National Cancer Institute

Division of Cancer Prevention

The Early Detection Research Network

Conducting Research to Identify, Test, and Validate Cancer Biomarkers

FIFTH REPORT • DECEMBER 2011

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

Acknowledgments

This report was composed by the Cancer Biomarkers Research Group of the National Cancer Institute Division of Cancer Prevention Program Staff:

Sudhir Srivastava, Ph.D., M.P.H., M.S., Chief of the NCI Cancer Biomarkers Research Group, EDRN Program Coordinator, Director of Administrative Activities

Annalisa Gnoleba, M.S.A., Program Specialist, Cancer Biomarkers Research Group

Jacob Kagan, Ph.D., M.Sc., Program Director, Prostate and Other Urological Cancers

Karl Krueger, Ph.D., Program Director, Lung and Upper Aerodigestive Cancers

Kathy Marshall, M.A., Project Manager

Richard Mazurchuk, Ph.D., Program Director, Prostate and Other Urological Cancers

Christos Patriotis, Ph.D., Program Director, Breast and Gynecological Cancers

Jo Ann Rinaudo, Ph.D., Program Director, Colorectal and Other Gastrointestinal Cancers

Lynn Sorbara, Ph.D., Program Director, Lung and Upper Aerodigestive Cancers

Paul Wagner, Ph.D., Program Director, Colorectal and Other Gastrointestinal Cancers

Wendy Wang, Ph.D., M.Sc., Program Director, Breast and Gynecological Cancers

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Foreword

The essential purpose of early detection of cancer is to reduce mortality and morbidity while minimizing the risks of screening and associated treatment. As molecularly-informed research moves us closer to progressively more specific interventions with less toxicity, the Early Detection Research Network (EDRN) is focused on finding new and improved methods to noninvasively and accurately detect potentially life-threatening cancers at their earliest stages.

As genomic knowledge accumulates, general guidelines for cancer screening may be supplanted by more targeted testing methods using validated cancer biomarkers to screen individuals at differing levels of risk. However, the deluge of molecular data increasingly challenges the ability of researchers and medical practitioners to find reliable ways to stratify the many kinds of cancer and degrees of cancer risk.

Accordingly, the EDRN laboratories are bridging the critical steps in the process of moving a biomarker from discovery to clinical use by validating both its analytical and clinical performance. Analytical performance measures the reproducibility, precision, and accuracy of the assays. Clinical validation of a biomarker or panel of biomarkers determines its capacity to accurately distinguish patients with cancer from those without, or to detect preclinical cancer. The full range of expertise for the development of early detection biomarkers requires a multidisciplinary effort, and the EDRN was created to integrate the efforts of the necessary spectrum of disciplines.

The EDRN established an innovative five-phase approach for cancer biomarker development and delineated a coherent and comprehensive set of guidelines for study design in the discovery, evaluation, and initial clinical development of biomarkers. In order to be of clinical value biomarkers must be reliable and reproducible in testing; highly sensitive and specific; quantitative; readily obtained by non-invasive methods; part of the causal pathway for disease; and have high predictive values for clinical disease. Ideally, identified biomarkers may also serve as targets for chemopreventive interventions.

An important goal is to identify molecular fingerprints of tumors and preneoplastic tissue abnormalities that can be used to predict clinical behavior and distinguish harmful progressive tumors detected by cancer screening tests from indolent tumors that do not require therapy. This would maximize the benefits of cancer screening while limiting the harms. All screening tests can introduce risks, and biomarkers will undoubtedly as well. But with the rigorous framework of the EDRN laboratories and clinical centers, the known harms of screening, such as overdiagnosis and false positive and false negative results, can be minimized. The multidisciplinary structure and rigorous process of the EDRN provide an innovative, integrated approach to biomarker discovery and validation of biomarkers for early cancer detection. The fruits of this process over the relatively short history of the EDRN come through in this progress report.

Barnett Kramer, M.D., M.P.H.
Director, Division of Cancer Prevention
National Cancer Institute

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Introduction

Early detection of cancer can dramatically improve outcomes. Finding breast and colon cancers when they remain localized results in a significant decrease in mortality. The Early Detection Research Network (EDRN) is helping make this an achievable goal for more cancers.

In 2000, NCI's Division of Cancer Prevention created EDRN, an investigator-driven network designed to conduct translational research that identified markers both for the early detection of cancer and for cancer risk. EDRN focuses on the goal of creating validated biomarkers ready for large-scale clinical testing and eventual application. Without a doubt, real progress has been made—and is being made, by this consortium of more than 300 investigators and 40 private sector and academic institutions. These scientists represent diverse disciplines, including genomics, proteomics, metabolomics, bioinformatics and public health.

EDRN is at the forefront of technology-driven research on the discovery and use of biomarkers for the early detection of cancer. By identifying and validating biomarkers, such as novel proteins or changes in gene expression, it is possible to measure an individual's disease risk, the development and progression of disease, and the response to therapy. Ultimately, EDRN research will aid in prevention and in early therapeutic intervention, based on early detection of disease.

Today, EDRN is a nationwide, interdisciplinary group of established partnerships among scores of institutions and hundreds of individuals working to advance the science for public health benefit.

Research collaborations take place within an environment of teamwork across different disciplines and laboratories focused on achieving common goals, such as:

- Developing and testing promising biomarkers and technologies to obtain preliminary information to guide further testing;
- Evaluating promising, analytically proven biomarkers and technologies, such as measures of accuracy, sensitivity, specificity and, when possible, potential predictors of outcomes or surrogate endpoints for clinical trials;
- Analyzing biomarkers and their expression patterns to serve as background for large, definitive validation studies;
- Collaborating with academic and industrial leaders to develop high-throughput, sensitive assay methods;
- Conducting early phases of clinical and epidemiological biomarker studies; and
- Encouraging collaboration and dissemination of information to ensure progress and avoid fragmentation of effort.

EDRN is a leader in defining and using criteria for the validation of biomarkers—an essential condition for scientific progress. While myriad proteins and genes have been linked to a variety of cancers, acceptable biomarkers must be: reliable and reproducible in testing; highly sensitive and specific; quantitative; readily obtained by non-invasive methods; part of the causal pathway for disease; capable of being modulated by a chemopreventive agent; and a high predictive value for clinical disease.

Executive Summary

The National Cancer Institute's Early Detection Research Network (EDRN) is the leading national initiative for finding cancer early using molecularly informed research. Since it was created in 2000, the EDRN has been setting standards for how to discover, validate appropriate samples, and translate biomarkers into clinical use for risk and early cancer detection, diagnosis and prognosis.

Improved methods for early detection are vital to reducing morbidity and mortality due to cancer. A primary cause of poor survivability is that many cancers are detected late, after they have metastasized to distant sites. Once a cancer has spread, it is more difficult to eradicate; therapies for late-stage disease are still not successful for nearly all cancer types. The mortality rates from cancers where screening tools are available are lower than from cancers for which no viable screening tools exist.

Even for cancers where screening tools currently exist, there is room for improvement, either in the accuracy of the tests or in making them more acceptable to patients. For instance, despite widely available screening methods that can detect early stage colon cancer, only about 40% of newly diagnosed colon cancers are localized. Consequently, EDRN investigators are committed to finding new and improved methods to noninvasively and accurately detect cancers at their earliest stages.

Thousands of research articles published in the last decade have fueled expectations that effective biomarker-based diagnostics would rapidly take form. However, much of the literature includes studies, which were conducted without appropriate study designs and population statistics. In reality, only a handful of biomarkers were approved by the Food and Drug Administration (FDA) during that time, several of which had completed the EDRN validation process. It is therefore important to have an infrastructure such as EDRN, which systematically assesses reported biomarkers and selects the truly promising ones for transition through rigorous validation for clearly defined clinical utilities. Today, at least 300 candidate biomarkers are positioned to move forward through the EDRN validation process. The state of biomarker research has changed dramatically since the inception of EDRN (see Box).

State of Biomarker Research Prior to and After EDRN

Prior to EDRN

- Fragmented studies, with discoveries using convenience samples
- Results of studies not generalizable
- Lack of Standard Operating Procedures for sample collection and study designs
- Studies compromised by chance, bias and confounders
- Lack of evidence for the claimed clinical use

After EDRN

- Clinically annotated samples for discovery
- Roadmap for biomarker discovery and validation using EDRN five-phase guidelines and PRoBE design
- Well-designed multi-center, multi-disciplinary validation studies to minimize chance, bias, confounders
- Well-designed Standard Reference Sample Sets to quickly evaluate biomarkers for intended clinical uses
- Adoption of EDRN-developed guidelines and concept of validation throughout the biomarker research community
- Adoption of EDRN-developed study-design evaluation criteria by the biomarker community and the NIH study sections

Beyond the issue of safety, the field had been comparatively unregulated until recently, and numerous potential markers emerge in this environment. The EDRN has adopted a series of validation benchmarks to effectively compare one candidate/technology against another. This helps the field to avoid numerous competing claims of being "the biomarker of choice," the notion of which arises simply from marketplace competition or differences between laboratories. The EDRN approach facilitates well-designed clinical studies that have an increasing hierarchy of complexity.

EDRN developed the first roadmap for guiding the scientific process of discovery, development and validation of cancer biomarkers. These guidelines are needed because molecules specific for early stage cancer that are "easy" to detect are very hard to find and verify. The EDRN adopted a five-phase schema in 2001[1] and its comprehensive infrastructure gave researchers with promising biomarkers a place to accurately assess them from the point of discovery to clinical validation. Strategies and methods for finding biomarkers with next generation technologies that query at the systems level with more sensitive detection capabilities moved forward.

Work by EDRN investigators to validate putative biomarkers has shown that many of the assays used in previous years were either not reproducible, or not sensitive and specific enough to warrant further consideration. EDRN has developed and standardized a number of assays commonly used in research and clinical application (Table 1). Biomarkers with both high sensitivity and specificity are desired for accurate diagnosis of disease. The sensitivity of a medical test refers to the fraction of individuals with a disease who test positive for the disease. The specificity of a medical test is the fraction of individuals without the disease who test negative for the disease. No medical tests are 100% sensitive or specific, and some people with or without the disease will not be identified correctly.

Some of the EDRN's major accomplishments to date include:

- More than 1,000 biomarkers identified. For example, for Triple-Negative Breast Cancer alone, more than 100 new biomarkers have been identified, including circulating proteins, autoantibodies and miRNAs.
- More than 300 prioritized biomarkers ready for verification and validation studies. For example, 125 to 150 candidate biomarkers for early ovarian cancer detection, not previously tested, are being subjected to verification and validation (see Part II, Chapter 1).
- Eight validation trials completed: MSA for bladder cancer; DCP and AFP-alpha 3 for liver cancer; ProPSA, PCA3, and MS proteomic profile assay for prostate cancer; methylation marker panel for esophageal cancer; Annexin 1 and 2 for lung cancer; and circulating protein markers for ovarian cancer (see Part III, Chapter 2). A number of EDRN-developed biomarkers have been adopted by the diagnostic community for clinical use (Table 2).
- Eight large validation studies underway (see Part III, Chapter 2).

[1] Pepe M S, Etzioni R, Feng Z, et al. Phases of biomarker development for early detection of cancer. J Natl Cancer Inst. 2001;93:1054-61.

Table 1. Biomarker Assays Developed and Standardized

Assays	Application
Validation of bleomycin-induced chromosomal breakage in lymphocytes	Biomarker of Lung Cancer susceptibility
Validation of 3.4KB mitochondrial DNA deletion	Biomarker for Prostate Cancer Risk
Development of high-density breast and prostate tissue microarrays	Testing of ISH and other molecular probes
Validation of SOPs for Microsatellite Instability (MSA) and DNA methylation assays	Biomarkers for Bladder Cancer
Validation of saliva-based mRNA assay	Biomarkers of Oral Cancer
Validation of proteomic prostate-specific biomarkers, including percent proPSA and other PSA isoforms	Biomarkers for Improving PSA Screening of Prostate Cancer
Urine PCA3 assay	Detection of Prostate Cancer
Urine/TMA assay for T2S:ERG fusion	Detection of Prostate Cancer
FISH assay for T2S:ERG fusion	Detection of Prostate Cancer
Aptamer-based assay	Detection of Lung Cancer
Proteomic panel for Lung Cancer	Detection of Lung Cancer
OVA1™ test for Ovarian Cancer	Differential Diagnosis of Benign Pelvic Mass from Ovarian Cancer
ROMA algorithm for Ovarian Cancer	Differential Diagnosis of Benign Pelvic Mass from Ovarian Cancer
Vimentin methylation in stool	Detection of Colon Cancer
SOPs for blood, sera, plasma, urine, stool	Standard Reference Sample Sets
8-oxyguanine DNA glycosylase (8OGG); Alkyl-adenine DNA Gycosylase; APE1 Endonuclease	Measuring DNA repair capacity for Lung Cancer risk

- Two validation studies planned (see Part III, Chapter 2).
- Ten valuable clinical specimen reference sets available for rapid and inexpensive testing of biomarkers (see Part IV, Chapter 3).
- More than 28 patents and licenses developed, indicative of the robustness of studies in which diagnostic companies are willing to invest further.
- More than 15 collaborations with biotechnology and diagnostic companies.
- More than 1,450 collaborative papers generated.
- Seven major workshops conducted, attracting on average 400 participants each (see Significant Activities table in Appendix).

Future short-term goals of the research network are to:

- Augment the ability of commonly used screening tests to detect major epithelial cancers (colon, breast, cervical, lung and prostate), and facilitate co-development of diagnostic tests for prevention or therapeutic interventions (theranostics).
- Evaluate biomarker discovery, development and validation, and collaborate with the NCI Cancer Intervention and Surveillance Modeling Network (CISNET) on integrating cost-benefit models in discovery and development.
- Create well-defined consensus standards and guidelines for biomarker development, validation and qualification using the Translational Research Working Group-developed Device Pathway schema to reduce uncertainty in discovery and development.

Testing Biomarkers to Make Sure They Work: EDRN as a "Brake" and an "Accelerator"

The OvaSure screening test for ovarian cancer which made its way to market use in June 2008 had to be withdrawn from the market for the lack of scientific rigor during testing.

After a week on the market, the Society of Gynecologic Oncology called for additional research on the effectiveness of the test, which was manufactured by LabCorp. Scientists at the Canary Foundation said that the reported LabCorp findings were overly optimistic, in part because they failed to take into account effects of a key protein in the panel, prolactin. An EDRN investigator also could not replicate the findings in a well-designed study using an appropriate cohort of samples.

The primary mistake made by the OvaSure producers was to calculate the PPV using the prevalence of cancer only from the study population, when it should have been determined based on the accepted prevalence of ovarian cancer for all post-menopausal women. By August 2008, the Food and Drug Administration declared that the test could harm public health because of the lack of adequate validation. OvaSure was removed from the market in October 2008.

Buchen L. Cancer: missing the mark. Nature. 2011 Mar 24;471(7339):428-32.

Visintin I, Feng Z, Longton G, et al. Diagnostic markers for early detection of ovarian cancer. Clin Cancer Res. 2008 Feb 15;14(4): 1065-72.

McIntosh M, Anderson G, Drescher C, et al. Ovarian cancer early detection claims are biased. Clin Cancer Res. 2008 Nov 15;14(22):7574; author reply 7577-9. Epub 2008 Oct 23.

Greene MH, Feng Z, Gail MH. The importance of test positive predictive value in ovarian cancer screening. Clin Cancer Res. 2008 Nov 15;14(22):7574; author reply 7577-9. Epub 2008 Oct 23.

Table 2. Adaption of EDRN-Supported Assays

Detection/ Biomarker Assay	Discovery	Refine/ Adapt for Clinical Use	Clinical Validation	Clinical Translation
Blood proPSA		✓	✓	FDA IVD pending review
Urine PCA3		✓	✓	FDA IVD pending review
Urine/TMA assay for T2S:ERG fusion for Prostate Cancer	✓	✓	✓	CLIA in process
FISH for T2S:ERG fusion for Prostate Cancer	✓	✓	✓	In CLIA Lab
Aptamer-based markers for Lung Cancer		✓	✓	In CLIA Lab
Proteomic panel for Lung Cancer		✓	✓	In CLIA Lab
OVA1™ for Ovarian Cancer		✓	✓	FDA Approved
SOPs for Blood (Serum, Plasma), Urine, Stool,		✓		Frequently used by biomarker research community
Vimentin methylation marker for Colon Cancer		✓	✓	In CLIA Lab
ROMA algorithm for CA125 and HE4 Tests for Pelvic Mass Malignancies		✓	✓	FDA Approved
Blood/DCP and AFP-L3 for Hepatocellular Carcinoma		✓	✓	FDA Approved
Blood GP73 for Hepatocellular Carcinoma	✓	✓	✓	Together with AFP-L3 used in China for monitoring/ risk assessment of cirrhotic patients for HCC

In the longer term, the EDRN aims to:

- Integrate the genetic, cell signaling and biochemical pathways with biomarker discovery efforts to have a broader applicability across different tumor types.
- Determine the potential of novel network- and pathway-based markers to detect and diagnose cancer, using a systems approach to diagnosis, prevention and therapeutic strategies.
- Develop new serum- and tissue-related methods for early detection and diagnosis to identify clinically significant diseases and predictions of clinical outcomes, with or without conventional tissue examination, by utilizing currently available biomarker tests.
- Expand collaborative efforts and shared resources to improve the capacity to conduct biomarker development and validation trials.
- Examine genome-wide chromosomal instability (i.e., chromosome copy gain or loss and loss of heterozygosity) and genome-wide association studies to predict progression from benign to malignant cancers via characterization of these regions of the genome.
- Integrate The Cancer Genome Atlas (TCGA) findings into biomarker discovery, verification and validation.
- Address the issue of over-diagnosis.

Improving Tumor Modeling with Biomarkers: Flexible Sigmoidoscopy

A model used to estimate the cost-effectiveness of flexible sigmoidoscopy screening for colorectal cancer in the U.S. will likely become more common as new biomarkers are introduced. Such mathematical models are built to simulate tumor growth over time, either at the cellular level or as representative of tumor size.

The models are calibrated to observed data, typically from longitudinal population studies of tumor growth and clinical disease. Real or hypothetical screening or diagnostic tests are then introduced, with sensitivity and specificity based on their ability to detect either markers that increase in the blood in proportion to tumor burden, resolution for detecting tumors of a particular size, or ability to detect metabolic changes.

Based on known or hypothesized distributions representing ranges in the rates of tumor onset and growth, the models are then run for a population either with or without disease to generate presumed life histories both in the absence and presence of testing. The outcomes (such as stage at diagnosis, tumor response rate, cancer-specific or overall survival, and medical care costs) are then compared under the test and no-test scenarios.

Knudsen A B, Lansdorp-Vogelaar I, Rutter C M, et al. Cost-effectiveness of computed tomographic colonography screening for colorectal cancer in the Medicare population. J Natl Cancer Inst. 2010;102,1238-52

In summary, EDRN has made significant progress in biomarker discovery, development and validation, and established a number of study standards that have been adopted by the greater biomarker research community. Standard Reference Sets have become a valuable tool for cheaply and effectively triaging promising biomarkers from those that are not clinically useful. EDRN has established a number of productive collaborations with extramural scientists, overseas investigators, and research foundations. In collaboration with stakeholders, EDRN is constantly evaluating the program focus to include research on companion imaging with molecular markers, as well as to address challenges related to over-diagnosis and the detection of interval cancers. Finally, EDRN is poised to bring many biomarkers to clinical fruition by working with industrial partners and the Food and Drug Administration.

NCI's Early Detection Research Network Wins NASA Award

The National Aeronautics and Space Administration (NASA) has awarded NCI's Early Detection Research Network Informatics Team the NASA Honors Award for Group Achievement for the innovative and pioneering use of NASA data system technologies to promote data and specimen sharing among cancer biomarker researchers.

The award was presented to EDRN teams from NASA's Jet Propulsion Laboratory, NCI, Dartmouth, and the EDRN Data Management and Coordinating Center at Fred Hutchinson Cancer Research Center.

Under the leadership of Dr. Sudhir Srivastava, chief of NCI's Cancer Biomarkers Research Group in the Division of Cancer Prevention, the EDRN Informatics Team pioneered and deployed a highly distributed national data system to implement cancer biomarker data analysis and research. This project is a flagship example of successful technology infusion and transfer between agencies.

The informatics platform has enabled EDRN science teams to share and integrate diverse datasets and software platforms for complex biomarker research, including discovery and validation of cancer biomarkers.

Incorporating a Systems Approach to Biomarker Research: Discovery of Gene Fusions in Solid Tumors

As data rapidly accumulate from genomic and proteomic analyses, algorithms to combine information from multiple biomarkers are in development.

The systems approach was successfully used to discover biomarkers from high-volume expression data along with a metabolite whose expression was also regulated by gene fusion products. In this case, it pertained to chromosomal translocations, which have been long known to occur in various types of hematopoietic and soft tissue tumors, but only a few have been documented in solid cancers.

A chromosomal rearrangement implicated as a potential cause of some prostate cancers was discovered through analyzing a database called Oncomine, a collection of data from cancer studies across the globe that screened the activity of thousands of genes.

By surveying Oncomine for over-expressed genes in prostate tumors, investigators identified two candidate genes that encode transcription factors, ERG and ETV1. The Oncomine database integrates 132 gene expression data sets representing 10,486 microarray experiments.

While the specific roles of these gene fusions are unknown, they may be important in prostate cancer development or progression. This belief is based on data obtained from cultured primary prostate cancer cell lines that showed this gene fusion in 23 of the 29 cell lines examined in the study.

These findings led EDRN investigators to study the downstream effect of these gene fusions on phenotypic expression and identified a metabolite, sarcosine, likely involved in the etiology, progression, and aggressive behavior of prostate cancer.

Additionally, components of the sarcosine synthesis pathway were found to have potential, not only as diagnostic/prognostic biomarkers of prostate cancer, but also as targets for developing new therapeutic modalities.

Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science. 2005;310,644-8.

Hampton T. Tool helps cancer scientists mine genes. JAMA. 2004;292,2073.

Part One

Evidence-Based Research on Biomarkers

EDRN is the first comprehensive network to develop and validate early detection biomarkers for cancer. It is one of the few NCI programs dedicated to biomarker research. It represents different scientific disciplines including genomics, proteomics, informatics and public health, and benefits from agreements with experts in molecular biology, technology development, bioinformatics, computational biology, clinical studies, epidemiology and other areas. EDRN is a driving force behind governmental, institutional and public-private collaborations that work together to encourage the rapid development of biomarkers and early detection science, and to make those developments applicable in medical practice.

Researchers in EDRN take biomarkers discovered in the laboratory and conduct studies to determine if they are effective for specific clinical applications and needs. Serendipity played a larger role in past biomarker discovery, but in the wake of evolving molecular tools and technologies, mathematical modeling may leave less to chance in deciphering biochemical mechanisms, such as control of the cell cycle[2], and correlating glycomic profiles with the expression[3] of all genes participating in glycan synthesis[4].

EDRN is incorporating the systems approach into their traditional approach to discovery and validation of multiple, highly predictive, early-stage disease biomarkers. The systems approach evaluates molecular processes as a group of networks and pathways connected to one another and working together to develop models of interdependent networks and pathways. Investigators can examine thousands of different biological molecules in a single experiment or in a series of high-throughput studies that rapidly compare samples from many different patients. The approach[5] generally comprises five features:

1. Measuring and quantifying biological information on a global scale;
2. Integrating distinct modalities of biological information such as DNA, RNA and proteins;
3. Capturing the dynamics of biological systems and networks;
4. Using these sources of information to model the system; and then
5. Iteratively testing and refining the model.

[2] Novak B, Tyson JJ. A model for restriction point control of the mammalian cell cycle. J Theor Biol. 2004 Oct 21;230(4):563-79.

[3] Marathe DD, Chandrasekaran E V, Lau JT, et al. Systems-level studies of glycosyltransferase gene expression and enzyme activity that are associated with the selectin binding function of human leukocytes. FASEB J. 2008 Dec;22(12):4154-67. Epub 2008 Aug 20.

[4] Liu G, Marathe DD, Matta KL, et al. Systems-level modeling of cellular glycosylation reaction networks: O-linked glycan formation on natural selectin ligands. Bioinformatics. 2008 Dec 1;24(23):2740-7. Epub 2008 Oct 7.

[5] Wang K, Lee I, Carlson G, et al. Systems biology and the discovery of diagnostic biomarkers. Dis Markers. 2010;28, 199-207.

EDRN has made significant progress in building resources to support the discovery of new candidate biomarkers, biomarker verification, and the undertaking of multi-center validation studies. Before the research network was established, each part of the biomarker research process was disconnected and progress was slow. Within EDRN, new discoveries made by research scientists and clinicians are combined through a strong administrative support system and knowledge databases for information sharing to shift effective biomarkers more quickly into clinical practice.

Increasingly, it is believed that a single diagnostic biomarker will be unlikely to distinguish disease from non-disease states because of the high degree of heterogeneity among cancers across large populations. Future diagnostic tests will more likely analyze groupings of multiple molecules that together distinguish "abnormalities" – i.e., developing a panel of several biomarkers for concurrent use. The multiplexing approach can eliminate time-consuming manual processing of samples and allow real-time data acquisition and more efficient sample comparison. Flexible technology platforms are being built by diagnostic companies to simultaneously analyze a panel of protein or nucleic acid biomarkers, or more than one kind of biomarker. To support multiplexing of biomarkers there is a need for standardized reagents, quality sample reference sets, robust study designs, computational methodologies and data sharing portals to be used across multiple research centers for the purposes of accessibility, precision, and reproducibility.

Projects are underway for major cancer sites, including: breast, colon, lung, prostate, liver, ovary, and pancreas. Future projects may encompass others, including blood and lymphoid cancers. More information can be found at http://edrn.nci.nih.gov/.

