The role of Molecular Markers in Thyroid Nodule Management: Then and Now

Yuri E. Nikiforov, MD, PhD
Division of Molecular & Genomic Pathology
University of Pittsburgh Medical Center
Disclosures

• Quest Diagnostics (consultant)
• Service agreement between UPMC and CBLPath to offer ThyroSeq for commercial use
Progress in Identifying Driver Mutations in Thyroid Cancer
Beginning of the molecular era…


A new oncogene in human thyroid papillary carcinomas and their lymph-nodal metastases.

Fusco A, Grieco M, Santoro M, Berlingieri MT, Pilotti S, Pierotti MA, Della Porta G, Vecchio G.

**PTC Is a Novel Rearranged Form of the ret Proto-Oncogene and Is Frequently Detected In Vivo in Human Thyroid Papillary Carcinomas**

Michele Grieco,* Massimo Santoro,* Maria Teresa Berlingieri,* Rosa Marina Melillo,† Rosangela Donghi,† Italia Bongarzone,† Marco A. Pierotti,‡ Giuseppe Della Porta,‡ Alfredo Fusco,§ and Giancarlo Vecchio†

We previously reported that a new oncogene was frequently activated in human thyroid papillary carcinomas (Fusco et al., 1967), on the basis of a transfection assay on NIH 3T3 mouse fibroblasts (Graham and van der Eb, 1973). The transforming gene that we detected was acti-

**Activated ras Oncogenes in Human Thyroid Cancers**

Nick R. Lemoine, Edward S. Mayall, Fiona S. Wyllie, Christine J. Farr, David Hughes, Rose Anne Padua, Valerie Thurston, E. Dilwyn Williams, and David Wynford-Thomas

[Cancer Research 48, 4459–4463, August 15, 1988]
Advances in Brief

High Prevalence of \textit{BRAF} Mutations in Thyroid Cancer: Genetic Evidence for Constitutive Activation of the RET/PTC-RAS-BRAF Signaling Pathway in Papillary Thyroid Carcinoma$^1$

Edna T. Kimura, Marina N. Nikiforova, Zhaowen Zhu, Jeffrey A. Knauf, Yuri E. Nikiforov, and James A. Fagin$^2$

\textit{Division of Endocrinology and Metabolism [E. T. K., J. A. K., J. A. F.] and Department of Pathology [M. N. N., Z. Z., Y. E. N.], University of Cincinnati College of Medicine, Cincinnati, Ohio 45267}

Table 2 \textit{Lack of overlap among BRAF, RAS, and RET/PTC mutations in papillary carcinomas}

<table>
<thead>
<tr>
<th>Mutation prevalence</th>
<th>BRAF</th>
<th>RAS</th>
<th>RET/PTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>32.8%</td>
<td>22/67</td>
<td>0</td>
</tr>
<tr>
<td>RAS</td>
<td>16.4%</td>
<td>0</td>
<td>11/67</td>
</tr>
<tr>
<td>RET/PTC</td>
<td>16.4%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>65.6%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Genetic Basis of Thyroid Cancer

**Major pathways involved:**

MAPK, PI3K

**Main mutation mechanisms:**

Point mutations and chr. rearrangements
Genomic Landscape of PTC

Point mutations: 74%
Gene fusions: 15%
Copy number variations: 9%

Driver mutations define carcinogenic mechanisms

**BRAFV600E-like and RAS-like scores**

Thyroid Differentiation Status in PTC

Frequencies of Somatic Mutation in Different Tumors

Molecular Markers for Cancer Diagnosis
## 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>Repat 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>

Molecular Markers for Thyroid Cytology

- Gene mutations
- Gene expression (mRNA)
- Circulating TSHR mRNA
- miRNAs
- Proteomics
- Combination of marker types
Progress in Composition of Gene Mutation Panels

**Single genes**
Conventional sequencing

**7-gene panel**
Conventional sequencing

**Multi-gene panel**
Next-Generation sequencing
Single gene tests - \textit{BRAF V600E}

Meta-analysis of \textit{BRAF} in Fine Needle Aspiration Biopsies of the Thyroid: A Potential Application for the Preoperative Assessment of Thyroid Nodules

\textbf{Conclusions:} \textit{BRAF} mutations are common in conventional PTCs, and they are specific for PTC. A \textit{BRAF} mutation can be reliably detected in cells aspirated from a thyroid nodule suggesting a role for this marker in the preoperative evaluation of thyroid nodules.


