Early
Detection
Research
Network

EDRN-NIST-FDA

Standards in Molecular Diagnostics

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Sudhir Srivastava
Chief, Cancer Biomarkers Research Group

Quality Assurance: Biomarkers

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- Scientific Validity
- Methodology
- Quality Control

Quality Assurance: Scientific Validity

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- A test used to detect biomarkers must be scientifically valid method in order for it to be associated with the occurrence of a disease;
- Analytical sensitivity, specificity, and positive predictive value must be appropriate to clinical practice;
- Data must be collected under investigative protocols to establish the clinical validity;
- Data must be collected to demonstrate the benefits and risks that accrue from both positive and negative results.

Quality Assurance: Methodology

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- Assay Design
- Assay Optimization
- Issues in Assay Validation
- Laboratory Evaluation

Quality Assurance: Assay design

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- **Rationale for selection of target sequence, primer, probe sequences, etc;**
- **Single versus multiple targets;**
- **Selection of specimen type;**
- **Design of internal controls, controls for contamination and quantitation standards, and well characterized panels of <u>reference reagents</u>.**

Quality Assurance: Assay Optimization

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- Extraction methods, internal controls for sample preparation, specimen storage and processing conditions;
- Length, sequence, efficiency and specificity of primers, probes, enzyme;
- Configuration and performance of controls, calibrators, capture probes, detectors;
- Assay conditions, e.g., length, temperature, storage (stability)

Quality Assurance: Assay Optimization

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- **Precision testing: multiple sites, different days, operators, kit lots;**
- **Proficiency testing: single operator, multiple days, kit lots**

Quality Assurance: Issues in Assay Validation

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- A test must be analytically validated for each biomarker that it is intended to measure;
- A test must be easy, cost-effective and not time consuming;
- For a test to be successful in population screening, serious consideration should be given to develop scale-up, automated methods with high throughput.

Quality Assurance: Laboratory Evaluation

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- Prior to beginning the routine patient testing, a laboratory must conduct a pilot phase (EDRN Phase II) in which it verifies the performance characteristics of its test, and then reviews and evaluates the data collected in the pilot phase;
- Research laboratories providing tests must validate their tests using the same internal and external reviews as other clinical laboratories;
- Multi-center cross-check for pooled specimens, and other inter- and intra-laboratory interfering factors.

Quality Assurance: Laboratory Evaluation*: continued*

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- **Specimen from known samples should be made available through a centralized system in order to facilitate analytical validation (EDRN Reference Sets);**
- **Test results must be written by the laboratories in a form that is understandable to health care providers.**

Verification of Biomarkers Using Reference Set

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Table I. Verification of colon cancer biomarkers using EDRN colon cancer reference set

Biomarker	Sensitivity	Specificity
Galectin-3 ligand	72%	80%
K-ras in urine	77%	65%
K-ras on FOBT card	14%	65%
GOS	77%	49%
GOS 1 FOBT	27%	95%
Proteomics-A	78%	88%
Proteomics-B	70%	66%
Proteomics-SELDI-TOF	19%	98%
Proteomics-MALDI-TOF	63%	48%
p53	40%	70%
CEA	40%	70%
Topoisomerase II	35%	70%
Cathepsin D	50%	70%
Cyclin B	40%	70%
IGF binding protein 2	35%	70%
TRAILR2	10%	96%
CIN248	12%	92%
P108	27%	94%
TRAILR2 and CIN248 and P108	29%	60%
FOBT TRAILR2 and CIN248 and P108	42%	97%

Abbreviations: CEA, carcinoembryonic antigen; FOBT, fecal occult blood test; GOS, galactose oxidase-Schiff test; IGF, insulin-like growth factor; MALDI-TOF, matrix-assisted laser desorption/ionization–time of flight; SELDI-TOF, surface-enhanced laser desorption/ionization–time of flight.

