Molecular Markers for Management of Patients with Thyroid Nodules and Cancer

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Disclosures

• Quest Diagnostics (consultant)
• UPMC/CBLPath contract (compensation from employer)
Thyroid Nodule and Cancer

Thyroid Cancer Incidence and Mortality

South Korea

Ahn HS et al. NEJM (2014)

United States; SEER data

Editorial

Thyroid cancer in thyroid nodules: Finding a needle in the haystack

Ernest L. Mazzaferri, M
Management of Patients with Thyroid Nodules

**FNA cytology**

- **Benign** (70%)
  - Cancer risk low
  - Observation

- **Indeterminate** (25%)
  - Cancer risk varies
  - Repeat FNA?
  - Observation?
  - Diagnostic surgery?

- **Cancer** (5%)
  - Cancer risk high
  - Thyroidectomy
  - (+/-) RAI

**Summary**

- **Benign** (70%): Low cancer risk, observation.
- **Indeterminate** (25%): Variable cancer risk, further evaluation needed.
- **Cancer** (5%): High cancer risk, surgical intervention.

**Note:**
- FNA (Fine-Needle Aspiration) is a procedure used to extract cells for diagnostic purposes.
- The percentages indicate the likelihood of each outcome based on FNA cytology results.
# The Bethesda System for Reporting Thyroid Cytopathology

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Risk of Cancer</th>
<th>Usual management</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Nondiagnostic or Unsatisfactory</td>
<td>1-4%</td>
<td>Repeat FNA with US</td>
</tr>
<tr>
<td>II. Benign</td>
<td>0-3%</td>
<td>Clinical follow-up</td>
</tr>
<tr>
<td>III. Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance (AUS/FLUS)</td>
<td>5-15%</td>
<td>Repeat FNA</td>
</tr>
<tr>
<td>IV. Follicular Neoplasm or Suspicious for a Follicular Neoplasm (FN/SFN)</td>
<td>15-30%</td>
<td>Surgical lobectomy</td>
</tr>
<tr>
<td>V. Suspicious for malignancy</td>
<td>60-75%</td>
<td>Total or lobectomy</td>
</tr>
<tr>
<td>VI. Malignant</td>
<td>97-99%</td>
<td>Total thyroidectomy</td>
</tr>
</tbody>
</table>

Thyroid cancer incidence and mortality

Thyroid Cancer Incidence and Mortality in the U.S.

Davies L & Welch HG. JAMA (2006)


Vaccarella S et al. NEJM (2016)

Data are age-adjusted to the 2000 U.S. Census with 95% CI.

Cramer JD et al. Surgery. 2010;148:1147
Ideal molecular markers should:

• provide accurate cancer diagnosis in nodules with indeterminate cytology

• predict cancer aggressiveness pre-operatively
Proof of the principle

Progress in molecular tests for thyroid nodules/cancer

SINGLE GENE TESTS

GENOMICS TESTS

Discovery of driver mutations in thyroid cancer

Progress in Thyroid Cancer Genetics and Molecular Markers

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>20%</td>
<td>30%</td>
<td>70%</td>
<td>&gt;90%</td>
<td></td>
</tr>
</tbody>
</table>
Genomic Landscape of PTC

Thyroid differentiation status in PTC and tumor groups

Molecular Markers for Cancer Diagnosis

• Mutational markers
• Gene expression (mRNA) markers
• miRNA markers
• Circulating TSHR mRNA
• Proteomics
Expansion of Diagnostic Mutational Panels for Thyroid FNA Samples

- **Single gene tests**
  - Conventional sequencing
  - \( \text{BRAF V600E} \)
  - 35-40%
  - Very high PPV
  - Low NPV

- **Small gene panels**
  - Conventional sequencing
  - 50-65%
  - High PPV
  - Intermediate NPV

- **Large gene panels**
  - NGS
  - \( \sim 90\% \)
  - High PPV
  - High NPV
# NGS-Based ThyroSeq® v2 Genomic Classifier

