Molecular Diagnosis of Follicular Cell Nodules

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Follicular Cell Derived Thyroid Cancer

- Follicular cell
- Follicular adenoma
- Follicular carcinoma
- Papillary carcinoma
- Poorly differentiated carcinoma
- Anaplastic carcinoma

Benign neoplasm | Well-differentiated cancer | Poorly differentiated cancer | Undifferentiated cancer
Genomic Revolution: Next Generation Sequencing

Conventional Sequencing
- Sequence up to $10^2$-$10^3$ bases
- Cost - $2400$ per $10^6$ bases

Next Generation Sequencing
- Sequence up to $10^6$-$10^9$ bases
- Cost - $0.05$-$1$ per $10^6$ bases
Progress in Identifying Driver Mutations in Thyroid Cancer

1990: 20% RAS, RET/PTC
2000: 30% RAS, RET/PTC, TP53, TRK, PTEN, b-catenin, PAX8/PPARg
2005: 70% RAS, RET/PTC, TP53, TRK, PTEN, b-catenin, PAX8/PPARg, BRAF, PIK3CA, BRAF/AKAP9
2014: >90% RAS, RET/PTC, TP53, TRK, PTEN, b-catenin, PAX8/PPARg, BRAF, PIK3CA, BRAF fusions, AKT1, STRN/ALK, ETV6/NTRK3, EIF1AX
Outline

• Molecular alterations in thyroid tumors
  • Individual lab studies
  • TCGA
• Diagnostic use of molecular markers
Molecular alterations in the Follicular Variant PTC are Different from Classic PTC

Zhu Z. et al. AJCP 2003

<table>
<thead>
<tr>
<th></th>
<th>RET/PTC</th>
<th>RAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>FV PTC (n=30)</td>
<td>3%</td>
<td>43%</td>
</tr>
<tr>
<td>Classic PTC (n=46)</td>
<td>28%</td>
<td>0</td>
</tr>
</tbody>
</table>

Rivera M. et al. Modern Pathol 2010

<table>
<thead>
<tr>
<th></th>
<th>RET/PTC</th>
<th>RAS</th>
<th>PAX8/PPARG</th>
<th>BRAF V600E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encapsulated FV PTC (n=28)</td>
<td>0</td>
<td>36%</td>
<td>4%</td>
<td>0</td>
</tr>
<tr>
<td>Infiltrative FV PTC (n=19)</td>
<td>10%</td>
<td>10%</td>
<td>0</td>
<td>26%</td>
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</tbody>
</table>

Howitt B. et al. Thyroid 2013

<table>
<thead>
<tr>
<th></th>
<th>RAS</th>
<th>BRAF V600E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partially encapsulated/ Well-circumscribed FVPTC (n=28)</td>
<td>46%</td>
<td>0</td>
</tr>
</tbody>
</table>
TCGA Thyroid Analysis Working Group

University of Michigan
Tom Giordano (co-chair)

MSKCC
Giovanni Ciriello

UCSC
Josh Stuart
Evan Paull
Matan Hofree
Trey Ideker

Brown
Ben Raphael
Fabio Vandin
Jonathon Eldridge

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Juok Cho (data coordinator)
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Mara Rosenberg
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Harindra Arachchi
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Mike Noble
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TCGA and BCRs
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Margi Sheth
Brad Ozenberger
Entire TCGA Network

239 total authors

T. Giordano
G. Getz
TCGA study of PTC (Cell, 2014)

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

TCGA. Integrated genomic characterization of papillary thyroid carcinoma Cell (2014)
TCGA study of PTC (Cell, 2014)

TCGA. Integrated genomic characterization of papillary thyroid carcinoma Cell (2014)
## Common Mutations in Various Types of Thyroid Cancer

<table>
<thead>
<tr>
<th>MUTATIONS</th>
<th>PTC</th>
<th>EFVPTC</th>
<th>FTC</th>
<th>PDTC</th>
<th>ATC</th>
<th>FA</th>
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<tbody>
<tr>
<td>BRAF V600E</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>BRAF K601E</td>
<td>+++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>NRAS</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>HRAS</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KRAS</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td></td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>PTEN</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>TSHR</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>++</td>
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<tr>
<td>GNAS</td>
<td></td>
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<td></td>
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<td></td>
<td>++</td>
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<tr>
<td>GENE FUSIONS</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>RET/PTC</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAX8/PPARG</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>ALK fusions</td>
<td>+</td>
<td></td>
<td></td>
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<td>++</td>
</tr>
<tr>
<td>BRAF fusions</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETV6/NTRK3</td>
<td>++</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NTRK1 fusion</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
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</table>
RAS Mutations in Thyroid Nodules