Clinical Chemistry 54:1
53–60 (2008)

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*]

Clinical Chemistry 51:1
102–112 (2005)

Cancer Diagnostics

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]

Disease Markers 19 (2003,2004) 185–195
IOS Press

Serum protein expression profiling for cancer detection: Validation of a SELDI-based approach for prostate cancer

William E. Grizzle[a,*], Bao-Ling Adam[b], William L. Bigbee[c], Thomas P. Conrads[d], Christopher Carroll[e], Ziding Feng[f], Elzbieta Izbicka[g], Moncef Jendoubi[h], Donald Johnsey[i], Jacob Kagan[i], Robin J. Leach[e], Diane B. McCarthy[j], O. John Semmes[b], Shiv Srivastava[k], Sudhir Srivastava[i], Ian M. Thompson[e], Mark D. Thornquist[f], Mukesh Verma[i], Zhen Zhang[l] and Zhiqiang Zou[k]

Quality Assurance: *Long-term*

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- **A national accreditation program for laboratories performing test must be established to promote standardization;**
- **Formal training programs in human and medical genetics for personnel associated with testing laboratories must be instituted.**

Translational Research

Volume 159, Issue 4, April 2012, Pages 343–353

Biomarkers: New Tools of Modern Medicine

New paradigms in translational science research in cancer biomarkers

PAUL D. WAGNER, and SUDHIR SRIVASTAVA

ROCKVILLE, MD

Investigator-Driven Consortium

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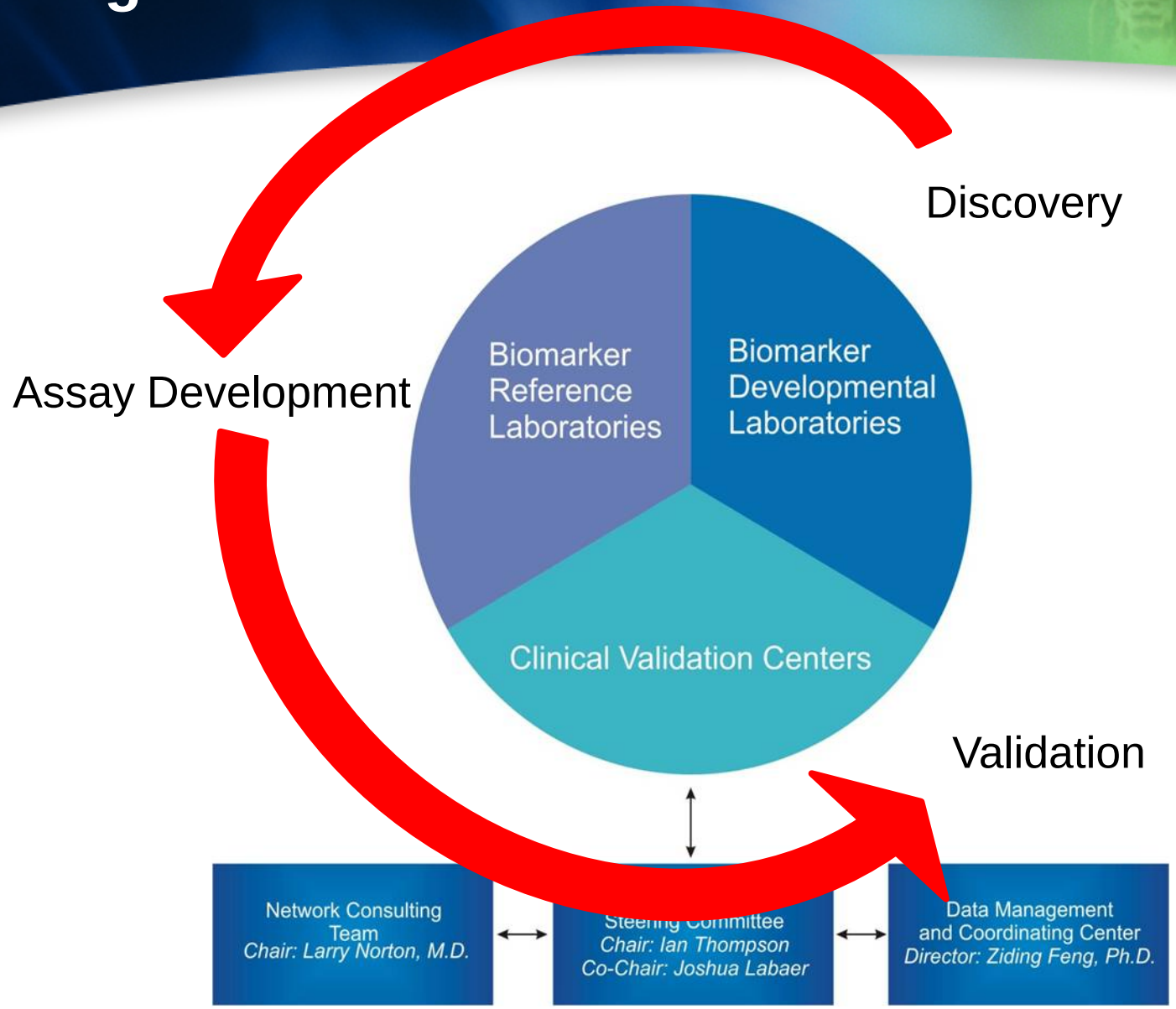

