Utilization of Molecular Markers

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Objectives

1. Discuss progress in understanding the genetic basis of thyroid cancer
2. Discuss diagnostic use of molecular markers for thyroid nodules
3. Discuss the use of molecular markers for thyroid cancer prognostication and patient management
Molecular Pathogenesis of Thyroid Cancer
Main Classes of Genomic Alterations in Thyroid Cancer

SNVs (point mutations)

Insertions/Deletions (Indels)

Gene fusions

Gene expression

Copy number Alterations (CNAs)

BRAF p.V600E
NRAS p.Q61R
TP53 p.E178*
PTEN c.9_30del22
RET/PTC1
EML4/ALK
CALCA
CHGA
MTC
22q loss
3p gain
Genetics of Papillary Thyroid Carcinoma

Figure 1. Landscape of Genomic Alterations in 402 Papillary Thyroid Carcinomas

- **Point mutations**: 75%
- **Gene fusions**: 15%
- **Copy number alterations**: 7%

**Copy number alterations**: 22q

**Point mutations** genes:
- BRAF
- NRAS
- HRAS
- KRAS
- EIF1AX
- TERT
- TP53
- PTEN
- PIK3CA

**Gene fusions** genes:
- RET
- PPARG
- NTRK1/3
- ALK
BRAF-like and RAS-like PTC

Mutations in PTC discovered post-TCGA

THADA fusion is a mechanism of IGF2BP3 activation and IGF1R signaling in thyroid cancer

Prevalence:
- 10/192 (5.2%) PTC
- Does not overlap with any other driver mutations
## Mutations in Follicular and Hurthle Cell Carcinomas

<table>
<thead>
<tr>
<th>Clinical Prevalence</th>
<th>PTC</th>
<th>FTC</th>
<th>PDTC</th>
<th>ATC</th>
<th>MTC</th>
<th>HCC</th>
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</thead>
<tbody>
<tr>
<td>~80%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>~15%</td>
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<tr>
<td>&lt;1%</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>&lt;2%</td>
<td>-</td>
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<tr>
<td>&lt;5%</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>2-3%</td>
<td>-</td>
<td>-</td>
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### Molecular Prevalence (%):

<table>
<thead>
<tr>
<th>Gene</th>
<th>PTC</th>
<th>FTC</th>
<th>PDTC</th>
<th>ATC</th>
<th>MTC</th>
<th>HCC</th>
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<tbody>
<tr>
<td>BRAF</td>
<td>45</td>
<td>-</td>
<td>15</td>
<td>38</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>RAS</td>
<td>20</td>
<td>40-50</td>
<td>30-54</td>
<td>50</td>
<td>~15§</td>
<td>16</td>
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<tr>
<td>RET/PTC</td>
<td>10</td>
<td>-</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>PAX8/PPARG</td>
<td>5</td>
<td>35</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>NTRK1</td>
<td>&lt;5%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CTNNB1</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>70</td>
<td>-</td>
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</tr>
<tr>
<td>TP53</td>
<td>3</td>
<td>-</td>
<td>20-30</td>
<td>50-80</td>
<td>-</td>
<td>22</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>-</td>
<td>&lt;10</td>
<td>6</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TERT</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5-30</td>
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<tr>
<td>PTEN</td>
<td>-</td>
<td>&lt;10</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>AKT1</td>
<td>-</td>
<td>-</td>
<td>5-10</td>
<td>5-10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RET</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- **Molecular Prevalence (%):**
- **BRAF:** 45 - 15 - 38 - 0
- **RAS:** 20 - 40-50 - 30-54 - 50 - ~15
- **RET/PTC:** 10 - 18 - - - 0
- **PAX8/PPARG:** 5 - 35 - - - 0
- **NTRK1:** <5% - - - - -
- **CTNNB1:** - - 20 - 70 - -
- **TP53:** 3 - 20-30 - 50-80 - 22
- **PIK3CA:** - <10 - 6 - 6 - -
- **TERT:** 5 - - - 5-30
- **PTEN:** - <10 - - - -
- **AKT1:** - - 5-10 - 5-10 - -
- **RET:** - - - - - -

*Mitochondrial mutation in complex I subunit genes (Gasparre et al. PNAS 2007: 104(21))

**Chromosomal gains and losses by CGH**

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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
Mourra MM *J Clin Endocrinol Metab* 2011; 96: E863
Genetic alterations in Hurthle cell tumors

