Early Detection Research Network

Bringing Discoveries to Clinical Application: Clinical Epidemiology and Validation Centers

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From the outside looking in, how are new cancer markers discovered & validated?

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- Most commonly, patients with and without cancer are selected.
- Biologic samples queried.
- Differences identified.
- Biomarkers identified.
- Publication of “new cancer test”

What are the problems with the approach?

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- First, individuals with expertise in molecular discovery rarely have an expertise in the clinical presentation of cancer or in clinical diagnostic needs.
- Clinicians generally know the questions but are not experts in biochemistry/technology; rarely have epidemiology/biostatistics expertise.
- Epidemiologists & biostatisticians are needed to fully understand analyses, mitigate bias, and to select appropriate populations for discovery and validation.

How do you achieve such an environment?

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- **You put the 'discoverers' together with the 'users' and supervise them with the 'methodologists'.**

  Discoverers – scientists

  Users – Clinician scientists

  Methodologists – Epidemiologists/statisticians

- **Together, they function as a *single team* with a single goal: to develop a valid test that will change the way medicine is practiced, preventing suffering and death from cancer.**

GU Group as a microcosm of the EDRN

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- How do we prioritize/select biomarkers?
- Regular meetings and conf calls, invited speakers, intra-EDRN and extra-EDRN discovery.
- Methodologic scrutiny.
- Biologic rationale.
- Concurrent development of appropriate reference sets/identification of appropriate specimens in biorepositories.

Three vignettes

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- A highly-promising technology we investigated, learned about methodology, and found was not valuable.
- An example of the *process* of prioritization.
- An example of a clinical success.

Vignette One.
SELDI for prostate cancer

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- The challenge of proteomics.
- Extremely promising data from multiple institutions.
- Multiple series suggested sensitivity and specificities exceeding 90%.
- EDRN GU group: “High Priority: Design the trial, now”

Trial Design

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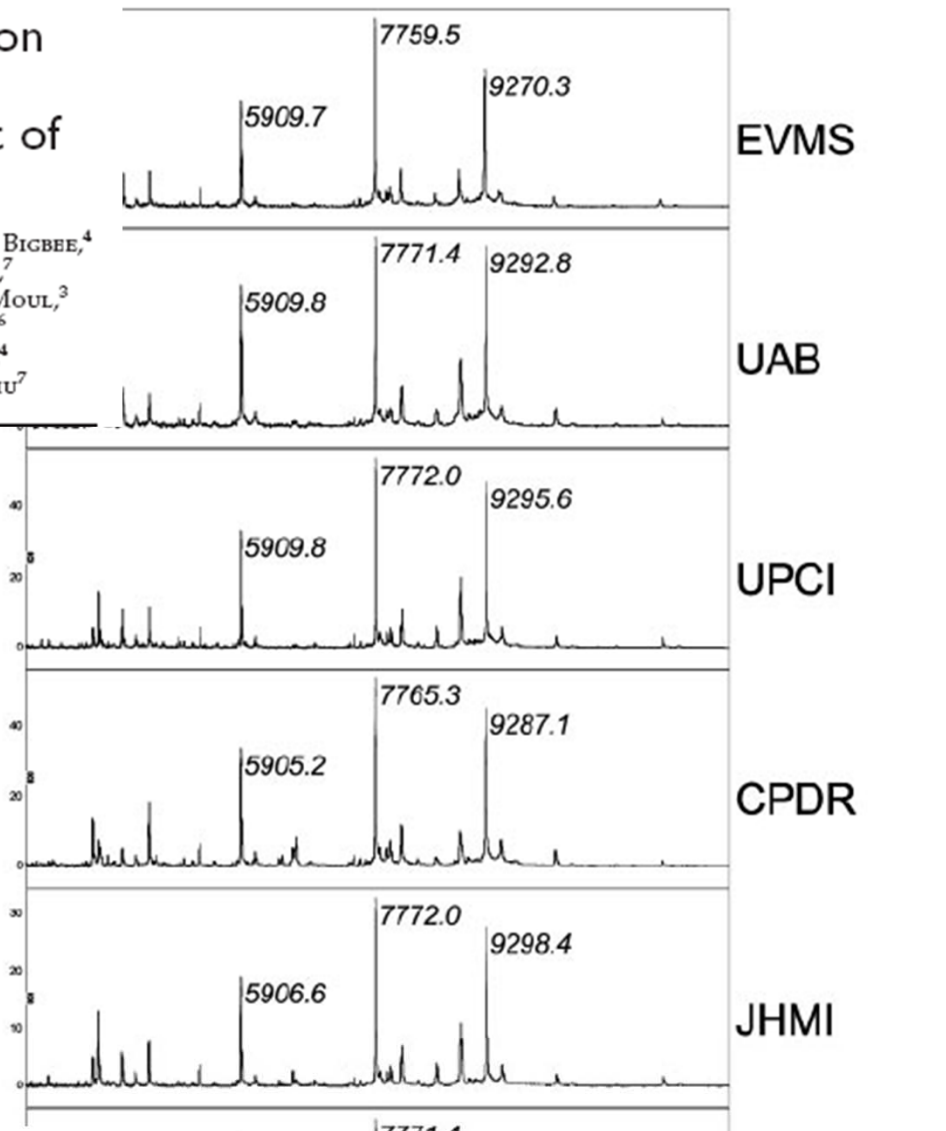
Three phases:

