Thyroid Nodule Issues with FNA Evaluation 2016: The Indeterminate Nodule

James V. Hennessey MD
Associate Professor of Medicine
Harvard Medical School
Objectives

At the completion of this talk the learner will:

1. Be able to articulate the basic types of genetic profiling to estimate the risk of malignancy in indeterminate thyroid nodules.

2. Identify the genetic pattern associated with non-invasive follicular variant papillary cancer.

3. Appreciate the potential of genetic profiling to predict the prognosis of thyroid cancer.
## Meta-analysis of Bethesda Classification of FNA

8 studies, 25,445 FNA. Surgery in 25%

<table>
<thead>
<tr>
<th>Category</th>
<th>Malignancy Risk (est. %)</th>
<th>Malignant % observed</th>
<th>Percent of FNAs</th>
<th>Percent with histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>&lt;1</td>
<td>3.7</td>
<td>59.0</td>
<td>10</td>
</tr>
<tr>
<td>AUS/FLUS</td>
<td>5-10</td>
<td>15.9</td>
<td>9.6</td>
<td>39</td>
</tr>
<tr>
<td>Follicular Neoplasm</td>
<td>20-30</td>
<td>16.1</td>
<td>10.1</td>
<td>70</td>
</tr>
<tr>
<td>Suspicious for malignancy</td>
<td>50-75</td>
<td>75.2</td>
<td>2.6</td>
<td>74</td>
</tr>
<tr>
<td>Malignant</td>
<td>100</td>
<td>98.6</td>
<td>5.4</td>
<td>74</td>
</tr>
<tr>
<td>Non-diagnostic</td>
<td></td>
<td>16.8</td>
<td>13.0</td>
<td>16</td>
</tr>
</tbody>
</table>

# FNA Malignancy Prediction with Bethesda System

<table>
<thead>
<tr>
<th>Category</th>
<th>Risk of Malig</th>
<th>What next?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Non-Dx</td>
<td>1-4+%</td>
<td>Re-do (U/S)</td>
</tr>
<tr>
<td>2. Benign</td>
<td>0-3%</td>
<td>Clinical F/U</td>
</tr>
<tr>
<td>3. Atypical</td>
<td>5-15%</td>
<td>Re-do (U/S)</td>
</tr>
<tr>
<td>4. Foll Neoplas.</td>
<td>15-30%</td>
<td>Lobectomy</td>
</tr>
<tr>
<td>5. Suspicious</td>
<td>60-75%</td>
<td>Total Tx</td>
</tr>
<tr>
<td>6. Malignant</td>
<td>97-99%</td>
<td>Total Tx</td>
</tr>
</tbody>
</table>

Ali SZ & Cibas E 2009 The Bethesda system for Reporting Thyroid Cytopathology. New York: Springer
Indeterminate: What Next?

- Chromosomal rearrangements of the RET protooncogene or the V600E point mutation of the BRAF gene seen in most papillary thyroid cancer (PTC)
- Mutations in RAS and rearrangements of the PPARγ genes seen in numerous cases of follicular thyroid cancer (FTC)

Mitsaides N, Fagin JA. Chapter 11, Genetic Diagnosis of Endocrine Disorders. 2010;117-138
Oncogene Studies for Diagnosis of Cancer on FNA Specimens
MAP kinase pathway

- B-type RAF kinase is abundant protein
- T1799A mutation results in BRAF(V600E) that is constitutively activated
<table>
<thead>
<tr>
<th>Cytologic Diagnosis</th>
<th>AUS/FLUS</th>
<th>FN/SFN</th>
<th>SMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer Risk Based on Cytology Only</td>
<td>14%</td>
<td>27%</td>
<td>54%</td>
</tr>
</tbody>
</table>

Testing for Panel of Mutations (BRAF, RAS, RET/PTC, PAX8/PPARγ)

<table>
<thead>
<tr>
<th>Mutational Status</th>
<th>Positive</th>
<th>Negative</th>
<th>Positive</th>
<th>Negative</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer Risk</td>
<td>88%</td>
<td>5.9%</td>
<td>87%</td>
<td>14%</td>
<td>95%</td>
<td>28%</td>
</tr>
</tbody>
</table>

Clinical Management:
- Total thyroidectomy
- Lobectomy vs. observation +/- repeat FNA
- Total thyroidectomy
- Lobectomy
- Total thyroidectomy
- Lobectomy

FIG. 3. Proposed clinical algorithm for management of patients with cytologically indeterminate thyroid FNA applying the results of mutational analysis.
Rule in Test Utility