Diagnostic value of fine needle aspiration \textit{BRAF(V600E)} mutation analysis in papillary thyroid cancer: a systematic review and meta-analysis.

Fna\textsuperscript{1}, Soobiah C\textsuperscript{2}, Al-Qahtani K\textsuperscript{1}, Hamid JS\textsuperscript{3}, Perrier L\textsuperscript{2}, Straus SE\textsuperscript{4}, Tricco AC\textsuperscript{5}.

- Meta-analysis of 47 studies
- 9,924 FNA tested for \textit{BRAF V600E}
- Specificity – ~100%
- Sensitivity – 52% (95%CI 39%-64%)

High specificity/PPV, very low sensitivity/NPV – good “rule in” test
Small (7-gene) panels

Molecular Testing for Mutations in Improving the Fine-Needle Aspiration Diagnosis of Thyroid Nodules


*J Clin Endocrinol Metab* 94: 2092–2098, 2009
Small (7-gene) panels

### TABLE 3. Comparison of Published Experiences With the 7-Gene Mutation/Fusion Panels on Thyroid Nodules With Indeterminate Cytology*

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Sample collection</strong></td>
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</tr>
<tr>
<td>No. of institutions</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Cytologic category</td>
<td>Prospective</td>
<td>Prospective</td>
<td>Prospective</td>
<td>Prospective</td>
<td>Retrospective</td>
<td>Retrospective</td>
<td>Prospective</td>
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<td>Single</td>
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<td>Multiple</td>
<td>Single</td>
<td>Single</td>
<td>Multiple</td>
</tr>
<tr>
<td></td>
<td>AUS/ FN/ FLUS</td>
<td>SFN</td>
<td>SFN</td>
<td>AUS/ FN/ SFN</td>
<td>SFN</td>
<td>AUS/ FN/ SFN</td>
<td>SFN/ SFN</td>
</tr>
<tr>
<td><strong>No. of cases</strong></td>
<td>21</td>
<td>23</td>
<td>41</td>
<td>247</td>
<td>22</td>
<td>19</td>
<td>141</td>
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<tr>
<td><strong>Prevalence of</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>malignancy based on</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>cytology^a</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Results</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>18</td>
<td>14</td>
<td>34</td>
<td>222</td>
<td>18</td>
<td>14</td>
<td>120</td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>9</td>
<td>7</td>
<td>25</td>
<td>5</td>
<td>21</td>
<td>31</td>
</tr>
<tr>
<td><strong>Test results^b</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>True-negative</td>
<td>18</td>
<td>11</td>
<td>33</td>
<td>209</td>
<td>9</td>
<td>12</td>
<td>102</td>
</tr>
<tr>
<td>False-negative</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>13</td>
<td>2</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>False-positive</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>True-positive</td>
<td>3</td>
<td>9</td>
<td>6</td>
<td>22</td>
<td>33</td>
<td>4</td>
<td>22</td>
</tr>
<tr>
<td><strong>Test performance^c</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
<td>75%</td>
<td>86%</td>
<td>63%</td>
<td>57%</td>
<td>36%</td>
<td>49%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
<td>100%</td>
<td>97%</td>
<td>99%</td>
<td>97%</td>
<td>82%</td>
<td>92%</td>
</tr>
<tr>
<td>NPV</td>
<td>100%</td>
<td>79%</td>
<td>97%</td>
<td>94%</td>
<td>86%</td>
<td>56%</td>
<td>85%</td>
</tr>
<tr>
<td>PPV</td>
<td>100%</td>
<td>100%</td>
<td>86%</td>
<td>88%</td>
<td>87%</td>
<td>67%</td>
<td>80%</td>
</tr>
</tbody>
</table>

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

*Nishino M. Cancer Cytopathol 2016;124:14-27*
Afirma Gene Expression Classifier (GEC)

Cytology performed at Veracyte

Molecular performed at Veracyte

25 Genes Screening Cassettes
1. Parathyroid
2. HCC
3. MTC
4. RCC
5. Breast
6. Melanoma

142 Gene Classifier

“Benign” or “Suspicious”
Afirma 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
Genomic Revolution: Next Generation (Deep) Sequencing