Gupta M et al. JCEM (2013)
RAS Mutations in Follicular Variant PTC

Gupta M et al. JCEM (2013)
NRAS, HRAS, KRAS Mutation

<table>
<thead>
<tr>
<th>Surgical Pathology Diagnosis/Features</th>
<th>NRAS61 n=136</th>
<th>HRAS61 n=44</th>
<th>KRAS12/13 n=24</th>
<th>Statistical Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodular hyperplasia</td>
<td>6 (4.4%)$^a$</td>
<td>2 (4.5%)$^b$</td>
<td>4 (16.7%)$^c$</td>
<td>a vs b: $P=0.03$</td>
</tr>
<tr>
<td>Follicular adenoma</td>
<td>12 (8.8%)$^a$</td>
<td>0 (0%)$^b$</td>
<td>10 (41.6%)$^c$</td>
<td>a vs c: $P&lt;0.001$</td>
</tr>
<tr>
<td>Follicular carcinoma</td>
<td>2 (1.5%)$^a$</td>
<td>2 (4.5%)$^b$</td>
<td>3 (12.5%)$^c$</td>
<td>b vs c: $P&lt;0.001$</td>
</tr>
<tr>
<td>Follicular variant papillary thyroid carcinoma</td>
<td>106 (78.0%)$^e$</td>
<td>37 (84.1%)$^b$</td>
<td>4 (16.7%)$^c$</td>
<td></td>
</tr>
<tr>
<td>Papillary thyroid carcinoma</td>
<td>9 (6.6%)$^a$</td>
<td>1 (2.3%)$^b$</td>
<td>2 (8.3%)$^c$</td>
<td></td>
</tr>
<tr>
<td>Poorly differentiated carcinoma</td>
<td>1 (0.7%)$^a$</td>
<td>0 (0%)$^b$</td>
<td>0 (0%)$^c$</td>
<td></td>
</tr>
<tr>
<td>Anaplastic carcinoma</td>
<td>0 (0%)$^a$</td>
<td>1 (2.3%)$^b$</td>
<td>0 (0%)$^c$</td>
<td></td>
</tr>
<tr>
<td>Medullary thyroid carcinoma</td>
<td>0 (0%)$^a$</td>
<td>1 (2.3%)$^b$</td>
<td>1 (4.2%)$^c$</td>
<td></td>
</tr>
<tr>
<td>Any carcinoma diagnosis</td>
<td>118 (86.8%)$^d$</td>
<td>42 (95.5%)$^e$</td>
<td>10 (41.7%)$^f$</td>
<td></td>
</tr>
</tbody>
</table>

Mean tumor size, cm

- NRAS61: 2.16$^g$
- HRAS61: 2.67$^h$
- KRAS12/13: 1.94$^i$

Oncocytic features$^j$

- NRAS61: 21 (15.4%)$^k$
- HRAS61: 2 (4.5%)$^l$
- KRAS12/13: 18 (75.0%)$^m$

Extrathyroidal extension

- NRAS61: 0 (0%)$^n$
- HRAS61: 1 (2.3%)$^o$
- KRAS12/13: 0 (0%)$^p$

$^a$ Cases with oncocytic features were comprised of those with the descriptor “oncocytic” mentioned in the diagnostic line (eg, oncocytic follicular variant papillary thyroid carcinoma, nodular hyperplasia with oncocytic changes, and oncocytic adenoma).

Radkay LA et al. Cancer Cytopathol (2014)
PAX8/PPARG Rearrangement

- t(2;3)(q13;p25)
- Fusion involves PAX8 and PPARγ genes
# PAX8/PPARG Rearrangement

<table>
<thead>
<tr>
<th></th>
<th>Follicular adenoma</th>
<th>Follicular Carcinoma</th>
<th>Papillary Carcinoma</th>
<th>PDCA and AC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Range</strong></td>
<td>0-20%</td>
<td>20-70%</td>
<td>0-25%</td>
<td>0</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>7%</td>
<td>30%</td>
<td>5%</td>
<td>0</td>
</tr>
</tbody>
</table>

* A single case of CREB3L2/PPARY fusion in follicular carcinoma reported (Lui WO et al., Cancer Res 2008)
20 PAX8/PPARG-positive nodules with surgical outcome:
  • 17 (85%) PTC (15 – EFV PTC)
  • 3 (15%) FTC
Spectrum of *BRAF* K601E Positive Thyroid Tumors