Creating a Translatable Product

The EDRN implemented a nationally recognized research schema designed to bring forward into clinical practice verifiably effective cancer biomarkers. The network[6] established a roadmap and standards for research that minimizes chance, bias and over-fitting, based on a protocol-driven, five-phase approach[7].

Phase 1, Preclinical Exploratory – Exploratory studies to identify potentially useful biomarkers

Phase 2, Clinical Assay and Validation – Studies to determine the capacity of biomarkers to distinguish between people with cancer and those without

Phase 3, Retrospective Longitudinal – Determine how well biomarkers detect preclinical disease by testing the markers against specimens collected longitudinally from research cohorts

Phase 4, Prospective Screening – Identify the extent and characteristics of disease detected by the test and determine the false referral rate

Phase 5, Cancer Control – Evaluate both the role of the biomarkers for detection of cancer and the overall impact of screening on the population through large-scale population studies

Objectives and Challenges

EDRN strives to make certain that good biomarkers are promoted without regard to pecuniary interests and to provide the best chance for promising assays to become future medical tests. A primary goal is to develop and implement diagnostic assays using multiple biomarkers, including gene expression patterns and epigenetic alterations.

EDRN facilitates the improvement of sensitive, high-throughput assay methods to identify and test cancer biomarkers; supports affiliations between academia and industry; and partners with other organizations to conduct clinical and epidemiological studies to evaluate the predictive value of biomarkers. Promoting expansion of the informatics infrastructure to assist pre-competitive data sharing is another key objective.

Incorporating up-and-coming technologies is essential to the network's success. While many EDRN investigators pioneer cutting-edge technologies, the network also invites collaboration from other scientists or companies to adapt their platforms for developing early detection diagnostic tests. Platforms being used for biomarker discovery involve genomics, epigenomics, glycomics, proteomics, metabolomics and nanotechnology.

Although the ultimate goal is to develop biomarkers ready for application in the clinic, bringing diagnostic markers to FDA for approval is beyond the scope of the EDRN due to limited resources. It is expected, however, that under agreements with lead investigators, the private sector will take responsibility for commercializing and seeking regulatory approval of EDRN-validated biomarkers. By the end of the current EDRN grant cycle in 2015, it is anticipated that at least one to three biomarkers could be submitted to the FDA. This number is likely to increase over the next 5 years as many biomarkers that are currently in development proceed through the validation pipeline.

[6] Pepe MS, Etzioni R, Feng Z, et.al. Phases of biomarker development for early detection of cancer. J Natl Cancer Inst. 2001;93:1054.

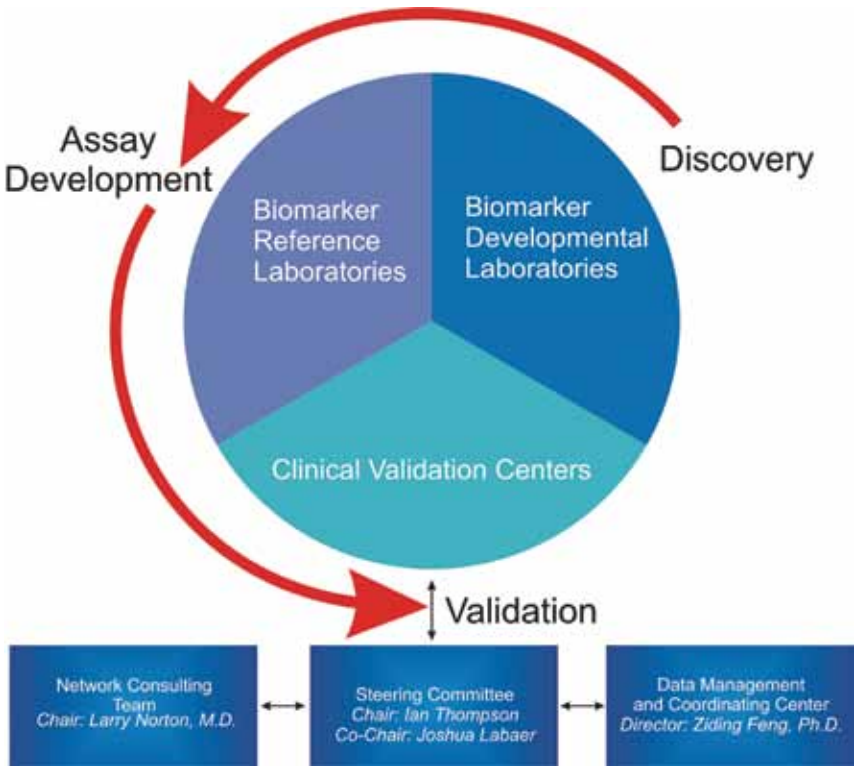
[7] Ziding Feng, Jacob Kagan and Sudhir Srivastava. In: Prostate Cancer Screening. Eds. Donna P. Ankerst, Catherine M. Tangen and Ian M. Thompson. Toward a Robust System for Biomarker Triage and Validation – EDRN Experience. Current Clinical Urology V, pp 297-306 (Totowa, NJ: Human Press) 2009.

Organizational Structure

The network is designed to accommodate rapidly evolving technologies, fortify the ever-growing knowledge base, empower investigators to meet scientific expectations, and reward collaborations and teamwork. As shown in Figure I-1, four scientific components make up the EDRN.[8] These components are:

1. Biomarker Developmental Laboratories (BDLs) – Twenty laboratories discover, develop and characterize new biomarkers or refine existing biomarkers. BDLs work to discover biomarkers that predict cancer progression, and determine prognosis. They also develop assays to detect candidate biomarkers; conduct pre-validation studies.
2. Biomarker Reference Laboratories (BRLs) – Three laboratories serve as the primary resource for analytical validation of biomarkers, technological development, standardization, assay refinement, and quality control and participate in biomarker clinical validation trials.
3. Clinical Validation Centers (CVCs) – Eight centers conduct clinical and epidemiological research on the validation of biomarkers for early cancer detection and risk assessment; serve as a resource center for EDRN Phase II and III biomarker research; lead or participate in collaborative biomarker validation studies; provide high quality biological specimens to other EDRN investigators for discovery research; partner with EDRN BDLs and BRLs.

Figure I-1: Structural Organization of EDRN



[8] Early detection research network in the US. Disease Markers. 1999 Dec;15(4):213-9.

4. Data Management and Coordinating Center (DMCC) – One center provides the computational and informatics needs of the network, including logistics for committee meetings; statistical and data management for protocol development; and analysis of clinical data. DMCC examines applied and theoretical approaches to the simultaneous analysis of multiple markers; and collaborates with EDRN investigators and the EDRN Informatics Center on the development of Common Data Elements (CDEs), analytical tools for data interpretation, and instruments for checking uniformity, consistency, accuracy, timeliness, reproducibility, and privacy of data.

Approximately 40% of current EDRN members are new in the 2010 re-competed network. A complete listing of funded groups is found in the Appendix. All EDRN investigators form the Steering Committee (SC) that provides scientific management, oversight and monitoring of ongoing biomarker research within the network. Two SC meetings are convened each year with an average of 170 attendees. The SC chair is elected from among the EDRN principal investigators, and the chair's institution serves as the network headquarters.

Salient Features of Newly Funded EDRN 2010-2015

- Mix of strong basic and translational scientists;
- More than 40% new grantees;
- Multi-PI approach;
- Multiple discovery platforms: genomics, proteomics, epigenomics, metabolomics and in silico discovery;
- Focused strategy on major epithelial cancers: breast, colon, lung, prostate, pancreas, ovary.

The headquarters is the locus of information dissemination to EDRN investigators and institutions and provides instrument management for the EDRN Associate Membership and EDRN-sponsored multi-center validation studies. The EDRN Executive Committee (EC) is formed by the SC chair and co-chair, the chairs of each organ-specific collaborative group, the Principal Investigator of the DMCC and one representative PI from the BRLs. The EDRN EC implements scientific, operational, and organizational policies and provides executive leadership, scientific direction and management.

EDRN Business Model and Project Management Approach: Effective Organization

The EDRN business model and project management approach has been reviewed and cited by the National Academy of Sciences Institute of Medicine as an effective team-based organization using informatics to manage and support biomarker research. The EDRN was praised for following best practices by the NCI Translational Research Working Group, and also by a series of scientific journals as a collaborative model for conducting discovery and validation research.

NAS large scale biomedical science. Institute of Medicine, 2001.

Biospecimen resources for the genomic and proteomic era. Rand Science and Technology Publication, 2003.

The Early Detection Research Network: translational research to identify early cancer risk; NIH Publication No. 01-4852, August 2001.

Buchen L. Cancer: missing the mark. Nature. 2011;471(7339) 428-32.

EDRN primarily focuses on the major epithelial cancers and a subgroup of cancers within these organ systems where a high potential exists for influencing morbidity and mortality. Overall, the network operates through four organ-system Collaborative Groups: Breast and Gynecologic, Colorectal and Other Gastrointestinal, Lung and Upper Aerodigestive, and Prostate and Other Urologic cancers.

Network Consulting Team Participation: An Extraordinary First 10 Years for the Early Detection Research Network

As Chair of the Early Detection Research Network's Network Consulting Team (NCT), I am enthusiastic about the progress it has made during the first 10 years and about the potential of this extraordinary consortium to help solve the cancer problem.

As both a physician who cares for patients and a researcher, I know firsthand the importance of reliability in tests in both clinical decision making, and in the generation of scientific insights. The skill and dedication of the investigators is impressive and encouraging. Through their work in the EDRN, these extremely competent researchers have committed to a vital activity that is not often rewarded by the academic community: the confirmatory evaluation of clinically relevant biomarkers.

In reviewing the EDRN, the NCT brings two points of view: that of conducting the research and that of overseeing the research. In this capacity the Team considers questions such as: Where is the EDRN at the present time? Where is the EDRN headed and how should it get there? What is being delivered? In September 2010, the NCT developed recommendations as the basis for reviews and discussions going forward.

The EDRN inherently recognizes the need to develop, rather than solely discover, important biomarkers, particularly those that may deal with currently unmet medical needs. The two-way flow of information between the laboratory and the clinic is recognized as essential to clinical and scientific progress. An advantage of this network is that the EDRN is broad in its outlook across disease types and organ systems. As these areas mature further, it is my hope that EDRN's value will be clear to all stakeholders in the scientific community and the general public.

***Larry Norton**, M.D., is Deputy Physician-in-Chief for Breast Cancer Programs, Medical Director, Evelyn H. Lauder Breast Center, Norma S. Sarofim Chair of Clinical Oncology, Memorial Sloan-Kettering Cancer Center, and Professor of Medicine, Weill Medical College of Cornell University. He is also Principal Investigator of a program project grant from the National Cancer Institute (NCI), which is aimed at better understanding breast cancer in the laboratory and bringing these advances into clinical practice. On a national level, Dr. Norton was formerly Chair of the Breast Committee of the NCI's Cancer and Leukemia Group B and President of the National Alliance of Breast Cancer Organizations. He was President of the American Society of Clinical Oncology from 2001 to 2002 and was appointed by President Clinton to serve on the NCI's National Cancer Advisory Board.*

A separate advisory group, the Network Consulting Team (NCT), ensures the responsiveness to promising opportunities; exhibits flexibility in its composition and decision-making; and makes prioritization decisions free from conflicts of interest.

Re-establishment of the NCT was recommended by the NCI's Board of Scientific Advisors (BSA) review in 2008. The NCT is composed of 12 members based on expertise and diversity drawn from the BSA, other institutions and groups.

Accomplishments

EDRN uses several intermediate measures to define success. For instance, a new biomarker with incremental improvement over existing diagnostic assays is considered an important measure of success. Annexin 1&2, %[-2] proPSA, and DCP, all had incremental performance improvement over the currently used serum-based tests. In contrast, TSP1, EPCA-2, and SELDI profiling all demonstrated very high levels of performance in preliminary verification studies, but failed in subsequent validation studies. These examples illustrate that in the absence of any other similar infrastructure for biomarker validation studies, EDRN also plays a very important "brake" role by defining new biomarker performance criteria in line with realistic clinical applications and use endpoints.

Importantly, prior to FDA filing and approval, another measure of success of network is the offering clinically and commercially relevant tests of biomarker assays developed within EDRN through Clinical Laboratories Improvement Amendments (CLIA)-certified laboratories. For example, EDRN-supported markers GST P1 methylation and PCA-3 as biomarkers for prostate cancer, and methylation of Vimentin as a biomarker for colon cancer are currently available in the clinic through various commercial, CLIA-certified diagnostic laboratories.

Among the significant results derived from EDRN efforts are the following:

- Percent [-2]proPSA was validated as a test for the detection of early stage prostate cancer with a potential prognostic value in determining aggressive and clinically significant prostate cancer.[9]
- Three markers for ovarian cancer, CA-125, HE4, and mesothelin, were found to be more informative in combination than CA125 alone.[10]
- Several prostate cancer specific gene rearrangements, which result in fusion transcripts (TMPRSS2-ETV1 and TMPRSS2-ERG), were identified. The fused genes may play a role in the development and progression of the disease.[11] Additional fusion transcripts were identified through integrative transcriptome sequencing.[12]
- Sarcosine was determined useful for differentiating between benign prostate tissue vs. localized and metastatic prostate cancer and may be associated with prostate cancer invasiveness and aggressiveness.[13]
- Proteins in blood were discovered that reliably indicate early stage pancreatic cancer, a breakthrough in the application of advanced proteomic technologies and mouse models to cancer-biomarker discovery.[14]

[9] Sokoll LJ, Sandra MG, Feng Z, et al. A prospective, multicenter, National Cancer Institute Early Detection Research Network Study of [-2]proPSA: improving prostate cancer detection and correlating with cancer aggressiveness. Cancer Epidemiol Biomarkers Prev. 2010;19(5); 1193-200.

[10] Anderson GL, McIntosh M, Wu L, et al. Assessing lead time of selected ovarian cancer biomarkers: a nested case-control study. J Natl Cancer Inst. 2010 Jan 6;102(1):26-38. Epub 2009 Dec 30.

[11] Tomlins SA, Rhodes DR, Perner S. Recurrent Fusion of TMPRSS2 and ETS Transcription factor genes in prostate cancer. Science. 2005 Oct 28;310(5748):644-8.

[12] Maher CA, Kumar-Sinha C, Cao X, et al. Transcriptome sequencing to detect gene fusions in cancer. Nature. 2009 Mar 5;458(7234):97-101. Epub 2009 Jan 11.

[13] Sreekumar A, Poisson LM, Rajendiran TM, et al. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature. 2009 Feb 12;457(7231):910-4.

[14] Faca VM, Song KS, Wang H, et al. A mouse to human search for plasma proteome changes associated with pancreatic tumor development. PLoS Med. 2008 Jun 10;5(6):e123.

•Autoantibodies to three proteins, Annexin I, LAMR1 and 14-3-3 theta, were shown to be effective in detecting lung cancer within one year prior to diagnosis or onset of symptoms. A search for additional autoantibody markers continues in order to assemble a larger panel for increased sensitivity. Annexin I was further validated on pre-clinical samples from the Carotene and Retinol Efficacy Trial (CARET) cohort.[15] This marker was subsequently used by an English group as a member of a panel for lung cancer detection.

- Repair mechanisms for oxidative damage of DNA prevent accumulations of somatic mutations. In heavy smokers, relative levels of activity for 8-oxoguanine DNA glycosylase in peripheral blood lymphocytes inversely correlate with the risk for lung cancer. Other repair enzymes being investigated as potential risk factors include alkyl-adenine DNA glycosylase and the endonuclease APE1. Assays for all three enzymes are being optimized for the clinical setting.[16]

- The Prostate Cancer Risk Calculator was published using results from the Prostate Cancer Prevention Trial (PCPT).[17]

- Although more than 100 biomarkers have been stopped from further development due to lack of performance, more than 200 have moved forward. Furthermore, several potential biomarker-based tests are under discussion with the FDA.[18]

[15] Qiu J, Choi G, Li L, et al. Occurrence of autoantibodies to annexin I, 14-3-3 theta and LAMR1 in prediagnostic lung cancer sera. J Clin Oncol. 2008 Nov 1;26(31):5060-6. Epub 2008 Sep 15.

[16] Paz-Elizur T, Sevilya Z, Leitner-Dagan Y, et al. DNA repair of oxidative DNA damage in human carcinogenesis: potential application for cancer risk assessment and prevention. Cancer Lett. 2008 Jul 18;266(1):60-72. Epub 2008 Apr 18. Review.

[17] Thompson IM, Ankerst DP, Chi C, et al. Assessing prostate cancer risk: results from the Prostate Cancer Prevention Trial. J Natl Cancer Inst. 2006;98:529-34.

[18] Maruvada P, Srivastava S. Joint National Cancer Institute-Food and Drug Administration workshop on research strategies, study designs, and statistical approaches to biomarker validation for cancer diagnosis and detection. Epidemiol Biomarkers Prev. 2006; Jun; 15(6):1078-82.

Table I-1: Measures of EDRN Productivity

Measure	Productivity
Biomarkers identified	More than 1,000
Prioritized markers ready for pre-validation and validation studies	More than 300
Validation studies	Eight completed studies (MSA for bladder cancer, DCP and AFP-alpha 3 for liver cancer; %[-2]proPSA, PCA3, and MS proteomic profile assay for prostate cancer; protein markers for ovarian cancers; gene methylation for esophageal cancer; Annexin 1 & 2 for lung cancer); Eight validation trials underway and two planned
Standard Reference Specimen Sets	10 valuable sets available
Patents and licenses developed	More than 28
Collaborations with biotechnology, diagnostic companies	More than 15
Publications	More than 1,450 collaborative papers have been generated, 15% of which are in high impact journals with an impact factor of 20 or above or a citation index of more than 300
Scientific Workshops	Seven conducted, attracting on average 300 participants

In summary, since its inception in 1999 EDRN has achieved several key milestones, summarized below:

1998 through 2000: Inception and Inauguration of EDRN

2001 to 2003: Meeting the Challenges to Harness and Share Emerging Scientific Knowledge

- EDRN Second Report, Translational Research to Identify Early Cancer and Cancer Risk, October 2002, http://edrn.nci.nih.gov/docs.) published.
- EDRN joined the Gordon Research Conferences to co-host New Frontiers in Cancer Detection and Diagnosis in 2002.
- Guidelines Set for Studies Measuring Biomarker Predictive Power Journal of National Cancer Institute (Vol. 93, No. 14, July 18, 2001).
- EDRN Associate Membership Program Initiated: This novel approach to make EDRN inclusive has been extremely successful. EDRN now has more than 120 Associate Members who are significantly contributing to EDRN efforts in biomarker discovery, development and validation.

2003 to 2004: Network Surges Ahead in Real-time

- Collaborative Discovery and Validation Projects: More than 100 collaborative projects spanned the various organ sites. These projects are monitored through the EDRN Study Information System (eSIS).
- EDRN Virtual Specimen Bank and Validation Management System Launched: The EDRN Virtual Specimen Bank, also known as ERNE knowledge system, was deployed to 10 institutions in early 2003, allowing a common web-based query to search for available specimens across the EDRN Clinical Epidemiology and Validation Centers (https://ginger.fhcrc.org/edrn/imp/GateServlet?pwd.)
- The Validation Study Information Management System (VSIMS) was created to allow multiple studies to be administered efficiently by minimizing development time with standardization of information and data management across multiple activities and research sites. This system encompasses all the security features of Food and Drug Administration (FDA)-required auditing systems.
- Partnership on the Plasma Proteome Project (PPP) Initiative of the Human Proteome Organization (HUPO): PPP project was initiated to evaluate multiple technology platforms, develop bioinformatic tools and standards for protein identification, and create a database of the plasma proteome. The entire study was published in the August issue of the journal Proteomics August 2005, Volume 4 (4), pp 1045-1450.

2005 to 2008: An Investment in Prevention

- In late 2006, EDRN's Program for Rapid, Independent Diagnostic Evaluation (PRIDE), was established (http://grants.nih.gov/grants/guide/notice-files/NOT-CA-07-003.html) as an administrative means to assist extramural investigators in successfully conducting cross-laboratory validation of biomarkers. Ten applications have been reviewed and five are being supported.
- EDRN underwent external reviews in 2007 and 2008.
- The Canary Foundation, Palo Alto, CA signed a Memorandum of Understanding with EDRN, NCI on supporting prostate cancer surveillance network of investigators from seven institutions. The tissue and serum will be collected during a three-year period and will be made available to extramural scientists for discovery and validation research.
- The Lustgarten Foundation, N.Y., funded six institutions to generate monoclonal antibodies and associated hybridoma cell lines for pancreatic cancer antigens (biomarkers) identified by EDRN and non-EDRN investigators. These resources will be stored at the NCI-Frederick Facility for distribution to extramural investigators.

2009 to 2011: Realizing Investment for Clinical Use

- Three biomarker tests approved by FDA and two IVDs pending FDA review.
- Six biomarker tests offered by CLIA labs.
- One biomarker test approved for clinical use outside the USA.

Partnerships

EDRN IS A CATALYST FOR BUILDING inter-governmental, inter-institutional and public-private collaborations aimed at rapidly translating early detection biomarkers to medical applications. The EDRN accounts for more than 100 collaborative efforts within the network itself, as well as with outside research groups, clinical trial communities, private foundations, Federal agencies, and others.

EDRN and FDA co-organize biennial meetings with the participation of the Centers for Medicare and Medicaid Services, where basic guidelines and requirements for FDA filing of diagnostic devices are discussed. In addition, EDRN-sponsored biomarker clinical validation trials are being presented and discussed at these meetings.

EDRN maintains Federal Interagency Agreements with: the Jet Propulsion Laboratory, NASA, which serves as an Informatics Center; the Pacific Northwest National Laboratory of the Department of Energy and the National Institute of Standards and Technology, both of which serve as BRLs. Other NIH institutes partnering with EDRN are the National Institute of Environmental Health Sciences as part of the Genes, Environment and Health Initiative (http://gei.nih.gov); and the National Institute of General Medical Sciences through their joint involvement in the Glycomics Alliance Initiative (http://glycomics.cancer.gov).

The EDRN has strong partnerships with other NCI consortia such as the Cooperative Groups, Specialized Program of Research Excellence (SPORE), Community Clinical Oncology Program, and designated cancer centers. The research network offers ongoing productive interactions among basic and clinical scientists; shares expertise; and curates a well-annotated biorepository for certain tumor sites.

Working with Prostate Cancer Prevention Trial investigators, EDRN helped create the Prostate Cancer Risk Calculator, a tool used to estimate the risk of prostate cancer based on multiple biomarkers and variables related to risk.[19] The tool has been validated in multiple populations and subsequently further improved with the incorporation of a range of other biomarkers.[20]

The SPORE and EDRN programs for the first time brought together disparate investigators and private sector partners to validate more than 70 candidate markers for the early detection of ovarian cancer in a Phase II/Phase III trial.[21] Almost half of those biomarkers, 32, were tested in 1,000 pre-diagnostic samples provided by the Prostate, Lung, Colon and Ovary Screening Trial cohort.[22]

Long-standing relationships are in place with industry and private sector partners, who have readily changed their business models to accommodate support for the EDRN process. Perpetual components of all partnering agreements relate to confidentiality, publications, data rights, specimen sharing, and intellectual property.

EDRN's transparent process for creating well-defined consensus standards and guidelines for biomarker development, validation and qualification has spurred strategic alliances with non-profits, including the Canary Foundation and the Lustgarten Foundation, and with professional organizations, such as the American Society of Clinical Oncology.

[19] Liang Y, Ankerst DP, Sanchez M, et al. Body mass index adjusted prostate-specific antigen and its application for prostate cancer screening. Urology. 2010 Nov;76(5):1268.e1-6. Epub 2010 Aug 24.
[20] Ankerst DP, Groskopf J, Day JR, et al. Predicting prostate cancer risk through incorporation of prostate cancer gene 3. J Urol. 2008 Oct;180(4):1303-8; discussion 1308. Epub 2008 Aug 15.
[21] Cramer DW, Bast RC Jr, Berg CD, et al. Cancer Prev Res. Ovarian cancer biomarker performance in prostate, lung, colorectal, and ovarian cancer screening trial specimens. 2011 Mar;4(3):365-74.
[22] Zhu CS, Pinsky PF, Cramer DW, et al. A framework for evaluating biomarkers for early detection: validation of biomarker panels for ovarian cancer. Cancer Prev Res. 2011 Mar;4(3):375-83.

A Multi-Platform Approach: Biomarker Discovery in Lung Cancer in Never Smokers

Through collaboration between the EDRN and the Canary Foundation for the study of lung cancer in never smokers, EDRN supports the study of lung tumor tissues, while the Canary Foundation is funding research using lung cancer cells lines. The collaboration between the Canary Foundation and the NCI adds value and synergy to the study.

The unique aspect of this study is that the same samples will be analyzed using a multi-platform approach. This extensive multi-platform (gene expression profiling, plasma proteomic profiling, methylation profiling, aCGH, SNPs, microRNAs, mitochondrial DNA mutations, and sequencing) approach is being used to identify biomarkers for early detection and risk assessment of lung cancer in never smokers and utilizing both clinical samples and cell lines across all platforms.

The results of this study will be integrated to develop a network-based approach to the analysis of the data for identification of biomarkers for lung cancer. This is a unique opportunity to leverage resources of multiple participating investigators, each bringing in expertise from different platforms.

EDRN outreach is vital to the program and speaks to the health of the network. Outreach activities include participation in activities of cancer advocacy groups and professional societies, such as the American Association for Cancer Research, the American Society of Clinical Oncology, the American Society of Molecular Pathologists, and others. EDRN has appointed a number of investigators to liaise with professional societies and has cancer advocates serving on its Steering Committee.