56-genes: 14 genes for mutations; 42 fusion types; 16 genes for expression

## Gene Mutations (DNA)

<table>
<thead>
<tr>
<th>Gene</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>RET</td>
</tr>
<tr>
<td>NRAS</td>
<td>TSHR</td>
</tr>
<tr>
<td>HRAS</td>
<td>AKT1</td>
</tr>
<tr>
<td>KRAS</td>
<td>TP53</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>GNAS</td>
</tr>
<tr>
<td>PTEN</td>
<td>CTNNB1</td>
</tr>
<tr>
<td>TERT</td>
<td>EIF1AX</td>
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</table>

## Gene Fusions (mRNA)

<table>
<thead>
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<th>Gene</th>
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</thead>
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<td>RET</td>
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</tr>
<tr>
<td>PPARPG</td>
<td></td>
</tr>
<tr>
<td>NTRK1</td>
<td></td>
</tr>
<tr>
<td>NTRK3</td>
<td></td>
</tr>
<tr>
<td>BRAF</td>
<td></td>
</tr>
<tr>
<td>ALK</td>
<td></td>
</tr>
<tr>
<td>Other (proprietary)</td>
<td></td>
</tr>
</tbody>
</table>

## Gene expression (mRNA)

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>PGK1</td>
<td>pan-cell marker</td>
</tr>
<tr>
<td>KRT7</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>Thyroid epithelial cells</td>
</tr>
<tr>
<td>TTF1</td>
<td></td>
</tr>
<tr>
<td>NIS</td>
<td></td>
</tr>
<tr>
<td>Calcitonin</td>
<td>MTC</td>
</tr>
<tr>
<td>PTH</td>
<td>parathyroid</td>
</tr>
<tr>
<td>KRT20</td>
<td>metastatic</td>
</tr>
<tr>
<td>Other (proprietary)</td>
<td></td>
</tr>
</tbody>
</table>
NGS-Based ThyroSeq® v2 Genomic Classifier

Step 1: Detection of:
- gene mutations
- gene fusions
- gene expression

Step 2: Cancer probability assessment:
- mutated/fused gene
- mutation hotspot
- AF of mutation
- gene expression profile
- sample adequacy

Step 3: Cancer risk assessment
- Combination of mutations
Patients with FN/SFN (Bethesda IV) cytology and known surgical outcome

143 consecutive FNA samples

Retrospective and prospective arms

Cancer prevalence – 27.3%

Sensitivity 90% (CI: 80-99%)
Specificity 93% (CI: 88-98%)
NPV 96% (CI: 92-95%)
PPV 83% (CI: 72-95%)
Accuracy 92% (CI: 88-97%)

Nikiforov et al. Cancer 2014, 120:3627-34
ThyroSeq performance in Bethesda III nodules

Impact of the Multi-Gene ThyroSeq Next-Generation Sequencing Assay on Cancer Diagnosis in Thyroid Nodules with Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance Cytology


- 465 consecutive FNA samples with AUS/FLUS (Bethesda III) cytology
- 96 patients with surgical outcome
- Prospectively evaluated
- Cancer prevalence – 22.5%

High sensitivity/NPV, high specificity/PPV – good “rule out” and “rule in” test

ThyroSeq performance:

- Sensitivity: 91% (CI: 78-100%)
- Specificity: 92% (CI: 86-98%)
- NPV: 97% (CI: 93-100%)
- PPV: 77% (CI: 61-93%)
- Accuracy: 92% (CI: 86-97%)

ThyroSeq nodule classification:

- Thyroid follicular cells present: Mutation negative: Surgery no n=459, Surgery yes n=72
- Mutation positive: Surgery no n=31, Surgery yes n=26

Non-thyroid cells (PTH): Mutation negative n=3, Mutation positive n=3

Surgery No n=3
Independent validation of ThyroSeq v2 performance

- 156 nodules with Bethesda III and IV cytology from Boston medical Center
- Sensitivity – 95%, specificity – 60%, PPV – 66%, NPV – 94%