Genome Haploidisation with Chromosome 7 Retention in Oncocytic Follicular Thyroid Carcinoma

Cover et al. 2012 PLoS One 7(6)

- Distinct pattern of genomic alterations was found: genome-wide DNA near-haploidisation
- 10 tumors were studied, 5 (50%) had extensive chromosome-wide monosomy (allelic state [A] and 5 (50%) had allelic states [AA] (diploid)
- Chromosome 7 retain heterozygous allelic state [AB]
- Mutations in mtDNA disturb normal function of the mitotic spindle, resulting in unbalanced mitosis
- Loss of chromosomes during several rounds of cell division and near-haploid [A or AA] genome
- These cells develop growth advantage and selected during tumor development
Genetic alterations in Hurthle cell tumors

- Exome NGS, 71 primary and metastatic HCC
- Mutations in DAXX, TP53, NRAS, NF1, and TERT promoter
- Disruptive mutations in complex I of the mitochondrial electron transport chain
- Copy number analysis showed widespread chromosomal losses, near-haploid state in a subset of tumors
- Complex I mitochondrial DNA mutations arise early during tumorigenesis and lead to widespread chromosomal loss

- Exome NGS, 56 HCC (minimally or widely invasive)
- TERT mutations found in 22% tumors; more common in the widely invasive phenotype (32% vs 5%)
- 63% HCCs had non-silent mutation to mitochondrial DNA with enrichment for Complex I subunits
- Copy number analysis identified near haploid or polysomic in majority of HCC
- Whole chromosomal duplication of chromosome 7 was associated with poorer outcome
Genetics of Poorly Differentiated and Anaplastic Carcinomas

BRAF/RAS Mutations

EIF1AX

FUSIONS

TERT

TP53

Landa I et al. JCI 2016
Novel Genetic Alterations in Medullary Carcinoma

Identification of Driving ALK Fusion Genes and Genomic Landscape of Medullary Thyroid Cancer.

Ji JH¹, Oh YL², Hong M², Yun JW³, Lee HW², Kim D⁴, Ji Y⁴, Kim DH⁴, Park WY³, Shin HT³, Kim KM², Ahn MJ⁵, Park K⁵, Sun JM⁵.

- Fusions detected in 2/98 (2%) MTC:
  - EML4-ALK (E13;A20)
  - GFPT1-ALK

- Patient with EML4-ALK positive metastatic MTC enrolled in a Phase I crizotinib trial (NCT01121588)
- Showed response to crizotinib, tumor lesions in the lung, liver, and bone shrank, and plasma calcitonin levels decreased
Progress in understanding genetic mechanisms of thyroid cancer

Summary

• Accelerated discovery phase; genomic landscape of thyroid cancer largely deciphered

• Different classes of genetic alterations (point mutations, indels, gene fusions, copy number alterations, gene expression alterations) serve as drivers in thyroid cancer

• Can be used for cancer diagnosis, prognostication and treatment
Diagnostic utility of molecular markers
Management of Patients with Thyroid Nodules

- **FNA**
  - Benign (70%)
  - Indeterminate (20-30%)
  - Malignant (5%)

- **Benign histology**  (70%)
  - Clinical Follow-up

- **Malignant histology** (30%)
  - Diagnostic Surgery
  - Therapeutic thyroidectomy

Modified from Nishino M. *Cancer Cytopathology* 2015
Management of Patients with Thyroid Nodules

FNA

~70%
Benign

Indeterminate

20-30%
High NPV (-)
High PPV (+)
Dx Test

~5%
Malignant

Diagnostic Surgery

Clinical Follow-up

Therapeutic thyroidectomy

Modified from Nishino M. Cancer Cytopathology 2015
Molecular Thyroid Tests for FNA Available in US

- **ThyroSeq: (UPMC/CBLPath)**
  - Multi-gene NGS assay, recently transitioned to v3 (mutations/indels, fusions, CNV, gene expression)
- **Afirma GEC: (Veracyte)**
  - 167 gene RNA-based Gene Expression Classifier (GEC)
  - Recently replaced by Gene Sequencing Classifier (GSC)
- **ThyGenX +ThyraMIR: (Interpace Diagnostics)**
  - 8 gene panel screen with reflex to a 10 miRNA panel
- **Rosetta GX Reveal: (Rosetta Genomics)**
  - 24 miRNA panel classifier
Evolution of ThyroSeq Test