AUS–FLUS

FN–SFN

Suspicious (suggestive of cancer)

Cytologically indeterminate

Onco Gene Expression

Negative
Hemi-Thyroidectomy

Positive
Total Thyroidectomy

Jameson L 2012 NEJM
Asuragen® Validation Study

- **Aim:** Evaluate multisite performance of:
  - Local FNA cytology
  - Molecular analysis; 17 oncogenic gene alterations
    - Centralized clinical laboratory
  - Reference diagnosis local pathology/management
- Nodules ≥ 1 cm, 5 academic MCs (3 States)
- Usual local FNA preparation
- 1 needle pass in RNARetain (preservation and stabilization of intracellular nucleic acids)

Beaudenon-Huibregtse S et al. 2014 Thyroid 24(10):1479-1487
## Correct Operation Predicted?

<table>
<thead>
<tr>
<th></th>
<th>Hemi-Tx</th>
<th>Total Tx</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AUS (n = 22)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histopath Malig</td>
<td>7/11(64%)</td>
<td>4/11(36%)</td>
</tr>
<tr>
<td>Histopath Benign</td>
<td>9/11(82%)</td>
<td>2/11(18%)</td>
</tr>
<tr>
<td><strong>FN/SFN (n = 19)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histopath Malig</td>
<td>2/6(33%)</td>
<td>4/6(66%)</td>
</tr>
<tr>
<td>Histopath Benign</td>
<td>12/13(92%)</td>
<td>1/13(8%)</td>
</tr>
<tr>
<td><strong>Susp Malig (n = 12)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histopath Malig</td>
<td>5/8(63%)</td>
<td>3/8(38%)</td>
</tr>
<tr>
<td>Histopath Benign</td>
<td>4/4(100%)</td>
<td>0/4(0%)</td>
</tr>
</tbody>
</table>

Beaudenon-Huibregtse S et al. 2014 Thyroid 24(10):1479-1487
53% of nodules having surgery were benign.

56% of malignant nodules had the wrong operation.

Beaudenon-Huibregtse S et al. 2014 Thyroid 24(10):1479-1487
ThyroSeq v2 Next Gen Sequencing

- 143 with FNA FN/SFN and surgical pathology.
  - 91 retrospective (Pathologist aware of basic MP)
  - 52 prospective (Pathologist aware of TSMP v1)

- Point mutations and indels:
  - AKT1, BRAF, NRAS, HRAS, KRAS, PTEN, TP53, TSHR, GNAS, CTNNB1, RET, PIK3CA, (C228T and C250T) TERT Hotspots

- Gene Fusions:
  - 38 RETs, BRAF, NTRK1, NTRK3, PPARG, THADA

- Cell type: PGK1, TG, TTF1, NIS, KRT7, CALCA, PTH

ThyroSeq v2 and FN/SFN

143 consecutive FN/SFN nodules with surgery

Retrospective group
n=91

Mutation NEGATIVE
n=64

CANCER n=2
BENIGN n=62

Mutation POSITIVE
n=27

CANCER n=23
BENIGN n=4

Prospective group
n=52

Mutation NEGATIVE
n=37

CANCER n=2
BENIGN n=35

Mutation POSITIVE
n=15

CANCER n=12
BENIGN n=3

False Negatives
FV-PTC
Hürthle cell

False Positives
FA, HP, HC

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

False Negatives
PTC
FV PTC

Impact of Cancer Prevalence

Current FN/SFN Results
Cancer Prevalence 27%

AUS/FLUS nodules
n=465

Thyroid follicular cells
n=462

- Mutation NEGATIVE
  n=431
    - Surgery no
      n=362
      Cancer n=2
      Benign n=67
    - Surgery yes
      n=69
      Cancer n=22
      Benign n=47

- Mutation POSITIVE
  n=31
    - Surgery no
      n=5
      Cancer n=20
      Benign n=6
    - Surgery yes
      n=26

93%
7%

Non-thyroid cells (PTH)
n=3

- Mutation NEGATIVE
  n=3
    - Surgery no
      n=2
      Clinically Primary Hyperparathyroidism n=2
    - Surgery yes
      n=1
      Parathyroid n=1

NRAS = 2
HRAS = 1
EIF1AX = 1
PTEN = 1
THADA = 1

84%
16%

3% FNeg
23% FPos

Nikiforov YE et al. 2015 Thyroid 25(11):1217-1223
Sens 90.7%
Spec 92.1%
PPV 76.9%
NPV 97.2%