I: Portability and reproducibility. ***Can SELDI as a clinical test provide comparable serum protein profiles in multiple laboratories? (3 sub-aims)***

Evaluation of Serum Protein Profiling by Surface-Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry for the Detection of Prostate Cancer: I. Assessment of Platform Reproducibility

O. John Semmes,[1*] Ziding Feng,[2] Bao-Ling Adam,[1] Lionel L. Banez,[3] William L. Bigbee,[4] David Campos,[5] Lisa H. Cazares,[1] Daniel W. Chan,[6] William E. Grizzle,[7] Elzbieta Izbicka,[5] Jacob Kagan,[8] Gunjan Malik,[1] Dale McLerran,[2] Judd W. Moul,[3] Alan Partin,[6] Premkala Prasanna,[3] Jason Rosenzweig,[6] Lori J. Sokoll,[6] Shiv Srivastava,[3] Sudhir Srivastava,[8] Ian Thompson,[9] Manda J. Welsh,[4] Nicole White,[6] Marcy Winget,[2] Yutaka Yasui,[2] Zhen Zhang,[6] and Liu Zhu[7]

Clinical Chemistry 51:1
102–112 (2005)



Conclusions: These results demonstrate that "between-laboratory" reproducibility of SELDI-TOF-MS serum profiling approaches that of "within-laboratory" reproducibility as determined by measuring discrete *m/z* peaks over time and across laboratories.

Phase Two

Refinement of predictive algorithm in multi-institutional case-control population.

Original plan for Phase II study

Stage II sample and data collection requirements.

Sample collection requirements for cases and controls:
- Minimum sample size: 600 μl
- Samples stored at -70°C or colder
- No more than one freeze/thaw
- Sample not more than 3 years old

Patient-specific data elements (required):
- Ethnicity/race
- Date of birth
- Date of biopsy
- Biopsy institution (where biopsy was performed)
- Date of specimen collection
- PSA
- DRE
- Biopsy results (pathology report required)
- Gleason score

Patient-specific data elements (optional):
- Confirmed presence (absence) of PIA or PIN from biopsy report (required for controls)
- Number of cores *(as a minimum, sextant cores will be accepted)*
- Family history (corresponding to family history common data elements (CDEs) part of the EDRN Core Baseline CDEs)
- Time from blood draw to freezer

Case-control definitions:

Controls ($n = 250$)
- No previous prostate biopsy
- Serum drawn before current prostate biopsy
- Serum drawn 6 months or less before to current prostate biopsy
- No evidence of prostate cancer
- No evidence of PIN or PIA
- No hormonal therapy, chemotherapy, or prior radiation therapy
- PSA $<$ 10.0 ng/ml (stratify by PSA of 0–4 vs. 4–10 ng/ml)

Cases: ($n = 500$) [Gleason < 7 ($n = 250$); Gleason ≥ 7 ($n = 250$)]
- No previous prostate biopsy
- Serum drawn before current prostate biopsy
- Serum drawn 6 months or less before current prostate biopsy
- Prostate adenocarcinoma (verified by pathology report)
- Clinical T1-2N0M0 disease

Revised study design (from phase 1)

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- Rigorous sample requirements – disease definition, processing, storage, age, # freeze/thaws.
- 125 samples from high grade, 125 low grade, 125 biopsy-negative controls, 50 with inflammatory disease, 50 with other cancer.
- Analysis at 2 EDRN laboratories. Obsessive-compulsive QC. Age/race-matched.