FNA is important in predicting the risk of thyroid malignancy. Molecular markers can potentially improve the diagnostic accuracy of FNA and reduce the number of operations. In this large prospective study we evaluated the performance of the ThyroSeq® analysis to detect oncogenic mutations in thyroid cancers. 469 FNA were performed between 02/15 - 02/16, were evaluated. 59 cases were excluded because of poor yield and 408 were sent for oncogenic mutational analysis with ThyroSeq® multigene next-generation sequencing analysis (NGS). Of the 156 indeterminate cytologies (Bethesda III-V), 148 (95%) underwent genetic analysis. Oncogenic mutations were detected in 51 patients (35%), of whom 29 (57%) had a thyroidectomy. Of the 29 patients, 19 (66%) had thyroid cancer and 10 had benign nodules (34%). The most frequently mutated genes were NRAS (p.Q61K, c.181C>A) in the benign nodules (45%), NRAS (p.Q61R, c.182A>G) in the follicular variant of papillary thyroid cancers (43%), BRAF (p.V600e, c.1799 T>A) in the papillary thyroid cancers (37%), and calcitonin gene in the medullary thyroid cancers (100%). Of the 92 patients with negative genetic markers, 16 had surgery (31%), of whom 15 were benign (94%) and 1 had thyroid cancer (6%). ThyroSeq® showed a sensitivity to detect cancer of 95% [confidence interval (CI) 75.1% – 99.9%], specificity of 60.0% [CI 38.7% – 78.9%], positive predictive value of 65.5% [CI 45.7% – 82.1%], and negative predictive value of 93.7% [CI 69.8% – 99.8%], with an overall accuracy of 75% [CI 67.6% – 91.5%]. ThyroSeq® provides a high sensitivity to detect thyroid malignancy in nodules with indeterminate cytology but the specificity was low due to the detection of an array of mutations in benign nodules. ThyroSeq® is useful to reduce unnecessary operations, but the current assay is not a “positive predictor” of malignancy because of the large number of mutations in benign nodules. Further studies will determine if the use of imaging studies in addition to cytology and oncogenic mutations will better define which patients need surgery for thyroid cancer.

Toraldo et al. ATA 2016
Preoperative Diagnosis of Benign Thyroid Nodules with Indeterminate Cytology


Aferma Gene Expression Classifier

- Multi-institutional double-blind prospective study of indeterminate cytology FNA samples
- Sample size – 265 FNAs

<table>
<thead>
<tr>
<th>Cytologic diagnosis</th>
<th>n</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>NPV</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUS/FLUS</td>
<td>129</td>
<td>90%</td>
<td>53%</td>
<td>95%</td>
<td>38%</td>
</tr>
<tr>
<td>FN/SFN</td>
<td>81</td>
<td>90%</td>
<td>49%</td>
<td>94%</td>
<td>37%</td>
</tr>
<tr>
<td>SUSP</td>
<td>55</td>
<td>94%</td>
<td>52%</td>
<td>85%</td>
<td>76%</td>
</tr>
</tbody>
</table>

High sensitivity/NPV, low specificity/PPV – good “rule out” test

Afirma Gene Expression Classifier

Gene expression classifier for the diagnosis of indeterminate thyroid nodules: a meta-analysis

Prasanna Santhanam¹ · Rodhan Khthir¹ · Todd Gress² · Ayman Elkadry¹ · Omolola Olajide¹ · Abid Yaqub³ · Henry Driscoll¹

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Site of study</th>
<th>GEC method</th>
<th>Research method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alexander et al.</td>
<td>Local, regional, and tertiary</td>
<td>RNA</td>
<td>Prospective validation</td>
</tr>
<tr>
<td>Melver et al.</td>
<td>Tertiary</td>
<td>RNA</td>
<td>Prospective</td>
</tr>
<tr>
<td>Harrell et al.</td>
<td>Local center</td>
<td>RNA</td>
<td>Not known? Retrospective</td>
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<tr>
<td>Lastra et al.</td>
<td>Tertiary</td>
<td>RNA</td>
<td>Retrospective</td>
</tr>
<tr>
<td>Marti et al.</td>
<td>Tertiary</td>
<td>RNA</td>
<td>Retrospective</td>
</tr>
<tr>
<td>Han et al.</td>
<td>Tertiary</td>
<td>RNA, DNA, BRAF</td>
<td>Retrospective</td>
</tr>
<tr>
<td>EKA et al.</td>
<td>Tertiary</td>
<td>RNA</td>
<td>Retrospective</td>
</tr>
</tbody>
</table>

