A Next-Gen Sequencing Assay for the Simultaneous Detection of Bladder Cancer-Associated Protein and DNA Markers

Anthony P. Shuber, CTO, Co-Founder
AACC Oak Ridge Conference
April 2013

Agenda

• “Why” combine DNA and Protein Biomarkers
  – CIDD Approach (Clinical Intervention Determining Diagnostic)

• “How” we combine DNA and Protein Biomarkers
  – MADR Approach (Multiple Analyte Diagnostic Readout)
  – Application to Bladder Cancer

• Simultaneous Analysis of Protein and DNA on a Next Gen Seq Platform

Disease Heterogeneity Creates Ambiguity

<table>
<thead>
<tr>
<th>Biomarker Results</th>
<th>Disease Free</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60% Sene, 80% Spec.</td>
<td>80% Sene, 60% Spec.</td>
</tr>
</tbody>
</table>

Standard Analytical Approach
Stratification of Patient Population

Clinical Intervention Determining Diagnostic (CIDD) Approach

- No Intervention (90% Sens.)
- Standard Intervention (High Sens. NPV)
- Maximum Intervention (High Spec. PPV)

MADR Reduces Population Overlap

- DNA plus protein markers can result in an increase in Sensitivity and Specificity simultaneously, maximizing NPV and PPV

CIDD/MADR Application to Triaging Hematuria Population
Hematuria Triage (PBS-002) Marker Panel

- **Matrix Metalloproteinase 2 (MMP-2)**
  - Involved with Angiogenesis, Tissue-remodeling (Tumor Growth) and Metastasis
  - Demonstrated Association with Multiple Cancers
  - Quantitative (Can achieve high sensitivity)

- **Fibroblast Growth Receptor 3 (qFGFR3)**
  - Cell surface Receptor Tyrosine Kinase for Fibroblast Growth Factor
  - Binary results
  - High specificity
  - Associated with genetically stable bladder tumors of low grade and stage

- **Twist1 and Nidogen2**
  - Twist1: transcription factor involved in multiple developmental pathways
  - Nidogen2: basement membrane protein
  - Binary or quantitative

- Performance Established in Urine

---

**PBS 002: Hematuria Triage Study**

- 27 Clinical Sites (2 Academic, 25 Community Practices)
- Total Number of Evaluable Subjects 748
  - Cancers 58
  - Hematuria+/Cystoscopy- 690

**PBS-002 Version 1**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Negative</th>
<th>Intermediate</th>
<th>Positive</th>
<th>PBS-002 Cutoffs</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGFR3</td>
<td>MMP-2&lt;1.100</td>
<td>Twist1&lt;139k</td>
<td>Nid2&lt;680k</td>
<td>98.2% (388/395) [96-99%]</td>
<td>87.9% (51/58) [76-95%]</td>
<td>56.2% (388/690) [52-60%]</td>
<td>N/A</td>
<td>95.2% (20/21) [76-100%]</td>
</tr>
</tbody>
</table>

*all marker negative for FGFR3, MMP-2, Twist and Nid2


---

**Application of Next Gen Sequencing**
Next Gen Sequencing of FGFR3 Increases Sensitivity in Urine

- 43 urine samples from cancer patients were tested by ngsFGFR3

<table>
<thead>
<tr>
<th>Stage</th>
<th>qPCR</th>
<th>Deep Sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ta</td>
<td>1/1 (100%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>T1</td>
<td>2/2 (100%)</td>
<td>0/2 (0%)</td>
</tr>
<tr>
<td>≥T2</td>
<td>0/1 (0%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>All</td>
<td>11/36 (30.5%)</td>
<td>5/36 (13.9%)</td>
</tr>
</tbody>
</table>

- 5 samples were previously positive by qPCR. All were positive by ngsFGFR3.
- ngsFGFR3 detected an additional 19 cancers that are as low as 0.02% mutant


TP53 Complements FGFR3 Sensitivity in Bladder Cancer

- TP53 mutations found in ~30% of all bladder tumors
  - Stage: pTa: 18%, pT1: 47%, ≥pT2: 52%
  - Grade: Low: 10%, High: 51%
- TP53 mutations have very little overlap with FGFR3 mutations


ngsTP53 Increases Clinical Sensitivity

- 57 tissues were analyzed both for ngsFGFR3 and ngsTP53
- 17 Ta, 21 T1, 3 Tis, 16 ≥T2

<table>
<thead>
<tr>
<th>Stage</th>
<th>ngsFGFR3</th>
<th>ngsTP53</th>
<th>ngsFGFR3/ngsTP53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ta</td>
<td>10/17 (59%)</td>
<td>5/17 (29%)</td>
<td>15/17 (88%)</td>
</tr>
<tr>
<td>T1</td>
<td>8/21 (38%)</td>
<td>3/21 (14%)</td>
<td>11/21 (52%)</td>
</tr>
<tr>
<td>≥T2</td>
<td>0/3 (0%)</td>
<td>1/3 (33%)</td>
<td>1/3 (33%)</td>
</tr>
<tr>
<td>Total</td>
<td>20/37 (54%)</td>
<td>11/37 (30%)</td>
<td>31/37 (84%)</td>
</tr>
</tbody>
</table>
Independent MADR Workflow

- Urine Sample Incubation
- Substrate Solution Addition + Incubation
- Conjugate Addition + Incubation
- Wash
- Wash
- Read Optical Density

FGFR3/TP53 NGS Workflow

- DNA Prep
- FGFR3/TP53 Multiplex Primary PCR (7 exons, 1 rx)
- FGFR3/TP53 Mutant Analysis (143 Mutations)

Platform-Agnostic MADR Technology

- DNA Prep
- Sequence – specific amplification
- Protein (Urine)
- Aptamer Selection

Protein Marker Detection by NGS (Model System)

- MMP-2 Detection by ELISA
- MMP-2 Detection by qPCR
- MMP-2 Detection by NGS

- blue: 6.25ng/ml MMP-2
- red: 3.125ng/ml MMP-2
- green: 1.56ng/ml MMP-2
- pink: 0.78ng/ml MMP-2
- grey: 0.39ng/ml MMP-2

*All analyses done in triplicate
Protein Marker Detection by NGS (Urine)

MMP-2 Detection by ELISA

MMP-2 Detection by qPCR

MMP-2 Detection by NGS

R² = 0.9894

R² = 0.9745

R² = 0.9593

Simultaneous Protein and DNA Detection by NGS

MMP-2/Aptamer

ngsFGFR3

MMP-2/Aptamer + ngsFGFR3

Single Marker Analysis

Multplex Protein/DNA Marker Analysis

Conclusions

• Combining Protein and DNA markers in a single assay improves clinical performance

• Application of NGS increases analytical and clinical sensitivity

• NGS associated protein and DNA analysis reduces assay complexity and reduces cost
Acknowledgements

- Cecilia Fernandez
- John Millholland
- Andrew Dunn
- Autumn Duchesne
- Lydia Anderson
- Maria Campo
- Maria Muraca
- Carol Ahearn
- Holly Gettler
- Alisha Josey

- Jeff Karnes
  — Mayo Clinic