Conventional Sequencing

- Sequence up to $10^2$-$10^3$ bases
- Cost - $2400$ per $10^6$ bases
- Sensitivity - 20-30% of mutant allele

Next Generation Sequencing

- Sequence up to $10^6$-$10^9$ bases
- Cost - $0.05$-$1$ per $10^6$ bases
- Sensitivity - 3-5% of mutant allele
Expansion of Molecular Panels Using Next-Gen Sequencing

12% Novel gene fusions (eg. ALK, NTRK3)
13% Novel mutations (eg. TERT, EIF1AX)

- PAX8/PPARγ
- RET/PTC1
- RET/PTC3
- BRAF
- BRAF other
- PTEN
- RET
- RAS
- CTNNB1
- PIK3CA
- TSHR
- AKT1
- TP53

90% 65%
**ThyroSeq® v.2 NGS Mutation Panel**

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

<table>
<thead>
<tr>
<th>Gene Mutations (DNA)</th>
<th>Gene Fusions (mRNA)</th>
<th>Gene expression (mRNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>RET</td>
<td>PGK1 – <em>pan-cell marker</em></td>
</tr>
<tr>
<td>NRAS</td>
<td>TSHR</td>
<td>KRT7</td>
</tr>
<tr>
<td>HRAS</td>
<td>AKT1</td>
<td>TG</td>
</tr>
<tr>
<td>KRAS</td>
<td>TP53</td>
<td>TTF1</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>GNAS</td>
<td>NIS</td>
</tr>
<tr>
<td>PTEN</td>
<td>CTNNB1</td>
<td>Calcitonin – MTC</td>
</tr>
<tr>
<td>TERT</td>
<td>EIF1AX</td>
<td>PTH – parathyroid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KRT20 – metastatic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other</td>
</tr>
</tbody>
</table>

- Thyroid epithelial cells
- Metastatic
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

Yuri E. Nikiforov,1 Sally E. Carty,2 Simon I. Chiosea,1 Christopher Coyne,3 Umamaheswar Duvvuri,4 Robert L. Ferris,4 William E. Gooding,5 Shane O. LeBeau,3 N. Paul Ohori,1 Raja R. Seethala,1 Mitchell E. Tublin,6 Linwah Yip,2 and Marina N. Nikiforova1

• 465 consecutive FNA samples with AUS/FLUS (Bethesda III) cytology at UPMC from May 2014-March 2015

• Prospective study

• Surgical outcome known for 96 patients

• Cancer prevalence after surgery – 22.5%

Nikiforov et al. Thyroid 2015;25:1217-23
ThyroSeq v2 Performance in AUS/FLUS (Bethesda III) Cytology Nodules

Nikiforov et al. *Thyroid* 2015;25:1217-23

High specificity/PPV and high sensitivity/NPV – good “rule in” and “rule out” test
Highly Accurate Diagnosis of Cancer in Thyroid Nodules With Follicular Neoplasm/Suspicious for a Follicular Neoplasm Cytology by ThyroSeq v2 Next-Generation Sequencing Assay

Yuri E. Nikiforov, MD, PhD; Sally E. Carty, MD; Simon I. Chiosea, MD; Christopher Coyne, MD; Umamaheswar Duvvuri, MD; Robert L. Ferris, MD, PhD; William E. Gooding, MS; Steven P. Hodak, MD; Shane O. LeBeau, MD; N. Paul Ohori, MD; Raja R. Seethala, MD; Mitchell E. Tublin, MD; Linwah Yip, MD; and Marina N. Nikiforova, MD

• Patients with FN/SFN (Bethesda IV) cytology and known surgical outcome seen at UPMC from Oct. 2013-May 2014
• Retrospective and prospective groups
• Sample size: 143 consecutive FNA samples
• Cancer prevalence after surgery – 39/143 (27.3%)
143 consecutive FN/SFN nodules with surgery

**Retrospective group n=91**
- Mutation NEGATIVE n=64
  - CANCER n=2
  - BENIGN n=62
  - Sensitivity 92%
  - Specificity 94%
  - PPV 85%
  - NPV 97%
  - Accuracy 93%
- Mutation POSITIVE n=27
  - CANCER n=23
  - BENIGN n=4

**Prospective group n=52**
- Mutation NEGATIVE n=37
  - CANCER n=2
  - BENIGN n=35
  - Sensitivity 86%
  - Specificity 92%
  - PPV 80%
  - NPV 95%
  - Accuracy 90%
- Mutation POSITIVE n=15
  - CANCER n=12
  - BENIGN n=3