Table 3 Pooled sensitivities—confidence intervals and heterogeneity results (REM)

<table>
<thead>
<tr>
<th>Test parameter (pooled values)</th>
<th>Value</th>
<th>Confidence interval</th>
<th>Heterogeneity Chi-square</th>
<th>p value</th>
<th>Inconsistency ($I^2$, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>0.957</td>
<td>0.922–0.979</td>
<td>10.99</td>
<td>0.09</td>
<td>45.40</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.305</td>
<td>0.260–0.353</td>
<td>76.29</td>
<td>&lt;0.01</td>
<td>92.10</td>
</tr>
<tr>
<td>Positive likelihood ratio</td>
<td>1.198</td>
<td>0.996–1.440</td>
<td>56.57</td>
<td>&lt;0.01</td>
<td>89.40</td>
</tr>
<tr>
<td>Negative likelihood ratio</td>
<td>0.2</td>
<td>0.111–0.357</td>
<td>4.85</td>
<td>0.56</td>
<td>0.00</td>
</tr>
<tr>
<td>Diagnostic odds ratio</td>
<td>7.857</td>
<td>4.100–15.057</td>
<td>6</td>
<td>0.42</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Comparison of Afirma and ThyroSeq Test Performance in Bethesda III (AUS) Cytology

FIG. 4. Predicted NPV and PPV of ThyroSeq v2.1 compared to the Afirma gene expression classifier test in AUS/FLUS nodules based on the sensitivity and specificity of ThyroSeq (solid lines) identified in this study and of Afirma (dashed lines) reported by Alexander et al. (14).

Nikiforov et al. *Thyroid* 2015;25:1217-23
ThyraMIR+ThyGenX Test

- 7 gene panel + 10 miRNA classifier
- Validation: 109 non consecutive cross-sectional specimens from 12 US sites
- Bethesda III and IV cytology only, with corresponding histology

<table>
<thead>
<tr>
<th>Cytology (Bethesda)</th>
<th>Cases (N)</th>
<th>Cancers (N)</th>
<th>Missed Cancers (%)</th>
<th>NPV (%)</th>
<th>PPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>58</td>
<td>19</td>
<td>2</td>
<td>(11%)</td>
<td>97</td>
</tr>
<tr>
<td>IV</td>
<td>51</td>
<td>16</td>
<td>2</td>
<td>(13%)</td>
<td>91</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>109</strong></td>
<td><strong>35</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Labourier E *J Clin Endocrinol Metab* 2015; 100: 2743
Rosetta GX Reveal miRNA classifier

- 24 miRNA Classifier
- Training set of 375 FNA specimens
- Validation with 201 of which 189 stained microscope slide specimens met RNA control
- No Hurtle cell cancers in validation set
- “Agreement” data - 17 of 31 cancers (55%) and 8 of 9 (89%) false negatives excluded