- Encapsulated follicular variant: 17%
- Unencapsulated follicular variant: 4%
- Papillary Thyroid Microcarcinoma: 3%
- Solid variant: 3%
- Classic variant: 3%
- Follicular Thyroid Carcinoma: 66%
- Follicular Adenoma
Impact of Mutational Markers of Cancer Diagnosis in Thyroid Nodules
Evolution of Mutational Panels for Thyroid Nodules at UPMC

7-gene panel conventional 15-gene panel NGS, ThyroSeq v1 56-gene panel NGS, ThyroSeq v2

- 2007: 65%
- 2013: 78%
- 2014: 90%
7-Gene Mutational Panel

- BRAF: 65%
- PAX8/PPARγ
- RET/PTC1
- RET/PTC3
- NRAS
- HRAS
- KRAS
## Cancer Risk in Mutation-Positive Thyroid Nodules Using 7-gene Panel

<table>
<thead>
<tr>
<th>Prospective FNA Studies</th>
<th>BRAF (n=123)</th>
<th>RAS (n=79)</th>
<th>RET/PTC (n=20)</th>
<th>PAX8/PPARγ (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nikiforov et al, <em>JCEM</em> 2009</td>
<td>100%</td>
<td>87%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Cantara et al, <em>JCEM</em> 2010</td>
<td>100%</td>
<td>74%</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Nikiforov et al, <em>JCEM, 2011</em></td>
<td>100%</td>
<td>85%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>100%</strong></td>
<td><strong>83%</strong></td>
<td><strong>100%</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>
## Performance of the 7-gene panel (conventional sequencing)

### Cancer Risk by Indeterminate Category

<table>
<thead>
<tr>
<th></th>
<th>FLUS</th>
<th>FN</th>
<th>SMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytology only</td>
<td>14%</td>
<td>27%</td>
<td>54%</td>
</tr>
<tr>
<td>Mutational Status</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
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<td></td>
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<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

### Cancer Risk

- **Total Thyroidectomy:**
  - **FLUS:** 88%
  - **FN:** 6%
  - **SMC:** 87%

### Clinical Management

- Total Thyroidectomy vs. Observation vs. Repeat FNA
- Diagnostic Lobectomy vs. Repeat FNA

*Nikiforov et al. J Clin Endocrinol Metab 2011; 96: 3390*
Further Expansion of NGS-Based Mutational Panel

- **12%** Novel gene fusions (eg. ALK, NTRK3)
- **13%** Novel mutations (eg. TERT, EIF1AX)
- **65%** Other categories: PAX8/PPARγ, RET/PTC1, RET/PTC3, RAS, BRAF, NTRK rearrangements, TP53, PIK3CA, TSHR, CTNNB1, EIF1AX, TERT, ALK, NTRK3, other.
## 56-Gene Panel (ThyroSeq v.2)

- 14 genes for mutations, >1000 hotspots
- 42 fusion types
- 16 genes for expression

<table>
<thead>
<tr>
<th>Gene Mutations (DNA)</th>
<th>Gene Fusions (RNA)</th>
<th>Gene expression (RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRAS</td>
<td>RET</td>
<td>PGK1</td>
</tr>
<tr>
<td>HRAS</td>
<td>TSHR</td>
<td>KRT7</td>
</tr>
<tr>
<td>KRAS</td>
<td>AKT1</td>
<td>TG</td>
</tr>
<tr>
<td>BRAF</td>
<td>TP53</td>
<td>TTF1</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>GNAS</td>
<td>SLC5A5 (NIS)</td>
</tr>
<tr>
<td>PTEN</td>
<td>CTNNB1</td>
<td>Calcitonin</td>
</tr>
<tr>
<td>TERT</td>
<td>EIF1AX</td>
<td>PTH</td>
</tr>
</tbody>
</table>