Nikiforov YE et al. 2015 Thyroid 25(11):1217-1223
Same Assays, Different Institution

- 261 operated thyroid nodules U Minn. MC
  - Histopathologic malignancy rate 48%
- AUS/FLUS 73/261 (28%)
  - Malignant Histopathology rate 30%
- FN/SFN 29/261 (11%)
  - Malignant Histopathology rate 38%
- SUSP Malig 23/261 (9%)
  - Malignant Histopathology rate 83%

Shrestha et al. 2016 Thyroid 26(8):1068-76
Methods

- 2013-2014 a dedicated FNA sample was collected and held pending cytopathology.
- Sent for molecular testing if:
  - AUS/FLUS, FN/SFN, SUSP for Malignancy
  - Small number were NOT sent for Molecular
    - Inadequate material for Molecular testing
    - FNAs from outside institutions
    - Logistical issue with 2nd MD verification required
- Mutational analysis done at U Pitt MC

Shrestha et al. 2016 Thyroid 26(8):1068-76
Molecular Testing Panels

- **01-09 2013: 7 gene Panel**
  - BRAF<sup>V600E</sup>, NRAS(61), HRAS(61), KRAS(12 & 13), RET/PTC1, RET/PTC3, PAX8/PPARG.

- **Sept. 2013 onwards, Next Gen Sequencing:**
  - **Thyroseq V1** (284 mutations)
    - AKT1, BRAF<sup>V600E</sup>, CTNNB1, GNAS, NRAS, KRAS, PIK3CA, PTEN, RET, TP53, TSH-R, RET/PTC1, RET/PTC3, PAX8/PPARG
  - **Thyroseq V2** (All of V1 Plus)
    - EIF1AX, TERT, and 42 further fusions of RET, PPARG, NTRK1, NTRK3, ALK, BRAF, IGFBP3

Shrestha et al. 2016 Thyroid 26(8):1068-76
Mutational Testing: Indeterminates

- 73/125 Indeterminates tested:
  - 5/73 (6.8%) UNSAT for Molecular testing
- 68 Satisfactory specimen pairs:
  - 44/68 (65%) AUS/FLUS
  - 12/68 (18%) FN/SFN
  - 12/68 (18%) SUSP for malignancy
    - Reflex discontinued early 2° to high malignancy rate
- 23/68 7 Gene panel, 45/68 ThyroSeq V1/2
  - Results of ThyroSeq are reported together.

Shrestha et al. 2016 Thyroid 26(8):1068-76
44 Nodules profiled
  - 13/44 (30%) Malignant
29 Nodules NOT profiled
  - 9/29 (31%) Malignant (NS)

Predicted Outcomes
  - False (+) 11/44 (25%)
  - False (-) 2/44 (4.5%)
  - True (+) 11/44 (25%)
  - True (-) 20/44 (46%)

Shrestha et al. 2016 Thyroid 26(8):1068-76
12 Nodules profiled
- 5/12 (42%) Malignant

17 Nodules NOT profiled
- 6/17 (35%) Malignant (NS)

Predicted Outcomes
- False (+) 3/12 (25%)
- False (-) 0/12 (0%)
- True (+) 5/12 (42%)
- True (-) 4/12 (33%)

Shrestha et al. 2016 Thyroid 26(8):1068-76
NIFTP and Thyroseq v2

- Consensus diagnostic criteria for Encapsulated FV papillary thyroid cancer (EFVPTC)

- 109 Non-invasive EFVPTC (Gr 1)
  - (67 Rx lobectomy only, No 131-I)
  - Median 13 [10-26] year follow up, all alive and NED
  - Noninvasive follicular thyroid neoplasm with papillary like nuclear features (NIFTP)

- 101 Invasive EFVPTC (Gr 2)
  - Adverse events in 12/101 (12%) invasive cases
    - 5/12 distant metastases (2 deaths)

### eTable 2. Results of molecular analysis of cases initially submitted to Group 1 (NIFTP)

<table>
<thead>
<tr>
<th>Gene mutation</th>
<th>Accepted to final Group 1</th>
<th>Excluded due to insufficient nuclear features</th>
<th>Excluded due to the presence of higher-grade exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAS*</td>
<td>8</td>
<td>n=5</td>
<td>n=5</td>
</tr>
<tr>
<td>NRAS</td>
<td>(5)</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>HRAS</td>
<td>(2)</td>
<td></td>
<td>(1)</td>
</tr>
<tr>
<td>KRAS</td>
<td>(1)</td>
<td></td>
<td>(1)</td>
</tr>
<tr>
<td>BRAF K601E</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TERT</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>PPARG fusion</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALK fusion</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>THADA fusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL MUTATION POSITIVE</strong></td>
<td><strong>21 (78%)</strong></td>
<td>0</td>
<td>4 (80%)</td>
</tr>
<tr>
<td><strong>TOTAL MUTATION NEGATIVE</strong></td>
<td>6 (22%)</td>
<td>5 (100%)</td>
<td>1 (20%)</td>
</tr>
</tbody>
</table>