ThyroSeq v.0
7-gene panel
2007
65%

ThyroSeq v.1
15-gene panel
2013
78%

ThyroSeq v.2
56-gene panel
2014
90%

ThyroSeq v.3
112-gene GC
11/1/2017
94%

Pre-NGS
Next Generation Sequencing Approaches
Next-generation sequencing of DNA and RNA

112 genes

Five classes of genetic alterations detected:

- SNVs (eg. BRAF, RAS, RET)
- Indels (eg. PTEN; TP53)
- Gene fusions (eg. RET, BRAF, PPARG, NTRK1/3, THADA)
- Gene expression alterations (GEA) (eg. CALCA, PTH, MET)
- Copy number alterations (CNA) (eg. 22q loss, 3p gain)

Genomic Classifier (GC): Algorithmic analysis of all detected genetic alterations is performed and reported as a categorical result (positive/negative)
ThyroSeq v3 flow

FNA Sample QA

Acellular

STOP

FNA Cellular Composition

Parathyroid cells
C-cells
Non-Thyroidal

Parathyroid Lesion
Medullary Carcinoma
Inadequate/Non-thyroid

NGS Analysis, DNA and RNA, 112 genes

GENOMIC CLASSIFIER

SNV Indels Gene Fusions
Gene Expression CNV

Detailed Genomic Findings

Negative Positive

## Training of ThyroSeq v3 Genomic Classifier using Tissue Set

<table>
<thead>
<tr>
<th>Tissue type/Pathology Diagnosis</th>
<th>n</th>
<th>Genetic alterations</th>
<th>Correctly detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SNV/indels</td>
<td>Gene fusions</td>
</tr>
<tr>
<td>Normal thyroid tissue</td>
<td>19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hashimoto Thyroiditis</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hyperplastic nodule</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hurthle cell hyperplasia</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Follicular adenoma</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hurthle cell adenoma</td>
<td>15</td>
<td>1 (7%)</td>
<td>0</td>
</tr>
<tr>
<td>NIFTP</td>
<td>2</td>
<td>2 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>Papillary thyroid carcinoma</td>
<td>45</td>
<td>18 (40%)</td>
<td>22 (49%)</td>
</tr>
<tr>
<td>PTC, Hurthle cell variant</td>
<td>12</td>
<td>4 (33%)</td>
<td>2 (17%)</td>
</tr>
<tr>
<td>Hurthle cell carcinoma</td>
<td>29</td>
<td>9 (31%)</td>
<td>4 (14%)</td>
</tr>
<tr>
<td>Follicular thyroid carcinoma</td>
<td>11</td>
<td>4 (36%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>PDTC, ATC</td>
<td>6</td>
<td>5 (83%)</td>
<td>1 (17%)</td>
</tr>
<tr>
<td>Medullary carcinoma</td>
<td>15</td>
<td>12 (80%)</td>
<td>0</td>
</tr>
<tr>
<td>Parathyroid lesion</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non-thyroidal tissue</td>
<td>20</td>
<td>na</td>
<td>5 (25%)</td>
</tr>
</tbody>
</table>

ThyroSeq v3 Genomic Classifier: ROC curves

Tissue Training Set (n=238)

- AUC: 93.1%
- Cross Validation AUC: 91.7%
- Sensitivity: 93.9%
- Specificity: 89.4%
- Accuracy: 92.1%

FNA Validation Set (n=175)

- AUC: 90.6%
- Cross Validation AUC: 89.9%
- Sensitivity: 98.0%
- Specificity: 81.8%
- Accuracy: 90.9%

Requirements for specimen cellularity

- ThyroSeq v3: Minimal acceptable thyroid cell content in FNA sample is 6-12%

<table>
<thead>
<tr>
<th>Thyroid Cells (%)</th>
<th>Blood Cells (%)</th>
<th>Set 1 GC Results</th>
<th>Set 2 GC Results</th>
<th>Set 3 GC Results</th>
<th>Sample Adequacy</th>
</tr>
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<tbody>
<tr>
<td>100</td>
<td>0</td>
<td>POS</td>
<td>POS</td>
<td>POS</td>
<td>Adequate</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>POS</td>
<td>POS</td>
<td>POS</td>
<td>Adequate</td>
</tr>
<tr>
<td>25</td>
<td>75</td>
<td>POS</td>
<td>POS</td>
<td>POS</td>
<td>Adequate</td>
</tr>
<tr>
<td><strong>12%</strong></td>
<td><strong>88</strong></td>
<td><strong>POS</strong></td>
<td><strong>POS</strong></td>
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<tr>
<td><strong>6%</strong></td>
<td>94</td>
<td>POSIX</td>
<td>POS</td>
<td>NEG</td>
<td>Limited</td>
</tr>
<tr>
<td>3</td>
<td>97</td>
<td>NEG</td>
<td>POS</td>
<td>NEG</td>
<td>Inadequate</td>
</tr>
<tr>
<td>2</td>
<td>98</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>Inadequate</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>NEG</td>
<td>NEG</td>
<td>NEG</td>
<td>Inadequate</td>
</tr>
</tbody>
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ThyroSeq v3
Multicenter Clinical Validation Study

