

## Moving Biomarkers from Discovery through Translation



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## Outline

- Introduction
- An integrated approach for biomarker discovery, validation and translation – *Johns Hopkins Clinical Chemistry Division.*
- The road map for biomarker translation: *The 4Bs, 4Gs & 4Ps case studies.*
- Conclusion.

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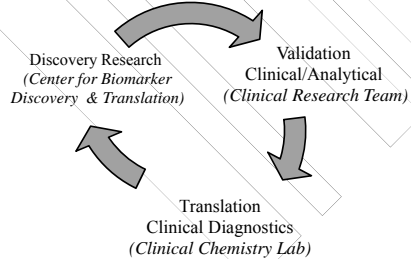
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## An Integrated Approach for Biomarker Discovery, Validation and Translation – *Johns Hopkins Clinical Chemistry Division*



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### Our Strategies

#### Cancer biomarker discovery, validation & translation

- Select the right technologies: Protein/Lectin array and/or mass spectrometry.
- Use well characterized clinical specimens – plasma, serum, urine, body fluid, tissue, cell: Pathology.
- Develop bioinformatics tools for data analysis and multiplexing of biomarkers: Engineering.
- Design multi-center case control study with extensive clinical validation to minimize the impact of possible confounding variables: Statistics.
- Discover and identify biomarkers (profile is not sufficient) with biological (clinical) significance: Cancer Biology.
- Translation of biomarker into multiplex clinical diagnostics: Clinical Chemistry.

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### Why are we interested in glycoproteomics?

- Glycoproteins modified by complex carbohydrates are extracellular proteins - membrane proteins, cell surface proteins, and secreted proteins.
- Most likely detected as surrogate serum biomarkers.
- Glycosylation are associated with disease progression.
- Most FDA approved serum cancer biomarkers are glycoproteins.
- Zhang H and Chan DW. *Cancer biomarker discovery in plasma using a tissue-targeted proteomic approach. Cancer Epidemiology, Biomarkers and Prevention (2007).*

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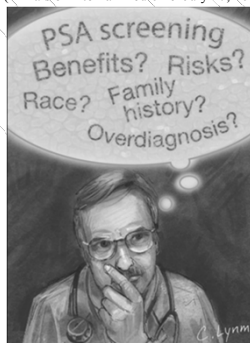
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### PSA Screening ?

The U.S. Preventive Services Task Force (USPSTF) recommends against PSA-based prostate cancer screening and gave the guidance a Grade D recommendation (Annals of Internal Medicine July 17, 2012).



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## Clinical unmet needs: Prostate cancer

- Opportunity for biomarkers to detect aggressive prostate cancer.
- One of the current definition for aggressive prostate cancer is based on tissue diagnosis using Gleason score = 7 and above. In addition, the assessment of prostate cancer recurrence (5-10 years after radical prostatectomy) is useful.
- Better biomarkers are needed for aggressive prostate cancer.

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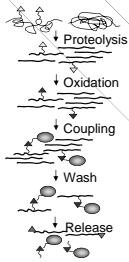
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## Solid phase extraction of glycopeptides (SPEG) and quantitative glycoproteomics



Zhang, H. Li, X-J, Martin, D. M., and Aebersold, R. *Nature Biotechnology* (2003).  
 Tian, Y., Zhou, Y., Elliott, S., Aebersold, R., and Zhang, H. *Nature Protocols* (2007).  
 Zhou, Y. et al. Isolation of N-linked glycopeptides from plasma. *Analytical Chemistry* (2007).  
 Zou, Z., Ibsaite, M., Zhou, Y., Aebersold, R. Xia, Y., and Zhang, H. Synthesis and Evaluation of Superparamagnetic Nanoparticles for Extraction of Glycopeptides in the Microtiter Plate Format *Analytical Chemistry* (2008).

April 11, 2013

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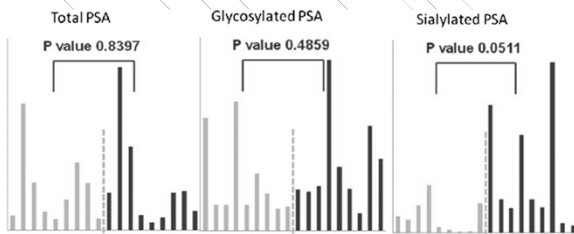
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## Simultaneous Analysis of Glycosylated and Sialylated PSA in Prostate Cancer Tissues



Y: Total PSA (ng/mL), Glycosylated and sialylated PSA (native to heavy peptide ratio\*100). X: Non-cancer tissues (green). Cancer tissues (red).  
 Yan Li, Yuan Tian, Taha Rezai, Amol Prakash, Mary F. Lopez, Daniel W. Chan and Hui Zhang. *Anal. Chem.*, 2011, 83 (1), pp 240–245

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## High-density Lectin Array

- 94 lectins were immobilized on the array surface
- Lectin-antibody immunoassay
- Fluorescent detection

Glass slide with N-Hydroxysuccinimide (NHS) esters coating

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### Lectin microarrays: Identification of lectins interacting with PSA and MME for aggressive prostate cancer.

