Translating "-omics" Biomarkers from Bench to Bedside

Y. Victoria Zhang, PhD, DABCC
University of Rochester Medical Center

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None related to this presentation

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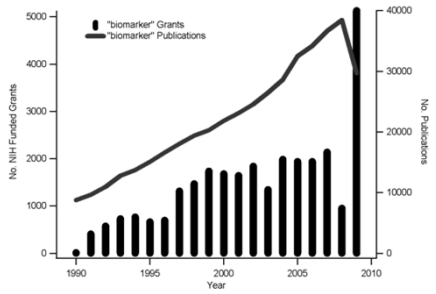
Outline

- Biomarker Research Is an Interdisciplinary Field and Has Great Challenges Moving from Bench to Bedside.
- Mass Spec Bridges Discovery Research and Clinical Applications.
- Clinical Chemists Can Make a Difference in Transitional "-omics" Biomarkers from Bench to Bedside.

Ping Pong Diplomacy

The Boom of Biomarker Era



Ptolemy, A. S., and Rifai, N. (2010) *Scandinavian Journal of Clinical & Laboratory Investigation 70*, 6-14.

Current Status:

Big Investments Small Returns

2008 – 2009 NIH funding

$2.5 billion | 5,943 Grants

1993 – 2008 FDA approved protein markers

Avg. 1.5 per year

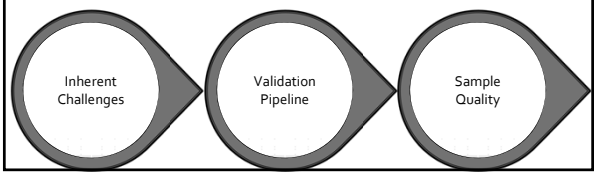
e.g., Troponin I/T, BNP, Cystatin C

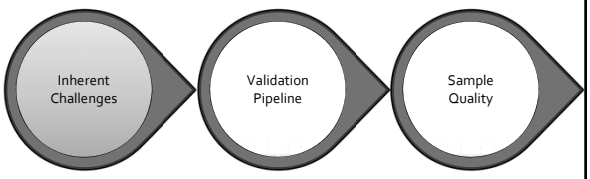
None from proteomics study

Ptolemy, A. S., and Rifai, N. (2010) *Scandinavian Journal of Clinical & Laboratory Investigation 70*, 6-14.
Anderson, N. L. (2009), *Clinical Chemistry 56*, 177-185

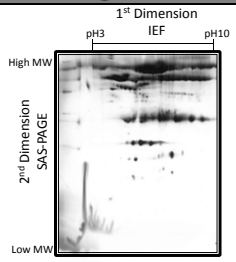
Challenges?

- Complexity of human proteome
  - 1 million proteins, 21,000 genes
  - Dynamic range
- Lack of coherent pipelines from discovery to validation and validation is a very expensive process.
- Lack of standardization in sample collection
  - Rely on whatever specimens conveniently available

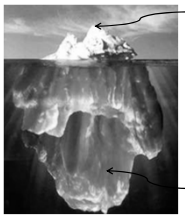




Low Abundance Provides Inherent Challenges for Protein Analysis



10uL hSerum, 15% gel, Silver stain



- Na+ concentration: 140 mmol/L
- Testosterone: 300 ng/dL → 10 nmol/L
- Vitamin D: 20 ng/mL → 50 nmol/L

- Albumin: 65 kDa
- Tg: 660 kDa (two 330 kDa monomers)

Biomarker Research is a Multi-Step System

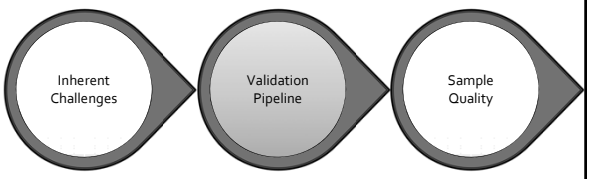
Phase I	Phase II	Phase III	Phase IV	Phase V
Candidate Discovery	Qualification	Verification	Assay Optimization Clinical Validation	FDA Approval Commercialization

Mass spectrometrists
"-omics"
Biochemists

Mass spectrometrists
Lab Medicine
Clinical Chemists

Bench → Bedside

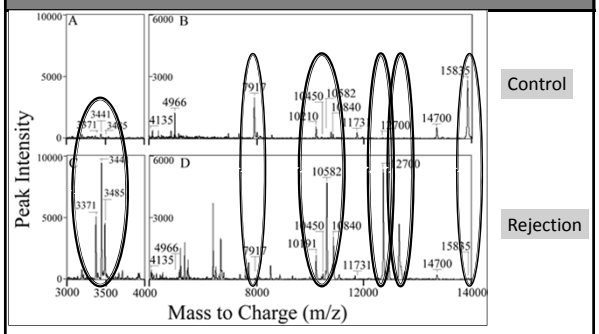
Modified from Rifai, N., Gillette, M. A., and Carr, S. A. (2006), *Nature Biotechnology 24*, 971-983