*Two cases had double mutations: RAS and EIF1AX*
Further Testing Options

- **MicroRNA (miRNA)**
  - Small, endogenous, noncoding RNA
  - Negative regulators of gene expression
    - Impact on cell growth, differentiation, apoptosis and adhesion all of which are implicated in carcinogenesis.

- miRNA expression is dysregulated in many types of human cancer including thyroid cancer.

- May be measured in FNA or blood (circulating)
  - Have significant PPV and NPV

Diagnostic value of serum let-7e, miR-151-5p, and miR-222. A and B ROC curve analyses of serum let-7e, miR-151-5p, and miR-222

Correlation with Clinical Status

- miR-151-5p/ miR-222 levels significantly higher (p=0.012) in node positive patients.
- Overexpression of miR151-5p strongly (p<0.001) associated with tumor size.
- Overexpression of miR-222 associated (p=0.015) with advanced tumor stage.
- Higher levels of let-7e were seen in patients with multifocal lesions (p<0.001)

Combining Mutation Identification and MIR (Multiplatform Mutation Test)

- Consecutive FNAs submitted to Asuragen®
- Sourced from across the USA
  - January 2011 to October 2013
    - 282 AUS/FLUS or FN/SFN
    - 20 physicians at 14 sites
    - 113 (40%) had traceable surgical pathology result
      - Local pathologists blind to the result of the MPT
  - All had adequate nucleic acids for testing
    - 4 cases with known cancer diagnosis excluded

Labourier E et al. 2015 JCEM 100(7):2743-50
MPT Molecular Analyses

- BRAF, HRAS, KRAS, NRAS, RET, RET-PTC1, RET-PTC3, PAX-8-PPARG
- miRNA expression by RT-qPCR
  - Reported as qualitatively positive or negative
- miRNAs tested
  - 29b-1-5p, 31-5p, 138-1-3p, 139-5p, 146b-5p, 155, 204-5p, 222-3p, 375, 551b-3p
- Samples positive for any marker = Positive
- Samples negative for all markers = Negative

Labourier E et al. 2015 JCEM 100(7):2743-50
AUS/FLUS n=58

Labourier E et al. 2015 JCEM 100(7):2743-50
Labourier E et al. 2015 JCEM 100(7):2743-50

FN/SFN n=51

A  Positive predictive value

82%

B  Negative predictive value

91%
Another Option to Identify Benign Nodules
GEC: The Rule Out Test

- AUS–FLUS
- FN–SFN
- Suspicious (suggestive of cancer)

Cytologically indeterminate

Gene-Expression Classifier Profile

- Benign
  - Monitor
- Suspicious
  - Surgery

Jameson L 2012 NEJM
Pre-op GEC Dx of Benign Indeterminate Nodules

- 3789 patients, 4812 FNAs, all nodules ≥ 1cm
- 577 indeterminate (suspicious) FNAs
  - 413 patients underwent surgery (Histopathology)
  - After exclusion criteria were met:
- 265 nodules underwent GEC analysis
  - Classified as still suspicious OR
  - Classified as likely to be benign

Alexander E et al. NEJM 2012;367:705-715
## MGC Test Performance

### Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance (N=129, 48.7%)

<table>
<thead>
<tr>
<th>GEC result</th>
<th>Malignant reference standard (N=31)</th>
<th>Benign reference standard (N=98)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(57%) Suspicious</td>
<td>28</td>
<td>46</td>
</tr>
<tr>
<td>(43%) Benign</td>
<td>3</td>
<td>52</td>
</tr>
</tbody>
</table>

- Sensitivity, 90% (74–98); specificity, 53% (43–63); PPV, 38% (27–50); NPV, 95% (85–99); prevalence of malignant lesions, 24%