In collaboration with others (See EDRN-Related Meetings), EDRN conducts meetings and workshops that are transparent and open to the extramural community; communicates its progress through the biennial reports and presentations to the NCI Executive Committee and the NCI Advisory Committees; has more than 21 listservs where the extramural community can subscribe and join EDRN collaborative groups; and provides opportunities for the extramural community to join EDRN through its Associate Membership Program, a unique concept among the NIH-supported networks and consortia. EDRN seeks input from extramural experts on research directions that may not be available within the network. The NCI-designated Cancer Center Directors recommendations noted, "With relatively small funding, experts note that this mechanism (EDRN) has done a commendable job developing collaborations across Cancer Centers, SPOREs, and Program Projects."[23]

The EDRN Associate Membership Program is a novel mechanism through which non-EDRN investigators can collaboratively pre-validate and/or validate their biomarkers. There are three types of Associate Membership: category A is geared toward investigators who have biomarkers ready for pre-validation or validation; category B is geared toward investigators who participate in ongoing or planned validation studies and who provide resources necessary for a specific validation study; and category C is designed to allow non-EDRN investigators, industrial partners and cancer advocates to participate in EDRN's mission, activities, and conferences. There are more than 150 Associate Members: 42 category A members have been approved to date, more than 75 category B involved in various EDRN-sponsored validation studies, and at least 40 category C members, among whom are many biotechnology companies, private foundations and international partners.

Associate members establish active collaborations with EDRN investigators and utilize network support, resources, and infrastructure. They attend EDRN

[23] NCI-designated Cancer Center Directors: Accelerating Successes Against Cancer (2007).

Steering Committee meetings and Collaborative Group teleconferences. Applicants are sponsored by an EDRN principal investigator. Applications are reviewed by the Standing Review Group and recommended to the EDRN Executive Committee on the basis of scientific merit, clinical potential and programmatic priorities.

In a partnership with the Canary Foundation, which provided funding support, EDRN investigators and the DMCC have provided major leadership, coordination and collaboration in the development of the Prostate Cancer Active Surveillance Study (PASS). Men with clinically localized prostate cancer are enrolled who have chosen to manage their cancer using active surveillance. Biospecimens (blood, urine, tissue) and associated clinical data are collected serially with rigorous quality control and deposited in a Central Biospecimen Repository. The ultimate goal is to utilize the resources created by PASS to develop biomarkers for prostate cancer progression.[24]

EDRN has partnered with the Lustgarten Foundation to make available a number of hybridomas producing monoclonal antibodies to potential biomarkers of pancreatic cancer. A collection of 40 hybridoma lines covering 20 different proteins were received from investigators associated with the Lustgarten Foundation and are stored within NCI facilities. The hybridomas are available upon request by researchers who propose to use the antibodies in their research projects.

International partners with EDRN include the Weizmann Institute of Science in Israel, which is looking into DNA repair biomarkers for risk assessment and early detection of lung cancer, and the University of Western Australia, which is a co-investigator in the American/Australian Mesothelioma Consortium.

[24] Newcomb LF, Brooks JD, Carroll PR, Feng Z, Gleave ME, Nelson PS, Thompson IM, Lin DW. Canary prostate active surveillance study: design of a multi-institutional active surveillance cohort and biorepository. Urology 2010;75:407-13.

EDRN-Related Meetings

February 15, 2007	EDRN-FDA Educational Workshop, Bethesda, MD
March 12-15, 2007	14th EDRN Steering Committee Meeting, Denver, CO
May 14-15, 2007	Stem Cell Workshop, Rockville, MD
July 16, 2007	Animal Models Workshop, Rockville, MD
September 17, 2007	EDRN-Public Private Partnership Workshop, Ann Arbor, MI
January 23-24, 2008	Barrett's Esophagus Meeting, Gaithersburg, MD
March 16, 2008	EDRN-Public Private Partnership Workshop II, Bethesda, MD
August 28, 2008	Workshop on Paradoxes in Cancer, Rockville, MD
November 7-10, 2008	Translational Research Meeting, Washington, DC
November 16-19, 2008	Frontiers in Cancer Prevention- AACR, Oxon Hill, MD
April 18-21, 2009	100th Annual AACR Meeting, Denver, CO
September 9-11, 2009	Circulating Tumor Cells: Emerging Technologies for Detection, Diagnosis, Prognosis and Treatment, Bethesda, MD
December 10-11, 2009	Rare Cancers with High Mortality: Challenges for Cancer Prevention and Treatment, Bethesda, MD
May 20-21, 2010	Stakeholder's Meeting on Biomarker Research Priorities, Rockville, MD
April 17-21, 2010	101st Annual AACR Meeting, Washington, DC
September 14, 2010	EDRN-FDA Meeting, Bethesda, MD
December 6-9, 2010	Frontiers in Cancer Prevention - AACR Meeting, Houston, TX
February 28, 2011	Molecular Imaging Think Tank, Rockville, MD
March 7, 2011	EDRN - Industry Forum, Los Angeles, CA
April 2-6, 2011	102nd Annual AACR Meeting, Orlando, FL

Biomarker Prioritization

The overarching goal of EDRN is to validate biomarkers for early cancer detection and risk assessment. Achieving this goal requires prioritization of biomarkers at all stages of their development, from initial discovery, through assay development to clinical validation. To optimize this process, EDRN established guidelines and metrics to determine which biomarkers should move ahead to the next phase. Most biomarkers do not progress beyond the discovery phase as they do not have sufficient sensitivity and specificity to warrant further testing.

Discovery: Most of the biomarker discovery and development within EDRN are conducted by the Biomarker Developmental Laboratories; these projects undergo peer review by an independent NCI Study Section.

Prevalidation/Verification: When investigators believe they have sufficient data to demonstrate the potential clinical use of their biomarker, they can apply for additional resources to verify their results. These resources can include help with assay development, independent verification of the biomarker performance, specimens and additional funding. Requests are reviewed by the appropriate EDRN organ-specific collaborative group using defined criteria, which include evaluation of data on the reproducibility, sensitivity, and specificity of the assay, study design and potential clinical or scientific impact. If the request is approved, help with assay development or independent verification of the biomarker performance can be done by an EDRN Biomarker Reference Laboratory, and specimens can be provided from the EDRN sample reference sets or by an EDRN Clinical Validation Center. If funding is required, the request must be reviewed by the EDRN Executive Committee. Investigators that are not part of the EDRN can also apply for these resources, and their requests are evaluated using the same process and criteria.

Validation: Results from the prevalidation/verification studies determine whether the biomarker or panel of biomarkers have sufficient performance to warrant a full validation trial. The same review process for prevalidation requests is used for validation trial requests, except that the bar is higher and reviews are also solicited from non-EDRN investigators. Review criteria include: scientific merit; study design; technical parameters: reproducibility, sensitivity, specificity, throughput, and automation; clinical impact; portfolio balance; practicality and feasibility, such as required sample size and amount of tissue; and collaborative strength, including contribution of resources and technology.

Most biomarkers evaluated in a prevalidation study do not progress to a validation trial, primarily due to lack of performance when assayed using independently collected specimens that represent the broad spectrum of the disease and its confounding conditions (e.g., benign prostatic hyperplasia for prostate cancer). If the validation trial is approved, the EDRN Data Management and Coordinating Center, Clinical Validation Centers and Biomarker Reference Laboratories will support the trial. Investigators who have prevalidation data obtained without EDRN support can also apply to the EDRN to support a validation trial, or to participate in an ongoing EDRN validation trial. These requests are evaluated using the same criteria as requests from EDRN investigators.

EDRN Advances Biomarker Science

Before EDRN:

- Non-existent Standard Operating Procedures (SOPs) for biospecimen collection
- No Common Data Elements (CDEs) exist for standardized sample annotation
- No roadmap for prioritization and study design for biomarker validation studies

After EDRN:

- SOPs and standardized management of biosample collection established. Disseminated beyond EDRN
- Epidemiological and population statistics research created and validated CDE repository for sample annotation.

Part Two

Moving Basic Science Discoveries into Biomarkers for Early Detection

The EDRN combines multidisciplinary, investigator-initiated projects with a strong national infrastructure and team science on collaborative projects. EDRN investigators study organ-specific cancers to identify early detection and surveillance biomarkers worthy of further research. Activities of four collaborative working groups, each with a multidisciplinary team, center on major epithelial cancers and a subgroup of diseases within those organ systems where the potential for influencing morbidity and mortality is greatest: breast and gynecological cancers, colorectal and other gastrointestinal cancers, lung and upper aerodigestive cancers, and prostate and other urologic cancers.

Building Teams via Collaborative Groups and Individual Study Areas

Each organ-specific group proposes strategic goals and a plan that includes the teaming up into collaborative projects and the assembly of standard reference specimen sets. A committee provides the administrative structure through which investigators team up, as shown in Figure II-1.

Figure II-1: EDRN committees provide the administrative structure for collaboration

Breast and Gynecological Cancers

Breast cancer is a highly prevalent disease with well-established early detection strategies: mammographic screening, clinical breast exams, and molecular tests to assist in determining treatment options after diagnosis. Such tests, however, are still unavailable for detecting the disease at a very early stage or for providing prognostic information that could help identify pathways to prevent malignant development of identified breast lesions. Ovarian cancer is a significantly less prevalent but more lethal disease, vastly due to the lack of any reliable strategies for its early detection at stages where treatment could be more effective. The Breast and Gynecological Cancers Collaborative Group has made significant progress addressing these challenges, a brief summary of which is provided below.

Major Accomplishments

- Completed multi-center (six EDRN and SPORE centers) Phase II/Phase III validation study of more than 75 candidate biomarkers for the early detection of ovarian cancer
- Longitudinal bank of quarterly collected serum/plasma from 2,500 high-risk women (EDRN-CVCs, CGN, SPOREs); 20,000+ serum samples banked with 20 Pre-diagnostic cases of ovarian cancer and 90 Pre-diagnostic cases of breast cancer
- Longitudinal bank of annually collected serum/plasma from 1,500 women (EDRN CVC at FCCC); 10 Pre-diagnostic cases of ovarian cancer
- Standard Reference Set for Breast Cancer – 1,000+ serum/plasma samples collected by multiple EDRN CVCs/BDLs at time of diagnosis of breast lesion; samples obtained prior to pathology – i.e., strongly unbiased PRoBE design
- Autoantibody panel identified as potential serum biomarker for triple-negative breast cancer

A list of the members of the Breast and Gynecological Cancers Collaborative Group can be found in the Laboratory Listing in the Appendix.

Breast Cancer - Strategic Goals

The increase in incidence of breast cancer observed over the past 20 years is almost entirely attributable to the detection of ductal carcinoma in situ (DCIS) and stage I cancer by imaging. The large majority of these lesions remain indolent. At the same time, there are many cancers that are being missed by the current screening modalities, many of which tend to be aggressive disease, such as "interval" and hormone-receptor negative or Triple-Negative breast cancers (TNBC). The incidence of the latter is significantly higher in premenopausal women where imaging screening modalities are significantly less effective.

The ultimate goal is to develop non-invasive, molecular methods for detecting and characterizing precancerous and cancerous breast lesions with certainty when they are small and more easily treatable. Specifically, biomarkers are needed that can either augment mammography in the short term, or replace mammography in the long term. Biomarkers are also sought for the assessment of risk of progression from Benign Breast Disease (BBD) or DCIS to invasive breast cancer (IBC), and for the detection of aggressive cancers, such as TNBC, the majority of which are not detected by routine imaging.

At the International Meeting on Ovarian Cancer, held on November 29 and 30, 2011 in London, EDRN proposed the creation of an international alliance on ovarian cancer

cohorts to improve early detection research. The proposal was accepted and U.S. and European scientists will be moving forward with this project.

Breast Cancer - The Plan

Investigators in the Breast and Gynecological Cancers Collaborative group are working toward the identification and validation of:

- Biomarkers to further improve the interpretation of conventional mammography or other computer-aided technologies;
- Biomarkers that detect characteristics of benign and malignant breast lesions and stratify benign disease into high and low risk for progression;
- Biomarkers which, in conjunction with mammography, can distinguish malignant from benign lesions in order to reduce or eliminate unnecessary biopsies;
- Biomarkers to detect highly proliferative early malignant lesions associated with increased mortality; and
- Tumor-specific biomarkers that could be used as contrast agents to improve the performance of existing imaging modalities.

Detecting and treating breast cancer at a precancerous stage will greatly increase the odds of reducing mortality. Breast and Gynecological Cancers Collaborative Group investigators conduct new biomarker discovery, pre-validation and validation projects for breast cancer by pursuing the strategic goals of the EDRN, as illustrated below.

- Investigators at the clinical validation center at the Fred Hutchinson Cancer Research Center in collaboration with investigators at the Swedish Medical Center are using a unique cohort of cases and controls with available detailed mammography information to systematically verify the clinical utility of hundreds of candidate protein biomarkers to add value to mammography screening. Biomarkers whose performance has been pre-validated will ultimately be validated in a clinical trial utilizing pre-clinical specimens obtained from the Women's Health Initiative (WHI) cohort.
- The Fox Chase Cancer Center clinical validation center is evaluating the utility of a number of DNA hypermethylation and protein biomarkers for risk stratification of women diagnosed with benign breast disease for progression to invasive breast cancer.
- Investigators at the Duke University clinical validation center together with their collaborators at Johns Hopkins University are developing immunohistochemistry and DNA methylation-based biomarkers for risk assessment for progression of pre-invasive breast disease (DCIS) to invasive carcinoma.
- The Nucleic Acid Programmable Protein Microarray (NAPPA) technology developed by EDRN investigators at Arizona State University has been successfully used to identify autoantibody-based biomarkers in sera for the non-invasive, early detection of breast cancer subtypes, including TNBC. The technology will also allow detection of protein post-translational modifications, such as glycosylation, which will increase significantly the type of tumor antigens being screened for auto-antibodies as candidate biomarkers.
- The Ohio State University BDL investigators are focusing on the development of a set of microRNAs as a novel class of biomarkers for the early detection of breast cancer, especially with regard to the receptor negative and triple-negative disease.

Ovarian Cancer - Strategic Goals

Currently, no reliable biomarkers exist for the detection of pre-clinical lesions or early stage disease that could be used for screening.

The absence of biomarkers, coupled with the typical late stage at diagnosis contributes to the significant lethality of ovarian cancer.

A recent clinical trial conducted by EDRN investigators in collaboration with SPORE investigators failed to identify any biomarkers from among more than 70 candidates tested that can detect ovarian cancer reliably more than 6 months before the manifestation of any clinical symptoms of the disease. The single best biomarker still remains CA-125, which has poor diagnostic performance for premalignant or early stage disease. Furthermore, its increased levels are found in about 3% of postmenopausal women, resulting in a significant number of false positives for this biomarker. The study confirmed that while the HE4 biomarker performs almost as well as CA125, it does not add value to it.

A few other biomarkers (CA15.3, CA72.4, mesothelin, and beta-2-microglobulin) were also shown to detect ovarian cancer as early as 12 to 18 months prior to clinical diagnosis, but their sensitivity is very low. New biomarkers for ovarian cancer are therefore needed to be developed urgently, some of which could be coupled with CA125 and/or HE4, CA15.3, CA72.4, mesothelin, and beta-2-microglobulin in highly accurate, multiplexed assays that can identify women at high risk of harboring premalignant or early malignant ovarian lesions.

Ovarian Cancer - The Plan

Investigators in the Breast and Gynecological Cancers Collaborative group are working toward the following:

- Identification and validation of biomarkers that can further improve the interpretation of conventional transvaginal ultra-sonography or other computer-aided imaging technologies;
- Identification and validation of biomarkers that can identify and stratify early ovarian lesions as benign and at high risk of progression to ovarian cancer;
- Building a consolidated pre-diagnostic specimen repository (PLCO, WHI, CARET, Nurses' Health Study, UKCTOCS), statistically powered for discovery and rapid pre-validation of candidate markers;
- Utilizing the NCI TCGA data on ovarian cancer somatic genetics to inform early detection biomarker development; and
- Focusing biomarker discovery efforts on a better understanding of the natural history of the disease and better characterization of putative pre-malignant ovarian lesions.

Biomarker discovery, pre-validation and validation projects for ovarian cancer are conducted by investigators along the lines of the EDRN strategic goals, as shown in the following examples.

- The Massachusetts General Hospital BDL is focusing on the discovery of new candidate biomarkers of ovarian cancer through proteomic and genomic analyses of multiple tissue sources associated with ovarian cancer development and progression, including ovarian cyst fluid, the fallopian tube system, and the ovarian secretome. Candidate biomarkers will be prioritized using Accurate Inclusion Mass Screening at the Broad Institute and enhanced bioinformatics analysis to select the 50 top-performing biomarkers to be tested using multiple reaction monitoring mass spectrometry to determine which of the candidates demonstrates the earliest possible (lead-time) sensitivity for ovarian cancer.
- The Mount Sinai, Toronto BDL investigators will continue the refinement of 40 newly discovered promising biomarkers for the early detection of ovarian cancer using a systems approach in combination with a bioinformatics and immuno-mass spectrometry approach. The investigators will also employ multi-parametric analysis to test if a panel of nine novel candidate biomarkers can improve the performance of CA125 to detect ovarian cancer at a very early stage.

- The FHCRC clinical validation center investigators will continue the Phase III evaluation of the top performing biomarkers tested in the recently completed EDRN/SPORE/PLCO validation study by examining their lead-time sensitivity in serial samples from the PLCO cohort and by employing longitudinal classification algorithms. The latter will be evaluated and trained on the PLCO serial samples and then validated using serial samples obtained from the UK Collaborative Trial of Ovarian Cancer Screening cohort.

In an effort to further promote collaboration among the members of the Breast and Gynecological Cancers Collaborative Group and to increase the efficiency of validating promising biomarkers for breast and ovarian cancer, three multi-institutional collaborative studies have been launched, two of which are focused on breast cancer and one on ovarian cancer.

Detection of Missed "Interval" Breast Cancer

Mammography, when used per guidelines, reduces breast cancer mortality by 20%. It is also known, however, that many breast cancers are missed in the interval between screening mammograms. These are often referred to as "interval cancers." Although some are missed due to technical problems or by error, true interval cancers are more likely to be at a more advanced stage and grade than screen-detected cancers.

It is not unlikely that interval cancers are molecularly different from screen-detected cancers, as in the case of pediatric neuroblastoma, where interval cancers were shown to be a molecularly distinct group from the screen-detected tumors. Interval breast cancers are a heterogeneous group of histologically distinct, exceptionally malignant, fast-growing cancers, which frequently are estrogen (ER) and/or progesterone (PR) receptor-negative or triple-negative (TNBC), which is negative for ER, PR and HER2neu.

While few interval cancers are detected in women over 50 years within the 2-year period between mammograms, in women ages 40 to 49 there is a significant increase in the rate of interval cancers between mammograms, especially when the latter are 2 or more years apart. In addition, while mammography is less than optimal in detecting malignant breast cancer in post-menopausal women, its' sensitivity is even poorer in pre-menopausal women under age 50 due mostly to the higher density of the breast tissue.

Hence, an alternative program, such as an interim blood biomarker test is urgently needed. Such a program in conjunction with mammography could improve the detection of aggressively growing, highly malignant interval breast cancers within a window of opportunity in which treatment may still be efficacious enough to reduce mortality.

Investigators in the EDRN Breast and Gynecological Cancers Collaborative Group have initiated a team project to address the early detection of TNBC. The goal of this collaborative study is to develop and validate a clinical biomarker blood test that can help identify women who are likely to have, or are at risk of developing TNBC, and who would benefit from more frequent (every 6 months) screening by an imaging modality (mammography or MRI).

See the online guidelines at: http://www.uspreventiveservicestaskforce.org/uspstf/uspsbrca.htm and at http://www.cancer.org/Healthy/FindCancerEarly/CancerScreeningGuidelines/american-cancer-society-guidelines-for-the-early-detection-of-cancer.

Ross JA, Davies SM. Screening for neuroblastoma: progress and pitfalls. Cancer Epidemiol Biomarkers Prev. 1999;8:189-94.

Team Project: Tissue Biomarkers for Risk of Invasive Breast Cancer

Background and Clinical Goals: Atypical ductal hyperplasia (ADH) and DCIS, lesions along a spectrum of increasing cellular and architectural abnormalities, are currently characterized by pathologic evaluation under a light microscope. While diagnosis remains an issue, the clinical management of these conditions presents the primary dilemma. About 1% to 2% of women diagnosed and treated for DCIS with surgery will have another DCIS or invasive breast cancer within 5 to 10 years.

The relative risk of developing invasive breast cancer after a diagnosis of ADH is two to four times greater than that of the general population. Since the vast majority do not progress or recur, the ability to stratify these frequently diagnosed lesions into high-risk and low-risk categories would form the basis for monitoring, chemoprevention, and primary treatment modalities tailored to the individual patient. The clinical goal of this team project is to identify women diagnosed with ADH or DCIS, who are at increased risk of developing invasive breast cancer and who might benefit from risk reduction with the use of chemoprevention agents such as tamoxifen.

Study: Biomarkers expressed in the tissues of women with these diagnoses who progress to IBC are quantitatively and/or qualitatively different from those expressed in tissue of matched women who do not progress to IBC. A nested case-control design will determine if available proteomic and methylation biomarkers predict risk for future IBC. Women with ADH or DCIS who are disease-free will be matched 2:1 to women with ADH or with DCIS who progress to IBC. Tissue specimens will be identified retrospectively using surgery/pathology records between 1994 and 2004-2005 by the three EDRN Breast Cancer Clinical Validation Centers (Fox Chase Cancer Center, Fred Hutchison Cancer Research Center, and the Duke/Johns Hopkins Universities). A wide range of promising risk assessment biomarkers will be tested to validate their performance on the corresponding common tissue specimen sets.

Team Project: Circulating Biomarkers for Triple-Negative Breast Cancer

Background and Clinical Goals: Breast cancers are classified into different subtypes based on the expression of estrogen and progesterone receptors and Her2 gene overexpression. Triple-negative breast cancers (TNBC) have none of these markers and make up 15% to 20% of breast cancers, including a majority of the aggressive tumors known as basal-like breast cancers. TNBCs are more rapidly proliferative, have p53 mutations in 44% of cases, and are associated with later stage at diagnosis and increased mortality.

TNBCs occur more frequently in younger women where mammographic screening is less reliable, especially in premenopausal African American women, Hispanic/Latina women, BRCA1 mutation carriers, and in association with an elevated waist-to-hip ratio. TNBCs are more likely to be diagnosed by physical exam than by mammographic screening and usually present as interval cancers between regular mammograms, likely due to a combination of rapid proliferation and presentation in younger women with higher breast density. There is an urgent clinical need for biomarkers for the early detection of TNBC. EDRN investigators have teamed up with the goal to develop a blood-based biomarker panel for the routine screening of women over the age of 40 to identify high-risk cases for TNBC for more frequent imaging by mammography and/or magnetic resonance imaging.

Study: The overall study design involves the identification of three distinct types of blood-based biomarkers: autoantibodies, protein antigens, and micro-RNAs (miRNA). These will be validated in a step-wise fashion using samples provided by the CVCs and multi-institutional cohorts, with study design and statistical sup-

port by the DMCC. Previously identified biomarkers associated with TNBC will be initially verified on a common set of clinical samples and the selected top-performing ones will be validated in a case-control study with a diagnostic set of plasma samples.

Given the very low incidence of TNBC, the top marker combinations must perform with a sensitivity of at least 30% to 40% at a very high specificity (98%) in order to achieve a clinically acceptable true positive ratio (TPR>0.38). The selected biomarker combination(s) will also be tested on the EDRN standard breast reference set for distinguishing TNBC from benign disease as well as for the detection of ER+ and HER2+ breast cancer. Finally, the top performing markers and their combinations will be validated in a Phase III retrospective longitudinal case-control study for the detection of TNBC using pre-diagnostic sera from the WHI, Risk of Ovarian Cancer Algorithm (ROCA) study, and the PLCO Screening trial cohorts.

Study Design Lessons Learned

Hundreds of candidate biomarkers of ovarian cancer have been discovered and more than 70 were recently subjected to rigorous validation in a Phase II/Phase III trial. The results indicate that while many of the biomarkers can distinguish between cases and controls in specimens obtained at diagnosis, the vast majority of them failed in distinguishing cancer from healthy control-associated specimens when the samples were obtained more than 6 months prior to clinical diagnosis.

This outcome emphasizes the importance of using appropriate specimens for biomarker research, from early discovery stages to clinical validation. Bias introduced by systematic differences in the case and control specimens during biomarker discovery, which can significantly inflate the performance of biomarkers, must be maximally avoided by adapting the principles of PRoBE (Prospective-specimen collection, Retrospective Blinded Evaluation) study design. Another factor for failure is the poor understanding of the natural history of the disease and the discovery of biomarkers in advanced cancer lesions, which may not be present in the preneoplastic and early neoplastic lesions that are the precursors of aggressively growing disease.

Pepe MS, Feng Z, Janes H, Bossuyt PM, Potter JD. Pivotal evaluation of the accuracy of a biomarker used for classification or prediction: standards for study design. J Natl Cancer Inst. 2008 Oct 15;100(20):1432-8. Epub 2008 Oct 7.

Team Project: Circulating Biomarkers for Early Detection of Ovarian Cancer

Background and Clinical Goals: Ovarian cancer is highly lethal because 80% of cases are detected at late stage. Despite recent therapeutic advances, there has been no reduction in ovarian cancer mortality. Since prognosis of ovarian cancer detected at early stage is excellent, an effective early detection program with regular screening tests may significantly reduce mortality. Approximately 20% of ovarian cancers appear to develop and disseminate to other organs very rapidly and are unlikely to be detected early enough by current screening methods. It is possible that the remaining 80% of cases could be identified early enough, thus resulting in a significant reduction in mortality from the disease. The goal of this team effort is to validate a large number of promising biomarkers to be used in a screening program, which will increase the proportion of ovarian cancers detected at an early stage to between 60% and 80%. Such a screening program will need to achieve an appropriate positive predictive value in order to minimize the number of unnecessary surgery referrals due to false positives: 10 or fewer surgeries for each ovarian cancer detected by the test, or a PPV of 0.1.