**Overall test performance**
- 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
Test Performance in AUS/FLUS (Bethesda III) Cytology Nodules

**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
## Cancer risk associated with individual mutations

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Cancer risk</th>
<th>NRAS</th>
<th>PTEN</th>
<th>TSHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF, ALK, NTRK1,3</td>
<td>&gt;95%</td>
<td>80%</td>
<td>20-40%</td>
<td>Very low*</td>
</tr>
<tr>
<td>PPARG, RET/PTC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TERT, TP53</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*Unless AF>30%*
Cancer Risk in Nodules with RAS Mutations

RAS mutation-positive “benign” nodules

Evidence for clonal neoplasm and early transformation to cancer

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

#### RAS mutations in AUS/FLUS Cytology: Does it Have an Additional Role in BRAF\textsuperscript{V600E} Mutation-Negative Nodules?

*Jung Hyun Yoon, MD, Hyeong Ju Kwon, MD, PhD, Hye Sun Lee, MS, Eun-Kyung Kim, MD, PhD, Hee Jung Moon, MD, PhD, and Jin Young Kwak, MD, PhD*

**Medicine** 2015;94:1-6

<table>
<thead>
<tr>
<th>Total FNA samples</th>
<th>198</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RAS+</td>
<td>31</td>
</tr>
<tr>
<td>RAS+ Cancer</td>
<td>7 (23%)</td>
</tr>
<tr>
<td>RAS+ Benign</td>
<td>24 (77%)</td>
</tr>
</tbody>
</table>

#### Preoperative RAS Mutational Analysis Is of Great Value in Predicting Follicular Variant of Papillary Thyroid Carcinoma

*Tae Sook Hwang,1 Wook Youn Kim,1 Hye Seung Han,1 So Dug Lim,1 Wan-Seop Kim,1 Young Bum Yoo,2 Kyung Sik Park,2 Seo Young Oh,3 Suk Kyong Kim,4 and Jung Hyun Yang4*


<table>
<thead>
<tr>
<th>Total FNA samples</th>
<th>132</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RAS+</td>
<td>27</td>
</tr>
<tr>
<td>RAS+ Cancer</td>
<td>26 (96%)</td>
</tr>
<tr>
<td>RAS+ Benign</td>
<td>1 (4%)</td>
</tr>
</tbody>
</table>

#### Clinical Outcomes and Molecular Profile of Differentiated Thyroid Cancers With Radioiodine-Avid Distant Metastases


**JCEM** 2013;98:E829

![Pie chart showing the distribution of different RAS mutations in all thyroid cancers](image)
Molecular Testing for miRNA, mRNA, and DNA on Fine-Needle Aspiration Improves the Preoperative Diagnosis of Thyroid Nodules With Indeterminate Cytology

Emmanuel Labourier, Alexander Shifrin, Anne E. Busseniers, Mark A. Lupo, Monique L. Manganelli, Bernard Andruss, Dennis Wylie, and Sylvie Beaudenon-Huibregtse

Asuragen, Inc (E.L., B.A., D.W., S.B.H.), Austin, Texas 78744; Jersey Shore University Medical Center (A.S.), Center for Thyroid, Parathyroid and Adrenal Diseases, Neptune, New Jersey 07753; Metropolitan Fine Needle Aspiration Service (A.E.B.), Washington, District of Columbia 20037 and Bethesda, Maryland 20814; Thyroid & Endocrine Center of Florida (M.A.L.), Sarasota, Florida 34231; and (M.L.M.) San Diego, California 92103

- Combination of 7-gene mutation panel (miRInform) and miRNA expression classifier based on 10 miRNA genes (ThyraMIR)
- Validated in 109 FNA samples with AUS/FLUS and FN/SFN cytology
- Cancer prevalence 32%
- Multi-center study (12 endocrinology centers)
Performance of combined 7-gene mutation panel (miRInform) and expression of 10 miRNA genes test