• Prospective double-blind multicenter international study (ClinicalTrials.gov identifier NCT02352766)
• Inclusion criteria: Patients with a thyroid nodule/s with Bethesda III-V cytology and known surgical outcome
• 10 study centers; patient recruitment 01/2015-12/2016
• ThyroSeq v3 testing performed at UPMC Molecular and Genomic Pathology (MGP) lab
• Central pathology sample review by a panel of 3 pathologist
• No post-unblind sample exclusion

# Multicenter ThyroSeq v3 Study

<table>
<thead>
<tr>
<th>Study Site</th>
<th>PI</th>
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<tbody>
<tr>
<td>1 Duke University</td>
<td>JA Sosa</td>
</tr>
<tr>
<td>2 Ohio State University</td>
<td>J Sipos</td>
</tr>
<tr>
<td>3 St. Peter’s Hospital, Albany, NY</td>
<td>JL Figge</td>
</tr>
<tr>
<td>4 National University Hospital of Singapore</td>
<td>P Yang</td>
</tr>
<tr>
<td>5 University of Cincinnati</td>
<td>DL Steward</td>
</tr>
<tr>
<td>6 University of Colorado</td>
<td>BR Haugen</td>
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<tr>
<td>7 University of Pennsylvania</td>
<td>SJ Mandel</td>
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<tr>
<td>8 University of Pittsburgh</td>
<td>SE Carty</td>
</tr>
<tr>
<td>9 University of Wisconsin</td>
<td>RS Sippel</td>
</tr>
<tr>
<td>10 Washington Center (MedStar)</td>
<td>KD Burman</td>
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</tbody>
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Overall study PI: YE Nikiforov, Univ. of Pittsburgh

Multicenter ThyroSeq v3 Study

Total enrolled
782 patients
1022 FNA samples

Bethesda III-V nodules with surgery
257 patients
286 FNA samples

Bethesda I, II or VI
No surgery

Insufficient/failed molecular testing

Final informative study set
234 patients
257 FNA samples

<table>
<thead>
<tr>
<th></th>
<th>Total enrollment n=1022</th>
<th>Bill-V and surgery n=286</th>
<th>Final study set n=257</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age of Patients (yr)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>53.1</td>
<td>51.7</td>
<td>51.6</td>
</tr>
<tr>
<td>median</td>
<td>54</td>
<td>53</td>
<td>53</td>
</tr>
<tr>
<td>range</td>
<td>16-90</td>
<td>18-90</td>
<td>18-90</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
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</tr>
<tr>
<td>Female</td>
<td>624</td>
<td>202</td>
<td>188</td>
</tr>
<tr>
<td>Male</td>
<td>158</td>
<td>55</td>
<td>46</td>
</tr>
<tr>
<td><strong>Nodule size by ultrasound (cm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>2.3</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>median</td>
<td>2.0</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>range</td>
<td>0.5-9.7</td>
<td>0.5-7</td>
<td>0.5-7</td>
</tr>
</tbody>
</table>

Central Pathology Review

- 274/286 (96%) of cases were available for review
- Three-pathologist review panel (R. Lloyd; Z. Baloch, R. Seethala)
- Blind review (blinded of results of diagnosis by contributing pathologist and another panel pathologist)
- Initial results of review: 55% complete agreement, 9% major disagreement (benign vs malignant) between reviewing pathologists
- Teleconference to discuss discrepant cases - consensus reached in 100% of cases
- Result of central review: contributing pathology diagnosis changed in 20 (7%) cases
## Multicenter ThyroSeq v3 Study
Composition of final validation set (n=257)