Biomarkers Development Pipeline

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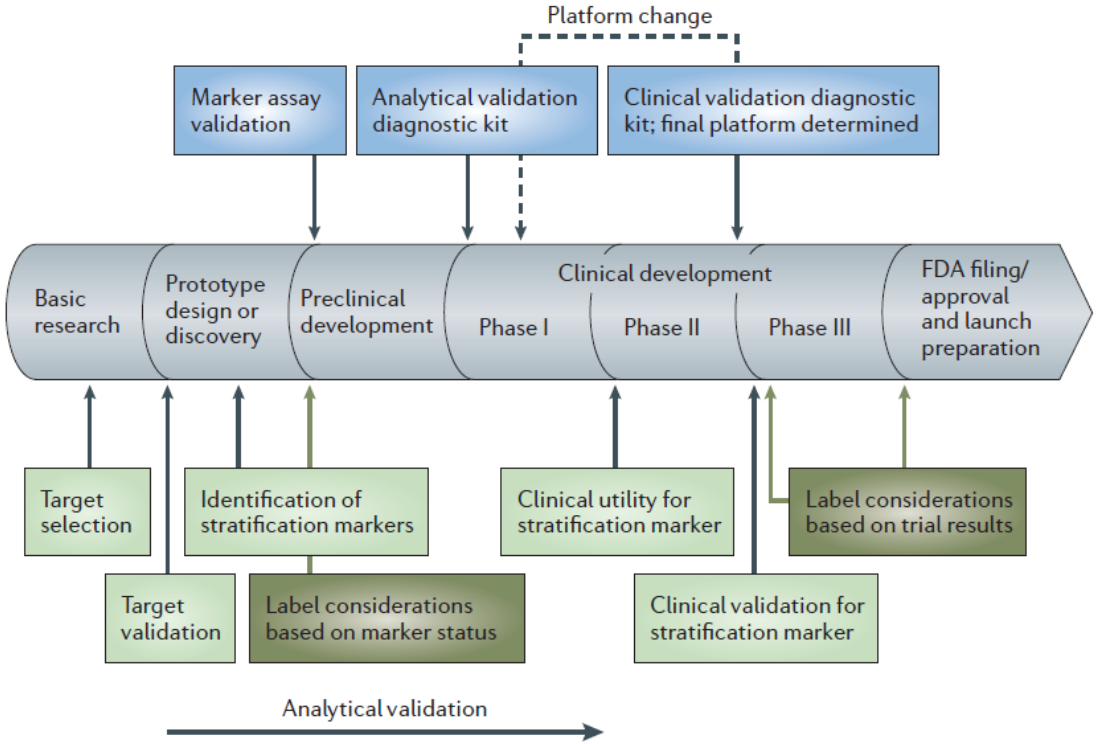

BDL → BRL → CVC

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Nature Reviews | Drug Discovery

