INTRODUCTION:
Mutational profiling of thyroid nodules using fine-needle aspiration (FNA) samples is a useful diagnostic tool. Multiple driver mutations are rarely found in differentiated thyroid cancer and may be associated with more aggressive tumors. However, the impact of genetic markers on preoperative cancer prognostication using FNA samples has not been well studied.

METHODS:
We analyzed a series of 63 nodules with multiple driver mutations (MDM) identified by ThyroSeq v2 56-gene panel in FNA (n=53) or surgically excised nodules (n=10), either prospectively or blinded to the final pathology diagnosis. Mutations were grouped as high risk for cancer (HR) mutations (e.g., BRAF V600E, RAS, TERT, PIK3CA, fusions) and low risk for cancer (LR) mutations (PTEN, EIF1AX). Aggressive tumor features were evaluated using clinical records and morphologic findings.

RESULTS:
Among 63 nodules with MDM, 50 had two and 13 more than two mutations. Thirty-five (55%) had BRAF plus one or more HR mutation(s), 18 (29%) RAS plus HR mutation(s), 3 (5%) had two other co-occurring HR mutations, and 7 (11%) had a HR mutation co-existing with LR mutation(s). The most common co-occurring mutation was TERT (n=43). Among 56 cases with BRAF or RAS and other co-occurring HR mutations, 55 (98%) were cancers and one tumor carrying RAS+TERT was benign. Among the 55 cancers, 51 (93%) had aggressive features including extrathyroidal extension (55%), vascular invasion (53%), lymph node macrometastasis (47%), poorly differentiated/anaplastic carcinoma areas (14%), or distant metastasis (8%). FNA cytology in these nodules was malignant in 51%, AUS (Bethesda III) in 21%, FN (Bethesda IV) in 21%, and SUSP (Bethesda V) in 7%. Among 7 samples with co-occurring HR and LR mutations, 4 (57%) were cancers, and 1 (25%) had aggressive features.

SUMMARY OF CONCLUSIONS:
- Among nodules with multiple driver mutations (MDM), co-occurrence of high cancer risk (HR) mutations, typically BRAF+TERT or RAS+TERT, is associated with very high probability of cancer with aggressive features.
- However, co-occurrence of high risk (HR) with low risk (LR) mutations, such as RAS+EIF1AX, correlates with lower cancer risk and rare aggressive features.
- Preoperative detection of multiple high-risk mutations may be important to optimize surgical management of these patients.

FIGURE 1. Thyroid nodules with multiple high risk (HR) driver mutations are associated with high probability of cancer with aggressive features

<table>
<thead>
<tr>
<th>Type of Mutations</th>
<th>Pathology Diagnosis* (n)</th>
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<tbody>
<tr>
<td>BRAF + Another HR Mutation</td>
<td>PTC (31), FTC (1), PDTC (2), ATC (1)</td>
</tr>
<tr>
<td>RAS + Another HR Mutation</td>
<td>PTC (7), FTC (4), PDTC (3), ATC (3), FA (1)</td>
</tr>
<tr>
<td>Other HR Mutations</td>
<td>PTC (2), PDTC (1)</td>
</tr>
<tr>
<td>HR + LR Mutations</td>
<td>PTC (4), FA (3)</td>
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</tbody>
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FIGURE 2. Pathology diagnoses in nodules with multiple mutations

- 35 (55%) BRAF + Another HR mutation
- 18 (29%) RAS + Another HR mutation
- 3 (5%) Other Multiple HR mutations
- 55 (98%) Thyroid Cancer

FIGURE 3. Cytology diagnoses in 55 thyroid nodules with multiple mutations and high-risk cancer on surgery

- 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%)

FIGURE 4. Potential management of patients with mutations detected by ThyroSeq analysis

- Negative: no mutations
- Positive: RAS-like mutation
- Positive: BRAF-like mutation
- Positive: multiple HR mutations

- Test result
- Probability of cancer or NIFTP
- Tumor type, risk of recurrence
- Patient management

- Indeterminate Cytology

- Observation
- Lobectomy
- Total thyroidectomy +/- CCLND