Clinical Chemistry 54:1
53–60 (2008)

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SELDI-TOF MS Whole Serum Proteomic Profiling with IMAC Surface Does Not Reliably Detect Prostate Cancer

Dale McLerran,[1] William E. Grizzle,[2] Ziding Feng,[1] Ian M. Thompson,[3] William L. Bigbee,[4] Lisa H. Cazares,[5] Daniel W. Chan,[6] Jackie Dahlgren,[1] Jose Diaz,[5] Jacob Kagan,[7] Daniel W. Lin,[8] Gunjan Malik,[5] Denise Oelschlager,[2] Alan Partin,[5] Timothy W. Randolph,[1] Lori Sokoll,[6] Shiv Srivastava,[9] Sudhir Srivastava,[7] Mark Thornquist,[1] Dean Troyer,[3] George L. Wright,[5] Zhen Zhang,[6] Liu Zhu,[2] and O. John Semmes[5*]

- **Performance of the SELDI classifier system:**
- **Cancer versus biopsy-negative controls – error rate 52% at EVMS and 50% at UAB.**
- **High grade versus ‘controls’ without high grade cancer – error rate 52% at EVMC and 48% at UAB**
- **Phase III study not pursued (validation in large prospective study, i.e., PCPT)**

Lessons learned

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- Previous studies use of suboptimal samples for discovery source of significant bias.
- Controls must be carefully selected – fully ascertained, include other cancers and/or inflammation (non-specific markers of disease).
- Sample size must be sufficient to reach clinically meaningful decisions. (We had an 86% power to confirm test benefit 965% specificity at 95% sensitivity) against a clinically unacceptable differentiation (50% specificity at 85% sensitivity).
- Also appropriate to include *biologic* issues related to tumor diagnosed (Gleason 7-10 versus Gleason $\leq$ 6).
- This publication is probably the current standard for validation of a disease biomarker

“The most important experiments are those that are not only worthwhile if the result is positive – but rather those that give major insights irrespective of whether or not they are positive or negative” Barnett S. Kramer

Vignette Two.
Biomarker 'cook-off'

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Multiple promising biomarkers related to prostate cancer risk.

Question: Which to pursue?

Answer: Develop standardized reference set.

- A reference set in which the question of cancer/no cancer is clinically-relevant.
- Offer the reference set to multiple competing opportunities.
- Develop standards that, if met or exceeded, might justify moving to the next stage of validation.
- Rigorous sample set but expeditiously respond to opportunities.

Description of the reference set

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- 123 specimens (63 PC, 60 non-malignant)
- 1 ml serum from each patient.
- Contributed from three EDRN CEVCs (Harvard, Johns Hopkins, UTHSC San Antonio).
- PSA > 2.5 ng/mL, rising PSA, %fPSA<15%, abnormal DRE. $\geq$ 10 cores. Rigorous specimen processing. Blinded labs. Data analyzed by EDRN DMCC.
- Specimen shipped to JHU reference lab for aliquoting, re-labeling, and shipping to four labs. Blinding by EDRN staff.

Pre-Validation (Beckman) Combination of BPHA, Testosterone, -2 ProPSA, fPSA, PSA, and %fPSA by LR

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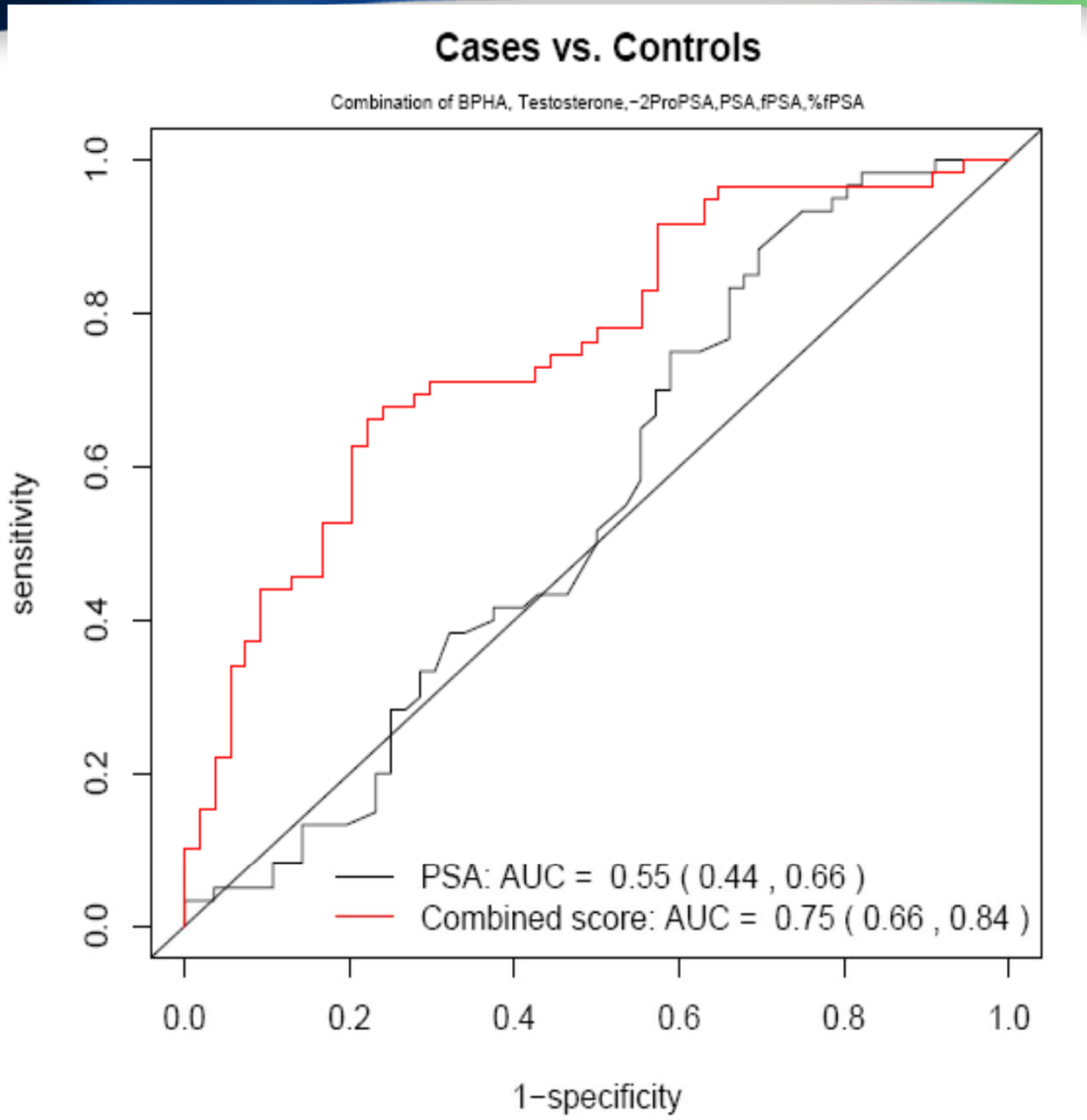