Study: To achieve ovarian cancer detection at such an early stage, the goal of this team project is to identify from among more than 150 candidates the biomarkers and their combinations through longitudinal (using multiple values from serial tests) biomarker algorithms; biomarkers would have to achieve sufficiently high sensitivity (>5%) at a fixed specificity of 98% at least one year prior to clinical detection of the disease. The plan is to develop highly accurate assays for 80 to 100 selected candidate protein markers previously identified by diverse approaches and platforms. Highly specific antibodies commercially available for many of these markers will be used to develop highly accurate immunoassays by EDRN's Johns Hopkins University BRL or alternatively using the NAPPA technology.

For those candidate biomarkers for which no antibodies are available, the EDRN Pacific Northwest National Laboratory BRL will utilize available resources to develop Selective Reaction Monitoring-Mass Spectromery (SRM-MS) assays for their pre-validation. To confirm the performance of all selected biomarkers at 98% specificity, they will be tested in a stepwise protocol, first using a common set of case/control samples obtained at diagnosis and on a limited number of samples obtained within one year from diagnosis. Verified biomarkers will be then tested on longitudinally collected samples obtained up to two or more years prior to diagnosis. EDRN is discussing with WHI, PLCO, CARET, and UKCTOCS cohort directors to provide samples for biomarker discovery and rapid triage.

Colorectal and Other Gastrointestinal Cancers

Cancers in the gastrointestinal tract include one of the most prevalent cancers in the United States (colorectal cancer), a cancer with the fastest rising incidence (liver cancer), and two of the most deadly malignancies (esophageal and pancreatic cancers).

The Colorectal and other Gastrointestinal Cancers Collaborative Group is focused on the discovery and validation of biomarkers for the early detection of colorectal and pancreatic cancers through independent research, collaborative team projects, and multi-site validation trials. This collaborative group also supports the validation of biomarkers for hepatocellular carcinoma (primary liver cancer).

Over the past several years this Collaborative group has made significant progress toward addressing the major challenges posed by these cancers. A brief account of the major accomplishments of the group is provided below.

A list of the members of the Colorectal and Other Gastrointestinal Cancers Collaborative group can be found in the Laboratory Listing in the Appendix.

Major Accomplishments

- Discovered galectin-3 ligand as a serum biomarker of colon cancer with sensitivity 72% and specificity 80% based on blinded validation in standard reference set
- Discovered Vimentin methylation in stool and demonstrated in controlled reference sets a sensitivity of 84% at 85% specificity
- Developed the first EDRN large, multi-center cross-sectional trial of biomarkers for the early detection of hepatocellular carcinoma (HCC); demonstrated that des-carboxyprothrombin (DCP) has a significant advantage over AFP for detecting HCC with viral etiology
- Collected a high quality biosample repository from high risk cirrhotic patients and from patients with early stage HCC; an invaluable resource for screening biomarkers for HCC
- Discovered GP73 as a classifier of HCC in high risk patients and demonstrated that it improves AFP performance in detecting early stage HCC. GP73 was approved for HCC screening in China in combination with AFP
- Identified DEAR1 and two miRNA profiles as potential biomarkers for early detection of pancreatic cancer.
- Seven EDRN sites participate in the construction of standard reference sets: serum/plasma and cystic fluid; reference sets to be used to screen DEAR1, miRNA profiles and other biomarkers to rule in or rule out biomarkers for larger validation study

Colorectal Cancer - Strategic Goals

Colorectal cancer (CRC) is the third most common cancer in the United States and the second most common cause of cancer-related deaths. The lifetime incidence of colorectal cancer is sufficiently high to justify population screening, and wide scale screening using fecal occult blood tests (FOBT) results in up to 30% reduction in colorectal adenocarcinoma mortality, but at the expense of many colonoscopies in patients without cancer.

Colonoscopy, while examining the complete colon, requires a thorough bowel preparation and sedation, and causes patient discomfort. Both FOBT and colonoscopy have low patient compliance. Consequently, there is need for alternate strategies to screen for colorectal cancer that can accurately identify those at greatest risk of having colorectal cancer and need to have a colonoscopy. EDRN investigators have identified genetic, epigenetic, and protein biomarkers that correlate with the presence of colorectal cancer using serum, stool and urine.

Major goals are to discover and validate biomarkers and novel imaging methods to identify patients at risk of colorectal cancer and, therefore, in need of colonoscopy; discover and validate biomarkers to stratify benign lesions into those at high- and low-risk for progression to cancer; and discover and validate minimally invasive biomarkers to be used in conjunction with imaging to improve performance.

Biomarker Selects Patients for Chemoprevention

An EDRN investigator, Dr. Sanford Markowitz, and his collaborators have found that the protein 15-PGDH (15-hydroxyprostaglandin dehydrogenase) can be used as a biomarker to predict whether an individual will benefit from taking celecoxib. Using specimens from the Adenomas Prevention with Celecoxib (APC) trial, these investigators found that individuals who developed new adenomas while receiving celecoxib treatment had low levels of colonic 15-PGDH. This suggests that it may be possible to determine which individuals might benefit from celecoxib by measuring the level of 15-PGDH in their colon.

Yan M, Myung SJ, Fink SP,et al. 15-Hydroxyprostaglandin dehydrogenase inactivation as a mechanism of resistance to celecoxib chemoprevention of colon tumors. Proc Natl Acad Sci U S A. 2009 Jun 9;106(23):9409-13. Epub 2009 May 22.

Colorectal Cancer - The Plan

Investigators are working toward the following:

- Conduct rigorous clinical validation of promising biomarkers for the detection of early stage colorectal cancer and adenomas, before implementation at the population level;
- Exploit data from genomic sequencing studies to identify novel biomarkers for colorectal cancer;
- Use proteomic, genomic and epigenomic techniques to identify biomarkers that determine which patients are at high-risk for adenocarcinoma and in need of having a colonoscopy;
- Develop and test novel optical imagining methods to detect colon cancer using brushings from rectal tissues; and
- Develop colon cancer reference sets comprised of serum, plasma, urine, DNA from white blood cells, and paraffin embedded tissues from normal colon, adenomas, inflammatory bowel disease, and colorectal cancer.

Investigators in the Colorectal and other Gastrointestinal Cancers Collaborative Group conduct new biomarker discovery, pre-validation and validation projects for colorectal cancer by pursuing the strategic goals of the EDRN as shown in the project descriptions below.

- The Fred Hutchinson Cancer Research Center BDL is working to identify and characterize proteins that can be measured in blood to determine which patients need to have a colonoscopy. The investigators are using both antibody and lectin arrays to identify proteomic and glycomic markers. Their microarray approach employs a technology with unparalleled sensitivity, enabling interrogation down to the low picomolar concentration of the circulating proteome. The best candidates will then be evaluated with more conventional ELISA and lectin-based methods.

- The Johns Hopkins University/University of Pittsburgh BDL is also working to identify and characterize proteins that can be measured in blood to determine which patients need to have a colonoscopy. The BDL has focused on the nuclear matrix to identify several sera markers, CCSA-2, 3 and 4, that are associated with colon cancer. Utilizing antibodies produced against these markers, they have demonstrated the ability to differentiate individuals with cancer or advanced adenomas from healthy individuals. This team is refining and optimizing the assays for these proteins to make them more robust and accurate.

- The Fred Hutchinson Cancer Research Center/Case Western Reserve University BDL is working to discover novel methylated genes that can be used as early detection markers and predictive markers. They propose to develop and validate epigenetic signatures of colon adenomas and early stage colon cancers to be used in a stool-based biomarker assay. Cutting-edge and complementary approaches of HumanMethylation27 DNA arrays and bisulfite deep sequencing will be used to determine the genome-wide methylation status of colon adenomas and colorectal cancers. This panel of methylated markers will then be further developed into a stool-based biomarker assay.

- The Evanston Northwestern Healthcare Corporation/Northwestern University BDL is developing an innovative light-scattering technology (partial wave spectroscopy or PWS) to identify the micro-architectural correlates of the genetic/epigenetic changes in field carcinogenesis that can be used to screen for early stage colorectal cancers. They propose to use PWS signatures of rectal tissue brushings to predict the presence of colon cancer or advanced adenomas. This should provide a highly accurate, minimally intrusive technique to allow risk-stratification and hence tailoring a CRC screening regimen.

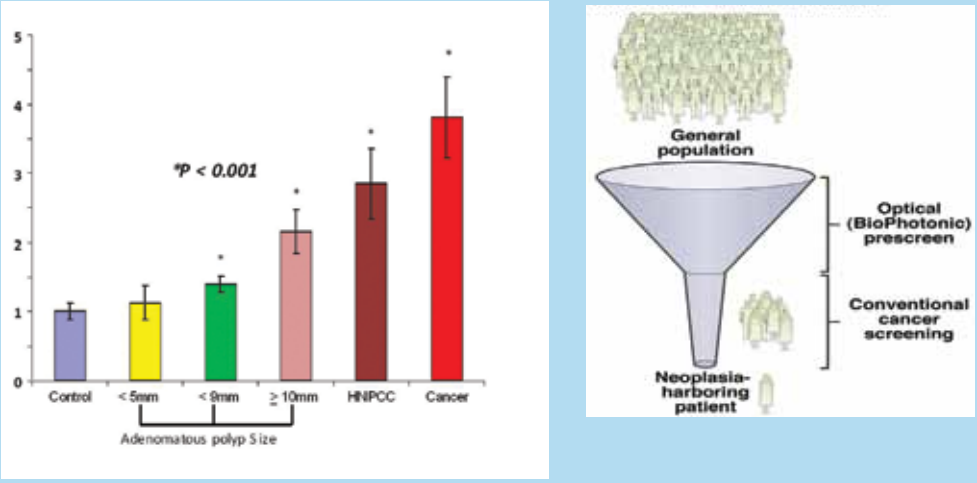
- The Great Lakes New England CVC has started a validation trial of several stool-based biomarkers and a blood-based biomarker to determine the sensitivity and specificity for detection of colorectal adenocarcinoma and high-grade dysplasia (this trial is described in more detail in Part III, Chapter 2). Multiple aliquots of stool, sera, plasma and urine will be collected from 6,000 patients prior to colonoscopy. All of the specimens should be collected within 3 years and will be available to validate markers being developed in the EDRN BDLs and by other investigators both within and outside the EDRN.

- The University of California Los Angeles BRL is working with investigators from the Great Lakes New England CVC to optimize and standardize the ELISA assay for the blood-based protein biomarker, galectin-3 binding protein to be tested in the biomarker validation trial being conducted by this CVC.

Novel Optical Technology Approach to Colon Cancer

The Evanston Northwestern Healthcare Corporation/Northwestern University EDRN BDL has developed a novel optical technology, partial wave spectroscopic microscopy (PWS) that enables probing of the nanoscale cellular structure for the micro-architectural correlates of the genetic/epigenetic alterations of field carcinogenesis. Using a simple brushing of the visually normal rectal mucosa, they were able to predict the presence of screen relevant neoplasia throughout the colon with approximately 90% accuracy (n=155). As demonstrated in Figure II-2, rectal PWS marker disorder strength (Ld) mirrored the neoplastic risk throughout the colon. The laboratory is continuing biomarker refinement and validation studies of this novel approach alone and also combining imaging with molecular biomarkers. Their vision is that the primary care physician could obtain samples via a simple digital rectal exam and could use this approach to personalize the CRC screening recommendations as illustrated.

Figure II- 2. Disorder strength for different patient populations



Pancreatic Cancer – Strategic Goals

Pancreatic cancer is the fourth most common cause of cancer death in the United States, although it comprises only approximately 2% of new cancer diagnoses. The median survival for all patients diagnosed with pancreatic cancer is less than six months while the 5-year survival is less than 5%. This dismal survival rate is largely due to being unable to diagnose this cancer at a stage when the option of curative surgery is still possible. The current standard for diagnosis of pancreatic cancer is the serum marker, CA 19-9. In an asymptomatic population, this biomarker has a positive predictive value below one percent. Better biomarkers need to be developed for the early detection and diagnosis of pancreatic cancer, thereby reducing its high mortality.

Commonly used imaging methods, such as endoscopic ultrasound, abdominal CT scan, or MRI are inadequate for the detection of early stage pancreatic cancer. These imaging methods are however, increasingly detecting mucinous cystic lesions in the pancreas. A significant proportion of these cystic lesions represent intraductal papillary mucinous neoplasias (IPMN), as 15% to 25% of IPMNs have the potential to progress toward adenocarcinoma. Study of these cystic lesions is important and has potential in early detection of pancreatic cancer in asymptomatic patients.

Primary goals are to identify and validate biomarkers to identify populations at risk for the development of pancreatic cancer; identify and validate biomarkers for early detection; and determine whether biomarkers and imaging can be used in tandem to better identify cysts with potential to progress toward pancreatic cancer.

Pancreatic Cancer – The Plan

Investigators in the Collaborative group are working toward the following:

- Determine whether the detection of glycans (sugar molecules) on proteins can improve biomarker performance;
- Utilize functional genomics to identify pathways altered in pancreatic cancer and use this information to develop cancer specific biomarkers;
- Combine biomarkers with imaging techniques; and
- Collect pancreatic cystic fluid samples to develop biomarkers that can distinguish benign cystic lesions from lesions that are likely to progress to pancreatic cancer.

Investigators in this Collaborative Group are performing a number of studies on discovery, verification and validation of molecular markers for pancreatic cancer in support of the EDRN strategic plan.

- Investigators at the University of Nebraska BDL are developing a broad based diagnostic test for pancreatic cancer that enhances the CA 19-9 test and incorporates elements of early detection of cancer by detecting autoantibodies to tumor specific glycopeptide structures found on mucins. Autoantibodies to these specific structures are produced at sufficiently high concentrations to be detected. The overall objective is the development of a set of three integrated tests for serum: to quantify autoantibodies, to quantify immune complexes, and to quantify circulating mucins for both oligosaccharide and core protein (on the same molecule).
- Investigators at the M. D. Anderson Cancer Center BDL utilize a functional genomics approach toward biomarker discovery by targeting the chromosome 3p12 pathway to tumorigenesis in pancreatic cancer. Three separate expression platforms have been used to develop a panel of genes differentially expressed in pancreatic tumor/normal samples and which represent potential genes in the 3p pathway, including a novel tumor suppressor/polarity regulator, DEAR1. These investigators are also exploring the use of miRNAs as early detection biomarkers and are using a panel of 1,536 SNPs and relevant covariates in 1,000 pancreatic cancer patients to develop a risk model to identify those individuals most likely to develop pancreatic cancer at an early age and who could be stratified for screening using the biomarker panels developed.
- The overall hypothesis of the Van Andel/University of Pittsburgh/Memorial Sloan-Kettering BDL is that the expression and glycosylation of specific proteins are significantly different between cysts with high malignant potential and cysts with low malignant potential, and that these molecules form accurate biomarkers for the diagnosis of pancreatic cysts. Their technological strategy is built on the powerful combination of novel glycoproteomics biomarker discovery methods and complementary antibody array methods for the high-throughput and precise profiling of multiple protein and glycan candidates.

In an effort to further promote collaboration among the members of the Colorectal and Other Gastrointestinal Cancers Collaborative Group and to increase the efficiency of validating promising biomarkers for colorectal and pancreatic cancer, five multi-institutional collaborative studies have been launched, two of which are focused on colon cancer and three on pancreatic cancer.

Team Project: Adenoma Detection

Background and Clinical Goals: Colonoscopy reduces incidence and mortality of colorectal cancer primarily through the removal of adenomas. However, as non-colonoscopic-based methods, such as FOBT, detect only about half of the high-risk adenomas (adenomas ≥1 cm), there is a need for biomarkers detectable in stool, blood, or urine with improved sensitivities for advanced adenomas. The goal of this team project is to discover and preliminarily validate biomarkers for the detection of advanced adenomatous polyps that can improve detection sensitivity by at least 20% over current non-invasive diagnostic technologies.

Design: This team project will employ a phased approach to the discovery, verification and validation of markers of advanced adenomas in bodily fluids. High-quality biosamples from tissue, plasma, or serum or DNA samples from the same 300 subjects will be distributed to multiple laboratories to permit discovery research on the same samples. Preliminary classifiers will be analyzed from data generated in each laboratory. In the next step, those laboratories with promising biomarkers will receive specimens from 475 different subjects to test biomarker performance. In this step, investigators will not know which specimens came from cases and which from controls, and will be required to use the results of their assays to predict which subjects had adenomas and which did not.

Discovery Phase: Adenomas have much less necrosis and cellular turnover than invasive cancers and, consequently, aberrant proteins, mutated or methylated DNA, or translated mutated DNA are in such low concentrations in body fluids that they are below the limits of detection by current technologies. EDRN laboratories have access to a diverse portfolio of new technologies that have the potential to identify and quantify protein and DNA markers at these very low concentrations.

Investigators at Case Western Reserve University will use a genome-wide profiling method known as Reduced Representation Bisulfite Sequencing (RRBS), which maps DNA methylation at single-nucleotide resolution. Investigators at the Fred Hutchinson Cancer Research Center (FHCRC) will use quantitative CpG methylation assessment with quantitative methylation specific PCR (qMSP or MethyLight). Genes will be assessed individually and in aggregate to assess for a locus-specific phenomenon as well as for global alterations in methylation. Other investigators at FHCRC will use high-density antibody microarrays to perform both broad proteomic and glycomic screens and specifically targeted analyses to discover/validate biomarkers of colon adenomas. Investigators at Johns Hopkins will focus on nuclear matrix proteins (NMPs). NMPs are tissue and tumor-specific and present a unique opportunity to develop tumor specific markers for screening, diagnostic and even prognostic assays.

Verification Phase: The performance of the promising biomarkers discovered in the first phase will be verified using blinded specimens. The investigators will be required to predict which subjects had adenomas and which did not.

Validation Phase: If any of these biomarkers or combination of biomarkers have sufficiently high sensitivity and specificity, they will be validated using the specimens being collected as part of the EDRN validation trial of biomarkers for colorectal cancer (see Part III, Chapter 2).

Team Project: Biomarkers to Predict Adenoma Recurrence

Background and Clinical Goals: Randomized trials of fecal occult blood testing and flexible sigmoidoscopy demonstrate that identification and removal of adenomatous polyps can reduce the subsequent incidence of and mortality from colorectal cancer. Individuals who have adenomas are at higher risk of forming recurrent adenomas and interval colorectal cancer, compared to individuals who have no history of adenomas. Consequently, subjects with a personal history of colon adenomas are recommended to undergo surveillance colonoscopy at a shorter interval (3 to 5 years) than people without a history of colon adenomas (10 years).

The burden of surveillance colonoscopy is substantial, accounting for at least one fourth of the colonoscopies performed annually, and surveillance is among the most commonly cited indications for colonoscopy. All subjects with adenomas undergo surveillance, but our ability to gauge their risk of subsequent neoplasia is crude and imprecise. Furthermore, surveillance colonoscopy is imperfectly applied, with evidence for overutilization in subjects at low risk and underutilization in subjects at higher risk. Some of this misapplied surveillance is a function of the fact that we need improved markers to determine patient risk, beyond simple characterization of whether adenomas were present or not, and what type of adenomas they were. Identification of markers that can better predict individual risk is needed so that surveillance colonoscopy, an expensive resource, can be targeted to the population that truly needs it. The goal of this team project is to identify markers that stratify or delineate individuals who may benefit from chemoprevention or require more intensified surveillance regimens.

Study: This team project will employ a phased approach to the discovery of biomarkers to predict the risk of adenoma recurrence and interval cancers. Four BDLs will participate in this project. The first phase will assess the variability of biomarker expression within individuals across the rectum, left colon and right colon (intra-individual variation). Those biomarkers with sufficient performance will then be examined to determine differences in their expression in normal colonic mucosa between subjects with normal colon, non-advanced adenoma, advanced adenoma, and cancer. After the markers are characterized and their expression within and across patients is well characterized, the markers that show an ability to distinguish people with adenomas or cancer from those without colon neoplasia will be tested for their predictive value in characterizing risk of developing adenomas.

Team Projects: Discriminating Early Lesions that Will Progress to Pancreatic Cancer

Background and Clinical Goals: Increasingly, cystic lesions of the pancreas are being detected with the widespread use of high resolution abdominal imaging. Although such cystic lesions are often benign, a significant portion of them may represent intraductal papillary mucinous neoplasias (IPMNs) as 15% to 25% of patients with such lesions go on to develop pancreatic adenocarcinomas. Hence, the reliable discrimination of IPMNs from benign lesions presents an opportunity for preventive intervention prior to their malignant progression. EDRN is currently in the process of verifying and validating a large number of biomarkers for the early detection of pancreatic cancer and for distinguishing cystic lesions that are likely to progress to pancreatic cancer.

Intraductal Papillary Mucinous Neoplasms: An Opportunity for Cancer Prevention

Intraductal Papillary Mucinous Neoplasms (IPMNs) are cystic tumors of the pancreas that are produced from the secretion of a thick fluid called mucin from abnormal cells lining the pancreatic ductal system. The cells continue to secrete the fluid, which builds up in the pancreatic ductal system and creates a cyst that can be seen on a CT scan. These lesions have the ability to turn into cancer. The primary clinical problem is that we do not know how quickly this process occurs, or in how many patients it will occur.

The cells producing these cysts go through a series of changes that can lead to cancer and are grouped into three categories: low grade, moderate grade, and high grade dysplasia. Cells with high-grade dysplasia look like pancreatic cancer cells under a microscope, but they have not yet invaded through the lining of the ductal system of the gland. The reason there is such an interest in IPMNs is the increasing incidental identification of these lesions on CT scans performed for non-specific abdominal complaints. Some of the cysts are benign, however, in some patients these lesions have the ability to progress into a cancer. An endoscopic ultrasound is done to sample the fluid that is in the cyst. The difficulty is determining if the cyst is benign or represents an IPMN.

These lesions represent a great opportunity for physicians to effectively intervene and prevent pancreatic cancer. However, the challenge in managing these patients is predicting who will develop cancer and how quickly that will occur. The treatment to remove an IPMN is a significant operation with substantial morbidity and measurable mortality. So we are faced with a dilemma: a patient has a lesion that might progress to pancreatic cancer, but the treatment to remove it has significant risks. This requires very careful discussion with the patients. What we need is the ability to determine when a patient's lesion has high grade dysplasia. Right now what we look for are findings on the CT scan or endoscopy that suggest that the lesion has high grade dysplasia. Unfortunately, these tests are currently not very accurate.

An important area of ongoing research is to develop biomarkers to identify patients with high grade dysplasia. We are using cystic fluid to look for various biomarkers of high grade dysplasia. One outcome we hope will come from this research effort is that in the future, we could pull out some of the cystic fluid and look for a marker that tells us whether high grade dysplasia is present. If it is absent, we would not need to even discuss whether there should be an operation.

Vignette provided by **Dr. Peter Allen**, a Principal Investigator on pancreatic cancer in the EDRN Biomarker Development Laboratory at Memorial Sloan-Kettering Cancer Center.

1. Prioritization of Biomarkers for Early Detection of Pancreatic Cancer

Study: Many promising candidate biomarkers have been discovered and are being developed by EDRN investigators. EDRN has constructed a high-quality reference set (serum and plasma) consisting of well-characterized samples from early stage pancreatic cancer. Controls include healthy individuals, chronic pancreatitis, and benign biliary obstruction (see Part IV, Chapter 3 for details). Investigators within the Colorectal and other GI Cancers Collaborative Group have teamed up to utilize this reference set in order to prioritize and verify the performance of diverse types of biomarkers for the early detection of pancreatic cancer. These include autoantibodies to tumor specific glycopeptides; mucin type glycoproteins; glycosylation pattern of specific proteins associated with pancreatic cancer; miRNAs; genomic markers; and SNP's.

2. Development of Biomarkers that Distinguish Benign Pancreatic Cysts from Precancerous Lesions

Study: EDRN is currently in the process of establishing a second pancreatic cancer reference set for IPMNs. Top-performing biomarkers in distinguishing pancreatic cancer cases from controls using the pre-validation reference set, as described above, will be further tested on the IPMN reference set for their ability to distinguish between benign pancreatic cysts from premalignant lesions. Panels of diverse biomarkers will be also tested for improved predictive performance.

3. Spectral Markers for Risk Assessment of Pancreatic Cancer

Study: EDRN investigators will evaluate the possibility of combining the imaging modality partial wave spectroscopy (PWS) with molecular biomarkers, including tissue miRNAs to detect pancreatic cancer. This will be done by correlating cellular disorder with changes in miRNA expression in cells obtained from duodenal brushings.

Rapid Autopsy Sample Collections to Study Natural History of Pancreatic Cancer

The natural history of cancer development is being addressed by a unique rapid autopsy program at the University of Nebraska Medical Center. The purpose of the rapid autopsy is to obtain tissue as quickly as possible after death, thus minimizing post mortem nucleic acid (particularly RNA) degradation. Autopsies are performed within 2 to 3 hours of death. In addition to obtaining neoplastic tissue and adjacent benign pancreas, abdominal or thoracic viscera (in cases of metastatic lesions), lymph nodes, liver, lung, spleen, and all possible involved and uninvolved organs are collected.

The Hollingsworth laboratory has available over 40,000 high-quality, well-annotated samples (in gram quantities) from rapid autopsy of 34 patients with pancreatic cancer. Also collected are normal pancreatic tissues from appropriately consented patients with no history of pancreatic cancer who died of other causes. This has enabled the assembly of groups of tumors with known characteristics, including differentiation status, metastatic distribution and other clinical features. In addition, gene expression, microRNA and CGH data are available on many of these samples. This is a valuable resource for studying the natural history of pancreatic cancer.