<table>
<thead>
<tr>
<th></th>
<th>Cytology (Bethesda)</th>
<th>Cases (N)</th>
<th>Cancers (N)</th>
<th>Missed Cancers</th>
<th>NPV (%)</th>
<th>PPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire Data Set</td>
<td>III+IV</td>
<td>150</td>
<td>31</td>
<td>26%</td>
<td>92</td>
<td>43</td>
</tr>
<tr>
<td>“Agreement” Data</td>
<td>III+IV</td>
<td>116</td>
<td>14</td>
<td>&lt;1%</td>
<td>100</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Cytology (Bethesda)</td>
<td>Cases (N)</td>
<td>Cancers (N)</td>
<td>NPV (%)</td>
<td>PPV (%)</td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
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<td>-------------</td>
<td>---------</td>
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<tr>
<td>Afirma</td>
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<td>129</td>
<td>31</td>
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<td>38</td>
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<tr>
<td></td>
<td>IV</td>
<td>81</td>
<td>20</td>
<td>94</td>
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<tr>
<td><strong>Total</strong></td>
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<td><strong>210</strong></td>
<td><strong>51</strong></td>
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<tr>
<td>Rosetta ALL</td>
<td>III+IV</td>
<td>150</td>
<td>31</td>
<td>92</td>
<td>43</td>
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<td>Rosetta Agreement</td>
<td>III+IV</td>
<td>116</td>
<td>14</td>
<td>100</td>
<td>41</td>
<td></td>
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<tr>
<td>ThyGenX + ThyraMir</td>
<td>III</td>
<td>58</td>
<td>19</td>
<td>97</td>
<td>68</td>
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<td>IV</td>
<td>51</td>
<td>16</td>
<td>91</td>
<td>82</td>
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<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>109</strong></td>
<td><strong>35</strong></td>
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<tr>
<td>ThyroSeq</td>
<td>III</td>
<td>95</td>
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<td>97</td>
<td>77</td>
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<td></td>
<td>IV</td>
<td>143</td>
<td>39</td>
<td>96</td>
<td>83</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>239</strong></td>
<td><strong>61</strong></td>
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</tr>
</tbody>
</table>
Additional value of gene-based tests: Cancer risk associated with individual mutations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cancer risk</th>
<th>BRAF/ALK</th>
<th>NRAS/HRAS</th>
<th>PTEN/EIF1AX</th>
<th>TSHR/GNAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NR/HRAS/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRAF/ALK</td>
<td>&gt;95%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTRK1,3PPARG</td>
<td>80%</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>RET/PTC</td>
<td>5-10%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TERT/TP53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Cancer risk >95% 80% 5‐10% Very low*

*Unless AF>30%*

Nikiforov et al. *Cancer* 2014;120:3627-34
Nikiforov et al. *Thyroid* 2015;25:1217-23
Cancer Risk in Nodules with RAS Mutations

<table>
<thead>
<tr>
<th>Medicine 2015;94:1-6</th>
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<tbody>
<tr>
<td>Total FNA samples</td>
</tr>
<tr>
<td>Total RAS+</td>
</tr>
<tr>
<td>RAS+ Cancer</td>
</tr>
<tr>
<td>RAS+ Benign</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total FNA samples</td>
</tr>
<tr>
<td>Total RAS+</td>
</tr>
<tr>
<td>RAS+ Cancer</td>
</tr>
<tr>
<td>RAS+ Benign</td>
</tr>
</tbody>
</table>
Multi-step cancer progression

**Colon cancer**
- Normal epithelium → Adenoma → Carcinoma *in situ* → Invasive carcinoma
- APC, RAS, TGFβ, BAX

**Breast cancer**
- Normal breast epithelium → Initiated cells → Carcinoma *in situ* → Invasive carcinoma → Metastasis
- Amphiregulin, RANKL, WNT4, Cyclin D1, ID4

Multi-step cancer progression
Nomenclature Revision for Encapsulated Follicular Variant of Papillary Thyroid Carcinoma
A Paradigm Shift to Reduce Overtreatment of Indolent Tumors

Table. Summary of Follow-up Information for Patients in the Study Groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1 (Noninvasive EFVPTC) (n = 109)</th>
<th>Group 2 (Invasive EFVPTC) (n = 101)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (range), y</td>
<td>45.9 (21-81)</td>
<td>42.8 (8-78)</td>
</tr>
<tr>
<td>Sex, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>91 (83)</td>
<td>71 (70)</td>
</tr>
<tr>
<td>Male</td>
<td>18 (17)</td>
<td>30 (30)</td>
</tr>
<tr>
<td>Tumor size, mean (range), cm</td>
<td>3.1 (1.1-9.0)</td>
<td>2.5 (0.6-5.5)</td>
</tr>
<tr>
<td>Extent of surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lobectomy</td>
<td>67</td>
<td>15</td>
</tr>
<tr>
<td>Total thyroidectomy</td>
<td>42</td>
<td>86</td>
</tr>
<tr>
<td>Follow-up, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range)</td>
<td>14.4 (10-26)</td>
<td>5.6 (1-18)</td>
</tr>
<tr>
<td>Median</td>
<td>13.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Adverse events during follow-up, No. (%)</td>
<td>0</td>
<td>12 (12)</td>
</tr>
</tbody>
</table>