### Table 3. Performance of the Multiplatform miRNA and Mutation Test

<table>
<thead>
<tr>
<th></th>
<th>Cohort, % (95% CI)</th>
<th>AUS/FLUS, % (95% CI)</th>
<th>FN/SFN, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>109</td>
<td>58</td>
<td>51</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>89 (73–97)</td>
<td>94 (73–100)</td>
<td>82 (57–96)</td>
</tr>
<tr>
<td>Specificity</td>
<td>85 (75–92)</td>
<td>80 (64–91)</td>
<td>91 (76–98)</td>
</tr>
<tr>
<td>PPV</td>
<td>74 (58–86)</td>
<td>68 (46–85)</td>
<td>82 (57–96)</td>
</tr>
<tr>
<td>NPV</td>
<td>94 (85–98)</td>
<td>97 (84–100)</td>
<td>91 (76–98)</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>44 (13–151)</td>
<td>68 (8–590)</td>
<td>48 (9–269)</td>
</tr>
</tbody>
</table>

*J Clin Endocrinol Metab 100: 2743–2750, 2015*
# Molecular Cytopathology for Thyroid Nodules: A Review of Methodology and Test Performance

Michiyo Nishino, MD, PhD

## Table 1. Overview of 3 Commercially Available Molecular Tests for Indeterminate Thyroid Fine-Needle Aspiration

<table>
<thead>
<tr>
<th>Strengths</th>
<th>Affirma</th>
<th>ThyGenX/ThyraMIR</th>
<th>ThyroSeq</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Company</strong></td>
<td>Veracyte</td>
<td>Interface Diagnostics</td>
<td>University of Pittsburgh Medical Center, via CBLPath</td>
</tr>
<tr>
<td><strong>Methodology</strong></td>
<td>mRNA (gene expression) microarray analysis; classification as either &quot;benign&quot; or &quot;suspicious&quot; gene expression profile by a proprietary algorithm</td>
<td>ThyGenX: multiplex PCR and detection of mutations (BRAF, HRAS, NRAS, and KRAF) and rearrangements (RET-PTC1, RET-PTC3, and PAX8-PPARG) by sequence-specific probes ThyraMIR: microRNA expression analysis; classification as either &quot;negative&quot; or &quot;positive&quot; by a proprietary algorithm</td>
<td>Next-generation sequencing to detect mutations (AKT1, BRAF, CTNNB1, GNAS, HRAS, KRAS, NRAS, PIK3CA, PTEN, RET, TP53, TSHR, TERT, and EGF1AX) and rearrangements (RET, PPARG, NTRK1, NTRK3, BRAF, and ALK)</td>
</tr>
<tr>
<td><strong>Limitations</strong></td>
<td>Low PPV; concern about performance for Hurthle cell lesions</td>
<td>ThyraMIR is a new test with limited real-world experience to date</td>
<td>New test with limited real-world experience to date; histology diagnosis not blinded to prior molecular testing results in validation study</td>
</tr>
<tr>
<td><strong>Cytology interpretation</strong></td>
<td>Performed by centralized cytopathology laboratory</td>
<td>Performed by local cytopathologists</td>
<td>Performed by local cytopathologists or by a centralized laboratory (CBLPath)</td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td>$4875 for Affirma GEC and MTC $975 for Affirma MTC alone $475 for Affirma BRAF alone</td>
<td>$1675 for ThyGenX alone $3000 for ThyraMIR (reflex test)</td>
<td>$3200</td>
</tr>
<tr>
<td><strong>Out-of-network maximum cost</strong></td>
<td>$800 for Affirma GEC and MTC $80 for Affirma MTC alone $60 for Affirma BRAF alone</td>
<td>$500 for both tests</td>
<td>$800</td>
</tr>
</tbody>
</table>
TABLE 4. Comparison of NGS-Based Mutational Panels Versus Combined MicroRNA/Mutational Panela