Lung and Upper Aerodigestive Cancers

Lung cancer is the most common lethal cancer, often attributed to the advanced stage of disease at time of diagnosis. Helical computer tomography (CT) scanning has gained increased use to screen individuals at high-risk for lung cancer. This technology has shown promise as a sensitive test in the detection of early-stage cancer, however, helical CT scans also exhibit a high false-positive rate. To exclude unneeded intervention on benign conditions, evaluation of these indeterminate lung nodules often requires additional invasive procedures, such as bronchoscopy, percutaneous needle biopsy and/or surgery.

The Lung and Upper Aerodigestive Cancers Collaborative group has made considerable progress in addressing the challenging questions of early detection of lung cancer and mesothelioma and for identifying clinically significant lung cancer, as illustrated below.

A list of the members of the Lung and Upper Aerodigestive Cancers Collaborative group can be found in the Laboratory Listing in the Appendix.

Major Accomplishments

- Identification of an autoantibody panel of markers (c-Myc, Cyclin A, Cyclin B1, Cyclin D1, CDK2 and Suvivin) for early detection of lung cancer
- Identification of miRNA profile in sputum for early lung cancer detection
- Identification of TCF21 and Ras-related GTPase gene methylation as diagnostic markers of lung cancer
- Development of 8-oxyguanine DNA glycosylase (8OGG), Alkyl-adenine DNA Gycosylase and APE1 Endonuclease DNA repair capacity-based assays as risk measures of lung cancer
- Demonstrated decreased osteopontin levels after NSCLC resection and rise with disease recurrence for monitoring of relapse
- Established three standard reference sets for rapid triage and validation of markers for lung cancer detection
- Identified and validated SMRP (Mesothelin) as a diagnostic marker of mesothelioma
- Identified mir29c miRNA as a prognostic marker for mesothelioma
- Establishing a nasal epithelial expression profile as a surrogate marker for lung cancer

Lung Cancer - Strategic Goals

Lung cancer continues to be the most lethal cancer in the United States with more than 160,000 deaths per year. The incidence is driven predominantly by smoking, with a prevalence of lung cancer in the smoker and former-smoker population in the range of 10% to 15%. About 80% of patients with lung cancer have a significant history of smoking.

CT imaging or chest X-rays are currently used on patients suspected of having lung cancer or, in some cases, are being used to screen high-risk cohorts. Although the sensitivity of CT imaging is very high, the false-positive rate is also high. In the EDRN, a variety of lung cancer markers have been pursued, including panels of gene methylation markers, mitochondrial DNA mutations, mitochondrial number, and chromosomal abnormalities. Within a high-risk population of smokers, these markers were not able to distinguish non-diseased smokers from lung cancer patients, even though the markers clearly differentiate smokers from nonsmokers. These markers persist later in life as evidenced in cohorts of former smokers, who stopped smoking 5 years previously. These findings highlight how smoking induces profound molecular alterations in the epithelial linings of the airway, setting molecular alterations on a path towards neoplasia.

The strategic goals to address current clinical needs are: determine if a test for early detection of lung cancer be developed that achieves a performance above the overriding risk factor that smoking presents; determine whether biomarkers can be developed for use in conjunction with CT imaging in order to identify which patients with an indeterminate nodule of ≤3 cm should receive further work-up for diagnosis of lung cancer; and determine whether a test can be devised to improve the negative predictive value and better classify true positives to enhance the performance of imaging techniques in samples from the National Lung Screening Trial (NLST).

Lung Cancer - The Plan

Previously, studies were performed testing an autoantibody panel in conjunction with CT imaging that showed considerable promise. These autoantibodies proved their utility in a blinded validation study of prediagnostic lung cancer specimens from CARET. Future plans are being made to include additional autoantibody panels from other investigators within and outside of the EDRN in order to maximize the potential outcome and determine whether different markers are complementary and thus improve the sensitivity/specificity of the panel. The clinical objective is to determine if an autoantibody marker panel can be developed to augment CT diagnosis of lung cancer in asymptomatic individuals by:

- Testing a longitudinal collection of sera from a number of CT-detected cases from the Clinical Validation Centers at New York University, Vanderbilt University, and possibly other sites in conjunction with matched controls that were also subjected to CT imaging. This validation will reveal whether the biomarker panel augments CT imaging in predicting which patients are likely to have early stage lung cancer thus requiring follow-up. It is anticipated that with the completed NLST this study will be expanded to make use of the wealth of samples collected under a CT-screening protocol from that long-term study.
- Examining ground-glass opacities in lungs of high-risk smokers. More than half of high-risk (>20 pack-years) smokers present with non-calcified nodules 4-8 mm in size, while approximately another 10% of the subjects have ground-glass opacities. Both types of lesions require follow-up to determine whether they are cancerous. The purpose of the study is to identify biomarkers in blood or sputum that are applicable to the diagnosis of the above suspicious abnormalities and complement CT-screening.

- Testing markers in prospectively collected samples from NLST cohorts to determine how early a lung cancer can be detected prior to diagnosis.

- Embarking on biomarker discovery in tumors from nonsmokers or distant former smokers. This study may offer a window into early diagnosis in this smaller but growing subset of lung cancers. This study is being conducted in partnership with the Canary Foundation.

In support of the plan investigators in the Lung Cancer Collaborative Group conduct a number of studies on discovery, verification and validation of molecular markers along the EDRN strategic goals:

- A multi-site team at the UCLA/Boston University BDL is studying chronic injury, inflammation and aberrant epithelial cell repair that can result in a field of injury that supports lung carcinogenesis. Elucidating the molecular, biochemical and cellular events associated with these processes will allow for the detection of early stage lung cancer in non-invasively collected biospecimens from the mouth, nose and blood.

- The assessment of the methylation status of several key genes is another approach for the detection of lung cancer in high-risk patients. This work done at the Johns Hopkins BDL is directed toward determining the global methylation status in lung cancer cell lines and primary lung tumors using new tiled arrays to identify genome-wide regions of methylation. These studies will be complementary to other genomic efforts being conducted by the members of this collaborative group. Following this approach, investigators at the Johns Hopkins BDL identified a panel of genes with promoter hypermethylation present in plasma of lung cancer patients that can be used for the detection of the disease.

- The New York University CVC noted increased plasma S-adenosylmethionine, a key substrate required for methylation, in lung cancer patients compared to other high-risk smokers. In other studies performed by the NYU Clinical Validation Center in collaboration with the Wistar Institute, a 29-gene expression pattern in white blood cells was identified that could discriminate lung cancer from high-risk controls .

- Also at NYU, BDL investigators utilize a multifaceted approach for the rapid discovery and eventual validation of early biomarkers for malignant pleural mesothelioma, an asbestos-related malignancy that is typically detected at an advanced stage when curative options are not feasible. This discovery approach involves three separate but related platforms: genomic expression, miRNA expression, and glycan arrays to detect anti-glycan autoantibodies in blood. Based on this approach, patients with asbestos exposure without mesothelioma can be segregated from those with malignant mesothelioma. The approach can be also used to predict progression and survival outcomes for patients with the cancer.

- A number of studies are being conducted by investigators at the Boston University and the Ohio State University BDLs to identify mRNA and miRNA expression profiles in the airway and blood that can serve as non-invasive diagnostic biomarkers for preclinical detection of lung cancer among high-risk current and former smokers. These biomarkers will be validated using prospectively collected specimen cohorts. Furthermore, EDRN BRLs at NIST and the University of Maryland are developing diagnostic miRNA assays and a miRNA biomarker panel in sputum for the early detection of non-small-cell lung cancer. One of the BRLs has found that 26 miRNA genes were differentially expressed between NSCLC tissues and normal tissues. To further aid these studies, the University of Maryland BRL will develop and standardize a real time reverse transcriptase

PCR assay to measure multiple miRNAs in sputum; optimize a panel of sputum miRNA markers; and validate the miRNA panel for early detection of lung cancer. These studies should provide a profound impact for the early detection of NSCLC.

- The Vanderbilt CVC will perform repeated measurements of clinical imaging (chest CT) variables as well as molecular biomarker signatures, some of which are obtained from bronchoscopic procedures. The hypothesis is that increased levels of the biomarkers are associated with lung cancer and that measurement of the biomarker over time is likely to provide an advantage over the epidemiological and imaging data to detect the disease earlier. This in turn can lead to a larger number of patient candidates for diagnostic surgery intervention. The CVC will work closely with its partnering BDL also at Vanderbilt to facilitate the discovery and verification of protein biomarker candidates for plasma-based detection of lung cancers. This work will apply standardized "shotgun" proteomic analyses for biomarker discovery in tissues and a standardized mass spectrometry-based platform that this lab has developed for quantitation of biomarker candidates in plasma.

- In collaboration with the non-profit Canary Foundation, investigators are studying why certain genes, such as MGMT, BHLHB5, and BOLL, are more frequently methylated in lung cancers in nonsmokers than in smokers. A rising provocative question is: why are these genes targeted by methylation in nonsmokers, but not in smokers? Investigators co-funded by the EDRN and the Canary Foundation are conducting genome-wide interrogation of DNA methylation to focus on etiology-specific pathways of gene activation and identify DNA methylation markers for plasma-based early detection of all lung cancers. An array of genomic and proteomic platforms are being used to comprehensively delineate significant events, including mutational hot-spots, epigenomic changes, and copy-number changes in human cell lines and tissues. Whole-genome sequencing of lung cancer specimens is also being performed by a collaborator in China.

Team Project: Development of biomarkers to discriminate benign from malignant indeterminate pulmonary nodules

The results from the National Lung Screening Trial (NLST) have refocused the search for new approaches to early diagnosis of lung cancer. This eight-year NCI study of "high-risk" current and former smokers found that low dose CT scans decreased lung cancer mortality by 20%, pointing to the benefits that might be achieved as a result of more frequent detection of early stage lung cancers. Despite this positive outcome, about 25% of subjects in the study had CT abnormalities, 96% of which represented non-cancerous changes. The current management of abnormalities found on chest CT leads to over-diagnosis characterized by expensive follow-up, diagnostic procedures and potentially serious complications resulting from unnecessary treatment.

The goal of this Lung Collaborative Group team project is to improve the diagnostic evaluation of patients with indeterminate pulmonary nodules. Molecular biomarkers diagnostic for lung cancer will be measured in minimally invasive and non-invasive biospecimens and examined for their ability to distinguish between patients with malignant or benign indeterminate pulmonary nodules that are incidentally detected in high risk smokers. Achieving this goal would suggest that these biomarkers could also be applied in the setting of indeterminate pulmonary nodules that are detected as part of radiographic screening for lung cancer. Sensitive and specific biomarkers that discriminate

between patients with malignant or benign indeterminate pulmonary nodules could spur early treatment and thereby improve lung cancer outcomes, while minimizing complications from over-diagnosis in patients without lung cancer. Thus, this team project seeks to validate a series of molecular biomarkers (including mRNA, miRNA, proteomic, and antibody signatures in the airway and blood) that have been developed by EDRN investigators as effective diagnostic tools of lung cancer for patients with indeterminate pulmonary nodules.

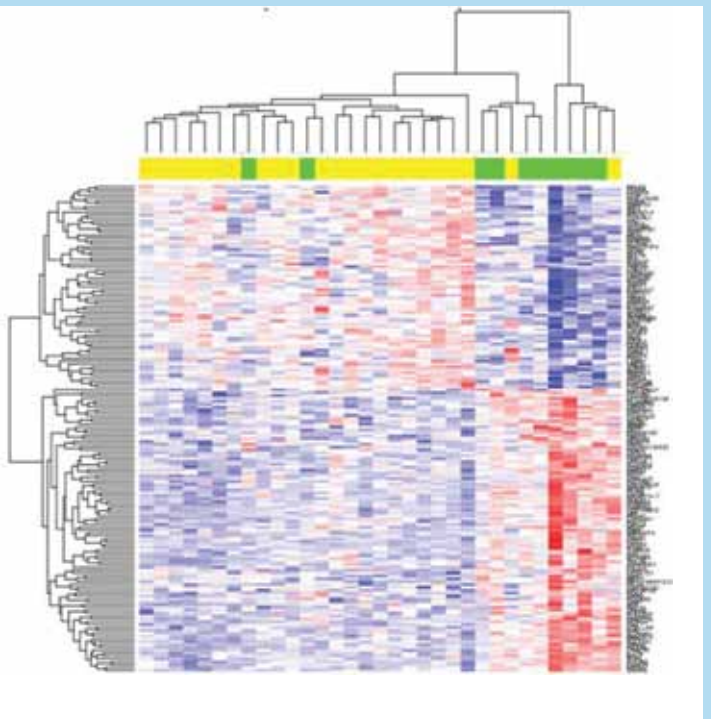
Gene Expression Changes in Nasal Cells: May Be Early Diagnostic Biomarker for Lung Cancer

A simple, minimally-invasive technique using cells from the interior of the nose could help clinicians detect lung cancer in its earliest – and most treatable – stages.

The team at Boston University wanted to determine if a minimally invasive site like the nose could be used to diagnose cancer in its early stages, when there is a much greater chance to reduce mortality from the disease. This study is motivated by their earlier work, which found gene expression differences in cells lining the mainstem bronchus in patients with cancer. The nose can be considered an extension of the same respiratory tract.

Their recent studies reveal that the vast majority of gene expression differences associated with smoking, the major risk factor for lung cancer, are similarly altered in both the bronchial and nasal airway. They identified 170 genes that were differentially expressed between patients with and without lung cancer.

They also found that genes linked to colon cancer, as well as genes that trigger cell division and blood vessel growth, were expressed at higher levels in patients with cancer. Genes involved in tumor suppression were expressed at lower levels in these patients. The results of the current study are an initial indication that simple nasal brushings could offer an alternative to lung biopsy and other invasive techniques aimed at identifying lung cancer in its early stages.



Genes differentially expressed in nasal epithelium samples from patients with and without lung cancer (yellow and green respectively). Blue indicates lower relative expression while red indicates higher relative expression.

Prostate and Other Urological Cancers

Prostate Cancer - Strategic Goals

Prostate cancer is the most frequently diagnosed non-skin cancer in men in the United States. In 2010, there were 218,000 men diagnosed with prostate cancer. The prevalence of the diagnosis makes the disease a major health burden. While the majority of the diagnosed men will survive the disease, about 15% will die from it, a rate that is affected by over-diagnosis and the consequent over-treatment. The major objectives of the EDRN Prostate Collaborative Group are to identify, develop and validate biomarkers for cancer risk and earlier detection, especially of aggressive forms of prostate cancer. In addition, the group is developing biomarkers to assist clinicians in decision making for questions such as who needs primary and secondary biopsies, which may help to reduce the number of unnecessary procedures.

The Prostate and other Urological Cancers Collaborative Group has made considerable progress in addressing the challenging questions of early detection of prostate cancer and especially for identifying clinically significant disease, as illustrated below.

A list of the members of the Prostate and Other Urological Cancers Collaborative Group can be found in the Laboratory Listing in the Appendix.

Major Accomplishments

- Discovery of cancer specific gene fusions (TMPRSS2-ETV1; TMPRSS2:ERG) for the first time in solid epithelial tumors - in prostate cancer and associated with aggressiveness of disease
- Completed MSA validation study for early detection of bladder cancer
- Completed validation of %[-2]proPSA to improve PSA-based detection of prostate cancer; IVD pending FDA review
- Completed validation of PCA3 as a urine-based test for early detection and diagnosis of prostate cancer
- Developed molecular assay and FISH for detection of T2-ERG fusion in urine and tissue, respectively. Both assays are offered by CLIA labs
- Constructed standard reference sets for rapid triage and validation of biomarkers for prostate cancer
- Discovered sarcosine as a metabolite biomarker in tissue and urine
- Developed Prostate Cancer Risk Calculator (http://www.prostate-cancer-risk-calculator.com)
- Developed non-coding RNA biomarkers

Prostate Cancer - The Plan

To accomplish the strategic goals the collaborative group proposed the following plan:

- Develop biomarkers, which could discriminate between indolent and aggressive prostate cancers based on a variety of "omics" approaches (genomics, epigenomics, proteomics and metabolomics);
- Develop urine- and blood-based assays for all promising prostate cancer biomarkers;
- Conduct rigorous clinical evaluation of promising biomarkers for early detection and of aggressive prostate based on recently discovered cancer specific fusion transcripts such as TMPRSS2-ERG, TMPRSS2-ETV1, etc.;
- Perform a meta-analysis on the performance of all validated promising prostate cancer biomarkers to select the best markers for earlier detection of clinically significant prostate cancers;
- Evaluate reactive cancer stromal markers in tissue and urine samples; and
- Combine biomarkers with imaging modalities for better and earlier detection of aggressive prostate cancer.

Investigators in the Prostate and Other Urological Cancers Collaborative Group conduct new biomarker discovery, pre-validation and validation projects by pursuing the EDRN strategic goals.

- Researchers at the University of Texas Health Science Center San Antonio CVC are conducting a validation study on gene polymorphisms, which are associated with critical pathways, such as enzymes involved in androgen metabolism, DNA repair and hereditary susceptibility genes. If validated, such markers will be added to the prostate cancer risk calculator (http://www.prostate-cancer-risk-calculator.com). EDRN investigators discovered that ~55% of all prostate cancers harbor genetic rearrangements such as fusion genes (e.g., TMPRSS2-ERG, SLC45A3-ETV1 etc.). Such cancer specific markers provide an important opportunity to develop a non-invasive, highly specific diagnostic test for the early detection of prostate cancer.
- At the Beth Israel Deaconess Medical Center (BIDMC) CVC, researchers are comparing the accuracy of detection of TMPRSS2-ERG fusion in post-digital rectal exam (DRE) urine with the detection of the fusion from biopsy. The prevalence of TMPRSS2-ERG rearrangements will be also examined in a community-based study with emphasis on African Americans. Finally, a correlation between this genetic rearrangement and the development of aggressive cancer will be examined in a longitudinal study based on active surveillance of 300 men at BIDMC.
- PCA3 is a non-coding prostate-specific RNA that was reported to be frequently overexpressed in prostate tumor cells. EDRN investigators at the University of Michigan BDL are collaborating with Gen-Probe, Inc. on a multi-institutional validation study testing the utility of a PCA3 urine test to assist clinicians in decision making for biopsy or repeat biopsy. Interim analysis of data from this study shows great promise. In addition, a quantitative PCA3 urine test demonstrated potential as an adjunct to current methods for prostate cancer diagnosis.
- Epigenetic modification of DNA (particularly within the 5' promoter region and the first exon) is a common alteration in cancer-related genes and is often associated with complete or partial repression of transcription. This is one of the mechanisms resulting in inactivation of tumor suppressor genes of cancers. Scientists at the Johns Hopkins University BDL developed a panel of promoter methylated genes which could serve as biomarkers of prostate cancer and high-grade prostatic intraepithelial neoplasia (HGPIN). The team developed an assay based on the percentage of methylated alleles and determined that these values are higher for the genes APC and RARß2 in HGPIN and carcinoma as compared to normal prostate tissue.

- Using gene expression microarrays and selected reaction monitoring-mass spectrometry (SRM-MS) investigators at the Fred Hutchinson Cancer Research Center BDL identified stromal-based biomarkers. For example, CD90 (Thy-1) is a cell surface protein frequently over-expressed in prostate cancer stroma. Furthermore, increased levels of CD90 peptide fragments have been detected in all urine samples of prostate cancer patients tested so far. Another stomal marker, CD10, is frequently absent in the majority of prostate tumors. Gleason 4 and CD10+ cancer cells are associated with poor outcomes. The laboratory is also developing tests to detect AGRA2, an overexpressed protein in tumor tissue.

- Validation of tissue expression of candidate biomarkers such as CCL3, CCL4, AGRA2, and others is being carried out using the intelligent prostate cancer tissue microarray TMA by the UCLA BRL. The University of California, Irvine BDL developed a classifier composed of 114 differentially expressed genes in reactive stromal cells adjacent to the cancer epithelial cells. The classifier was recently tested on 364 samples and showed promising results (sensitivity=98% and specificity= 88%). These results indicate that the prostate cancer microenvironment exhibits reproducible changes useful for categorizing the presence of tumors in patients when a prostate biopsy is derived from near the tumor site. The laboratory plans to further refine this classifier and test it on a larger cohort.

- Investigators at another BDL at Johns Hopkins University are applying a mass spectrometry-based analytical method to measure different glycosylated forms of glycoproteins from complex biological samples by coupling a glycopeptide extraction strategy for specific glycosylation with SRM-MS. Using this approach, they have recently demonstrated that the relative abundance of glycosylated PSA isoforms did not correlate with total PSA protein levels. Interestingly, a sialylated PSA was differentially distributed between tumor and non-tumor tissues. These findings suggest that glycosylated isoforms of glycoproteins can be quantitatively

Metabolomic Biomarkers for Non-Invasive Detection of Prostate Cancer

The "Warburg effect" is an observation that most cancer cells produce energy via a high rate of glycolysis followed by lactic acid fermentation, rather than through a comparatively low rate of glycolysis followed by oxidation of pyruvate in the mitochondria as typically observed in normal cells. This observation pointed to the fact that there is a different profile of certain metabolites in cancer cells. EDRN Investigators at the University of Michigan hypothesized that unbiased metabolomic profiling of prostate cancers could result in detection of differential metabolite levels and perturbed metabolic pathways.

A combination of high-throughput liquid-and-gas-chromatography-based mass spectrometry was used to profile 1,126 metabolites across 262 clinical samples of prostate cancer (42 tissues and 110 each of urine and plasma). These unbiased metabolomic profiles were able to distinguish benign prostate, clinically localized prostate cancer and metastatic disease. Sarcosine, an N-methyl derivative of the amino acid glycine, was identified as a metabolite whose concentration was highly elevated in metastatic prostate cancer. Interestingly, initial observations suggest that this metabolite can be also detected non-invasively in urine.

Sreekumar A, Poisson LM, Rajendiran TM, et al. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature. 2009 Feb 12;457(7231):910-4.

Fusion Transcripts as Prostate Cancer Specific Biomarkers

Until recently it was thought that activation or inactivation of oncogenes through genomic rearrangements is limited to hematopoietic malignances and soft tissue sarcomas. However, recent discoveries by the EDRN BDL lead by Arul Chinnaiyan at the University of Michigan reveal that genomic rearrangements resulting in fusion transcripts are a common mechanism of activation of oncogenes in prostate cancers.

The majority of prostate cancers harbor gene fusions of the 5'-untranslated promoter region of the androgen-regulated transmembrane protease serine 2 (TMPRSS2) with genes of the erythroblast transformation-specific (ETS) transcription factor family. The most common fusion is between TMPRSS2 and v-ets erythroblastosis virus E26 oncogene homolog (ERG) resulting in TMPRSS2-ERG, which is detected in approximately 50% prostate cancers. ETS fusions can be detected noninvasively in the urine of men with prostate cancer.

The BDL identified over 25 different fusion transcripts associated with chromosomal rearrangements, each of which could serve as a potential cancer biomarker. In addition, tissue and functional studies suggest a specific role for ETS fusions in the progression to invasive carcinoma. Currently, the laboratory is developing a validation trial where TMPRSS2-ERG is tested as a biomarker for earlier detection as well as diagnosis of prostate cancer.

Tomlins SA, Aubin SM, Siddiqui J, et al. Urine TMPRSS2: ERG Fusion Transcript Stratifies Prostate Cancer Risk in Men with Elevated Serum PSA. Sci Transl Med. 2011 Aug 3;3(94):94ra72.

Tomlins SA, Laxman B, Dhanasekaran SM, et al. Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. Nature. 2007 Aug 2;448(7153):595-9.

Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science. 2005 Oct 28;310(5748):644-8.

analyzed and have the potential to serve as cancer biomarkers. The laboratory is currently pursuing new candidate biomarkers based on their differential glycosylation in prostate cancer specimens. Their partnering BRL at Johns Hopkins University is validating 15 promising prostate glycoproteins that were previously identified as candidate biomarkers. They are developing ELISA and glycan-linked immunosorbent assays (GLISA) for each of the candidate biomarkers to be followed up by construction of multiplex assays for analytical and clinical validation. In addition, the BRL will develop and validate multivariate predictive models which will be applied to identify aggressive prostate cancer.

- By combining the latest ultrasound technology with tumor angiogenesis-targeted microbubbles, the BDL at Stanford University is improving the imaging of prostate cancer. Microbubbles are gaseous bubbles encased by lipid shells functionalized with antibodies specific for the target tumor-associated angiogenesis marker VEGFR2. When introduced into the bloodstream, VEGFR2-targeted microbubbles bind to the endothelial cells of the tumor neovasculature to provide enhanced contrast during ultrasound. Incorporated into transrectal ultrasound, this approach will increase the accuracy of detection during the screening process. With the emergence of new prostate cancer cell surface associated markers this approach may enable targeted screening for earlier detection of prostate cancer.

Team Projects

Background and Clinical Goals: Given the high prevalence of non-aggressive prostate cancer, the team projects focus on the early detection of cancers that are likely to progress. Investigators developed highly interactive experiments that will eventually validate biomarkers in Phases II and III in urine and in tissue samples. The 3-year projects were developed around two major independent themes: pre-validation/validation of urine-based biomarkers for early detection of clinically significant prostate cancer, and for assisting clinicians in decision making; and molecular classification and diagnosis of clinically significant prostate cancer based on tissue-discovered markers.

Project 1: Evaluation of Urine PCA3 and TMPRSS2-ERG

Study: The project will determine whether multiplex combination of urinary measurement of TMPRSS2-ERG fusion and PCA3, together with serum PSA and percent-proPSA can improve the early detection of histopathologically aggressive prostate cancer.

