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

Anthony P. Shuber, CTO, Co-Founder
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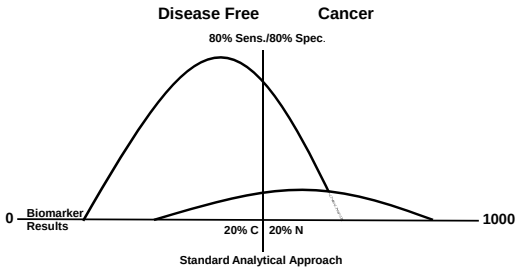
Agenda

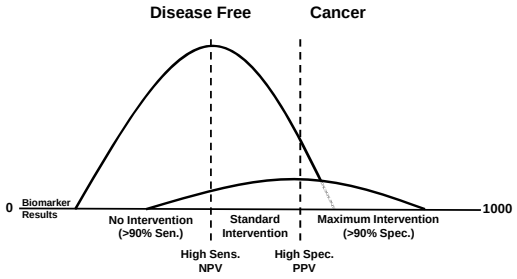
- "Why" combine DNA and Protein Biomarkers
  - CIDD Approach (Clinical Intervention Determining Diagnostic)
- "How" we combine DNA and Protein Biomarkers
  - MADR Approach (Multiple Analyte Diagnostic Readout)
  - Application to Bladder Cancer
- Simultaneous Analysis of Protein and DNA on a Next Gen Seq Platform

Disease Heterogeneity Creates Ambiguity



Stratification of Patient Population

Clinical Intervention Determining Diagnostic (CIDD) Approach



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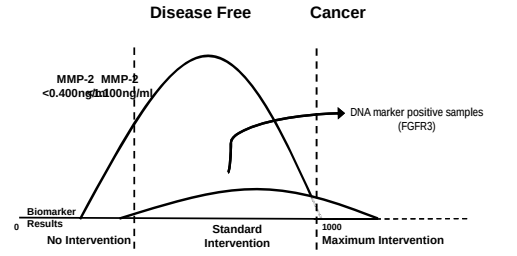
MADR Reduces Population Overlap



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

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CIDD/MADR Application to Triaging Hematuria Population

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Hematuria Triage (PBS-002) Marker Panel

MMP2

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

FGFR3

- 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/NID2

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

- Performance Established in Urine

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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

Study	Markers	Negative*			Intermediate	Positive (qFGFR3 pos)		
PBS-002	Cutoffs	NPV	Sensitivity	Specificity		PPV	Sensitivity	Specificity
58 cancers 690 H+/C-	FGFR3 MMP-2<1,100 Twist1 <139k Nid2<680k	98.2% (388/395) [96-99%]	87.9% (51/58) [76-95%]	56.2% (388/690) [52-60%]	N/A	95.2% (20/21) [76-100%]	34.5% (20/58) [22-48%]	99.9% (689/690) [99-100%]
Patient Distribution		53% (12% cancer, 56% HC)			44% (53% cancer, 44% HC)	3% (35% cancer, .1% HC)		

* all marker negative for FGFR3, MMP-2, Twist1 and Nid2

Karnes *et al.*, *Mayo Clinic Proceedings*, 2012;87(9):835-42.

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Application of Next Gen Sequencing

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Next Gen Sequencing of FGFR3 Increases Sensitivity in Urine

- 43 urine samples from cancer patients were tested by ngsFGFR3

	Sensitivity	
Stage	**qPCR**	**Deep Sequencing**
Ta	11.1% (3/27)	63.0% (17/27)
T1	22.2% (2/9)	55.6% (5/9)
≥T2	0.0% (0/7)	28.6% (2/7)
All Stages	**11.6% (5/43) [5-24%]**	**55.8% (24/43) [40-71%]**

- 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

Millholland *et al.*, *Research and Reports in Urology*, 2012;4(1):33-40.