(A) lectins that interact with PSA from pooled tissue samples of different groups  
 (B) lectins that interact with MME (membrane metallo-endopeptidase) from pooled tissue samples of different groups.

Yan Li, Sheng-Ce Tao, G. Steven Bova, Alvin Y. Liu, Daniel W. Chan, Heng Zhu, Hui Zhang. *Anal. Chem.* **2011**, *83*, 8509-8516.

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### Lectin-based immunosorbent assays

(A) A lectin-based immunosorbent assay.  
 (B) PSA\_SNA & PSA\_Jacalin  
 (C) MME\_GS-II & MME\_MAL-I

Yan Li, Sheng-Ce Tao, G. Steven Bova, Alvin Y. Liu, Daniel W. Chan, Heng Zhu, Hui Zhang. *Anal. Chem.* **2011**, *83*, 8509-8516.

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## Case Study: Ovarian Cancer

- A 50-year-old woman presented at the Johns Hopkins Hospital clinic. Physical examination revealed masses in the pelvic area. An ultrasonography was performed, however, the result was not diagnostic. Her serum CA125 was 105 U/mL. What was the diagnosis?

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## OVA1 – *Wall Street Journal* 3-9-2010

### Cancer Clues

A new test measures five proteins that increase or decrease in your blood if you have ovarian cancer.

PROTEIN	Apolipoprotein A1	Beta 2 microglobulin	CA125	Prealbumin	Transferrin
Function	Cholesterol Transport	The body's immune response	Released by tumor cells	Hormone and vitamin transport	Iron transport
LIKELY CHANGE IF YOU HAVE CANCER	DOWN	UP	UP	DOWN	DOWN

Source: Verillion Inc.

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## OVA1 – Intended Use (FDA)

- The OVA1 test is a qualitative serum test that combines the results of 5 immunoassays into a single numerical score.
- It is indicated for women who meet the following criteria: over age 18, ovarian adnexal mass present for which surgery is planned, and not yet referred to an oncologist.
- The OVA1 test is an aid to further assess the likelihood that malignancy is present when the physician's independent clinical and radiological evaluation does not indicate malignancy.
- The test is not intended as a screening or stand-alone diagnostic assay.

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## The Wall Street Journal

### Test to Help Determine If Ovarian Masses Are Cancer - 3/9/2010

- “Doctors and hospitals are getting a new test that many think will help fight ovarian cancer... by helping them to more quickly distinguish cancerous from benign growths.”
- The test, which is called OVA1 ... was shown to correctly flag 92% of cancers, when used along with radiological imaging and a standard patient work-up, in a study of 27 hospitals, doctors' offices and clinics. physicians using their usual detection methods but not OVA1 had previously found 72% of the cancers.”

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## Biomarker discovery for Ovarian Cancer

### ■ Proteomic Approaches to Tumor Marker Discovery: Identification of Biomarkers for Ovarian Cancer.

Alex J. Rai, Zhen Zhang, Jason Rosenzweig, Ie-ming Shih, Thang Pham, Eric T. Fung, Lori J. Sokoll and Daniel W. Chan. (Johns Hopkins University) Archives of Pathology and Laboratory Medicine. 126:1518-1526, 2002.

### ■ Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer.

Z Zhang, RC Bast, Y Yu, J Li, LJ Sokoll, A Rai, J Rosenzweig, B Cameron, Y Wang, X Meng, A Berchuck, C van Haafren-Day, NF Hacker, HW de Bruijn, AGJ van der Zee, JJ Jacobs, ET Fung & DW Chan. (Johns Hopkins Univ, MD Anderson, Duke Univ, Royal Hosp, Sydney, Australia; Groningen Univ Hosp, Netherlands, Ciphergen, London Univ, UK). Cancer Research 64, 5882-5890, 2004.