<table>
<thead>
<tr>
<th>Cytology Dx</th>
<th>Number of samples</th>
<th>Final Pathology</th>
<th>Rate of Cancer+NIFTP</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Benign</td>
<td>NIFTP</td>
</tr>
<tr>
<td>Bethesda III</td>
<td>154 (60%)</td>
<td>119</td>
<td>6</td>
</tr>
<tr>
<td>Bethesda IV</td>
<td>93 (36%)</td>
<td>60</td>
<td>5</td>
</tr>
<tr>
<td>Bethesda V</td>
<td>10 (4%)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>257 (100%)</td>
<td>181</td>
<td>11</td>
</tr>
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</table>

**ThyroSeq v3 Performance in Multicenter Study**

<table>
<thead>
<tr>
<th></th>
<th>Cohort % (95% CI)</th>
<th>Bethesda III % (95% CI)</th>
<th>Bethesda IV % (95% CI)</th>
<th>Bethesda III+IV % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of cases</td>
<td>257</td>
<td>154</td>
<td>93</td>
<td>247</td>
</tr>
<tr>
<td>Disease prevalence</td>
<td>30</td>
<td>23</td>
<td>35</td>
<td>28</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>93 (86-97)</td>
<td>91 (77-97)</td>
<td>97 (85-100)</td>
<td>94 (86-98)</td>
</tr>
<tr>
<td>Specificity</td>
<td>81 (75-86)</td>
<td>85 (77-90)</td>
<td>75 (63-84)</td>
<td>82 (75-87)</td>
</tr>
<tr>
<td>PPV</td>
<td>68 (58-76)</td>
<td>64 (50-77)</td>
<td>68 (54-80)</td>
<td>66 (56-75)</td>
</tr>
<tr>
<td>NPV</td>
<td>97 (93-99)</td>
<td>97 (92-99)</td>
<td>98 (89-100)</td>
<td>97 (93-99)</td>
</tr>
</tbody>
</table>

ThyroSeq v3
Performance in Bethesda III and IV Nodules

n=247

<table>
<thead>
<tr>
<th></th>
<th>Histology Cancer/NIFTP</th>
<th>Histology Benign</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test POSITIVE</td>
<td>64</td>
<td>33</td>
</tr>
<tr>
<td>Test NEGATIVE</td>
<td>4</td>
<td>146</td>
</tr>
</tbody>
</table>

Disease prevalence 28%

Sensitivity: 94%
Specificity: 82%
Positive Predictive Value: 66%
Negative Predictive value: 97%

ThyroSeq v3 Performance Across Various Cancers and NIFTP

- **NIFTP**
  - 11/11

- **PTC**
  - 24/27

- **Hurthle Cell Carcinomas**
  - 10/10

- **Medullary Thyroid Carcinoma**
  - 1/1

- **PTC, FV**
  - 21/22

- **Follicular Carcinomas**
  - 3/4

- **Metastatic Carcinoma**
  - 1/1

**ThyroSeq v3 Performance in Hurthle Cell Nodules in Multicenter Study**

<table>
<thead>
<tr>
<th>Histopathology diagnosis</th>
<th>N of nodules</th>
<th>Test positive</th>
<th>Test negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>HN, Hurthle-cell predominance</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Hurthle-cell adenoma</td>
<td>34</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td>Hurthle-cell carcinoma</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>49</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Benign Call Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% (69.2-100)</td>
<td>66.7% (49.8-80.9)</td>
<td>53.1%</td>
</tr>
</tbody>
</table>

Characteristics of False-Negative Cases

- All cancer missed intrathyroidal, low stage, no aggressive histology

<table>
<thead>
<tr>
<th>Pt</th>
<th>Age</th>
<th>Sex</th>
<th>Nodule Size by US</th>
<th>Cytology (Bethesda group)</th>
<th>Contributing Pathology Diagnosis</th>
<th>Consensus Pathology Diagnosis</th>
<th>Vascular invasion</th>
<th>Extrathyroidal extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52</td>
<td>F</td>
<td>4.0 cm</td>
<td>3</td>
<td>HCC</td>
<td>PTC</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>M</td>
<td>3.0 cm</td>
<td>3</td>
<td>FA</td>
<td>PTC, EFV</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>F</td>
<td>3.2 cm</td>
<td>3</td>
<td>FTC, MI</td>
<td>FTC, MI</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>F</td>
<td>4.0 cm</td>
<td>4</td>
<td>PTC</td>
<td>PTC</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