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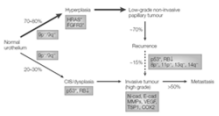
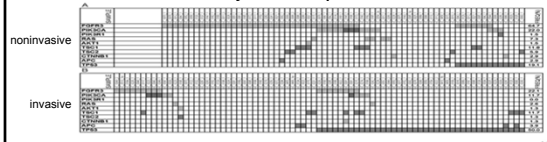
TP53 Complements FGFR3 Sensitivity in Bladder Cancer



- **TP53 mutations found in ~30% of all bladder tumors**
  - Stage: pTa: 18%, pT1: 47%, ≥pT2: 52%
  - Grade: Low: 19%, High: 51%
- **TP53 mutations have very little overlap with FGFR3 mutations**



van Rhijn et al. JCO May 2003 and Sjodahl et al. PLoS1 6(4):e18583, 2011

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ngsTP53 Increases Clinical Sensitivity

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

Stage	ngsFGFR3	ngsTP53	ngsFGFR3/ngsTP53
Ta	10/17 (59%)	1/17 (6%)	11/17 (65%)
T1	8/21 (38%)	1/21 (5%)	9/21 (43%)
Tis	0/3 (0%)	1/3 (33%)	1/3 (33%)
≥T2	2/16 (13%)	8/16 (50%)	10/16 (63%)
Total	**20/57 (35%)**	**11/57 (19%)**	**31/57 (54%)**

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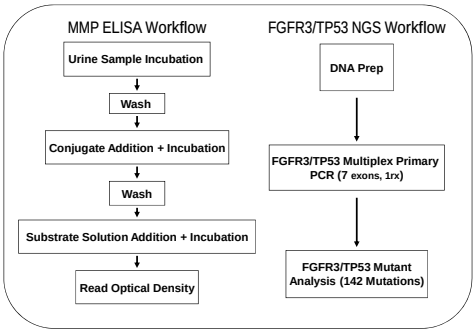
Independent MADR Workflow



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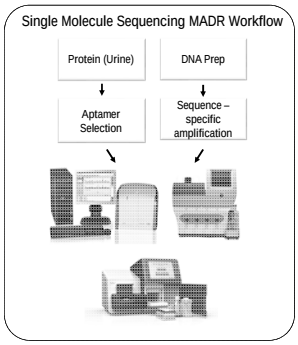
Platform-Agnostic MADR Technology



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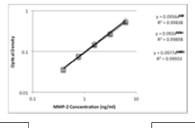
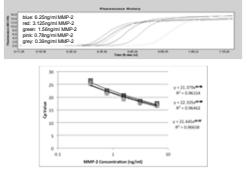
Protein Marker Detection by NGS (Model System)

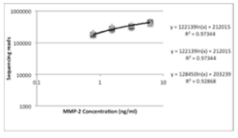
MMP-2 Detection by ELISA



MMP-2 Detection by qPCR



MMP-2 Detection by NGS



*All analyses done in triplicate

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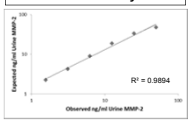
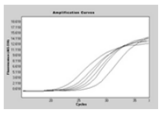
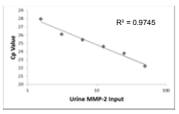
Protein Marker Detection by NGS (Urine)

MMP-2 Detection by ELISA

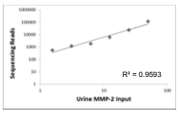


MMP-2 Detection by qPCR





MMP-2 Detection by NGS



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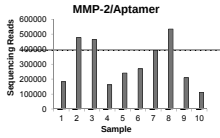
Simultaneous Protein and DNA Detection by NGS

Single Marker Analysis

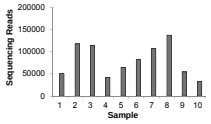


ngsFGFR3

Sample	1	2	3	4	5	6	7	8	9	10
% Mutant	0%	0%	0%	0%	0%	48.3%	3.6%	3.6%	4.5%	10.8%

Multiplex Protein/DNA Marker Analysis

MMP-2/Aptamer + ngsFGFR3



Sample	1	2	3	4	5	6	7	8	9	10
% Mutant	0%	0%	0%	0%	0%	49.3%	2.4%	2.3%	3.2%	7.9%

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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

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