- Other
ThyroSeq Workflow

Annotation, Risk Assessment, Reporting

<table>
<thead>
<tr>
<th>Chr</th>
<th>Gene</th>
<th>cDNA</th>
<th>Protein</th>
<th>Freq</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>TERT</td>
<td>c.1-124C&gt;T</td>
<td>p.?</td>
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</tr>
<tr>
<td>7</td>
<td>BRAF</td>
<td>c.1799T&gt;A</td>
<td>p.V600E</td>
<td>37</td>
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<tr>
<td>3</td>
<td>PIK3CA</td>
<td>c.3140A&gt;G</td>
<td>p.H1047R</td>
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</tr>
<tr>
<td>14</td>
<td>AKT1</td>
<td>c.49G&gt;A</td>
<td>p.E17K</td>
<td>6</td>
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</tbody>
</table>
Highly Accurate Diagnosis of Cancer in Thyroid Nodules With Follicular Neoplasm/Suspicious for a Follicular Neoplasm Cytology by ThyroSeq v2 Next-Generation Sequencing Assay

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

- **Sensitivity** 92%
- **Specificity** 94%
- **PPV** 85%
- **NPV** 97%
- **Accuracy** 93%

- **Sensitivity** 86%
- **Specificity** 92%
- **PPV** 80%
- **NPV** 95%
- **Accuracy** 90%

**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%)

Nikiforov et al. Cancer (2014)
Molecular Markers for Tumor Prognostication and Targeted Therapies

<table>
<thead>
<tr>
<th>Gene Mutations (DNA)</th>
<th>Gene Fusions (RNA)</th>
<th>Gene expression (RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRAS</td>
<td>RET</td>
<td>PGK1</td>
</tr>
<tr>
<td>HRAS</td>
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<tr>
<td>KRAS</td>
<td>AKT1</td>
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<td>TP53</td>
<td>TTF1</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>GNAS</td>
<td>SLC5A5 (NIS)</td>
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<tr>
<td>PTEN</td>
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<td>EIF1AX</td>
<td>PTH</td>
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<td></td>
<td></td>
<td>KRT20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other</td>
</tr>
</tbody>
</table>

- **RNA Fusions**: RET, PPARG, NTRK1, NTRK3, BRAF, ALK
- **RNA Expression**: PGK1, KRT7, TG, TTF1, SLC5A5 (NIS), Calcitonin, PTH, KRT20, Other
74 yo female with solitary right lobe thyroid mass, present for years and 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)

FLUS/FN:
- Cancer risk for FLUS 5-15%, FN – 20-30%
- Repeat FNA?
- Diagnostic lobectomy?
74 yo female with solitary right lobe thyroid mass, present for years and recently increased in size

**FLUS/FN:**
- Cancer risk for FLUS 5-15%, FN – 20-30%
- *Repeat FNA?*
- *Diagnostic lobectomy?*

**ThyroSeq:**

Positive for NRAS (Q61R); TP53 (R175H); PIK3CA (E545K)

<table>
<thead>
<tr>
<th>Chr</th>
<th>Gene</th>
<th>Pos</th>
<th>Type</th>
<th>cDNA</th>
<th>Protein</th>
<th>Freq</th>
<th>Zyg</th>
<th>Cov</th>
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<tbody>
<tr>
<td>1</td>
<td>NRAS</td>
<td>115256520</td>
<td>SNV missense</td>
<td>c.182A&gt;G</td>
<td>p.Q61R</td>
<td>31</td>
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<td>848</td>
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<td>p.E545K</td>
<td>7</td>
<td>Het</td>
<td>1166</td>
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<td>17</td>
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<td>SNV missense</td>
<td>c.524G&gt;A</td>
<td>p.R175H</td>
<td>7</td>
<td>Het</td>
<td>526</td>
</tr>
</tbody>
</table>
74 yo female with solitary right lobe thyroid mass, present for years and recently increased in size

**Total thyroidectomy**

- **Oncocytic follicular carcinoma with capsular and multifocal vascular invasion (3 foci)**
74 yo female with solitary right lobe thyroid mass, present for years and recently increased in size

**Cytology:**
- FLUS/FN (cancer risk for FLUS 5-15%, FN – 20-30%)

**ThyroSeq:**
- Positive for NRAS (Q61R), TP53 (R175H), PIK3CA (E545K)

**Total thyroidectomy:**
- Oncocytic follicular carcinoma with capsular and multifocal vascular invasion (3 foci)
Conclusions

• Molecular profile of encapsulated FV of PTC is different from classic PTC

• **BRAF V600E, RET/PTC** fusions are common in classic PTC

• **RAS, PAX8/PPARG, BRAF K601E** are common in encapsulated FV PTC

• Molecular profiling of thyroid nodules can aid tumor diagnosis, prognostication, and targeted therapies for thyroid cancer
Moving Forward

Personalized Medicine for Thyroid Cancer: Delivery of the right treatment to the right patient at the right time
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