Different Approaches for Discovery

- Mass spectrometry is the major platform
  - MALDI Based
  - Electro-spray based
- Specimen from post transplant patients
  - BALF (Bronchoalveolar Lavage Fluid)
  - Control: collected >100 months before diagnosis
  - Rejection: diagnosed with biopsy

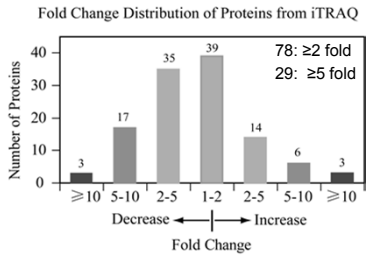
Marker Candidates from MALDI



Zhang, Y., Wroblewski, M., et.al., (2006) Proteomics 6: 1001-1010

Marker Candidates from iTRAQ

Total of 117 Proteins

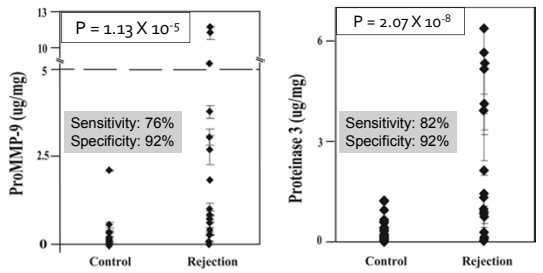


Zhang, Y., Wendt, C., et.al., (2007) Clinical Proteomics 3: 3-12

Validation on iTRAQ results

Confirm Real Differences; Remove False Positives

Total Sample: 65 (48 controls and 17 rejection)

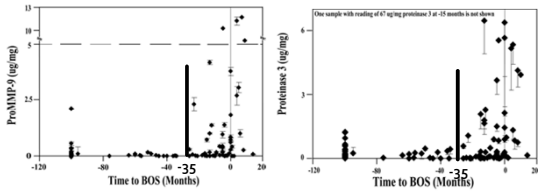


Replotted based on Zhang, Y., Wendt, C., et.al., (2007) Clinical Proteomics 3: 3-12

Further Step: Prediction

Total Sample: 142 (48 controls, 17 rejection, and 77 intermediates)

Statistical model based on multiple markers allows early detection **35 months sooner**

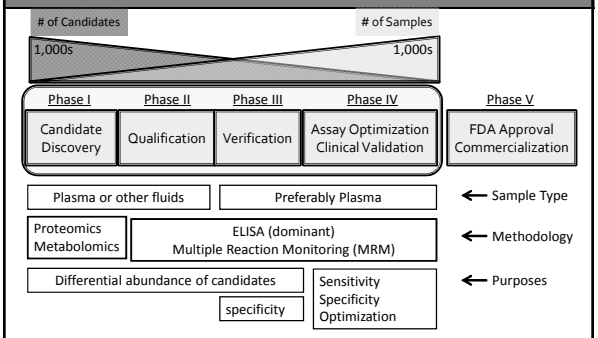


Zhang, Y., Wendt, C., et.al., (2007) Clinical Proteomics 3: 3-12

Challenges in Validation Phase

- How to multiplex 100s candidates for validation?
- Current Method of Choice: ELISA
  - Available for < 2000 proteins
  - Time consuming for new assay development
- 150,000 papers generated thousands of claimed biomarkers
  - <100 have been validated

Validation is an Expensive Process



Modified from Rifai, N., Gillette, M. A., and Carr, S. A. (2006), *Nature Biotechnology 24*, 971-983

Validation pipeline

nature biotechnology
July 2011

A pipeline that integrates the discovery and verification of plasma protein biomarkers reveals candidate markers for cardiovascular disease

Terri A Addona[1], Xu Shi[2,7], Hasmik Keshishian[1,7], D R Mani[1], Michael Burgess[1], Michael A Gillette[1,3], Karl R Clauser[1], Dongxiao Shen[2], Gregory D Lewis[2,4,5], Laurie A Farrell[2], Michael A Fifer[2], Marc S Sabatine[2,5,6], Robert E Gerszten[1,2,4,5] & Steven A Carr[1]