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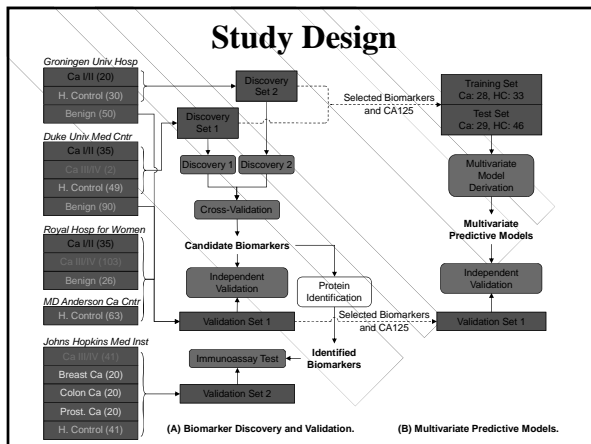
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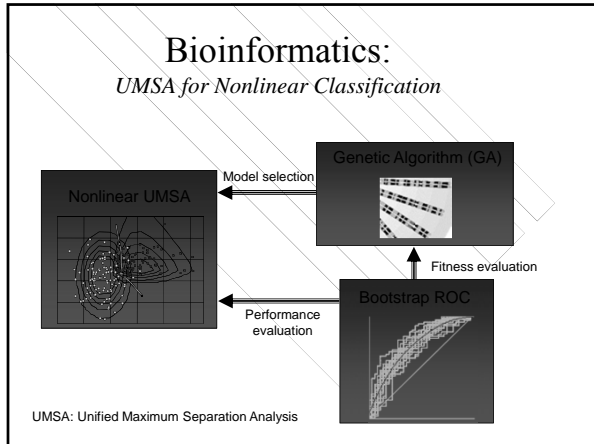
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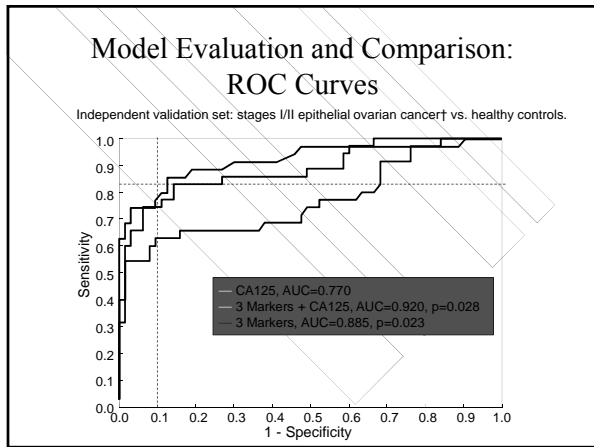
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- ### OVA 1: Choice of Assay
- Discovery assay: SELDI mass spectrometry
  - Clinical assay: ELISA

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### Translation into clinical diagnostics

- Vermillion Inc. licensed the invention, conducted clinical trial and cleared by the FDA for clinical use on September 11, 2009 as the OVA1 test - the 1<sup>st</sup> proteomics IVDMIA (*in vitro* diagnostic multivariate index assays) cleared by the US FDA.

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Lesson learned: Do we have the right approach for biomarker discovery and validation?

	<b>Traditional Approach</b>	<b>New Approach</b>
Discovery Study	Small convenient set of specimens (n=10-100)	Large well defined set of specimens (n=100-1000)
Validation Study	Large, well defined set of specimens (n=100-1000)	Large, well defined set of specimens (n=100-1000)

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Do you have the right specimens for biomarker discovery?

- To obtain the right specimens from the right targeted population for biomarker discovery.
- For example, if the goal is to discover biomarkers for aggressive prostate cancer, specimens should be obtained from patients with and without aggressive prostate cancer.

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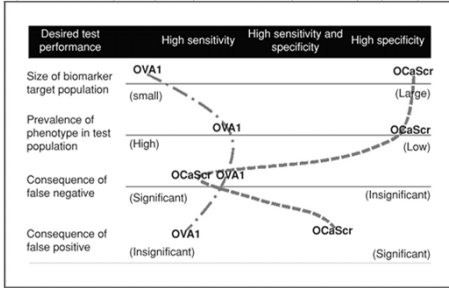
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Lesson learned: The intended use define clinical performance and the target population.