MI – minimally-invasive

## Cancer Probability Associated with Individual Mutations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cancer probability</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BRAF V600E</strong></td>
<td>&gt;99%</td>
</tr>
<tr>
<td><strong>RET</strong></td>
<td>Very high</td>
</tr>
<tr>
<td><strong>ALK</strong></td>
<td>Intermediate</td>
</tr>
<tr>
<td><strong>NTRK1,3</strong></td>
<td>Low</td>
</tr>
<tr>
<td><strong>RET/PTC</strong></td>
<td>Very low</td>
</tr>
<tr>
<td><strong>RAS</strong></td>
<td>20-90%</td>
</tr>
<tr>
<td><strong>DICER1</strong></td>
<td></td>
</tr>
<tr>
<td><strong>PPARG</strong></td>
<td></td>
</tr>
<tr>
<td><strong>THADA</strong></td>
<td></td>
</tr>
<tr>
<td><strong>BRAF K601E</strong></td>
<td></td>
</tr>
<tr>
<td><strong>PTEN</strong></td>
<td>5-10%</td>
</tr>
<tr>
<td><strong>EIF1AX</strong></td>
<td></td>
</tr>
<tr>
<td><strong>TSHR</strong></td>
<td>&lt;3%</td>
</tr>
<tr>
<td><strong>GNAS</strong></td>
<td></td>
</tr>
</tbody>
</table>

Why cancer incidence in RAS-positive nodules varies significantly in different studies?

<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>

<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>

Nuclear features of Papillary Carcinoma

Clearly absent

Clearly present
Multistep Cancer Progression and Existence of Borderline Tumors

Colon cancer

Normal epithelium  
Adenoma  
Carcinoma in situ  
Invasive carcinoma  

APC  
RAS  
BRAF  
SMAD4  
TP53  
TGFB  
BAX  
CSC  
EMT  

NIFTP: Non-Invasive Follicular Thyroid Neoplasm with Papillary-like Nuclear Features

- Previously known as encapsulated follicular variant of papillary carcinoma
- If no invasion upon removal – very low (<1%) risk of recurrence
- Best viewed as “pre-malignant”, equivalent of “carcinoma in situ”
- Still requires surgical resection, but lobectomy likely sufficient surgery
- RAS and RAS-like mutations common, but BRAF V600E, TERT not seen

Characteristics of false-positive cases in multi-center study

- 68% diagnosed on pathology as OA or FA
- 10% initially diagnosed by contributing pathologist as cancer/NIFTP
- 94% had one or more clonal oncogenic molecular alterations (mutation, gene fusion, or CNV) indicating that they are clonal tumors (not hyperplasia)

<table>
<thead>
<tr>
<th>Cytology diagnosis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bethesda III</td>
<td>18</td>
</tr>
<tr>
<td>Bethesda IV</td>
<td>15</td>
</tr>
<tr>
<td>Bethesda V</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pathology findings</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>OFA</td>
<td>13</td>
</tr>
<tr>
<td>FA</td>
<td>10</td>
</tr>
<tr>
<td>HN</td>
<td>11</td>
</tr>
<tr>
<td>Initial pathology dx of cancer or NIFTP</td>
<td>3 (10%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Molecular findings</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutations</td>
<td>21</td>
</tr>
<tr>
<td>RAS</td>
<td>18</td>
</tr>
<tr>
<td>BRAF K601E</td>
<td>1</td>
</tr>
<tr>
<td>EIF1AX</td>
<td>1</td>
</tr>
<tr>
<td>Fusions</td>
<td>2</td>
</tr>
<tr>
<td>THADA/IGF2BP3; PAX8/PPARG</td>
<td>2</td>
</tr>
<tr>
<td>Copy number alterations</td>
<td>15</td>
</tr>
<tr>
<td>Gene expression</td>
<td>14</td>
</tr>
<tr>
<td>Clonal mutations and/or fusions or CNV</td>
<td>32 (94%)</td>
</tr>
</tbody>
</table>

Steward DL et al. ATA 2017 abstract
Prognostic utility of molecular markers
Use of Molecular Markers for Cancer Risk Assessment

Tumor Genotype Determines Phenotype and Disease-related Outcomes in Thyroid Cancer: A Study of 1510 Patients

- 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 Signature and Risk for Recurrence and Mortality from Differentiated Cancer

DTC, n=469

Follow-up (years)

Cum Survival

Melo M et al. JCEM (2014)

DTC, n=551

Follow-up Time (years)

Disease-Free Survival (probability)

ATA high risk, TERT(-)

ATA high risk, TERT(+)