### Follicular or Hürthle-Cell Neoplasm or Suspicious for Follicular Neoplasm (N=81, 30.6%)

<table>
<thead>
<tr>
<th>GEC result</th>
<th>Malignant reference standard (N=20)</th>
<th>Benign reference standard (N=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(60%) Suspicious</td>
<td>18</td>
<td>31</td>
</tr>
<tr>
<td>(40%) Benign</td>
<td>2</td>
<td>30</td>
</tr>
</tbody>
</table>

- Sensitivity, 90% (68–99); specificity, 49% (36–62); PPV, 37% (23–52); NPV, 94% (79–99); prevalence of malignant lesions, 25%
Benign GEC Follow up

- 95 Cyto Indeterminate (I)/GEC Benign (B) nodules seen in 90 patients.
  - 5/95 immediate surgical resection
- 90 Cyto I/ GEC B followed
  - 58/90 (64.4%) had US F/U available (4-40 mos.)
- 1224 Cyto-Benign nodules followed (Control)
- Cyto I/ GEC B nodules vs. Cyto Benign: **Growth**
- **Surgical intervention**
  - 13.8% Cyto I/GEC B vs. 0.9% Cyto Benign

Angell TE et al. 2015 100(11):E1477-E1483
Bottomline

- 13 of 95 (13.7%) GEC B nodules to surgery:
  - 5/5 of those going directly were Benign
  - 1/13 (7.7%) going to surgery were malignant
    - 1/95 (1.1%) false negative rate

- 12/873 (1.4%) CytoB underwent surgery
  - 7/12 Re-FNA abnormal Cytopath, 2 Sx, 3 growth
  - 4/12 (33%) were Malignant
    - 4/876 (0.4%) false negative rate

Angell TE et al. 2015 100(11):E1477-E1483
Growth of Thyroid Nodules

Angell TE et al. 2015 100(11):E1477-E1483
NIFTP and GEC

- 249 FNAs sent for GEC 01/12- 10/14
- 63 cases AUS or SFN, GEC suspicious, + Surgery
  - 34/63 (54%) AUS/FLUS, 29/63 (46%) SFN
- Surgical resection results:
  - 16/63 (25%) FVPTC
    - 14/16 (88%) NIFVPTC (NIFTP) = 64% of ALL cancers
  - 5/63 (8%) FTC
  - 1/63 (2%) cPTC
  - 41/63 (65%) Benign nodules

Wong KS et al. 2016 Thyroid 26 (7):911-915
Background

- Well-differentiated PTCs usually indolent.
  - Many may have been “over treated” previously.
- 5-10% of PTCs behave aggressively.
  - Metastases
  - Death
- Identification of prognostic markers able to identify aggressive forms of PTC beyond histopathology would be clinically useful.
  - Focus aggressive treatments on those with risk.
  - Avoid over treatment among those who are not.
Can Cytogenetics Predict Clinical Outcomes?
BRAF Positivity

- Systematic review of the incremental accuracy (IA) of + FNA BRAFV600E and prognosis of PTC
- 67 studies included
- Pooled IA=2% (CI 0.5-4%)
- Relative risk (RR) of various clinical factors linked to prognosis

TERT present in 40/242 (16.5%)

BRAF present in 177/242 (73.1%)

FIG. 2. Kaplan–Meier survival curves with log-rank test of recurrence-free survival after classification into two groups based on the presence of a TERT$^{C228T}$ mutation (A) or a BRAF$^{V600E}$ mutation (B).
TERT (telomerase reverse transcriptase)

- **TERT** encodes the reverse transcriptase component of telomerase. Adds telomere repeats enabling cell replication.
- **Telomerase** activity is required for cell immortalization.
- Somatic **TERT mutations** identified as a frequent event in several cancers such as melanomas and gliomas.

Landa I et al. 2013 *JCEM* 98(9):E1562-6
TERT Mutations in DTC

- Two recurrent, non-overlapping mutations of TERT promoter identified: C228T and C250T
- TERT mutations conferred a 2-4 fold increase in TERT transcriptional activity.
- TERT promoter mutations were found in:
  - 22% of papillary thyroid cancers (PTCs)
  - 51% of advanced thyroid cancers
  - 23% of widely invasive Hürthle-cell cancers
  - 0/8 minimally invasive HCC

Landa I et al. 2013 *JCEM* 98(9):E1562-6
Table 1. *TERT* Promoter Mutations in Thyroid Tumors in a Chinese Cohort

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mutation C228T</th>
<th>Mutation C250T</th>
<th>Collective Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign tumor</td>
<td>0/44 (0)</td>
<td>0/44 (0)</td>
<td>0/44 (0)</td>
</tr>
<tr>
<td>PTC</td>
<td>39/408 (9.6)</td>
<td>7/408 (1.7)</td>
<td>46/408 (11.3)</td>
</tr>
<tr>
<td>FTC</td>
<td>7/22 (31.8)</td>
<td>1/22 (4.6)</td>
<td>8/22 (36.4)</td>
</tr>
</tbody>
</table>

Data are expressed as number of mutations/number of tumors (percentage).