Outcome of this process

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- Formal reference set with larger sample size being collected.
- proPSA being targeted for primary analysis in the same fashion as the ‘cook-off’ evaluation set.

Vignette Three.
Risk assessment in Prostate Cancer

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- Impact on mortality isn't known; nonetheless, 75% of men have had a PSA and 50% have on regularly.
- PSA cutoff of 4.0 ng/mL widely used for 20+ years.
- Fundamental basis for PSA cutoff was never validated.

PCPT Schema

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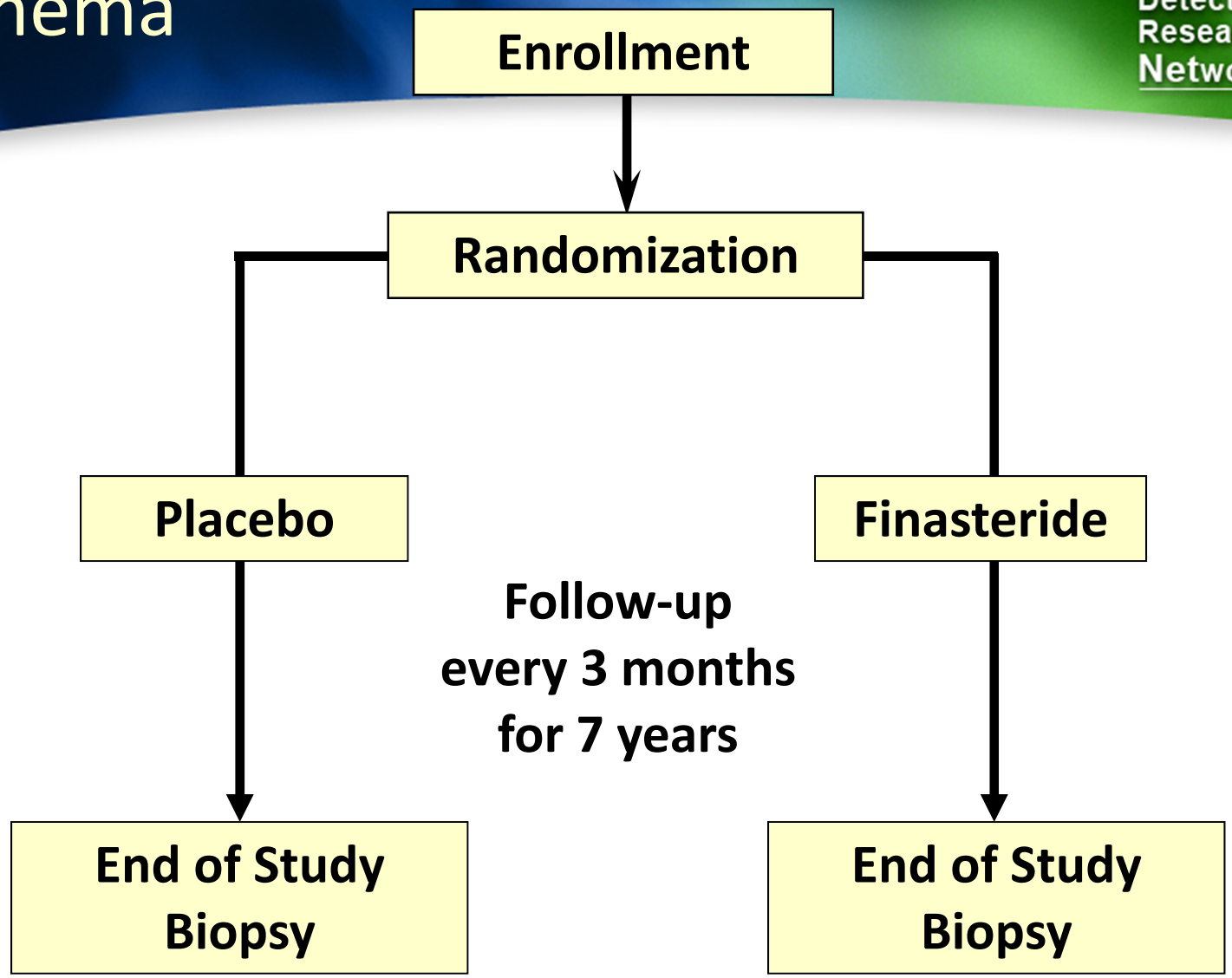
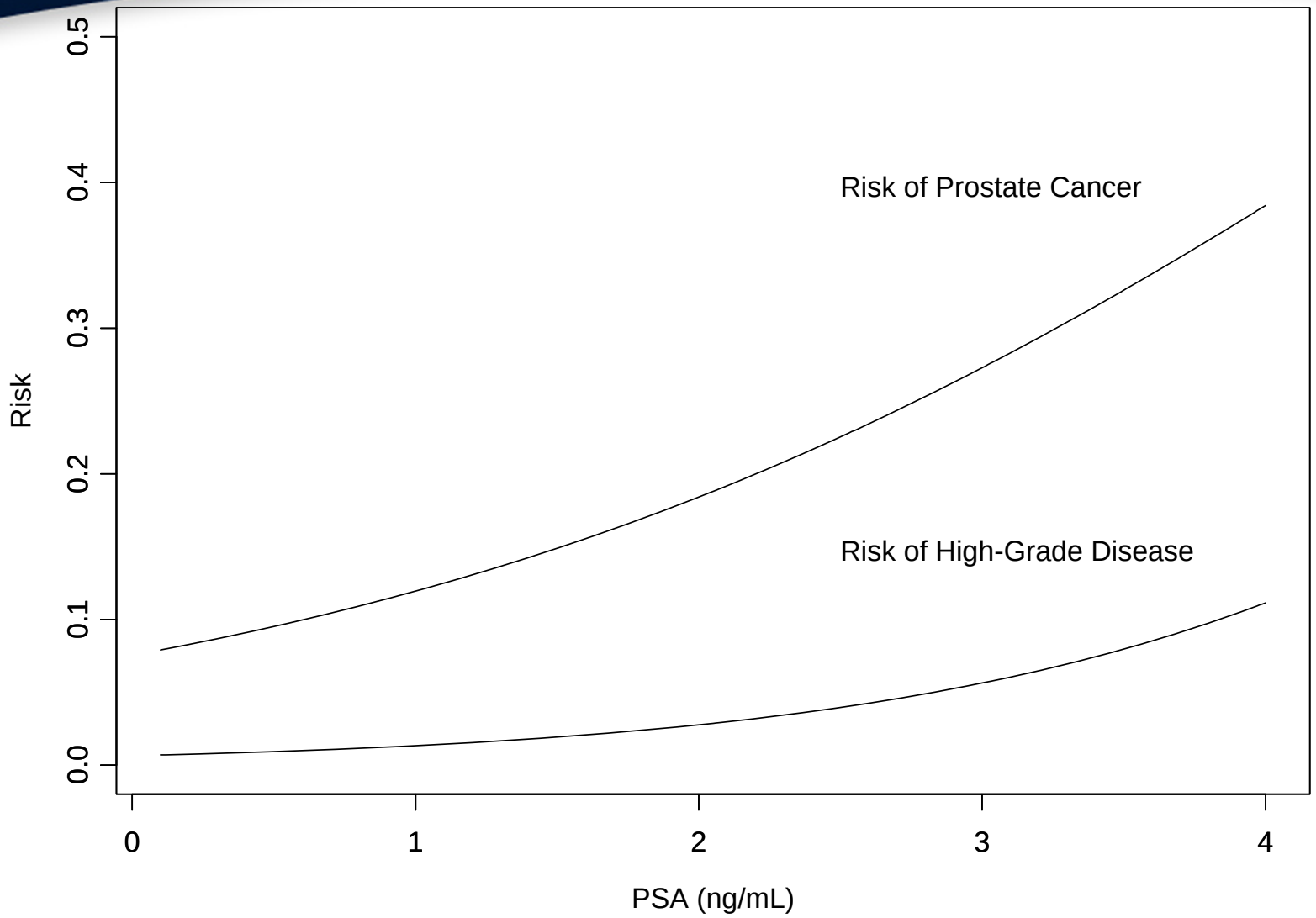
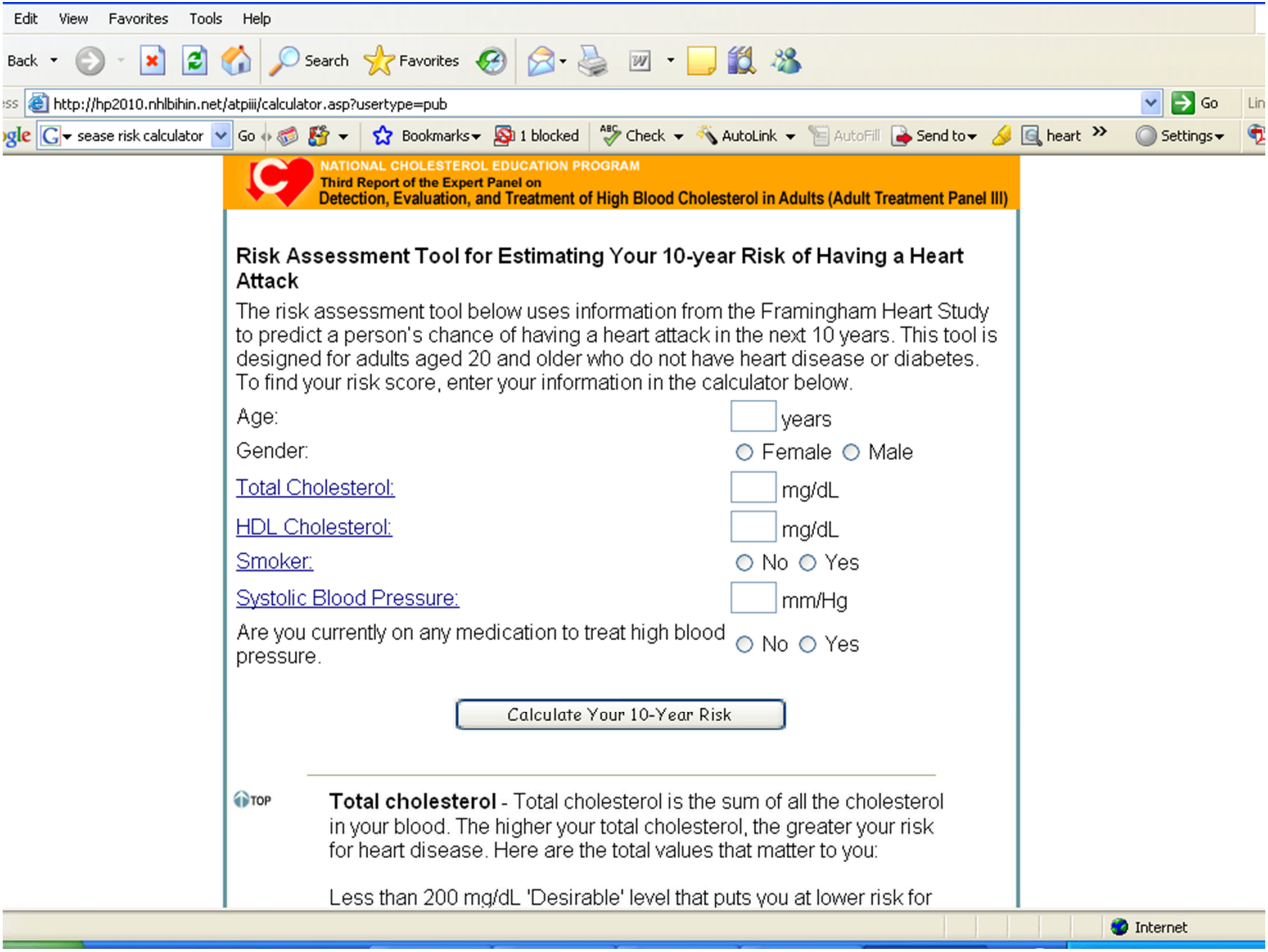