Song YS et al. Cancer (2016)

PTC, n=507

Duration of Follow-Up (years)

Recurrence-Free Survival (probability)

BRAF+TERT

Xing M et al. JCO (2014)

DTC, n=551

Follow-up Time (years)

Disease-Free Survival (probability)

RAS

BRAF

RAS+TERT

BRAF+TERT

Song YS et al. Cancer (2016)
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**

---

Haugen BR et al. *Thyroid.* 2016, 26:1-133
Molecular markers for cancer therapeutics
# Molecular Markers for Therapeutics of Thyroid Cancer

<table>
<thead>
<tr>
<th>Genetic Alteration</th>
<th>Tumor Type</th>
<th>Available Targeted Therapeutics</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF V600E</td>
<td>PTC, ATC</td>
<td>Vemurafenib, Dobrafenib</td>
</tr>
<tr>
<td>HRAS</td>
<td>PTC, FTC (OFTC), PDTC, ATC</td>
<td>Farnesyltransferase inhibitor tipifarnib</td>
</tr>
<tr>
<td>PAX8/PPARG</td>
<td>FTC (OFTC)</td>
<td>Pioglitazone</td>
</tr>
<tr>
<td>ALK fusions</td>
<td>PTC, ATC, PDTC</td>
<td>Crizotinib, ceritinib</td>
</tr>
<tr>
<td>NTRK1/2/3 fusions</td>
<td>PTC, ATC, PDTC</td>
<td>Entrectinib (RXDX-101), LOXO-101</td>
</tr>
<tr>
<td>RET</td>
<td>MTC</td>
<td>Vandetanib, cabozantinib</td>
</tr>
</tbody>
</table>

*Bible KC and Ryder M. 2016 Nat Rev Clin Oncol*
Multiple Mutations Detected Preoperatively
May Predict Aggressive Behavior of Papillary
Thyroid Cancer and Guide Management—A Case Report

Rupendra T. Shrestha,1 Arivarasan Karunamurthy,2 Khalid Amin,3 Yuri E. Nikiforov,2 and M. Luiza Caramori1

0.6 cm nodule

AUS cytology

BRAF+/TERT+AKT1+/PIK3CA+

<table>
<thead>
<tr>
<th>Gene</th>
<th>cDNA</th>
<th>Protein</th>
<th>Allelic Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>c.179T&gt;A</td>
<td>p.V600E</td>
<td>37%</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>c.3140A&gt;G</td>
<td>p.H1047R</td>
<td>21%</td>
</tr>
<tr>
<td>AKT1</td>
<td>c.49G&gt;A</td>
<td>p.E17K</td>
<td>6%</td>
</tr>
<tr>
<td>TERT</td>
<td>c.1-124C&gt;T</td>
<td>-</td>
<td>77%</td>
</tr>
</tbody>
</table>

mPTC with extrathyroid ext
Clinical management informed by molecular markers
Potential patient management informed by molecular testing

Bethesda III-IV Cytology

ThyroSeq

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

Probability of Cancer or NIFTP
- 3-4%
- <10%
- 80-90%
- 95-99%
- 98%

Tumor type, risk of recurrence
- N/A
- NIFTP or low-risk cancer
- NIFTP or low-risk cancer
- Intermediate-risk cancer
- High-risk cancer

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

Nikiforov YE. *Endocrine Practice* (2017)
Clinical performance of ThyroSeq in independent studies

ThyroSeq v2

ITN (n=156): Bethesda III (n=124), IV (n=32)

- Thyroseq-negative 65% (102/156)
  - No Surgery: 77% (79/102)
    - Benign: 96% (22/23)
      - NIFTP (n=1)
    - Malignant: 4% (1/23)
      - PTC, oncocytic variant: 100% (1/1)
  - Surgery: 23% (23/102)

- Thyroseq-inconclusive 2% (3/156)
  - Surgery: 100% (3/3)

- Thyroseq-positive 33% (51/156)
  - No Surgery: 27% (14/51)
    - Malignant: 22% (8/37)
      - FTC: 25% (2/8)
  - Surgery: 73% (37/51)
    - Malignant: 33% (1/3)
      - cPTC: 100% (1/1)
    - NIFTP (n=2)
      - cPTC: 50% (4/8)