Phases of Biomarker Validation

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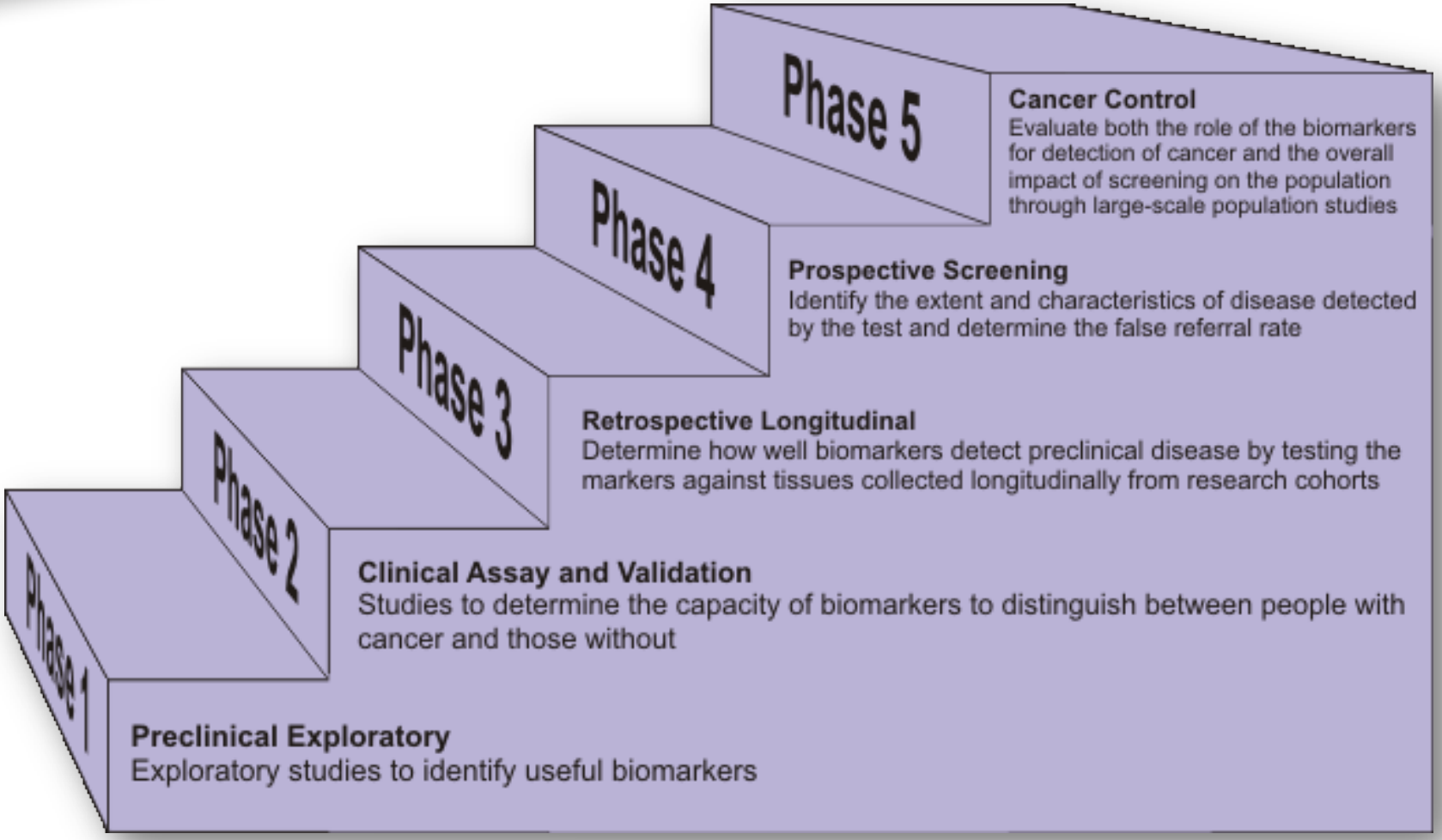