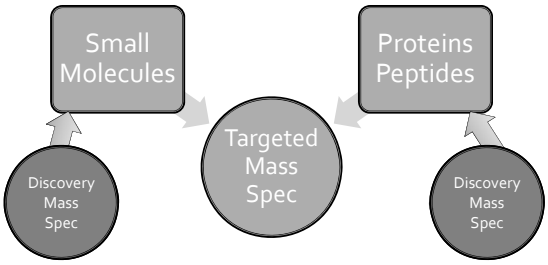
$4 million for 4 years

A targeted proteomics–based pipeline for verification of biomarkers in plasma

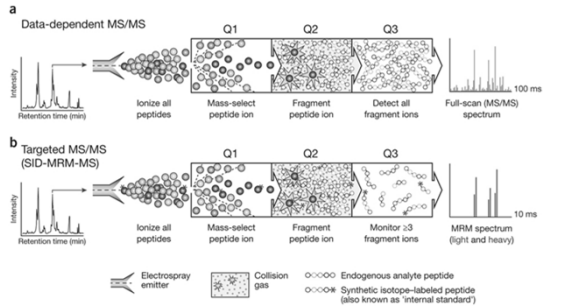
Jeffrey R Whiteaker[1,3], Chenwei Lin[1,3], Jacob Kennedy[1,3], Liming Hou[1,3], Mary Trute[1,3], Izabela Sokal[1,3], Ping Yan[1], Regine M Schoenherr[1], Lei Zhao[1], Uliana J Voytovich[1], Karen S Kelly-Spratt[1], Alexei Krasnoselsky[1], Philip R Gafken[1], Jason M Hogan[1], Lisa A Jones[1], Pei Wang[1], Lynn Amon[1], Lewis A Chodosh[2], Peter S Nelson[1], Martin W McIntosh[1], Christopher J Kemp[1] & Amanda G Paulovich[1]

Anderson Leigh (2012) Clinical Chemistry 58: 28-30

Transition from Discovery to Targeted Mass Spec

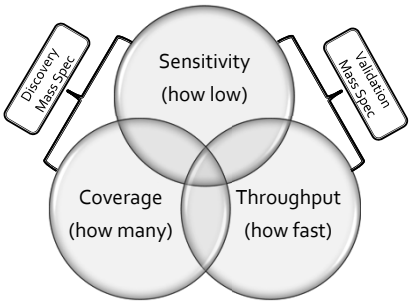


Discovery vs. Targeted MS

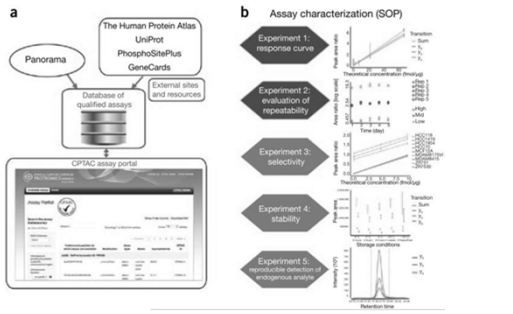


http://www.nature.com/nmeth/journal/v10/n1/images/nmeth.2309-F3.jpg

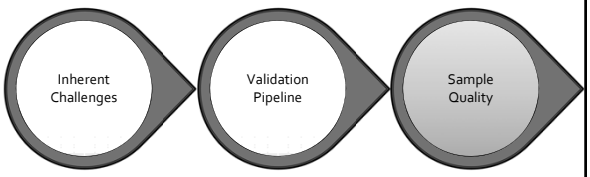
Balance in Discovery vs. Validation



CPTAC Assay Portal



Jeffrey R. Whiteaker, et al., Nat Methods, 2014, 11(7): 703 - 704

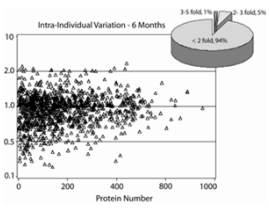


Sample Quality

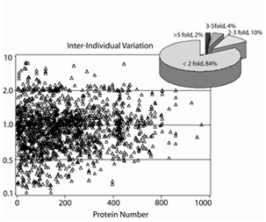
- Pre-analytical concerns
- Sample type
- Sample collection process
- Compound Stability
- Storage
- Freeze thaw cycles
- Inter/Intra-individual variability
- Et al

Big variations among individuals

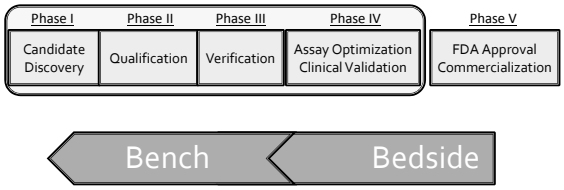
INTRA-INDIVIDUAL



INTER-INDIVIDUAL



Biomarker Research shall Begin with the End in Mind



Clinical Chemists Plays an Important Role in Translating Biomarkers from Bench to Bedside

Modified from Rifai, N., Gillette, M. A., and Carr, S. A. (2006), *Nature Biotechnology 24*, 971-983

Conclusions

- Biomarker Research is a complex system, which requires multiple disciplines to work together.
- Mass Spectrometry is a powerful platform to bridge discovery and validation process.
- Clinical chemists can play an important role in moving biomarkers from the bench to bedside.