Thompson IM et al. N Engl J Med 2004;350:2239-46


Edit View Favorites Tools Help
Back Search Favorites
http://hp2010.nhlbihin.net/atpiii/calculator.asp?usertype=pub
Go
sease risk calculator Go
Bookmarks 1 blocked Check AutoLink AutoFill Send to heart Settings
NATIONAL CHOLESTEROL EDUCATION PROGRAM
Third Report of the Expert Panel on
Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)
Risk Assessment Tool for Estimating Your 10-year Risk of Having a Heart Attack
The risk assessment tool below uses information from the Framingham Heart Study to predict a person's chance of having a heart attack in the next 10 years. This tool is designed for adults aged 20 and older who do not have heart disease or diabetes. To find your risk score, enter your information in the calculator below.
Age: years
Gender: Female Male
Total Cholesterol: mg/dL
HDL Cholesterol: mg/dL
Smoker: No Yes
Systolic Blood Pressure: mm/Hg
Are you currently on any medication to treat high blood pressure. No Yes
Calculate Your 10-Year Risk
TOP
Total cholesterol - Total cholesterol is the sum of all the cholesterol in your blood. The higher your total cholesterol, the greater your risk for heart disease. Here are the total values that matter to you:
Less than 200 mg/dL 'Desirable' level that puts you at lower risk for
Internet

Development of an individualized risk calculator.

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Assessing Prostate Cancer Risk: Results from the Prostate Cancer Prevention Trial

Ian M. Thompson, Donna Pauler Ankerst, Chen Chi, Phyllis J. Goodman, Catherine M. Tangen, M. Scott Lucia, Ziding Feng, Howard L. Parnes, Charles A. Coltman, Jr.

Journal of the National Cancer Institute, Vol. 98, No. 8, April 19, 2006 ARTICLES 529

5519 men in placebo group of PCPT

All had prostate biopsy and

- PSA and DRE at time of biopsy

- At least 2 prior PSA values

Tested the impact on cancer detection of:

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- ❑ Age*
- ❑ Family history of prostate cancer*
- ❑ PSA*
- ❑ Change in PSA (PSA velocity – 20 different methods of calculation)
- ❑ Prostate examination*
- ❑ Prior negative prostate biopsy*

- ❑ Tested impact on both cancer and aggressive (high-grade cancer) detection

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Predicting Likelihood Of Cancer If A Prostate Biopsy Is Performed

The fields with * sign are required.

Race: *	Choose one
Age: *	
PSA Level: *	ng/ml
Family History of Prostate Cancer: *	Choose one
Digital Rectal Examination Result: *	Choose one
Prior Negative Prostate Biopsy: *	Choose one

Submit

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Predicting Likelihood Of Cancer If A Prostate Biopsy Is Performed

The Result:

Based on the data provided, the person's estimated risk of biopsy-detectable cancer is **26 %** .

The **95%** Confidence Interval for this prediction is **24%** to **28%**. _
More information about confidence interval ...

The person's estimated risk of biopsy-detectable high grade prostate cancer is **4 %** .

The **95%** Confidence Interval for this prediction is **3.4%** to **5.1%**. _
More information about confidence interval ...

The result is based on:

Age:	65
Race:	Caucasian
PSA Level:	2.4 ng/ml
Family History of Prostate Cancer:	No
Digital Rectal Examination Result:	Normal
Prior Negative Prostate Biopsy:	No

RAPID COMMUNICATION

ELSEVIER

EXTERNAL VALIDATION OF THE PROSTATE CANCER PREVENTION TRIAL RISK CALCULATOR IN A SCREENED POPULATION

DIPEN J. PAREKH, DONNA PAULER ANKERST, BETSY A. HIGGINS, JAVIER HERNANDEZ, EDITH CANBY-HAGINO, TIMOTHY BRAND, DEAN A. TROYER, ROBIN J. LEACH, AND IAN M. THOMPSON

Conclusions. The results of our study have shown that the PCPT risk calculator, available from the Internet and incorporating the current best panel of risk factors, is valid in other, more diverse, populations. UROLOGY **68:** 1152–1155, 2006. Published by Elsevier Inc.

How do we make the calculator more accurate?