Zhang Z, Chan D W Cancer Epidemiol Biomarkers Prev 2010;19:2995-2999

© 2010 by American Association for Cancer Research

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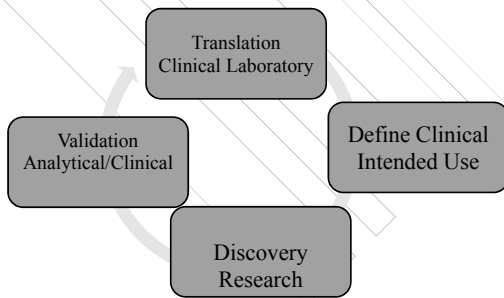
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### The 4Bs & 4Gs for Successful Biomarker Translation




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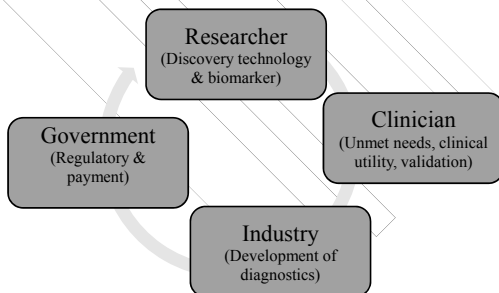
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### Moving Forward: 4Ps (*Partnership*)




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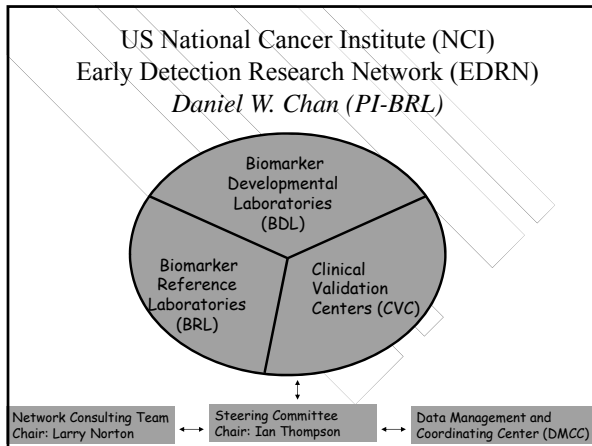
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**Case Study – Prostate cancer biomarkers decision gate**

- In 2005, 14 biomarkers were submitted to EDRN for consideration (Chair of prostate cancer group – Daniel W. Chan).
- Based on preliminary data from the investigators, the top 5 biomarkers were selected for pre-validation using the same prostate clinical specimen reference set (blinded): ProPSA, human Kallikreins, EPCA2, PCA3 and TSP1.
- Three biomarkers (human kallikreins, TSP1 and EPCA2) failed pre-validation. Pro-PSA and PCA3 were recommended for clinical validation.

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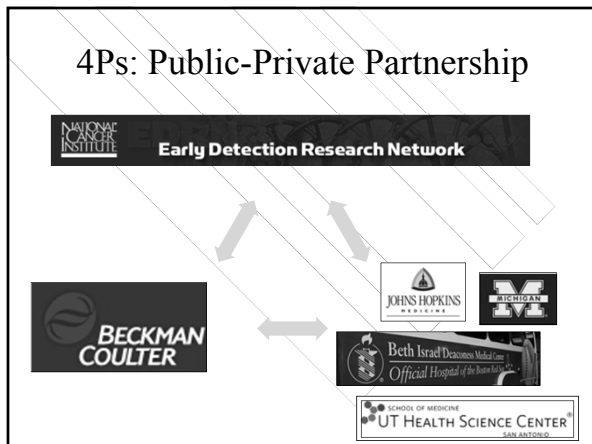
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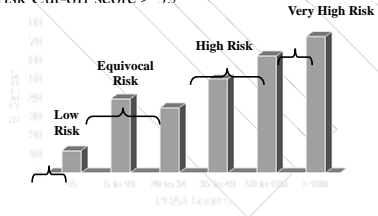
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## Intended Use for PCA3 (PROGENSA)

- To determine the need for repeat prostate biopsies in men who have had a previous negative biopsy: Gen-Probe, Inc.
- FDA approved 2/15/2012.
- High risk Cut-off score > 35



Early Detection Research Network

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## Conclusion

- The future of cancer diagnostics will be more individualized with a specific clinical intended use.
- It will most likely be a panel of multiple biomarkers.
- The 4Bs (bridge), 4Gs (gate) and 4Ps (partnership): To translate cancer biomarkers into clinical diagnostics, we need to construct a roadmap for the development of cancer diagnostics and with close collaboration between researchers, industry, clinicians and clinical chemists.
- We learned that the road from biomarker discovery, validation to clinical diagnostics could be long and winding, sometimes frustrating, however, we know that at the end of the road .....

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