Source: *J. Natl. Cancer Inst.* 93, 1054-1061, 2001

Ovarian Markers as an Example

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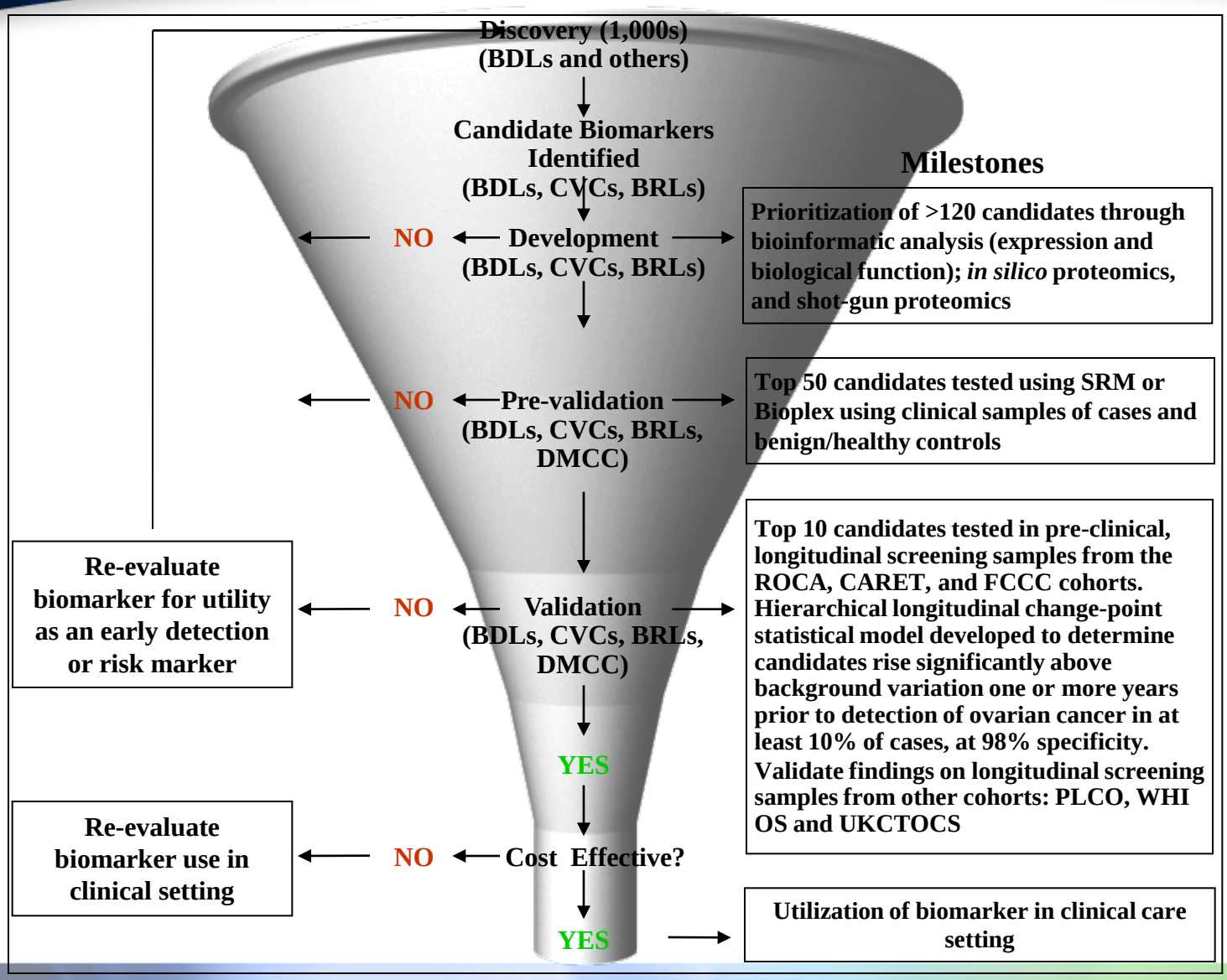

It takes a Village…

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Harvard U./FHCRC/MDACCC/U.Pittsburgh study:

- Phase 2 study: Compare the performance of 67-70 markers previously associated with OC using >800 clinical samples;
- Phase 3 study: Selected 10-15 markers with best performance (sensitivity >30% at 95% specificity) tested on pre-symptomatic PLCO samples: 119 cases collected on average at 6 months prior to D*x*, and up to 7 yrs; 475 GP healthy controls; 238 FP CA125 controls; 238 H*x* controls.