Liu X et al. 2014 JCEM 99(6):E1130-E1136
Relation of BRAF to TERT

- **BRAF** found in 250 (61%) of the 408 PTC
  - In this Chinese population
- **TERT** in 3.8% of BRAF-PTC, 16% BRAF+
- **TERT** but NOT **BRAF** associated with:
  - Older age
  - Larger Tumor size
  - Extra-Thyroidal-Invasion
  - Stage III/IV PTC

Liu X et al. 2014 JCEM 99(6):E1130-E1136
TERT Meta-Analysis

- Systematic search in PubMed, EMBASE, OVID and Web of Science databases.
- Eight eligible trials involving 2035 subjects.
- TERT Promoter mutation found in 10.3%.
- Review Manager (Version 5.2.1) used to calculate summary pooled odds ratios with 95% confidence intervals, adjustments made for heterogeneity and publication bias.

Yin D-T et al. 2016 Clin Endo 85:299-305
### TERT & Outcome (Persistence or Recurrence)

**Yin D-T et al. 2016 Clin Endo 85:299-305**

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>TERT Mutated</th>
<th></th>
<th>TERT Wild-type</th>
<th></th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Events</td>
<td>Total</td>
<td>M-H. Fixed</td>
</tr>
<tr>
<td>Mingzhao Xing 2014</td>
<td>29</td>
<td>61</td>
<td>51</td>
<td>446</td>
<td>7.02 [3.93, 12.55]</td>
</tr>
<tr>
<td>Marina Muzza 2015</td>
<td>10</td>
<td>22</td>
<td>29</td>
<td>160</td>
<td>3.76 [1.48, 9.55]</td>
</tr>
<tr>
<td>de Biase Dario 2015</td>
<td>1</td>
<td>12</td>
<td>6</td>
<td>276</td>
<td>4.09 [0.45, 36.96]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>95</td>
<td>882</td>
<td>100.0%</td>
<td></td>
<td>5.73 [3.55, 9.26]</td>
</tr>
<tr>
<td>Total events</td>
<td>40</td>
<td>86</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Heterogeneity: Chi² = 1.34, df = 2 (P = 0.51); I² = 0%**

**Test for overall effect: Z = 7.13 (P < 0.00001)**
### Association: TERT & Mortality

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>TERT mutated</th>
<th>TERT wild-type</th>
<th>Odds Ratio M-H, Fixed, 95% CI</th>
<th>Odds Ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tiantian Liu 2014</td>
<td>11 Events</td>
<td>38 Total</td>
<td>20.63 [3.78, 112.51]</td>
<td></td>
</tr>
<tr>
<td>Miguel Melo 2014</td>
<td>2 Events</td>
<td>265 Total</td>
<td>10.27 [1.61, 65.69]</td>
<td></td>
</tr>
<tr>
<td>Greta Gandolfi 2015</td>
<td>9 Events</td>
<td>100 Total</td>
<td>5.02 [1.77, 14.23]</td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>53 Events</td>
<td>403 Total</td>
<td>8.30 [3.78, 18.19]</td>
<td></td>
</tr>
</tbody>
</table>

- **Total events**: 22 Events, 24 Total
- **Heterogeneity**: Chi² = 2.05, df = 2 (P = 0.36); I² = 2%
- **Test for overall effect**: Z = 5.28 (P < 0.00001)

Yin D-T et al. 2016 Clin Endo 85:299-305
TERT and BRAF Status in PTC

Liu X et al. 2014 JCEM 99(6):E1130-E1136
Disease Specific Death: TERT & BRAF in 80 PTC Patients

Bullock M et al. 2016 Clin Endo 85:283-290
Tentative Conclusions

- For AUS/FLUS and Follicular Neoplasm, oncogene measurements are positive in ~80% which may include NIFTP as GEC suspicious.
- For patients in these categories, a benign GEC provides and now also the newer more extensive mutation panels reasonably rule out cancer and the nodule is most likely benign.
- MiRNAs and detection of mutations in the TERT promoter advance the diagnosis and may enhance our ability to predict prognosis.