Project 2: Establishing Community-Based Normal Distributions of Urinary PCA3 and TMPRSS2-ERG

Study: The frequency of detection of post-DRE urinary PCA3 and TMPRSS2-ERG fusion, and percent-free PSA and percent-proPSA in serum of a community-based sample of men undergoing prostate cancer screening will be determined by this project. The goal is to examine whether frequency differs between African-American men as compared to men of other racial backgrounds, and between Hispanic-American men as compared to men of non-Hispanic ethnicity. The broad representation of many EDRN sites in this project facilitates a strong accrual of racial and ethnic minorities, thus improving the generalizability of the anticipated outcome from this work.

Project 3: Measuring Multiple Cancer Secreted Proteins

Study: Selected Reaction Monitoring (SRM) quantitative mass spectrometry (MS) will be used to identify and quantify proteins in urine. The study will discern aggressive from indolent prostate cancers by measuring multiple proteins secreted or shedded by prostate cancer in voided urine.

Project 4: Tissue Microarrays for Biomarker Validation

Study: Identification and testing of available tissue microarrays (TMAs) is essential for evaluation and validation of diagnostic and prognostic biomarkers. The immediate goal is to identify TMAs that are available through participating sites. A list of available TMAs is being developed which should contain statistically powered clinically defined end points, such as organ confined versus cancers with extracapsular extension; or cancers with lymph node involvement or with distant metastasis; hormone refractory cancers; neuroendocrine carcinomas. In addition, they should be accompanied by clinical data such as survival. A "Google-Document" will be developed that will allow all participants to monitor the current status of available and potentially available TMAs.

Project 5: Upgrading Gleason Scores on Radical Prostatectomies

Study: This project will determine the clinical parameters associated with upgrading Gleason scores on radical prostatectomies. The current EDRN biopsy collection protocol records significant clinical and pathology data. A subset of these individuals has undergone prostatectomy at their respective participating institutions. At the University of Michigan and Weill Cornell Medical College sites alone, there will be approximately 500 cases where patients have entered an EDRN protocol for the collection of urine and blood samples prior to surgery by the end of the second year. The major goal of this project is to ensure that all clinical

parameters that can be used in conjunction with future biomarkers are assessed. This project will also include mandatory review of current literature and available data for modeling.

Project 6: Molecular Sub-Classification of ETS and Non-ETS Rearrangements

Study: This project addresses the molecular sub-classification of ETS and non-ETS rearrangements in prostate cancer. Significant data is developing to suggest that similar to the classification of acute myeloid leukemia (AML), prostate cancer can be molecularly classified to subcategories by specific genetic alterations including gene fusions and mutations. This project will try to determine the frequency of each category. This process is similar to developing a classification for AML even prior to known treatments. An immediate goal will be to identify individuals with targetable mutations. This study will use assays pioneered by EDRN investigators, which have been used in over 100 studies throughout the world and some of which are planned to be commercially available (e.g., the TMPRSS2-ERG in situ assay licensed and distributed by Ventana/Roche).

Project 7: Prostate Cancer Phenotypes and Clinical Outcomes

Study: This project is focused on prostate cancer phenotypes and clinical outcomes. Two markers, AGR2 and CD10, can group patients into three survival or disease risk categories: 1) AGR2hi/CD10lo for high survival/low risk; 2) AGR2lo/CD10hi for low survival/high risk; and 3) AGR2hi/CD10hi and AGR2lo/CD10lo for intermediate survival/risk. Additional informative sub-grouping will be provided by markers associated with Gleason pattern 4 vs. pattern 3 cells, and CD10+ vs. CD10– cancer cell types. Further reactive stroma markers will be added based on a recently developed classification.

Project 8: Vascular Tissue Biomarkers for Molecular Imaging

Study: This project, centers on the identification of cancer-related vascular tissue biomarkers for molecular imaging. This project directly addresses a need to develop molecular biomarkers that can identify tumor endothelium. Validated biomarkers will then be handed off for further development by the Stanford BDL group. These biomarkers will enhance the group's ability to monitor cancers over time and seek associations with biomarkers developed by other team projects of this collaborative group. A specific example might be a particular vascular pattern seen in ERG-rearranged prostate cancers as compared to non-rearranged cancers given the important role of ERG in vascular development.

Part Three

Meeting Clinical Needs

Biomarker Validation

The goal of both the core research projects conducted by the EDRN Biomarker Developmental Laboratories (BDLs), Clinical Validation Centers (CVCs) and Biomarker Reference Laboratories (BRLs), as well as by the EDRN team projects described in Part II, is to develop biomarkers for clinical use. A critical step in the process of moving a biomarker from discovery to clinical use is validation of both its analytical performance and its clinical performance.

Experience has taught us that an individual laboratory or center cannot be both the "prosecutor and the judge" of their biomarker. Rather, it is crucial that both the analytical and clinical validation (judgment) be performed by laboratories other than that of the discoverer (the prosecutor). The EDRN BRLs work with the biomarker discoverers to independently validate the analytical performance of their assays. Analytical performance concerns the reproducibility, precision and accuracy of the assays. The CVCs are the EDRN's primary resource for the clinical validation of biomarkers. Clinical validation of a biomarker or panel of biomarkers determines its capacity to accurately distinguish patients with cancer from those without, or to detect preclinical cancer. The CVCs work both with EDRN investigators and non-EDRN investigators, including companies that want to have their biomarkers validated. The concept of clinical validation asks:

- Whether a test is clearly described;
- Whether the spectrum of patients with and without disease is adequate;
- Whether the assessment of test and disease status is conducted in an unbiased manner;
- If the test performance is summarized by the important terms of sensitivity, specificity and positive and negative predictive values; and
- If the true presence or absence of disease can be established for all individuals.

The EDRN BRLs are the primary network resource for laboratory and clinical validation of early detection biomarkers, for technological development and standardization of assay methods, and for refinement of existing methods. The CVCs conduct Phase II and III validation and epidemiological research, and provide high-quality biological specimens for discovery research. The EDRN Data Management and Coordinating Center (DMCC) coordinates validation studies with respect to protocol and manual operation procedures development, by developing and maintaining study database and specimen tracking systems, and by providing necessary statistical support.

Section 1: Rigorous Validation Process

One of the chief pitfalls for effective biomarker discovery and development is bias. In addition to the establishment of the EDRN five-phase approach for biomarker development, a coherent and comprehensive set of guidelines for study design for the discovery and evaluation of biomarkers for use in screening and early cancer detection, diagnosis, or prognosis has been delineated.

Samples collected prospectively according to the "prospective-specimen collection, retrospective-blinded-evaluation" (PRoBE) study design, developed by EDRN investigators at the DMCC, aim to

eliminate primary sources of bias in specimens by collecting all samples from identical cohorts under standard operating protocols prior to diagnosis of disease. A set of rigorous study design standards and guidelines are described, which further address issues regarding the rate of false discovery due to the use of samples of convenience and introduction of bias and data over-fitting.

The PRoBE study design includes four key components, which relate to: 1) the clinical context and outcomes; 2) criteria for measuring biomarker performance; 3) the biomarker itself; and 4) the sample size included in the study.[25] Many EDRN reference sets described in Part IV were assembled using the PRoBE design, offering some of the highest standards for biomarker testing.

Section 2: Process for Prioritizing Clinical Validation of Biomarkers

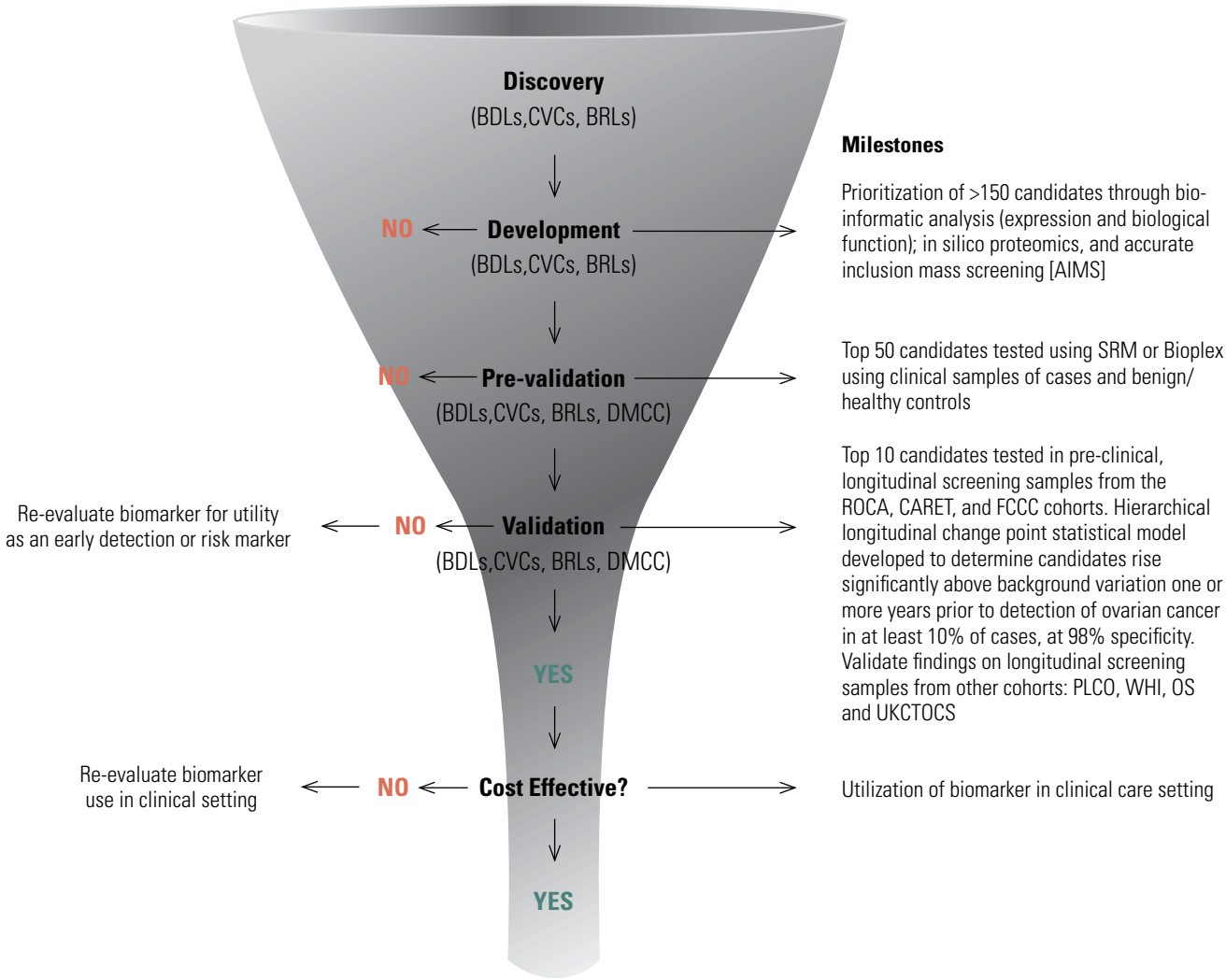
Because clinical biomarker validation studies are expensive and only a few biomarkers may succeed, prioritization is achieved through a triage system via a series of "go or no-go" decisions. EDRN developed a series of approaches for the rapid triage of hundreds of candidate biomarkers, first by in silico evaluation on the basis of a number of criteria, such as expression, biological function, tissue specificity, followed by a rapid targeted Mass Spectrometry screening approach for their presence and detectability in blood or other bodily fluids. The ultimate prioritization of the most promising candidates is conducted through testing in statistically powered EDRN standard reference sample sets, which have been constructed for the biomarkers' intended clinical use. Only when biomarkers successfully pass this rigorous process of prioritization and verification, a large clinical validation study is planned.

The "go/no go" decision point schema determines which biomarkers identified through discovery can progress to verification, and then to validation. This model has been developed throughout EDRN's existence and is refined as knowledge increases. The network has clear milestones for reaching a "go" or "no-go" decision during the development process, as shown in Figure III-1. Milestones for "go" or "no-go" decision are based on 1) biological strength; 2) statistical criteria for biomarker diagnostic performance characteristics; 3) meeting anticipated clinical use; and 4) projected economic impact (i.e., cost-benefit effect).

Most of the biomarkers identified in discovery do not meet the rigorous standards set for continuing development and therefore do not receive a "go" decision. The EDRN Network Consulting Team and FDA colleagues are consulted to review this information to see what adjustments must be made in the discovery process to focus future research only on those biomarkers likely to be able to move to validation.

[25]Pepe, MA, Feng, Z, Janes, H, et al. Pivotal evaluation of the accuracy of a biomarker used for classification or prediction: standards for study design. J Natl Cancer Inst. 2008 October 15; 100(20): 1432-8.

Figure III-1: An illustration of the "funnel effect" of the vertical integration of biomarker discovery, development, verification, and validation of ovarian markers



Biomarker Validation Studies

Section 1: Completed Validation Studies

Bladder Cancer

Microsatellite Analysis (MSA) Detection of Urinary Sediment: Multi-Institutional Study

The MSA validation study tested a panel of 15 microsatellite markers for its sensitivity and specificity for detecting bladder cancer. The analysis was performed in two phases:

a) A cross-sectional analysis was conducted with 266 individuals with a superficial urothelial bladder cancer, 83 healthy controls, and 93 individuals with potentially confounding conditions (benign prostatic hypertrophy, hematuria, infections in the genitourinary system, or the presence of foreign bodies, such as urinary stones or stents).

b) A longitudinal analysis was performed with the 266 individuals after their bladder tumors were excised, during 2 years of surveillance for tumor recurrence.

In the first phase of the study, completed in 2009, the primary analysis was performed for the pre-specified panel decision rule in which any of the 15 individual markers is positive. The results revealed that the minimum performance characteristics of at least 80% sensitivity and 90% specificity were not reached. Therefore, the 15-marker panel was considered insufficient to alter clinical practice.

In the second phase of the study, further laboratory assays were done on the follow-up samples. A positive assay for three or more individual markers was associated with a 2.4 fold greater odds of having bladder cancer. Although the panel demonstrated a statistically significant association with the presence of cancer, the sensitivity of the panel was 33% at a specificity of 83% - i.e., slightly different from the findings in the initial phase analysis. It was concluded that the panel could distinguish individuals with and without cancer, but did not have adequate performance to be useful as a screening test for bladder cancer. The MSA study has resulted in the creation of serum and urine DNA specimen reference sets relevant to screening biomarkers in the context of bladder cancer, and has provided EDRN with its first real-world data set to test statistical methods for the analysis of longitudinal marker data.

Colorectal Cancer

Serum-Based Protein Markers for the Detection of Colorectal Cancer

The aim of this study is to validate the sensitivity and specificity of two serum proteins (CCSA-3 and CCSA-4) in their ability to distinguish colon cancer from benign colon. The projected clinical use of the validated biomarkers is for general population screening for colorectal cancer.

Biomarkers to be tested by this study include Colon Cancer Specific Antigen (CCSA)-3 and -4, nuclear matrix proteins specific to colorectal cancer that can be measured in sera.

Preliminary results using the EDRN colorectal cancer reference set indicated that measurement of CCSA-3 and CCSA-4 either alone or in combination did not have sufficient sensitivity and specificity to warrant proceeding to a full validation trial. The investigators are currently optimizing the assays for CCSA-3 and CCSA-4 and performing prevalidation studies on additional biomarkers, including nuclear matrix protein CCSA-2. If a panel with sufficient performance characteristics can be identified, it will be validated using the specimens collected as part of the EDRN Colorectal Cancer Biomarker Validation Trial.

Esophageal Cancer

Barrett's Esophagus Progression Biomarkers

The aim of this study is to test a three-class stratification model for risk of progression from Barrett's esophagus to esophageal adenocarcinoma. Patients classified as at high risk for progression would undergo endoscopy more frequently than the currently recommended surveillance interval; those at intermediate risk, at the customary interval; and the low risk group would undergo endoscopy less frequently.

A multicenter, double-blinded validation study was performed to determine the ability of the methylation status of three tumor suppressor genes (p16, HPP1, RUNX3) in combination with four clinical parameters as biomarkers to predict progression from Barrett's esophagus to esophageal adenocarcinoma. Progression or non-progression was determined at 2 years and 4 years. Methylation was assayed in 145 non-progressors and 50 progressors using real-time quantitative methylation-specific PCR. The methylation biomarker-based panel was able to predict neoplastic progression in Barrett's esophagus but lacked sufficient predictive power to warrant a larger study. Adding other methylation markers to the panel to increase its predictiveness is being explored.

Liver Cancer

Alpha-fetoprotein, Des-Gamma-Carboxyprothrombin, and Lectin-Bound Alpha-Fetoprotein in Early Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the fourth most common cancer in the world. The incidence and mortality from liver cancer are virtually identical, indicating the high mortality and importance of early detection. Liver cirrhosis is the most important risk factor for the development HCC and patients with cirrhosis comprise a high-risk group recommended for surveillance.

Alpha-fetoprotein (AFP) is the standard biomarker for detecting HCC. EDRN initiated a biomarker validation study to test two biomarkers, des-gamma carboxyprothrombin (DCP) and lectin-bound AFP (AFP-L3%) as potential serum markers for detecting early stage HCC. EDRN conducted a Phase II biomarker validation study to compare the ability of DCP and AFP-L3% with that of AFP in differentiating cirrhosis from early HCC[26]. This study was important in determining the performance of AFP and DCP for early stage HCC. Seven centers across the country enrolled a total of 836 patients: 417 had cirrhosis and 419 had HCC. Within the HCC cases, 208 patients had early stage HCC. The results from this study concluded that AFP (at a cut-off of 10.9 ng/ml) was more sensitive than DCP or AFP-L3% for diagnosing early stage HCC. AFP had a sensitivity of 66% and a specificity of 82%. The study also determined that the performance of DCP is affected significantly by the underlying causes of liver disease for the diagnosis of early stage HCC, specifically for cases with a viral etiology. The sensitivity of DCP for early stage HCC with a viral etiology is 79%, at a specificity of 60%, as compared to a sensitivity of 67% for AFP, at a specificity of 79%. The combination of AFP and DCP, however, did not improve the overall performance.

[26] Marrero JA, Feng Z, Wang Y, et al. Alpha-fetoprotein, des-γ carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. Gastroenterology. 2009:137:110-118.
[27] Qiu J, Choi G, Li L, et al. J Clin Oncol. 2008 Nov 1;26(31):5060-6. Epub 2008 Sep 15. Occurrence of autoantibodies to annexin I, 14-3-3 theta and LAMR1 in prediagnostic lung cancer sera.

Lung Cancer

Lung cancer continues to be the most lethal cancer in the United States yet biomarkers to detect this disease at early stages are not currently available. Humoral response by the immune system during early stages of cancer is one potential means to exploit early detection through the identification of antibodies to discrete cancer antigens. The biomarker development laboratory of Dr. Samir Hanash had discovered autoantibodies in lung cancer patients to several proteins. To determine whether these autoantibodies are found in a greater number of patients and to determine how early prior to diagnosis of lung cancer they may be found, this laboratory conducted a validation study using samples prospectively collected prior to diagnosis from the Carotene Retinol Efficacy Trial (CARET).

Sera from the CARET study were selected among 85 lung cancer cases within one year prior to diagnosis and 85 matched controls. Autoantibodies tested on the sera were against Annexin 1, PGP 9.5, LAMR 1, and 14-3-3 theta.[27] This validation confirmed that three of the autoantibodies (annexin 1, LAMR 1 and 14-3-3 theta) are found prior to diagnosis in a subset of about one-third of the lung cancer patients. In particular, antibodies to annexin 1 may be found as early as 1 year prior to the onset of symptoms and diagnosis of lung cancer. Ongoing studies will search for additional autoantibodies to increase the proportion of the cancers that can be detected using this approach.

Mesothelioma

Malignant mesothelioma (MM) is an aggressive and debilitating cancer associated with exposure to asbestos or related fibers. Since it usually presents at a clinically advanced stage, the median survival is about 7-10 months. Earlier detection of MM in asymptomatic patients affords better therapeutic interventions and survival, but this would depend on the development of a non-invasive, sensitive and specific biomarker.

Two blood-based markers which show promise for detecting MM have been reported in recent years. Soluble Mesothelin Related Peptide (SMRP) is expressed by normal mesothelial cells but is highly overexpressed in MM. Several laboratories have published encouraging results for SMRP in the diagnosis of MM in asbestos-exposed cohorts. The BDL at NYU has identified osteopontin as another secreted protein highly expressed in MM. This laboratory headed a series of Phase II validation studies to test SMRP and osteopontin in the diagnosis and early detection of MM.

In the first stage of the validation study, sera from 817 individuals with a history of asbestos exposure (165 MM and 652 controls) were obtained from cohorts in Wittenoon, Western Australia, Mt. Sinai, New York, and Libby, Montana with additional mesothelioma cases provided from three additional cohorts at NCI, Wayne State University and NYU. In this blinded validation study SMRP was found to have a performance consistent with previous results for this marker; however, the performance of osteopontin was considerably poorer than found in earlier studies from the NYU lab and it is, most likely, related to the stability of the marker in serum. Thus, the data from these studies indicated that SMRP is a robust serum biomarker for MM whereas osteopontin appears susceptible to degradation.

The second phase of the validation study included serum samples from the Beta-Carotene and Retinol Efficacy Trial (CARET) that followed 4,060 asbestos-exposed subjects

EDRN Investigator Receives FDA Approval for Ovarian Cancer Test, OVA1™

Case Study: A 50 year old woman presented at a hospital clinic. Physical examination revealed masses in the pelvic area. While the result of ultrasonography was not diagnostic, her serum CA125 was elevated. Her physician was suspicious of ovarian cancer and a surgical procedure was planned.

To aid in the clinical assessment of cases such as this, the OVA1™ test offers additional relevant information regarding suspicion of ovarian cancer. The OVA1™ test is a qualitative serum test that combines the results of five biomarkers into a single numerical score. The five biomarkers are CA125, pre-albumin (transthyretin), Apolipoprotein A1, transferrin, and beta 2-microglobulin. The OVA1™ score is calculated using OvaCalc software with values between 0 and 10. The expected values for the probability of malignancy are: Pre-menopausal: High OVA1≥5.0; Low OVA1<5.0; Post-menopausal: High OVA1≥4.4; Low OVA1<4.4.

The intended use of OVA1™ test 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. The test is not intended as a screening or stand-alone diagnostic assay.

The invention of the biomarker panel together with the algorithm for the OvCalc software was developed by EDRN investigators at the Johns Hopkins University and has been licensed to Vermillion Inc. The company conducted a clinical trial with 516 patients from 27 clinical sites. It was cleared by the FDA for clinical use on September 11, 2009 as the OVA1™ test. This is the first proteomics IVDMIA (In vitro diagnostic multivariate index assay) ever cleared by the FDA.

Zhang Z, Bast RC, Yu Y, et al. Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. Cancer Research 2004; 64: 5882-5890.

for 9 to 17 years, 49 of whom developed MM. SMRP and osteopontin were again tested in samples from the 49 MM cases and 96 matched controls to determine if they could predict MM development years before clinical presentation. SMRP was found to be significantly elevated in the year prior to diagnosis. However, this elevation was not statistically significant for the time interval 1 to 2 years before diagnosis. Since only serum samples were available from CARET, osteopontin exhibited, as expected, poor performance in predicting MM. Hence, validation of osteopontin will require further testing in plasma. These results suggest SMRP is elevated at least within a year or possibly earlier prior to diagnosis and should receive further consideration as an early detection biomarker of MM.

Ovarian Cancer

EDRN-SPORE-PLCO Phase II/Phase III Study for Validation of a Biomarker Consensus Panel for Early Detection of Ovarian Cancer

Most cases of ovarian cancer are diagnosed late with poor survival. However, when an early diagnosis is made, survival can reach up to 90%. Pelvic examination and sonography along with CA125 remains the mainstay of ovarian cancer diagnosis. CA125 gives a true positive result for about 50% of stage I ovarian cancer patients, but is inadequate as an early detection biomarker when used alone. EDRN and SPORE investigators joined forces to develop a two-phase study to identify a panel of biomarkers that could be used to screen for ovarian cancer.

In the first phase, investigators at Brigham and Women's Hospital, Fred Hutchinson Cancer Research Center, University of Texas M. D. Anderson Cancer Center, the University of Pittsburgh Cancer Institute, and Yale University validated more than 70 biomarkers that performed well in preliminary studies in their respective laboratories. This Phase II study was done on a blinded set of sera from 80 early stage and 80 late stage ovarian cancer cases collected at diagnosis, 160 controls with benign disease and 480 healthy controls. Five of the markers and CA125 were evaluated by the Yale University investigators on a separate cohort of sera from 156 newly diagnosed ovarian cancer patients and 362 healthy women. Top-performing markers in Phase II specimens included CA125, HE4, transthyretin, CA15.3, and CA72.4 with a sensitivity ranging from 0.73 to 0.40 at 95% specificity.