- NPV 96%
- PPV 27%
Clinical performance of ThyroSeq in independent studies

ThyroSeq v2

Table III.
ThyroSeq-positive mutations/rearrangements and risk of malignancy

<table>
<thead>
<tr>
<th>Mutations/rearrangements*</th>
<th>Prevalence of mutation in ThyroSeq-positive nodules, % (n = 51)</th>
<th>Malignancy rate in resected nodules, %</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF V600E</td>
<td>10 (5)</td>
<td>100 (3/3)</td>
<td>Classic PTC</td>
</tr>
<tr>
<td>BRAF K601E</td>
<td>2 (1)</td>
<td>0 (0/1)</td>
<td>Follicular adenoma</td>
</tr>
<tr>
<td>PAX8/PPARG</td>
<td>8 (4)</td>
<td>33 (1/3)</td>
<td>FVPTC</td>
</tr>
<tr>
<td>RET/PTC1</td>
<td>2 (1)</td>
<td>100 (1/1)</td>
<td>Classic FTC</td>
</tr>
<tr>
<td>ETV6/NTRK3</td>
<td>2 (1)</td>
<td>100 (1/1)</td>
<td>FVPTC</td>
</tr>
<tr>
<td>THADA/IGF2BP3</td>
<td>4 (2)</td>
<td>0 (0/1)</td>
<td>NIFTP</td>
</tr>
<tr>
<td>TP53</td>
<td>2 (1)</td>
<td>0 (0/1)</td>
<td>Follicular adenoma</td>
</tr>
<tr>
<td>Overexpression of MET</td>
<td>6 (3)</td>
<td>0 (0/2)</td>
<td>Follicular adenoma</td>
</tr>
<tr>
<td>EIF1AX</td>
<td>10 (5)</td>
<td>0 (0/4)</td>
<td>Follicular adenoma</td>
</tr>
<tr>
<td>NRAS Q61R</td>
<td>37 (19)</td>
<td>7 (1/15)</td>
<td>FTC</td>
</tr>
<tr>
<td>KRAS Q61R, G12V, G12D</td>
<td>10 (5)</td>
<td>33 (1/3)</td>
<td>FTC</td>
</tr>
<tr>
<td>HRAS Q61R</td>
<td>10 (5)</td>
<td>0 (0/4)</td>
<td>NIFTP; follicular adenoma</td>
</tr>
<tr>
<td>All RAS mutations</td>
<td>57 (29)</td>
<td>9 (2/22)</td>
<td>FTC (n = 2)</td>
</tr>
</tbody>
</table>
Impact of Molecular Tests on Patient Management

ThyroSeq v3

Bethesda III-IV nodules (n=100)

ThyroSeq v3 negative (n=61)

ThyroSeq v3 positive (n=39)

-Benzin histopathology

-Cancer/NIFTP histopathology

Bethesda III and IV nodules; Disease prevalence 28%
58 yo F with R thyroid nodule on palpation

- 0.9 cm R upper pole hypoechoic nodule w/ clustered macrocalcifications
  - BRAF V600E
  - TERT C228T
- 2.4 x 1.7 x 2 cm solid hypoechoic nodule in R lower pole/isthmus
  - No mutations
- 1.2 x 1 x 1 cm L upper pole hypoechoic nodule
  - HRAS Q61R

Hyperplastic nodule

cPTC, extrathyroidal extension

Follicular Adenoma
Utilization of Molecular Markers

Summary

• Diagnostic use for nodules with indeterminate FNA results
• Provide prognostic information preoperatively
• Therapeutics for advanced thyroid cancer
• Help to inform more individualized/personalized management of patients with thyroid nodules and cancer
Thyroid Nodules: Evolution of Diagnostic Approaches

- Surgery
- FNA Cytology
- Ultrasound
- Molecular Markers
Day 1 - Pre-symposium Course 2
Friday, February 2nd, 2018 - 12PM-5PM
MOLECULAR BIOLOGY ESSENTIALS FOR PRACTICING CLINICIANS

Co-Directors: Marina Nikiforova, MD and Yuri Nikiforov, MD

This course will provide an overview of genetics of thyroid cancer and in-depth discussion of the ThyroSeq test. It will start with the review of basic principles of molecular biology pertinent to clinical practice. Next, the next generation sequencing (NGS) technology, its advantages and limitations, will be discussed. Detailed information on the ThyroSeq test will be provided including the new version of the test. Several clinical situations will be discussed with emphasis on the ThyroSeq report interpretation and implications for patient management. At the completion of the course, the attendees will receive a certificate of completion and a voucher for one free ThyroSeq v3 test.