*352 non-invasive EFVPTC reported in the literature, 2/352 (0.6%) recurred

A distinct class of thyroid tumors:
- Non-invasive, follicular-patterned, moderately to well developed nuclear features of PTC (nuclear score 2-3)
- Clonal process driven by distinct oncogenic mutations (RAS and RAS-like gene mutations)
- Highly favorable outcome (<1% risk of recurrence in 15 y)

Recommended new terminology:

“Non-Invasive Follicular Thyroid neoplasm with Papillary-like nuclear features“ (NIFTP)

Figure 2. Putative Scheme of Thyroid Carcinogenesis

<table>
<thead>
<tr>
<th>Growth Pattern</th>
<th>Nuclear Features of PTC</th>
<th>Main Oncogene</th>
<th>Disease Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillary</td>
<td>Yes</td>
<td>BRAF</td>
<td>Papillary microcarcinoma</td>
</tr>
<tr>
<td>Follicular</td>
<td>Yes</td>
<td>RAS</td>
<td>NIFTP</td>
</tr>
<tr>
<td>Follicular</td>
<td>No</td>
<td>RAS</td>
<td>Follicular adenoma</td>
</tr>
</tbody>
</table>

EFVPTC indicates encapsulated follicular variant of PTC; NIFTP, noninvasive follicular thyroid neoplasm with papillary-like nuclear features; PTC, papillary thyroid carcinoma.

Not all benign thyroid nodules are created equal: Variability in malignant potential

- **Non-clonal**
  - Benign hyperplastic nodule
  - No mutations

- **Clonal**
  - Benign follicular adenoma
  - Mutation + (e.g. RAS)
  - Atypical FA
    - NIFTP
    - FTC
  - PTC
**TERT** Promoter Mutation as an Early Genetic Event Activating Telomerase in Follicular Thyroid Adenoma (FTA) and Atypical FTA

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Mutation</th>
<th>Age at Diagnosis, y</th>
<th>Sex (M/F)</th>
<th>Primary Tumor</th>
<th>Disease Recurrence</th>
<th>Patient Outcome</th>
<th>Time, mo</th>
<th>Final Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTA-1</td>
<td>wt</td>
<td>55</td>
<td>F</td>
<td>FTA</td>
<td>no</td>
<td>DWOD</td>
<td>172</td>
<td>FTA</td>
</tr>
<tr>
<td>FTA-2</td>
<td>wt</td>
<td>40</td>
<td>F</td>
<td>FTA</td>
<td>no</td>
<td>AWOD</td>
<td>316</td>
<td>FTA</td>
</tr>
<tr>
<td>FTA-3</td>
<td>wt</td>
<td>52</td>
<td>M</td>
<td>FTA</td>
<td>no</td>
<td>DWOD</td>
<td>87</td>
<td>FTA</td>
</tr>
<tr>
<td>FTA-4</td>
<td>wt</td>
<td>32</td>
<td>F</td>
<td>FTA</td>
<td>no</td>
<td>AWOD</td>
<td>314</td>
<td>FTA</td>
</tr>
<tr>
<td>FTA-5</td>
<td>wt</td>
<td>46</td>
<td>F</td>
<td>FTA</td>
<td>no</td>
<td>AWOD</td>
<td>313</td>
<td>FTA</td>
</tr>
<tr>
<td>FTA-6</td>
<td>wt</td>
<td>40</td>
<td>M</td>
<td>FTA</td>
<td>no</td>
<td>DWOD</td>
<td>277</td>
<td>FTA</td>
</tr>
<tr>
<td>FTA-7</td>
<td>wt</td>
<td>48</td>
<td>M</td>
<td>FTA</td>
<td>no</td>
<td>AWOD</td>
<td>309</td>
<td>FTA</td>
</tr>
</tbody>
</table>