<table>
<thead>
<tr>
<th></th>
<th>Nikiforov 201448</th>
<th>Labourier 201538</th>
<th>Le Mercier 201551b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>NGS-based, thyroid-specific mutation/gene fusion panel (ThyroSeq)</td>
<td>MicroRNA-based expression classifier (ThyraMIR) and 7-gene mutation panel (ThyGenX)</td>
<td>NGS-based mutation panel of generic cancer genes (AmpliSeq Cancer Hotspot Panel)</td>
</tr>
<tr>
<td>Sample collection</td>
<td>Retrospective and prospective</td>
<td>Prospectively</td>
<td>Retrospective</td>
</tr>
<tr>
<td>No. of institutions</td>
<td>Single FN/SFN</td>
<td>Multiple AUS/FLUS and FN/SFN</td>
<td>Single Indeterminate (&quot;follicular proliferation&quot;)b</td>
</tr>
<tr>
<td>Cytologic category</td>
<td></td>
<td></td>
<td>34</td>
</tr>
<tr>
<td>Indeterminate FNA</td>
<td>143 (143)</td>
<td>109 (109)</td>
<td></td>
</tr>
<tr>
<td>with molecular test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevalence of malignancy based on cytologyc</td>
<td>27% (27%)</td>
<td>32% (32%)</td>
<td>21% (21%)</td>
</tr>
<tr>
<td>Results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negatived</td>
<td>101 (71%)</td>
<td>83 (76%)</td>
<td>67 (61%)</td>
</tr>
<tr>
<td>Positive</td>
<td>42 (29%)</td>
<td>26 (24%)</td>
<td>42 (39%)</td>
</tr>
<tr>
<td>miRNA Classifier</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-Gene Panel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both Tests Combined</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test resultsc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>True-negative</td>
<td>97 (97%)</td>
<td>68 (68%)</td>
<td>63 (63%)</td>
</tr>
<tr>
<td>False-negative</td>
<td>4 (4%)</td>
<td>15 (15%)</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>False-positive</td>
<td>7 (7%)</td>
<td>6 (6%)</td>
<td>11 (11%)</td>
</tr>
<tr>
<td>True-positive</td>
<td>35 (35%)</td>
<td>20 (20%)</td>
<td>31 (31%)</td>
</tr>
<tr>
<td>Test performancec</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>90% (90%)</td>
<td>57% (57%)</td>
<td>89% (89%)</td>
</tr>
<tr>
<td>Specificity</td>
<td>93% (93%)</td>
<td>92% (92%)</td>
<td>85% (85%)</td>
</tr>
<tr>
<td>NPV</td>
<td>96% (96%)</td>
<td>82% (82%)</td>
<td>94% (94%)</td>
</tr>
<tr>
<td>PPV</td>
<td>83% (83%)</td>
<td>77% (77%)</td>
<td>74% (74%)</td>
</tr>
</tbody>
</table>

Abbreviations: AUS/FLUS, atypia of undetermined significance/follicular lesion of undetermined significance; FN/SFN, follicular neoplasm/suspicious for a follicular neoplasm cytology; FNA, fine-needle aspiration; NGS, next-generation sequencing; NPV, negative predictive value; PPV, positive predictive value. 

a Only data for nodules with indeterminate cytology (AUS/FLUS, FN/SFN, or comparable categories), satisfactory molecular testing results, and follow-up reference histology were extracted from the listed studies.
Cancer risk and proposed patient management based on combination of cytology and ThyroSeq test

*Depending on the type of mutation

Nikiforov et al. *Cancer* 2014, 120:3627-34
Nikiforov et al. *Thyroid* 2015, 25:1217-23
Data on file; UPMC MGP lab
Molecular Markers for Cancer Prognostication
**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 >3 cm

**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)

- 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 PMC, BRAF mutated* (≈1-2%)
- Intrathyroidal, encapsulated, FV-PTC (≈1-2%)
- Unifocal PMC (≈1-2%)
Tumor Genotype Determines Phenotype and Disease-related Outcomes in Thyroid Cancer: A Study of 1510 Patients


Mean follow-up 33 ± 21.2 months
Gene Panels for Tumor Prognostication

**BRAF V600E mutation**

- All Variants of PTC included
- Conventional PTC Only
- All Patients with PTC

**BRAF V600E status and tumor recurrence and mortality**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>NPV</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>2167</td>
<td>83%</td>
<td>51%</td>
<td>99%</td>
<td>5%</td>
</tr>
<tr>
<td>(Tufano et al. Medicine (2012))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrence</td>
<td>1849</td>
<td>66%</td>
<td>54%</td>
<td>87%</td>
<td>25%</td>
</tr>
<tr>
<td>(Xing M et al. JAMA (2013))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Thyroid Cancer Prognostication

**Aggressive Tumors Have Multiple Mutations**


Papillary Carcinomas


- n=57
- BRAF
- NRAS
- HRAS
- KRAS
- PIK3CA
- TSHR
- TP53
Thyroid Cancer Prognostication