Yale U./LabCorp study:

- Six-marker panel: PRL, IGF-II, OPN, LPN, MIF, CA125;
- Phase 2 study: with 560 Yale U. clinical samples as initial training set to determine classification algorithm;
- Algorithm tested on blinded clinical samples from GOG (~400) and other independent institutions (~200); unblinded samples ultimately used to further adjust algorithm;
- Phase 3 study: Resulting classification algorithm tested on the same PLCO pre-symptomatic samples as above.

What Are the Considerations for Deciding Panel and Platforms?

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Comparison Of Markers By "Platform"

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Marker Name	CV
CA125	
Harvard	9.9%
FHCRC	12.1%
Pittsburgh	60.5%
CA15.3	
Harvard	11.6%
Pittsburgh	25.6%
CA19.9	
Harvard	9.2%
Pittsburgh	29.7%
Spondin-2	
Harvard	14.3%
FHCRC	19.2%

Marker Name	CV
HE4	
Harvard	5.7%
Pittsburgh	23.4%
FHCRC	25.1%
Mesothelin	
FHCRC	16.3%
Pittsburgh	17.6%
Harvard	41.7%
CA72.4	
Pittsburgh	14.3%
Harvard	19.2%

Top Markers – Area Under The Curve All Cases

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Marker Name	All controls		GP controls	
	AUC	Rank	AUC	Rank
CA 125	0.91	1	0.92	1
HE4	0.85	2	0.86	2
IGF2	0.79	3	0.80	3
TT	0.79	4	0.80	4
Apolipoprotein A1	0.76	5	0.77	5
Prolactin	0.75	6	0.77	6
CA 72.4	0.75	7	0.75	7
Soluble vascular cell adhesion molecule 1	0.74	8	0.75	8
Cytokeratin 19	0.74	9	0.74	13
Spondin-2	0.73	10	0.74	10
Chitinase (YKL 40)	0.72	12	0.74	9

Top Markers – Sensitivity At 95% Specificity All Cases

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Marker Name	All controls		GP controls	
	Sens.	Rank	Sens.	Rank
CA 125	0.69	1	0.73	1
HE4	0.57	2	0.57	2
TT	0.47	3	0.47	3
CA 15.3	0.45	4	0.46	4
CA 72.4	0.43	5	0.40	5
KLK6 (HK6)	0.37	6	0.36	9
OV-110 (B7-H4)	0.35	7	0.35	11
Cytokeratin 19	0.35	8	0.37	7
Apolipoprotein A1	0.33	9	0.34	12
IGF2	0.33	10	0.36	8
IGFBP2	0.31	12	0.38	6
Mesolthelin	0.33	11	0.35	10

Top Markers – CV

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Marker Name	CV
HE4	5.7%
Decoy receptor 3 (DD-C248)	6.0%
IGF2	6.8%
CA 19.9	9.2%
CA 125	9.9%
Beta 2 Microglobulin	10.1%
CEA	11.3%
CA 15.3	11.6%
Connective tissue activating protein 3	12.2%
EGFR	12.8%

Phase 3 Results:
by time to Dx (SN at 95% SP among GP controls)

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Marker Name/Panel	Cases Diagnosed ≤ 6 Mos After Draw (n=25)		Cases Diagnosed ≥ 6 Mos After Draw (n=35)	
	AUC	SENS	AUC	SENS
CA125 (PLCO)	0.94	0.79	0.62	0.14
FHCRC				
CA125	0.94	0.75	0.63	0.28
HE4	0.83	0.71	0.67	0.11
MMP7	0.63	0.25	0.58	0.22
Combined HE4&CA125	0.93	0.75	0.67	0.28
Harvard U.				
CA125	0.92	0.79	0.62	0.15
HE4	0.88	0.68	0.69	0.23
CA72.4	0.81	0.48	0.63	0.21
5-marker panel*	0.93	0.78	0.72	0.26
Yale U.				
CA125	0.91	0.80	0.59	0.17
MIF	0.67	0.20	0.58	0.014
OPN	0.58	0.16	0.45	0
6-marker panel**	0.77	0.52	0.49	0.09
* - CA125, HE4, CA72.4, B7-H4, CA15.3; ** - CA125, MIF, OPN, IGF-II, LPN, PRL				

Outcome of this meeting

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- **Better understanding and appreciation of standards issues in diagnostics**
- **Manuscript**
- **Research ideas**