**TABLE 1. Mutations and Follow-Up for the 58 Patients With a Primary FTA**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Mutation</th>
<th>Age at Diagnosis, y</th>
<th>Sex (M/F)</th>
<th>Primary Tumor</th>
<th>Disease Recurrence</th>
<th>Patient Outcome</th>
<th>Time, mo</th>
<th>Final Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTA-21</td>
<td>C228T</td>
<td>69</td>
<td>F</td>
<td>FTA</td>
<td>yes, FTC</td>
<td>DOD</td>
<td>250</td>
<td>FTC</td>
</tr>
<tr>
<td>FTA-22</td>
<td>wt</td>
<td>52</td>
<td>M</td>
<td>FTA</td>
<td>no</td>
<td>AWOD</td>
<td>301</td>
<td>FTA</td>
</tr>
<tr>
<td>FTA-23</td>
<td>wt</td>
<td>50</td>
<td>M</td>
<td>FTA</td>
<td>no</td>
<td>AWOD</td>
<td>301</td>
<td>FTA</td>
</tr>
<tr>
<td>FTA-24</td>
<td>wt</td>
<td>63</td>
<td>M</td>
<td>FTA</td>
<td>no</td>
<td>DWOD</td>
<td>255</td>
<td>FTA</td>
</tr>
<tr>
<td>FTA-26</td>
<td>wt</td>
<td>57</td>
<td>F</td>
<td>FTA</td>
<td>no</td>
<td>AWOD</td>
<td>297</td>
<td>FTA</td>
</tr>
<tr>
<td>FTA-27</td>
<td>wt</td>
<td>43</td>
<td>M</td>
<td>FTA</td>
<td>no</td>
<td>DWOD</td>
<td>289</td>
<td>FTA</td>
</tr>
</tbody>
</table>

The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary

David N. Louis¹ · Arie Perry² · Guido Reifenberger³,⁴ · Andreas von Deimling⁴,⁵ · Dominique Figarella-Branger⁶ · Webster K. Cavenee⁷ · Hiroko Ohgaki⁸ · Otmar D. Wiestler⁹ · Paul Kleihues¹⁰ · David W. Ellison¹¹

Diffuse astrocytic and oligodendroglial tumours
Diffuse astrocytoma, IDH-mutant 9400/3
Gemistocytic astrocytoma, IDH-mutant 9411/3
Diffuse astrocytoma, IDH-wildtype 9400/3
Diffuse astrocytoma, NOS 9400/3

“The current update breaks with the century-old principle of diagnosis based entirely on microscopy by incorporating molecular parameters into the classification of CNS tumors”
• 1510 patients, 97% with PTC
• Excised tumors tested for 7 common mutations
• 70% of tumors found mutation-positive
• Mean follow-up 33 ± 21.2 months with PTC
Molecular markers of cancer risk stratification

DTC, n=469

Melo M et al. JCEM (2014)

DTC, n=551

Song YS et al. Cancer (2016)

PTC, n=507

Xing M et al. JCO (2014)

DTC, n=551

ATA risk groups

Song YS et al. Cancer (2016)
Cancer risk assessment using ThyroSeq v2

Multiple Mutations Detected Preoperatively May Predict Aggressive Behavior of Papillary Thyroid Cancer and Guide Management—A Case Report

Rupendra T. Shrestha, Arivarasan Karunamurthy, Khalid Amin, Yuri E. Nikiforov, and M. Luiza Caramori

0.6 cm nodule

AUS cytology

BRAF+/TERT+/AKT1+/PIK3CA+

35 (55%) BRAF + Another HR mutation

18 (29%) RAS + Another HR mutation

3 (5%) Other Multiple HR mutations

55 (98%) Thyroid Cancer

51 (93%) Cancers with Aggressive Features:
- Extrathyroidal extension (55%)
- Vascular invasion (53%)
- Lymph node macrometastasis (47%)
- Poorly differentiated/anaplastic carcinoma areas (14%)
- Distant metastasis (8%)