Aggressive Tumors Have TERT Mutations

- 469 patients with FCDTC
- Mean follow-up 7.8 ± 5.8 years

<table>
<thead>
<tr>
<th>Distant metastases (n=294)</th>
<th>Persistent Disease (n=211)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariate analysis</td>
</tr>
<tr>
<td>Presence (%)</td>
<td>OR (95%CI)</td>
</tr>
<tr>
<td>TERT wt</td>
<td>34 (12.5)</td>
</tr>
<tr>
<td>TERT mut</td>
<td>10 (43.5)</td>
</tr>
</tbody>
</table>

Melo M et al. *JCEM* (2014)
Thyroid Cancer Prognostication

III. Aggressive Tumors Have TERT Mutations

Xing M. et al. JCO (2014)
Preoperative cancer risk stratification based on molecular profiling

Risk of Recurrence

- **High Risk**
  - BRAF + TERT
  - Multiple driver mutations (e.g., BRAF and PIK3CA)
  - TP53
  - TERT

- **Intermediate Risk**
  - ALK fusions
  - NTRK1 fusions
  - NTRK3 fusions
  - BRAF V600E

- **Low Risk**
  - RET/PTC
  - RAS
  - PTEN
  - BRAF K601E
  - PAX8/PPARG
Case #1
70 yo F with 1.2 cm nodule

Case #2
62 yo F with 2.1 cm nodule

Case #3
41 yo M with 1.6 cm nodule

Case #4
74 yo F with 3.7 cm nodule

FNA Cytology:
• Atypia of Undetermined significance
Case #1: 70 year-old woman with incidentally noted 1.2 cm thyroid nodule

Left lobe normal, no risk factors

Cytology:
Satisfactory/FLUS
• Cancer risk 5-15%

Molecular Testing:
7-gene panel (2010)
No mutations identified
• Cancer risk - 6%
ThyroSeq v2 (2014)
No mutations identified
• Cancer risk - 3%

Observation:
Neck exam and ultrasound stable at 5 years follow-up
Case #2: 62 year-old woman with 2.1 cm thyroid nodule

Cytology:
Follicular lesion of undetermined significance (FLUS) (Bethesda III) due to low cellularity
• Cancer risk 5-15%

ThyroSeq:
BRAF V600E mutation is identified
(9% AF/ 18% cells with mutation)
• Cancer risk ~99%

Total thyroidectomy:
PTC with extrathyroidal extension
Case #3: 41 yo male with left inferior pole thyroid nodule

**Cytology:**
Groups of microfollicles with atypia (Bethesda III-IV)
- *Cancer risk for FLUS* 5-15%, *FN* – 20-30%

**ThyroSeq:**
- Mutations NOT identified
- High expression (92%) of the parathyroid hormone (PTH) gene DETECTED
  - *Strong evidence for parathyroid tissue*

**US:**
1.6 x 1.2 x 1.2 cm predominantly solid, isoechoic nodule in left inferior pole, not convincingly border forming

**Parathyroid exploration, left thyroidectomy:**
- Parathyroid adenoma, left inferior, intrathyroidal
**Case #4: 74 yo female with solitary right lobe nodule, recently increased in size**

**US:**
Solitary 3.7 x 2.6 x 2.8 cm, solid, isoechoic, hypervascular, circumscribed nodule with calcifications

**Cytology:**
Hurthle cell nodule (Bethesda III/IV)
Cancer risk for FLUS 5-15%, FN – 20-30%

**ThyroSeq:**
Positive for NRAS (Q61R); TP53 (R175H); PIK3CA (E545K) mutations

**Total thyroidectomy:**
Oncocytic follicular carcinoma with capsular and multifocal vascular invasion (3 foci)
Case #1
70 yo F with 1.2 cm nodule
Observation

Case #2
62 yo F with 2.1 cm nodule
Therapeutic surgery

Case #3
41 yo M with 1.6 cm nodule
Dx of parathyroid disease

Case #4
74 yo F with 3.7 cm nodule
Therapeutic surgery
Risk prediction

FNA Cytology:
• Atypia of Undetermined significance
Precision Medicine

Precision Medicine refers to the tailoring of medical treatment to the individual characteristics of each patient. It does not literally mean the creation of drugs or medical devices that are unique to a patient, but rather the ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease, in the biology and/or prognosis of those diseases, or in their response to a specific treatment. (National Research Council)
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Christopher Coyne

Radiology
Mitchell Tublin

Pharmacology
Danny Altschuler

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