In the second phase of the study, 32 top-performing markers from Phase II were evaluated in a Phase III study using PLCO proximate specimens obtained from women within 6 months and up to 7 years prior to the diagnosis of ovarian cancer. These included 118 cases from women with ovarian cancer and 476 matched controls. With the exception of transthyretin, the tested markers had similar or better sensitivity in specimens drawn within 6 months of clinical diagnosis as compared with results from the previous, Phase II study. Unfortunately, the performance of all markers declined sharply in Phase III study specimens collected more than 6 months prior to diagnosis. The study results demonstrated that despite the many promising new markers for ovarian cancer, CA125 remains the single-best biomarker in the Phase II and Phase III specimens tested.[28]

In the Phase III validation study, five predictive models, each containing six to eight biomarkers, were evaluated according to a predetermined analysis plan. The sensitivity of each predictive model to distinguish ovarian cancer from controls at 98% specificity was first determined for the entire set of samples. In this study design, one model showed comparable performance with CA125 in terms of sensitivity and specificity, whereas the remaining models had poorer performance than CA125. A second component of this study involved unblinding half of the samples to perform training with each model, followed by testing on the remaining samples. In this case again, only one model showed performance that was merely comparable with CA125 alone. Thus, all tested markers failed to show improvement over CA125.[29]

A commentary published in the same issue of the journal as the two reports describing the data emphasized that this important EDRN Phase III biomarker study, nested in a large, randomized screening trial, will serve as the standard against which future analyses of this kind should be judged.[30] Another commentary said the study represents a major collaborative effort involving nine of the leading research centers for ovarian cancer research in the U.S. and is the first systematic and reliable comparison of a large number of candidate biomarkers for early detection of ovarian cancer in a sample set well-suited for this purpose.[31]

[28] Cramer DW, Bast RC Jr, Berg CD, et al. Ovarian cancer biomarker performance in prostate, lung, colorectal, and ovarian cancer screening trial specimens. Cancer Prev Res. 2011 Mar;4(3):365-74.
[29] Zhu CS, Pinsky PF, Cramer DW, et al. A framework for evaluating biomarkers for early detection: validation of biomarker panels for ovarian cancer. Cancer Prev Res. 2011 Mar;4(3):375-83.
[30] Mai PL, Wentzensen N, Greene MH. Challenges related to developing serum-based biomarkers for early ovarian cancer detection. Cancer Prev Res. 2011 Mar;4(3):303-6.
[31] Jacobs I, Menon U. The sine qua non of discovering novel biomarkers for early detection of ovarian cancer: carefully selected preclinical samples. Cancer Prev Res. 2011 Mar;4(3):299-302.

Prostate Cancer

Serum Protein Profiling Using Mass Spectrometry

The EDRN pioneered a systematic evaluation of protein profiles as a candidate biomarker using surface-enhanced laser desorption-time of flight mass spectrometry (SELDI-TOF-MS) and Matrix-assisted laser desorption/ionization (MALDI) platforms for diagnosis of prostate cancer. The study was initiated in 2003 to test published claims that mass spectrometry-based protein profiling in ovarian, prostate, lung and other cancers could serve as reliable biomarkers. Network investigators designed a three-stage study to: validate the reproducibility of the platform (Stage 1), validate diagnostic use of protein patterns (Stage 2), and conduct a clinical validation (Stage 3), using well-annotated, prospective specimens from stratified risk groups and prostate cancer cases and controls. The Stage 1 study confirmed the portability and reproducibility of the SELDI-TOF-MS platforms among the eight participating institutes. The stage 2 study could not substantiate previous claims that protein profiling significantly discriminates between prostate cancer and non-cancer. Thus, SELDI/MALDI-TOF-MS-based protein expression profiling approach did not perform well enough to warrant the launch of the stage 3 prospective study.

Validation of Serum %[-2]proPSA as a Biomarker

Free PSA consists of enzymatically inactive isoforms associated with either prostate cancer or benign prostatic hyperplasia. PSA with a truncated pro-leader sequence, [-2]proPSA, has been previously shown to be associated with prostate cancer tissue and is also found in serum. The primary objectives of this study were to validate the serum %[-2]proPSA ([-2]proPSA/free PSA) biomarker for the improvement of early detection, diagnosis and risk assessment of prostate cancer, and to test whether it could be used to predict who needs a biopsy.

Secondary objectives were: 1) to determine whether [-2]proPSA and/or %[-2]proPSA tests are complementary to other PSA derivatives in a multi-marker model to improve the diagnosis and risk assessment of prostate cancer and reduce unnecessary biopsies; 2) to validate the serum %[-2]proPSA marker for the improvement of diagnosis and risk assessment of prostate cancer and reduce unnecessary biopsies in patients with PSA ranges of 2-10 ng/mL and 2-4 ng/mL; and 3) to determine whether %[-2]proPSA or a combination of markers studied can aid in the identification of aggressive cancers as determined by Gleason score.

This study was carried out by EDRN investigators in collaboration with Beckman-Coulter, Inc. Serum PSA, free PSA, and [-2] proPSA were measured using the Beckman Coulter ACCESS II immunoassay analyzer. At four EDRN CVCs participating in the study, 566 subjects met the eligibility criteria, where 43% of the participants were determined to have prostate cancer on biopsy. At a specificity of 70%, the sensitivity of %[-2]proPSA was 54%; also, within the 4-10 ng/mL and 2-10 ng/mL PSA ranges, it had significant improvement over individual PSA forms. With increasing Gleason score the %[-2]proPSA levels also increased. Overall, this prospective multicenter study demonstrated potential clinical utility for the detection of prostate cancer and was associated with histopatholgically aggressive disease. The study was recently published[32] and Beckman-Coulter submitted the proPSA test for FDA approval.

[32] Sokoll LJ, Sanda MG, Feng Z, et al. A prospective, multicenter, National Cancer Institute Early Detection Research Network study of [-2]proPSA: improving prostate cancer detection and correlating with cancer aggressiveness. Cancer Epidemiol Biomarkers Prev. 2010 May;19(5):1193-200.

Section 2: Validation Studies Underway

Phase II/Phase III biomarker validation studies are underway at each of the EDRN Clinical Validation Centers, which is in line with their main responsibilities within the network. In addition, two large, multi-center validation studies have been initiated, one focused on colorectal cancer and one on prostate cancer.

Colorectal Cancer

Colorectal cancer remains the most common fatal cancer among non-smokers in the U.S. The lifetime incidence is sufficiently high at 6%, or 1 in 18, to justify population screening. Both colorectal cancer incidence and mortality have decreased in the past decade, developments that are attributed at least in part to more effective screening and surveillance.

Wide-scale screening using fecal occult blood tests (FOBT) results in up to a 30% reduction in colorectal adenocarcinoma mortality, but at the expense of many colonoscopies in patients without neoplasia. Also, colonoscopy has a significant cost and carries a small, but non-negligible risk of complications. Approximately half the eligible population does not undergo colonoscopy because of concerns about cost, discomfort, complications, embarrassment and accessibility. Consequently, patient-friendly approaches are needed with the combined features of high accuracy for cancer, high grade dysplasia, adenomas, broad acceptability to the general population, health care providers, and third party payers.

The EDRN has initiated a validation trial to determine the ability of several new biomarkers in either blood or stool to detect colorectal cancer and advanced adenomas and to compare their performance to the fecal immunochemical test (FIT). The goal is to develop a non-invasive test to determine who is likely to have cancer or advanced adenomas and needs to have a colonoscopy. The performance of the following biomarkers will be determined using colonoscopy as the gold standard: stool vimentin methylation; serum galectin-3 ligand; FIT; Exact Sciences Corp. (Madison, WI) stool DNA panel; and other biomarkers being developed by EDRN and non-EDRN investigators.

Six thousand asymptomatic subjects age 50 to 80 years undergoing routine colonoscopy screening for colorectal cancer from community and major medical center outpatient settings across multiple centers and consortia will be recruited. Subjects will visit or be visited by research staff prior to any colonoscopy preparative procedure. After completing informed consent, they will complete EDRN data element forms and a food frequency tool. The following biosamples will be collected from each participant prior to initiating the preparation procedure for colonoscopy: serum, plasma, urine, DNA from circulating white cells, and stool. In addition to the validation of the above mentioned biomarkers, the samples collected will form a colorectal cancer validation set. In this validation set, multiple aliquots of each sample type will be compiled and stored for future biomarker validations.

Prostate Cancer

PCA3 Validation Study for Prostate Cancer

Prostate cancer antigen 3 (PCA3) is a prostate-specific non-coding gene that was detected in over 90% of prostate cancers. PCA3 is significantly over-expressed in cancer tissues versus benign tissues. Clinically, RNA for this gene is detectable in the urine and prostatic fluid of men with prostate cancer. However, the RNA levels are independent of prostate volume. A urine-based assay was developed by GenProbe, Inc. based on the collection of urine after an attentive digital rectal examination to increase the number of prostate cells shed into the urine. The PCA3 assay score is based on a ratio between PCA3 RNA and PSA mRNA. In preliminary studies, with an initial cutoff value set at 50, this assay demonstrated a sensitivity of 69% and specificity of 79%.

The EDRN validation study is a multi-institutional study involving 11 institutes. The intended use of PCA3 is to supplement PSA in clinical prostate cancer detection to improve upon the clinician's ability to determine prostate cancer risk and thereby guide decisions for a prostate biopsy. It is intended to be used in men with an initial
or a repeat prostate biopsy. The primary aims of the study are:

- To evaluate the positive predictive value (PPV) of PCA3 for initial biopsy population and negative predictive value (NPV) of PCA3 for repeat biopsy population in a multi-center cohort of men without prior history of prostate cancer.
- To develop a novel urinary reference set consisting of whole urine/urine sediment as well as plasma/serum necessary for future pre-validation studies of urinary biomarkers.

The secondary aims are: to evaluate the sensitivity, specificity, PPV, NPV and absolute risk prediction by PCA3 alone and combined with other biomarkers and clinical variables in the detection of prostate cancer overall; and to evaluate the correlation between PCA3 and prostate biopsy tumor grade. Accrual of 928 patients with rising PSA has occurred; 273 had cancer on initial biopsy and 69 had cancer on repeat biopsy. The study will be completed in the fall of 2011.

Part Four

Creating Standards for Translating Biomarkers

Standard Specimen Reference Sets

EDRN is one of the first organizations to recognize the importance of standardized, prospective collection of biologic samples in the context of clinically-relevant circumstances to discover and accelerate the validation of biomarkers for cancer detection and prognosis. Samples from cancer cases and controls are collected prospectively for each major cancer organ site, including cancers of the ovary, breast, lung, colon, liver, prostate and pancreas.

The sample sets contain comprehensive clinical, demographic, and epidemiologic information that help in the rapid evaluation of technologies and biomarkers in pre-validation before initiating prospective, large, and expensive validation trials.

Material transfer agreements for reference sets are available to researchers with projects approved by the relevant EDRN Collaborative Group. The agreements permit NCI to distribute samples to qualified investigators and their institutions, limiting the use of samples to studies approved by the EDRN Collaborative Group.

Lung Cancer Reference Sets

Several reference sets for lung cancer have been assembled to serve different clinical purposes (Table IV-1). Two sets focus on pre-validation of biomarkers for the diagnosis of lung cancer and target individuals at high risk for lung cancer or abnormal chest x-ray (CXR) or chest computed tomography (CT), but outside of the context of a CT screening trial. In this category, set A was assembled using samples that existed in eight institutions, whereas set C was collected prospectively from four institutions using a uniform standard operating protocol according to the PRoBE study design.

A third set, B, focuses on pre-validation of biomarkers for early diagnosis (screening) of lung cancer by targeting lung cancer patients diagnosed in the context of a CT-based screening trial of high-risk individuals. The clinical question to be addressed using set B relates to whether a plasma biomarker has added diagnostic value to current tests (CT scan and/or positron emission tomography (PET) scan) for the diagnostic evaluation of CT-detected pulmonary nodules.

Prostate Cancer Reference Sets

The Prostate and other Urinary Cancers Collaborative Group developed several cohorts for clinical validation of promising cancer biomarkers.

Reference set #1: This reference set was developed for a rapid pre-validation and selection (triage) of candidate biomarkers. Promising biomarkers were then tested in a multi-institutional validation study. All samples were retrospectively collected at three EDRN Clinical Validation Centers (CVCs). Uniform inclusion/exclusion criteria were used to select the samples. Similar criteria were used to select the prospectively collected cohorts which are described below. The sample size was 123 serum specimens. This reference set, that now has been depleted, played an important triaging role in selecting biomarkers for multi-institutional validation studies.[33] This reference set was initially used to verify the %[-2]proPSA as a cancer biomarker by the EDRN.[34]

[33] Sokoll LJ, Sanda MG, Feng Z, et al. A prospective, multicenter, National Cancer Institute Early Detection Research Network study of [-2]proPSA: improving prostate cancer detection and correlating with cancer aggressiveness. Cancer Epidemiol Biomarkers Prev. 2010 May;19(5):1193-200.
[34] Sokoll LJ, Wang Y, Feng Z, et al. [-2]proenzyme prostate specific antigen for prostate cancer detection: a National Cancer Institute Early Detection Research Network validation study. J Urol. 2008 Aug;180(2):539-43; discussion 543. Epub 2008 Jun 11.

Table IV-1: Lung Reference Sets

Lung Reference Sets	Reference Sets A and C	Reference Set B
Clinical context	Diagnosis of lung cancer	Early diagnosis of lung cancer
Study design	Case-control study	Case-control study
Study population	All suspicious lung lesions on CXR or on CT	CT screening
Cases	Lung cancers, ≥50% Stage I	Detected by CT- lung cancer >0.5cm and <3cm
Controls	Clinically free of lung cancer 1 year after enrollment 75 patients with other cancers (25 breast, 25 colon, 25 prostate; set C has only 37 other cancers)	Detected by CT with a lung nodule >0.5cm and <3cm 10% are determined by CT to be without a lung nodule All free of disease at the 1 year F/U CT 25 patients with other cancers
Matching criteria	Age, sex, smoking status, PKYs	Age, sex, smoking status, PKYs
Sample size Rapid pre-validation (1) Combined pre-validation (2)	(1) 27 cases 50 controls, 25 other cancer controls (2) 180 cases and 180 controls, 75/37 other cancer controls)	(1) 38 cases 87 controls, 25 other cancer controls
Pulmonary diseases	Enriched across whole population	Enriched in controls with CT detected lung nodules
Institution provider candidates	Pittsburgh, Vanderbilt, MDACC, NYU, UCLA, Mayo, UCHSC, JHU	Pittsburgh, NYU, UCLA, Mayo

Reference set #2: This reference set was developed for validation of the most promising biomarkers for early detection and diagnosis of prostate cancer as well as to assist clinicians in decision making for those who need a biopsy. Blood (serum and plasma) samples were prospectively collected prior to prostate biopsy. Among men recommended by current practice for biopsy, an estimated 60% to 70% will have negative biopsies.

Specimens were collected in a strongly unbiased way for the intended clinical application according to the PRoBE design.[35] More than 600 men (about 40% of whom had positive biopsies) from four EDRN CVCs were accrued for this reference set. The reference set #2 was used to successfully validate the %[-2]proPSA biomarker.

Reference set #3: This cohort provides a validation reference set of prospectively collected blood and urine. This cohort was developed to validate the utility of promising urine-based biomarkers such as the non-coding gene PCA3 and the fusion transcript TMPRSS2-ERG. The intended clinical use is to evaluate the positive predictive value of PCA3 and TMPRSS2-ERG for initial biopsy population and the negative predictive value of PCA3 for the repeat biopsy population in a multicenter prostate biopsy cohort of men without prior history of prostate cancer.

Two specimen types were collected in the following order: pre-digital-rectal exam (DRE) blood and post-DRE urine. The post-DRE urine specimens were collected up to 1 month after the collection of the blood specimen but both prior to prostate biopsy. The biopsy specimens were collected within one month after the collection of the urine specimen. Patient accrual and sample collection was completed at 11 participating institutes. Biopsy is the primary outcome. The assembled reference sets and the study design for testing promising biomarkers were prepared according to the PRoBE design. Specimens were collected from 930 men, about 40% of whom had positive biopsies and about 300 patients had a repeat biopsy.

Pancreatic Cancer Reference Sets

An EDRN pancreatic cancer reference set was designed to create a set consisting of well-characterized serum/plasma specimens to use as a resource for the development of biomarkers for the early detection of pancreatic adenocarcinoma. Additionally, the testing of biomarkers on the same reference set permits direct comparison among markers, thereby allowing the development of a biomarker panel that can be evaluated in a future study. The pancreatic cancer reference set consists of replicate aliquots of serum and plasma from 12 patients with stage I; 39 patients with stage IIA; and 33 patients with stage IIB pancreatic cancer. In addition the reference set contains serum and plasma from benign conditions: 60 patients with chronic pancreatitis, 30 patients with acute benign biliary obstruction, and 60 healthy subjects.

Cystic lesions of the pancreas are increasingly being recognized due to the widespread use of high resolution abdominal imaging. Since certain cyst types are precursors to invasive cancer, this situation presents an opportunity to intervene prior to malignant progression. To address this type of lesion, EDRN investigators have initiated collection of another reference set to be composed of cystic fluid, serum, plasma, and urine which will be collected and characterized.

Hepatocellular Carcinoma Reference Sets

The NCI-funded EDRN Phase II validation study for the early diagnosis of hepatocellular carcinoma (HCC) described in Part III, Chapter 3 resulted in a large collection of samples from early and late-stage liver cancer and matched cirrhotic controls. A total of 424

[35] Pepe MA, Feng Z, Janes H, et al.Pivotal Evaluation of the Accuracy of a Biomarker Used for Classification or Prediction: Standards for Study Design. J Natl Cancer Inst. 2008 October 15; 100(20): 1432-1438.

cirrhotic controls and 422 cases of HCC (208 early stage cancers) were enrolled. Serum was collected and stored from all patients, and plasma and genomic DNA were also collected and stored from the majority of the patients. These specimens are available to investigators with promising biomarkers. The samples were divided into two sets: a prevalidation reference set composed of 50 early stage cases and 50 cirrhotic controls (50/50 set), and a validation set composed of 374 cirrhotic controls and 422 liver cancer cases, 158 of which are early stage HCC .

The prevalidation reference set is provided to investigators for analysis of candidate biomarkers for which there is preliminary data indicating that the markers perform as well as, or complement, alpha-fetoprotein (AFP). The samples will be blinded to the investigator, and the EDRN DMCC will perform the data analysis. If the biomarker performs at least as well as or complements the standard serum tests (AFP, AFP-L3, DCP) in the prevalidation reference set, the validation set will be made available to the investigator. The samples will remain blinded and the EDRN DMCC will perform the data analyses. This two-step procedure allows the efficient testing of potential biomarkers on a smaller prevalidation reference set to determine performance characteristics before access to the larger validation set. To date, the reference sets have been used to test candidate biomarkers from six labs (three from industry). Due to promising results from the 50/50 reference set, three labs (one from industry and two from academia) have been approved for access to the larger validation reference set.

Breast Cancer Reference Set

The clinical application of the assembled reference set is to rapidly test biomarkers which, in conjunction with mammography or other imaging modalities, can discriminate women with benign breast disease from women with malignant breast conditions for the early detection of breast cancer. Samples and specified common data elements (CDEs) were collected prospectively from eligible individuals referred to diagnostic radiology after mammography (and/or other imaging modality, e.g., ultra-sonography), but prior to surgery and diagnosis.

Alternatively, samples were collected at mammography screening clinics and confirmed as cases or controls following referral, biopsy, and diagnostic pathology. Each sample in the reference set is represented by plasma and serum. The breast cancer reference set contains samples from women with incident invasive cancer (n=207), carcinoma in situ (n=55), benign pathology with atypia (n=63), benign disease with no atypia (n=231), and women with no evidence of breast disease by screening mammography (BI-RADS 1 or 2, n=276).

Colon Cancer Reference Set

This reference set is for the evaluation of blood- or urine-based biomarkers for the detection of early stage colorectal cancer and advanced adenomas. Colonoscopic screening reduces both the incidence and mortality from colorectal cancer, primarily through the removal of adenomas. However, approximately half of the eligible population does not undergo colonoscopy because of concerns about cost, discomfort, complications, embarrassment and availability. Consequently, biomarkers for early stage colorectal cancer or adenomas that can be measured in blood or urine will enhance adherence to screening guidelines and ultimately further reduce mortality from colorectal adenocarcinoma.

The reference set consists of replicate aliquots of serum, plasma and urine from 50 subjects with colorectal adenocarcinoma, 50 subjects with adenomas confirmed by pathology, and 50 subjects with normal colons after colonoscopy. To date, this reference set has been used to test candidate biomarkers from one academic and two industry labs.

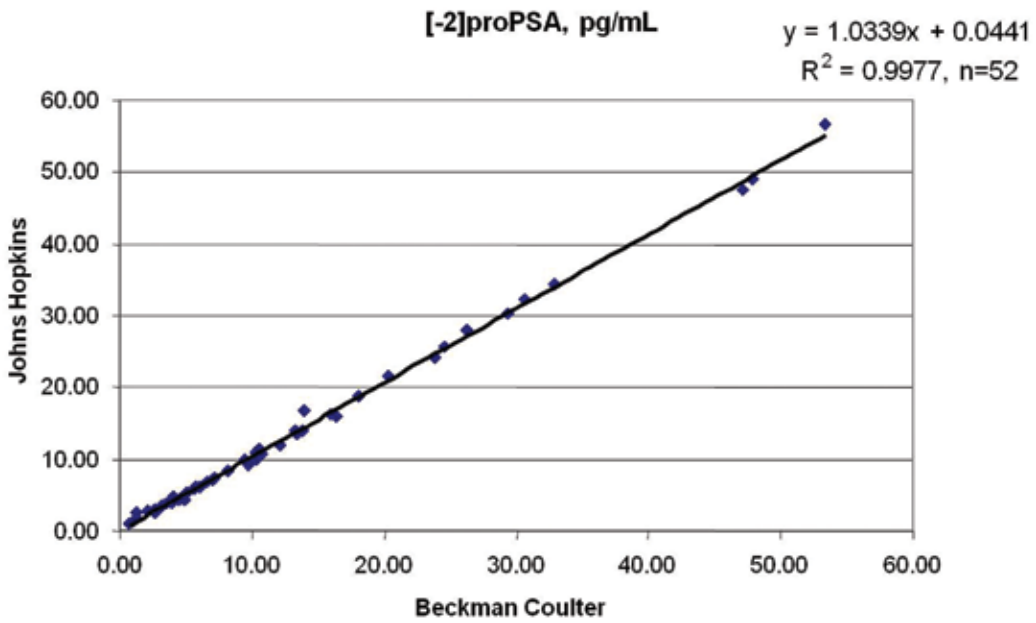
Laboratory Standards

Standardization of technologies

and procedures for handling biological samples, documenting data, and using proteomic technologies are necessary to produce identical results from multiple laboratories. All EDRN BRLs meet the requirements of the Clinical Laboratory Improvement Amendments for testing patient specimens.

One example from an EDRN validation study illustrates the importance of creating standard operating procedures and assay parameters in inter-laboratory and inter-institutional studies to ensure quality of test results. In the [-2]proPSA validation, analysis of 566 study specimens was performed at the Johns Hopkins BRL. To ensure the validity of results, a site-to-site quality assurance study was initially performed comparing assay results between the BRL and the manufacturer. As illustrated in Figure IV-4, essentially identical results were obtained (from a common set of 52 specimens), confirming the ability of the BRL to obtain consistently accurate results.

Figure IV-1: Correlation plot of [-2]proPSA immunoassay results illustrating site-to-site comparability



Regardless of the technology employed in cancer biomarker discovery and validation (PCR-detection, microarray expression assays, etc.), there are significant challenges in standardization of assay methods. For example, if an immunoassay such as ELISA is used, standardizing assay design, calibration of controls and reagents, and assay parameter are critical to obtain consistent assay results.

With the rapid development of mass spectrometry technologies, especially the recent introduction of targeted analysis of proteins using multiple-reaction monitoring (MRM), sensitivity and reproducibility have increased dramatically. The sensitivity of MS detection coupled with multidimensional chromatography and MRM is approaching that of immunoassays. The specificity of an MRM scan greatly reduces chemical background. This enables low levels of detection and limit of quantitation, and the ability to quantify the concentration over a wider dynamic range.

The Johns Hopkins BRL and the National Institute of Standards and Technology developed a standard reference material based on a pooled serum standard for benchmarking serum proteomics as a source of cancer biomarkers. Using healthy donor sera, nine FDA-approved cancer biomarkers were spiked into the base pool. The spiked material was assayed at the Johns Hopkins BRL for the added markers; recoveries were determined and the two materials were analyzed for about 50 other proteins to characterize and provide reference values.

In another study, four laboratories including three EDRN laboratories, (Johns Hopkins BDL, Toronto BDL, and Johns Hopkins BRL) used identical triple-quadruple mass spectrometers, standard protocols and instrumentation settings to analyze unknown samples and internal standards in a digested plasma matrix to quantify 51 peptides from 39 human proteins using a multiplexed SRM assay.

The inter-laboratory coefficient of variation was less than 10% for 25 of 39 peptides quantified (12 peptides were not quantified based upon hydrophobicity) and exhibited coefficients of variation of less than 20% for the remaining peptides. This study demonstrated that the development of standardized protocols and the use of standard reference materials are essential to produce reproducible and transferable results using SRM-MS assays between clinical research laboratories.

Part Five

Charting the Course of Biomarker Research

Recent advances in clinical diagnostic imaging and screening have increased our ability to detect lesions, but our inability to differentiate aggressive vs. indolent cancers has lead to over-diagnosis and over-treatment. Moreover, over-diagnosis has also lead to greatly increased health care costs due to the technologies used, procedures performed and number of patients examined. To address these problems, the EDRN has begun discussing the challenges and scientific opportunities that are available for lowering costs, minimizing over-diagnosis and for improving cancer detection, diagnosis and staging through the combined use of biomarkers and imaging.

Challenges with Over-Diagnosis

Many detectable lesions and cancers are asymptomatic and not life threatening. However, current inability to discern which lesions will lead to clinically significant morbidity and death vs. benign or slow-growing cancers leads to excessive testing, a phenomenon known as over-diagnosis. Because over-diagnosis leads to unnecessary medical procedures and treatment and increased risk of adverse side effects, it is important to develop strategies to quantify and manage its occurrence.

About 25% of breast cancers detected on mammograms and about 60% of prostate cancers detected with prostate-specific antigen (PSA) tests could represent over-diagnosis.[36] In a lung cancer screening trial of chest X-rays and sputum tests, they estimate that 50% of the suspicious abnormalities detected represented over-diagnosis. They argue that this estimate will only increase with spiral CT scanning, which, in one observational study, found almost as many tentative lung cancers in non-smokers as in smokers. In addition to screening, clinical diagnostic imaging may contribute to over-diagnosis. CT colonography (virtual colonoscopy) for instance, often detects abnormalities outside the colon that can lead to additional diagnostic tests.