2016 ATA abstract #210
Molecular markers to predict cancer risk

Risk of Structural Disease Recurrence
(In patients without structurally identifiable disease after initial therapy)

High Risk
Gross extrathyroidal extension, incomplete tumor resection, distant metastases, or lymph node >3cm

Intermediate Risk
Aggressive histology, minor extrathyroidal extension, vascular invasion, or > 5 involved lymph nodes (0.2-3 cm)

Low Risk
Intrathyroidal DTC ≤ 5 LN micrometastases (< 0.2 cm)

Molecular Signature

- BRAF+TERT, RAS+TERT
- Multiple driver mutations (eg. NRAS and PIK3CA or TP53)
- TERT
- ALK fusions
- NTRK1 fusions
- NTRK3 fusions
- BRAF V600E
- RET/PTC
- RAS
- BRAF K601E
- PAX8/PPARG

FTC, extensive vascular invasion (≈ 30-55%)
pT4a gross ETE (≈ 30-40%)
pN1 with extranodal extension, >3 LN involved (≈ 40%)
PTC, >1 cm, TERT mutated ± BRAF mutated* (≈40%)
pN1, any LN > 3 cm (≈ 30%)
PTC, extrathyroidal, BRAF mutated* (≈ 10-40%)
PTC, vascular invasion (≈ 15-30%)
Clinical N1 (≈20%)
pN1, > 5 LN involved (≈20%)
Intrathyroidal PTC, < 4 cm, BRAF mutated* (≈10%)
pT3 minor ETE (≈3-8%)
pN1, all LN < 0.2 cm (≈5%)
pN1, ≤ 5 LN involved (≈5%)
Intrathyroidal PTC, 2-4 cm (≈ 5%)
Multifocal PMC (≈ 4-6%)
pN1 without extranodal extension, ≤ 3 LN involved (2%)
Minimally invasive FTC (≈ 2-3%)
Intrathyroidal, < 4 cm, BRAF wild type* (≈ 1-2%)
Intrathyroidal unifocal PTMC, BRAF mutated*, (≈ 1-2%)
Intrathyroidal, encapsulated, FV-PTC (≈1-2%)
Unifocal PMC (≈ 1-2%)

NIFTP (<1%)

Haugen BR et al. Thyroid. 2016, 26:1-133
Summary: Clinical management based on the results of cytology and molecular testing

Bethesda III-IV Cytology

ThyroSeq v2

Test result
- Negative: no mutations
- Currently Negative: low level LR mutations
- Positive: RAS-like mutation
- Positive: BRAF-like mutation
- Positive: multiple HR mutations

Probability of Cancer or NIFTP
- Currently Negative: low level LR mutations
  - NIFTP or low-risk cancer
  - NIFTP or low-risk cancer
  - NIFTP or low-risk cancer

Tumor type, risk of recurrence
- Negative:
  - N/A
- Currently Negative:
  - N/A
- Positive:
  - N/A
- Positive:
  - N/A
- Positive:
  - N/A

Patient management
- Observation
- Active surveillance
- Lobectomy
- Total thyroidectomy or lobectomy
- Total thyroidectomy +/- CCLND

Test result
- Negative:
  - 3-4%
- Currently Negative:
  - <10%
- Positive:
  - 80-90%
- Positive:
  - 95-99%
- Positive:
  - 98%

Probability of Cancer or NIFTP
- NIFTP or low-risk cancer
- NIFTP or low-risk cancer
- NIFTP or low-risk cancer

Tumor type, risk of recurrence
- N/A
- N/A
- N/A

Patient management
- Observation
- Active surveillance
- Lobectomy
- Total thyroidectomy or lobectomy
- Total thyroidectomy +/- CCLND
Role of Molecular Markers in Thyroid Nodule Management

• Demise of indeterminate cytology
• Departure from the diagnosis based entirely on microscopic pathology
• Cancer prognostication preoperatively

Personalized management of patients with thyroid nodules or cancer
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Umamaheswar Duvvuri

Endocrinology
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Christopher Coyne

Radiology
Mitchell Tublin

Pharmacology
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