignant Cells

- In the context of a SMC diagnosis, the finding of \textit{BRAF} V600E appears to highly specific for PTC.
- For institutions which have a relatively conservative management practices, this may have an impact.

<table>
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<th>Molecular Panel</th>
<th>Cancer Risk</th>
<th>Management</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>95%</td>
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<td>Total Thyroidectomy</td>
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<td></td>
<td>Benign outcome: RAS+ FA.</td>
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<tr>
<td></td>
<td>Negative</td>
<td>28%</td>
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<tr>
<td></td>
<td>Lobectomy</td>
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</table>


Adding Molecular Testing Improves Diagnostic Yield

<table>
<thead>
<tr>
<th>Cancer Risk by Indeterminate Category</th>
<th>FUS</th>
<th>FN</th>
<th>SMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytology only</td>
<td>14%</td>
<td>27%</td>
<td>54%</td>
</tr>
<tr>
<td>\textit{BRAF}</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>\textit{PAK8}/\textit{PPARγ}</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>\textit{RET}/\textit{PTC}</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>\textit{RAS}</td>
<td>84</td>
<td>85</td>
<td>88</td>
</tr>
<tr>
<td>\textit{Any mutation}</td>
<td>88</td>
<td>87</td>
<td>95</td>
</tr>
<tr>
<td>\textit{No Mutation}</td>
<td>6</td>
<td>14</td>
<td>28</td>
</tr>
</tbody>
</table>

Nikiforov YE / J Clin Endocrinol Metab 2011; 96: 3390
Gene-expression Classifier

- Veracyte Afirma ®
  - Isolated mRNA from needle washings
  - Expression signature of 167 genes
  - Proprietary diagnostic algorithm
  - Validated for
    - AUS/FLUS
    - FN/SFN
    - Suspicious for Malignancy


Test Performance for AUS/FLUS Dx

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>NPV</th>
<th>PPV</th>
<th>Missed Malignancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afirma®</td>
<td>90%</td>
<td>53%</td>
<td>95%</td>
<td>38%</td>
<td>PTC</td>
</tr>
<tr>
<td>Mutational Panel®</td>
<td>63%</td>
<td>99%</td>
<td>94%</td>
<td>88%</td>
<td>PTC</td>
</tr>
</tbody>
</table>

Cancer Risk Management

- Positive → 88% → Total Thyroidectomy
- Negative → 5.9% → Observation vs Repeat FNA vs Lobectomy


Molecular Testing: work in progress

- Positive molecular results from a panel of BRAF, RAS, RET/PTC, and PAX8/PPARγ have high Positive Predictive Value (PPV).
- Benign outcomes from molecular positive cases are usually RAS positive Follicular Adenomas.
- False Positive cases resulting in a hyperplastic lesion are rare. (?demographics ?methodology)
- Indeterminate diagnoses with negative molecular results approach the NPV of a Benign case.
Summary

• **Practical Issues in Thyroid Cytology**
  – Indeterminate diagnoses still problematic

• **Common Molecular Alterations in Thyroid Neoplasia**
  – *BRAF, RAS, RET/PTC, PAX8/PPARγ*
  – 70% of common thyroid neoplasms

• **Performance Characteristics of Molecular Testing for Bethesda System Diagnoses**
  – Molecular results are helpful in sorting and adding or removing shades of gray.
## Mutations and Pathologic Correlates

<table>
<thead>
<tr>
<th>Test</th>
<th>Common Correlates</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF V600E</td>
<td>Papillary Thyroid Carcinoma (Classic, TCV); PD Ca; ACa</td>
</tr>
<tr>
<td>BRAF K601E</td>
<td>Follicular Patterned Neoplasms</td>
</tr>
<tr>
<td>RAS (N-, H-, K-)</td>
<td>Follicular Patterned Neoplasms (e.g. FVPTC, FC, FA)</td>
</tr>
<tr>
<td>RET/PTC 1 and 3</td>
<td>Papillary Thyroid Carcinoma (RET/PTC3 and solid var)</td>
</tr>
<tr>
<td>PAX-8/PPAR gamma</td>
<td>Follicular Patterned Neoplasms (e.g. FC, FA, rare FVPTC)</td>
</tr>
</tbody>
</table>