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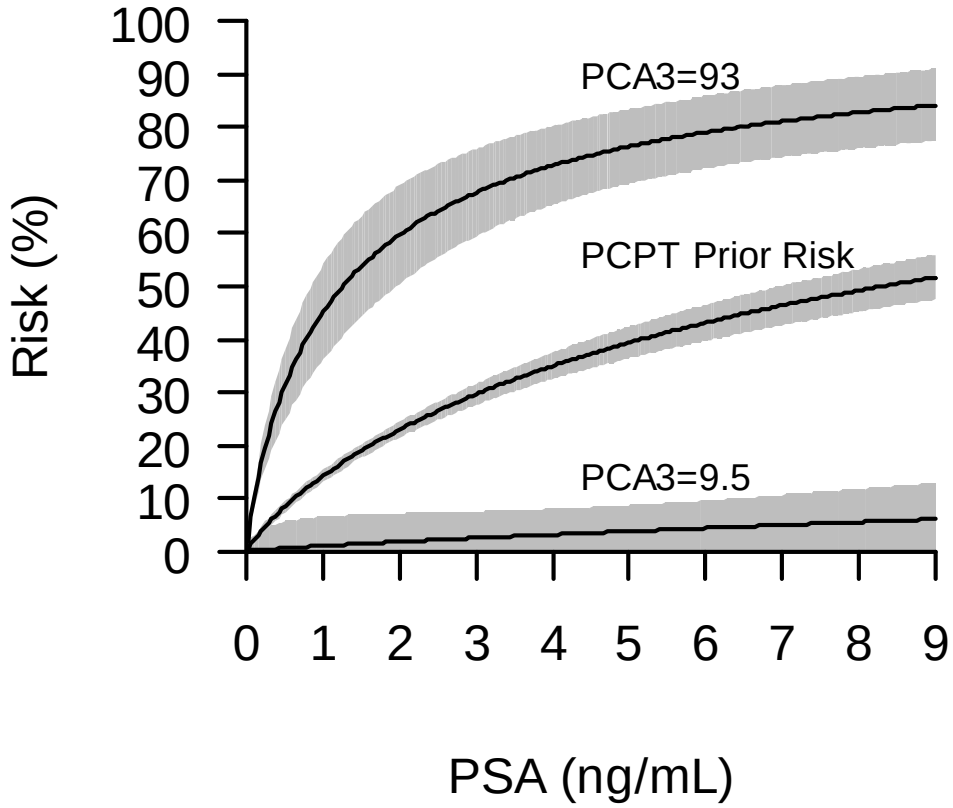
- Add new measures of risk
- Promising biomarker – PCA3. Gene upregulated in prostate cancer cells – detectable in urine.

65-year Caucasian with no prior biopsy, no family history of disease and a normal DRE the PCPT prior risk according to PSA value and updated posterior risks for PCA3 values of 9.5 (25th percentile) and 93 (90th percentile). Gray shades indicate 95% confidence intervals.



VALIDATION STUDIES IN PROGRESS: AFP versus DCP for Hepatocellular Carcinoma

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1. Determine the sensitivity and specificity of des-gamma carboxyprothrombin (DCP) for the diagnosis of early hepatocellular carcinoma (HCC).

VALIDATION STUDIES IN PROGRESS: EDRN-PLCO-SPORE Ovarian Markers

1. Identify a consensus panel comprised of biomarkers that are most informative in detecting early ovarian cancers (CA 72-4, CA 15-3, CEA, CA 19-9, SMRP-1, OV-1.10, HE-4, Osteopontin, HK-11, HK -10, Spondin-2, Prolactin and CA-125).

VALIDATION STUDIES IN PIPELINE

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Samir Hanash: Validation of Protein Markers of Lung Cancer.

Harvey Pass: Serum Protein Biomarkers for Early Detection of Mesothelioma.

David Sidransky : Circulating DNA Methylation Markers of Lung Cancer.

Alan Partin: GSTP1 Methylation Markers in Screen-Detected Prostate Biopsy as reflex markers

Stephen Meltzer : A panel of methylation markers to determine the risk of progression from Barrett's esophagus to esophageal adenocarcinoma

Robert Getzenberg and Robert Schoen: Novel serum based markers for detection of colorectal cancer.

Brian B. Haab : Discrimination of benign from malignant prostatic disease in men with elevated PSA using serum TSP-1.

Eleftherios Diamandis: Human Kallikreins, biomarkers for early detection and progression of prostate cancer.

Robert Getzenberg: EPCA (Early Prostate Cancer Antigen) as a markers for earlier detection of prostate cancer (sensitivity 92%, specificity is 94%).

The bottom line

- **Cancer biomarker discovery and validation requires the talents of multiple disciplines.**
- **Requires a culture of:**
- **Collaboration (the organizational objective and benefits and rewards to the organization are more important that those of the individual; a *radical departure from historical perspective*)**
- **Seeking opportunities wherever they may be (partnering with industry, outside EDRN)**

Focus on the primary objective: Discovery and validation of biomarkers/biomeasures that ultimately reduce morbidity and mortality from cancer.