Improved cancer screening and imaging sensitivities today detect abnormalities, lesions and tumors that previously were not detectable. However, our methodologies are not yet specific enough to discern which ones are or will develop into life threatening cancers. In fact, the vast majority of lesions detected by diagnostic imaging are not life-threatening. There is an urgent need to develop non-invasive procedures to determine which tumors represent significant disease.

There are ongoing debates regarding how to recognize and manage over-diagnosis. One proposed strategy is the development of disease-specific biomarkers that can distinguish aggressive from non-aggressive cancers. It is possible that new insights from genomics from The Cancer Genome Atlas and novel molecular approaches will ultimately allow us to more accurately predict tumor behavior at the tissue, cellular or molecular level. However, progress in early cancer detection and image-based diagnosis has been hampered by our lack of understanding regarding the natural history of the disease. Innovations in molecular biology, genomics, proteomics and immunology may provide insights.

Where do we begin to study the natural history of cancer? This is not a trivial question. It is well known that even some untreated tumors will not continue to grow or will only grow at an extremely slow rate over a period of years. In fact, some studies have shown that early stage prostate cancer is present in (almost) all older men (50 years or older). Benign prostatic hyperplasia or early stage cancer is present at the time of death even if they had not been diagnosed with cancer. This suggests that prostate cancer develops over a long period of time.

[36] Welch,Black. Overdiagnosis in cancer. J Natl Cancer Inst. 2010 May 5;102(9):605-13. Epub 2010 Apr 22.

One theory predicts all men have prostate cancer that grows so slowly that most likely die from something else before the cancer becomes problematic. Other men, for some unknown reason, develop a more aggressive phenotype that results in death. Since there is a large gap between the number of carcinomas known to be present at autopsy and those that present clinically, it is widely assumed that many carcinomas are dormant and pose no threat to patients and are, therefore, clinically insignificant. Despite the ability to detect prostate carcinomas, determining which early stage cancers will rapidly progress remains a problem. In general, this scenario holds true for most common cancers including breast, colon, ovarian, pancreas, lung and others.

EDRN's future direction aims to develop strategies for studying the natural history of cancer to aid in developing better tools for determining which cancers are clinically important. The surrogate markers of cancer aggression often used as evaluative criteria, including tumor grade, migration, invasion, and apoptosis, may or may not predict the clinical outcomes under assessment, such as metastasis or death. Unfortunately, current studies often ignore the potential role of a tumor's local environment. This is a significant limitation. How can researchers design relevant studies or establish implementable strategies to determine which cancers will likely lead to an undesirable clinical outcome?

To begin to address these issues, the EDRN is considering the following questions:

1. Why do some pre-neoplastic lesions progress rapidly and require intervention?
2. Therapeutic intervention is often triggered by assessment of a static picture of disease (histologic snapshot of observables assumed to be representative of the whole tumor), rather than knowledge of the inherently dynamic nature of the underlying disease. Current assessments are thus incomplete. What should be done to advance knowledge of cancer progression? Can information derived from the integration of imaging and molecular diagnostics further this understanding?
3. What types of molecular properties confer aggressiveness to some pre-neoplastic lesions? What should be done to study such behaviors to avoid over-diagnosis and over-treatment?
4. What should be done to define and characterize pre-neoplastic lesions in order to improve current standards in histology and cytology?

Imaging, Molecular Diagnostics and Early Cancer Detection

To exploit the advantages of imaging-based approaches, EDRN has uniquely positioned itself to facilitate the merging of biomarker and early detection imaging initiatives under a single umbrella of focused research called Companion Imaging and Molecular Diagnostics. EDRN's goal is to improve the sensitivity and specificity of individual biomarkers by developing multiplexed analysis methodology.

Recently published data suggest biomarker imaging can be used both as a probe to visualize cancer early during the course of the disease and to non-invasively assess altered metabolic processes in vivo. Specifically, imaging as a surrogate biomarker has been used to: detect the presence of cancer; determine tumor stage; assess tumor aggressiveness; assess response to therapy; and predict outcome. However, these developments have not yet been applied, systematically optimized and efficiently incorporated into the clinical arsenal of tools used for early cancer detection. EDRN aims to overcome these shortcomings.

Moving promising molecular diagnostics into the clinic subsequent to validation is a major undertaking in terms of expense, hours and resources required. In this regard, merging ex vivo and in vivo imaging methodologies with biomarker information offers:

1. Non-invasive near simultaneous detection of multiple targets and surrogate endpoints in an efficient, robust and cost effective manner;

2. Non-invasive assessment of dynamic physiologic processes in near real-time, such as rate constants of cellular processes, blood flow, oxygenation, pH, temperature, and diffusion; and

3. Ability to locate biomarker origins in 3D-space during a single exam. It is anticipated that imaging-based clinical validation studies could be used to significantly shorten the current time-line required for clinical validation and subsequent regulatory approval by the Food and Drug Administration and recommendations by the Centers for Medicare and Medicaid Services.

Imaging approaches will become increasingly important as the health informatics arena matures. Medical images will be directly captured into a patient's medical record and displayed on personal mobile electronic devices. If a picture is worth a thousand words, then volumes of subjective textual verbiage from radiologic impressions will be replaced with the original information from which it was derived. In this context, knowing with confidence the negative predictive value of results from a panel of biomarkers and/or image acquisition(s) will most likely be the most important piece of information used by clinicians to make informed decisions. The result would be to minimize over-diagnosis and over-treatment while maintaining favorable patient outcome.

To begin discussions for integrating imaging and molecular diagnostics, a number of activities were undertaken:

- Biomarkers Research Stakeholders Meeting (May 2010)
- FDA Workshop (September 2010)
- EDRN Steering Committee Meeting (November 2010)
- Monthly imaging interest group teleconference calls (since November 2010)
- NCI Sponsored Imaging Think Tank (February 2011)
- Co-Sponsor of Imaging in 2020 Conference (September 2011)
- Organized a session at the World Molecular Imaging Congress focused on Early Cancer Detection (September 2011)

Of these, the Think Tank has become the primary platform for discussing integration strategies. The Think Tank was chaired by Sanjiv Sam Gambhir, M.D., Ph.D. and endorsed by the American College of Radiology Imaging Network (ACRIN), Academy of Molecular Imaging (AMI) and the Society for Molecular Imaging (SMI). The Think Tank brought together imagers, biomarker researchers, clinicians, academicians and industrial consultants, as well as representatives from the NIH, NCI, NIST, FDA and other federal agencies to define common goals.

Recommendations

- Integration of imaging and biomarkers should be an NCI priority. NCI oversight is required to facilitate the process. EDRN was the logical and suggested platform for initiating integration and should be the leader for developing imaging applications specifically focused on early cancer detection because of its record of success, resources and infrastructure.

- Biomarkers identified specifically for early cancer detection are urgently needed. This is because a paucity of validated biomarkers for early detection exists; those that are currently under study are typically derived from late stage disease.
- Imaging researchers should be formally integrated into biomarker disease site groups to determine when mature diagnostic imaging acquisitions trigger a biomarker study, or conversely, when a mature molecular diagnostic panel triggers a subsequent imaging study.
- The EDRN should attempt to generate well-annotated tissue specimens from early stage cancers that are known to progress to advanced cancer.
- An urgent need was identified to discover and develop "image-able" biomarkers for blood, and urine, as well as tissue (cell surface and intracellular species).
- There is a need to develop decision analysis models, whenever possible, to assess comparative effectiveness research.

Several areas are actively being pursued to address the recommendations of the Think Tank as follows.

Liver Cancer - EDRN Collaborative Research with ACRIN

ACRIN has recently initiated a clinical trial comparing CT vs. MRI to improve current metrics for assessing patients requiring liver transplantation with known liver cirrhosis or suspected hepatocellular carcinoma (HCC). Because imaging is notoriously poor in predicting and measuring residual and recurrent disease following treatment (i.e., hemorrhaging, fibrosis, inflammation), there is a potentially significant role for biomarkers in assessing liver disease. For example, a positive biomarker correlation regarding tumor burden in the absence of imaging evidence for recurrent or residual disease may alter the frequency with which follow-up studies might be conducted, or biomarker results may compel clinicians to consider additional treatment cycles. EDRN's amendment adding a multiplexed panel of eight biomarkers was recently approved by senior ACRIN leadership. The clinical study now includes the collection of serum samples from patients with known cirrhosis and suspected HCC. Biomarker results will subsequently be analyzed and correlated to imaging metrics indicative of progressive HCC, subsequently confirmed by pathology.

National Lung Screening Trial (NLST) and Early Detection of Lung Cancer

The recently completed NCI-sponsored NLST resulted in a 20% decrease in mortality from a well-defined high risk cohort of ~ 53,000 patients when CT screening was compared to chest X-rays as the standard of care. The observed 20% reduction in mortality was significant. While both data acquisitions employed X-rays, the major difference between the two arms of the study was back-projection vs. tomographic imaging of lung nodules using CT.

Following the rationale in which structural information was primarily assessed, it is expected that greater successes would be realized if physiologic information were also incorporated into the study design. The EDRN aims to determine if a combined imaging and molecular diagnostics approach will improve current standards of care for early cancer detection and diagnosis. Currently, the NLST has established public access to the imaging data as well as a limited set of biological samples available for retrospective analysis. Ongoing research will focus on assessing the NLST imaging data in concert with biomarker data available from a subset of biological samples with the goal of gleaning additional information for subsequent design of future studies.

Prostate Cancer

In the current funding cycle, imaging-based biomarker approaches for early cancer detection were the focus of two grants. The first grant at Stanford University focuses on the development of two complementary strategies. One project seeks to develop magneto-nanosensor technology for multiplexed analysis of blood-based biomarkers. The second project uses ultrasound and photoaccoustic imaging for the discovery and verification of vascular biomarkers. The goal is to develop new low cost detection and assessment protocols for the management of prostate cancer.

Colon Cancer

A second imaging-based grant at the University of Chicago and Northwestern University focuses on developing innovative light-scattering technologies to identify the micro-architectural correlates of the genetic changes in field carcinogenesis. Interrogation of the microscopically normal rectal mucosa has been found to predict concurrent neoplasia throughout the colon. The approach is based on partial wave spectroscopy, which has been shown to enable unprecedented quantitative characterization of the nanoscale cellular architecture beyond that available from histology and immunohistochemistry. The development of this spectroscopic imaging-based approach combined with biomarker information could provide a highly accurate, minimally intrusive technique for risk-stratification and improved screening.

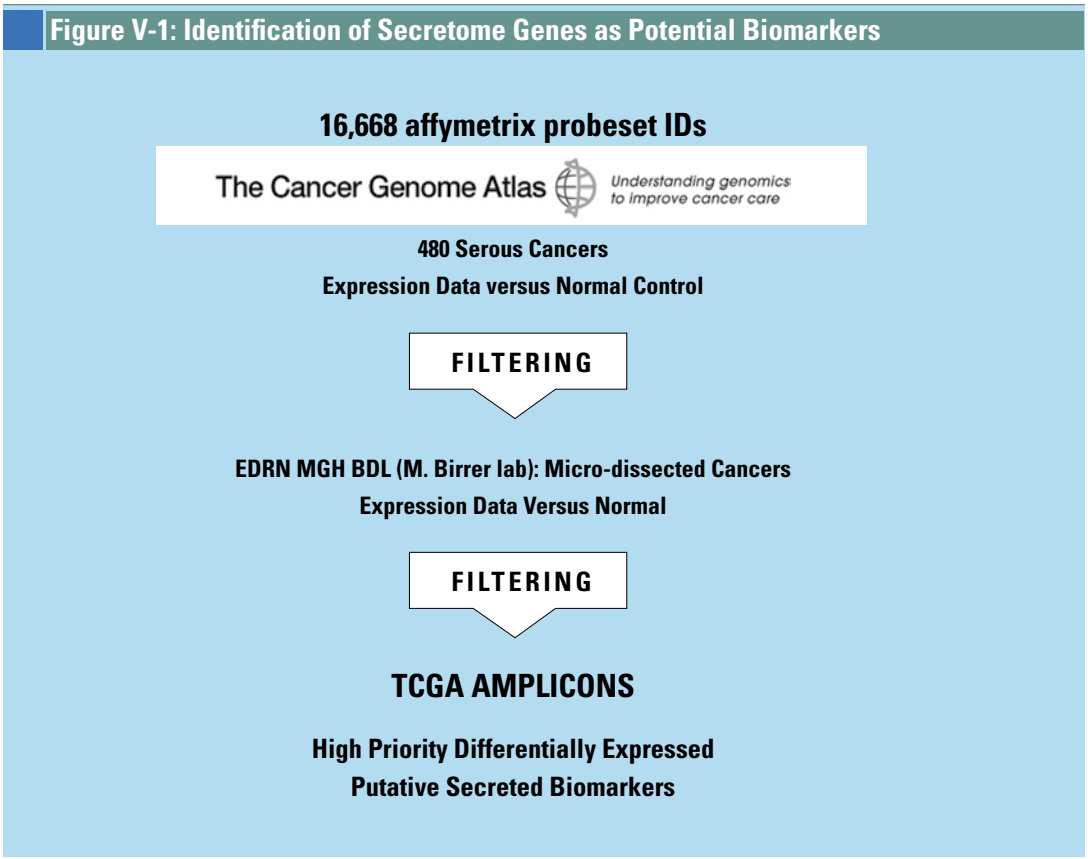
Leveraging Data Generated by TCGA in Biomarker Discovery

In 2006, the National Cancer Institute and the National Human Genome Research Institute established The Cancer Genome Atlas (TCGA) program, which is cataloguing genetic mutations responsible for cancer using recently developed high-throughput genome analysis techniques and is seeking to improve our ability to diagnose, treat, and prevent cancer through a better understanding of the molecular basis of this disease. Data that is being generated include gene expression profiling, copy number variation profiling, SNP genotyping, genome wide DNA methylation profiling, microRNA profiling, and exon sequencing of at least 6,000 genes, including microRNA.

The goal of the project is to provide systematic, comprehensive genomic characterization and sequence analysis of 20 different types of human cancers. TCGA recently published its findings on GBM and ovarian cancers. There is a large amount of data publically available for ovarian cancer including gene mutations, copy number changes, mRNA gene expression, miRNA gene expression, and CpG methylation status. However, this data has not yet been utilized in a clinically meaningful fashion. This presents an extraordinary opportunity for investigators interested in developing effective early detection assays for this disease.

EDRN investigators are proactively analyzing the data along with their own expression data to triage biomarker information relevant to early detection of cancer. An example as to the use of TCGA-generated data to guide the discovery of ovarian cancer-related secretome genes that can be further prioritized and subjected to scrutiny for clinical validation is illustrated in Figure V-1.

Figure V-1: Identification of Secretome Genes as Potential Biomarkers



EDRN investigators at the Massachusettes General Hospital BDL have developed a secretome consisting of all affymetrix probesets (found on 133 plus 2 arrays), which identified genes that potentially encode proteins found in the serum (secreted or transmembrane proteins). The secretome will be used to interrogate large numbers of microarrays performed on ovarian cancers and control tissues including TCGA. All 480 serous ovarian cancers (within the TCGA), which have mRNA expression data, will be utilized to identify differentially expressed transcripts between cancer and normal tissue (using fallopian tube or ovarian surface epithelium arrays available in the Birrer laboratory). Lists of genes with significant differential expression will then be filtered using a second set of expression profiling databases of microdissected ovarian cancers and normal control tissues (available in the Birrer laboratory). Any gene found in both datasets will be considered high priority epithelial biomarkers.

Genes that are excluded in this last step will be considered possible differentially expressed stromal genes and further developed (in parallel) using stromal microarray databases (available in the Birrer laboratory). Further filtering will include utilizing the TCGA characterized amplicons and selecting genes according to their amplification status. The final step in this process will be eliminating genes that are highly expressed in other tissues. This will be accomplished using publically available databases of affymetrix microarrays from normal human tissues. The final product will be a highly filtered list of differentially expressed genes (not found highly expressed in other tissues) that potentially encode proteins found in the serum. These candidates will then be provided to the BDL proteomic collaborators at the Broad Institute (Carr laboratory) and other EDRN proteomic investigators for specific validation in the sera of ovarian cancer patients but not in controls.

Part Six

Frequently Asked Questions

1. What are tumor markers?

Tumor markers are substances produced by tumor cells or by other cells of the body in response to cancer or certain benign (noncancerous) conditions. These substances can be found in the blood, in the urine, in the tumor tissue, or in other tissues. Different tumor markers are found in different types of cancer, and levels of the same tumor marker can be altered in more than one type of cancer. In addition, tumor marker levels are not altered in all people with cancer, especially if the cancer is early stage. Some tumor marker levels can also be altered in patients with noncancerous conditions.

To date, researchers have identified more than a dozen substances that seem to be expressed abnormally when some types of cancer are present. Some of these substances are also found in other conditions and diseases. Scientists have not found markers for every type of cancer.

2. What are risk markers?

Some people have a greater chance of developing certain types of cancer because of a change, known as a mutation or alteration, in specific genes. The presence of such a change is sometimes called a risk marker. Tests for risk markers can help the doctor estimate a person's chance of developing a certain cancer. Risk markers can indicate that cancer is more likely to occur, whereas tumor markers can indicate the presence of cancer.

3. How are tumor markers used in cancer care?

Tumor markers are used in the detection, diagnosis, and management of some types of cancer. Although an abnormal tumor marker level may suggest cancer, this alone is usually not enough to diagnose cancer. Therefore, measurements of tumor markers are usually combined with other tests, such as a biopsy, to diagnose cancer.

Tumor marker levels may be measured before treatment to help doctors plan appropriate therapy. In some types of cancer, tumor marker levels reflect the stage (extent) of the disease. (More information about staging is available in the National Cancer Institute (NCI) fact sheet Staging: Questions and Answers, which can be found at http://www.cancer.gov/cancertopics/factsheet/Detection/staging)

Tumor marker levels may be also used to check how a patient is responding to treatment. A decrease or return to a normal level may indicate that the cancer is responding to therapy, whereas an increase may indicate that the cancer is not responding. After treatment has ended, tumor marker levels may be used to check for recurrence (cancer that has returned).

4. How and when are tumor markers measured?

The doctor takes a blood, urine, or tissue sample and sends it to the laboratory, where various methods are used to measure the level of the tumor marker.

If the tumor marker is being used to determine whether a treatment is working or if there is recurrence, the tumor marker levels are often measured over a period of time to see if the levels are increasing or decreasing. Usually these "serial measurements" are more meaningful than a single measurement. Tumor marker levels may be checked at the time of diagnosis; before, during, and after therapy; and then periodically to monitor for recurrence.

5 What research is being done in this field?

Scientists are evaluating patterns of gene expression (the step required to translate to proteins what is in the genes) for their ability to predict a patient's prognosis (likely outcome or course of disease) or response to therapy, and proteomics (the study of protein shape, function, and patterns of expression) in hopes of developing better cancer screening and treatment options. NCI's Early Detection Research Network is developing a number of genomic- and proteomic-based biomarkers, some of which are being validated. More information about this program can be found at http://edrn.nci.nih.gov/ on the Internet.

6. Why is the EDRN important for biomarker research?

The EDRN is the first comprehensive network to develop and validate early detection biomarkers for cancer. It is one of the few NCI programs dedicated to biomarker research. It represents different scientific disciplines including genomics (the study of genes), proteomics (the study of proteins), informatics, and public health. The EDRN is a driving force behind governmental institutional and public-private collaborations that work together to encourage the rapid development of biomarkers and early detection science and to make those developments usable in medical practice.

The EDRN takes biomarkers discovered in the laboratory and performs clinical studies to determine if there are good clinical applications for these discoveries. EDRN has also made significant progress in building resources to support this effort, identifying candidate biomarkers and undertaking multi-center validation studies. Before EDRN, each part of the process was disconnected and progress was slow. Now, through the EDRN, discoveries by scientists and doctors are combined with a strong administrative support system and databases for information- sharing to move markers more quickly into clinical practice.

7. How does EDRN keep up with the new technologies and discovery programs?

Incorporating up-and-coming technologies into research devoted to the discovery and development of biomarkers is an essential ingredient to the success of the EDRN. While many EDRN investigators pioneer cutting-edge technologies, the Network also invites collaboration from other scientists or companies to adapt their platforms for developing early detection diagnostic tests. Platforms being used for biomarker discovery range from genomics to epigenomics to glycomics to proteomics to nanotechnology.

8. What cancers are being studied by EDRN researchers?

The EDRN has ongoing projects in many major cancer sites, including: breast, ovary, cervix, colon liver, esophagus, pancreas, lung, prostate, and bladder. Although these cancer sites represent the majority of cancers in the United States, future projects on other cancers, including blood and lymphoid cancers, are possible.

9. How do you define success?

EDRN uses several intermediate measures to define success, some of which are provided below. For instance, a new marker with incremental improvement over existing diagnostic assays, even if moderate but confirmed true, is considered an important measure of success. In the examples illustrated in previous sections, Annexin 1&2, %[-2] proPSA, and DCP, all had incremental performance improvement over the currently used serum based tests. On the other hand, TSP1, EPCA-2, and SELDI profiling all demonstrated very high level of performance based on preliminary data, but failed in subsequent validation. This illustrates two points: 1) EDRN plays a very important "brake" role (there is no infrastructure like EDRN to conduct such validation studies); and 2) We need to be realistic with regard to the performance criteria of a new biomarker.

It is also important to remember, that CLIA certified laboratories can offer clinically and commercially relevant tests. Increasingly, smaller companies are licensing diagnostic tests to larger clinical diagnostic laboratories to generate income and gain experience for eventual FDA filings. For example, EDRN-supported markers GST P1 methylation and PCA-3 as biomarkers for prostate cancer, and methylation of Vimentin as a biomarker for colon cancer are available through various commercial clinical diagnostic laboratories.

10. Has EDRN brought biomarkers to FDA for approval?

There is a misconception by some about the EDRN's role in bringing diagnostic markers to the FDA for approval. This is beyond the scope of EDRN. It is expected that the private sector will take the responsibility for commercializing and seeking FDA approval for EDRN-validated biomarkers under the agreement with the lead investigators. EDRN will assist in the process. At present, EDRN research has led to the development of three biomarker tests that have been approved by FDA and two new IVDs pending FDA review. It is anticipated that at least 2 to 3 additional biomarkers will have been submitted to the FDA for regulatory approval. The

number of biomarkers developed by the EDRN that are taken forward by the private sector to the FDA is likely to increase within the next 5 years as the biomarkers currently under development progress through the validation pipeline.

11. How does EDRN communicate with the public, cancer advocates, and other scientists?

EDRN outreach is vital to the program and speaks to the health of the existing Network. Outreach activities include participation in activities of the Advocacy groups, and professional societies, such as the American Association for Cancer Research (AACR), the American Society of Clinical Oncology (ASCO), the American Society of Molecular Pathologists (ASMP), and others. EDRN has appointed a number of investigators to liaise with profession societies and has cancer advocates serving on its Steering Committee.

EDRN conducts meetings and workshops that are transparent and open to extramural community, communicates its progress through the biennial reports and presentations to the NCI Executive Committee and the NCI Advisory Committees; has more than 21 listserv for the extramural community to subscribe to and join EDRN collaborative groups; and provides opportunity for the extramural community to join EDRN through its Associate Membership Program, a unique concept in the NIH-supported networks or consortia. EDRN seeks input from extramural experts on research directions that may not be available within the Network. As noted in the recommendations from the NCI-designated Cancer Center Directors: Accelerating Successes Against Cancer (2007)[37], "With relatively small funding, experts note that this mechanism (EDRN) has done a commendable job developing collaborations across Cancer Centers, SPORES, and Program Projects."

12. How can someone get involved in EDRN research?

As a result of the EDRN's work during the last 10 years, several clinical trials have been completed and more are ongoing or in planning. The ongoing studies are testing biomarkers for the early detection of liver, colon, and prostate cancer. These trials need diverse patient populations who are willing to contribute blood, urine, or tissue samples to help measure the incidence of early cancer, recurrence of cancer, diagnosis or prognosis of cancer. The trial may last from three to five years or more. For example, the biomarker PCA3 is being tested in a multi-site trial to determine its ability to detect prostate cancer early. This study will compare a measurement of PCA3 in urine to the measurement of PSA in blood to determine which most accurately detects early prostate cancer and to see if the tests can be used together to improve cancer detection.

[37]http://cancercenters.cancer.gov/documents/Accelerating_Successes_Against_Cancer_Report.pdf

Conclusion

The EDRN has made significant progress in: developing an organized effort for biomarker discovery and validation; building resources to support this effort; demonstrating the capabilities of several genomic and proteomic platforms; identifying candidate biomarkers; and undertaking multi-center validation studies.

Accomplishments: In its first 10 years, the EDRN went from a groundbreaking concept to an operational success. To date, the EDRN has developed a rich pipeline of more than 300 biomarkers subjected to rigorous Phase II validation and ready for large validation studies (see below); completed five validation studies; and has a number of prioritized markers ready for prevalidation and validation studies. More than 1450 publications, many of which have been published in high impact journals (impact factor 20-30), more than 28 patents and more than 14 licenses have been generated.

Prioritization: EDRN mandates a strong peer-review at each step from discovery to development to validation. Prior to becoming part of the EDRN, BDL applicants are peer-reviewed by an NCI-established Special Emphasis Panel and applications of high scientific merit are selected. Collaborative Groups and the EDRN Executive Committee employing statistical reasoning and clinical needs in mind. More than 300 biomarkers have been prioritized for further development and validation.

Outcomes and Metrics: EDRN has established clear milestones for reaching a decision of 'go' or 'no go' during the biomarker development process. Milestones are established based on statistical criteria, performance characteristics of biomarkers, and anticipated clinical use. More than 100 biomarkers have been stopped from further development. There are potential biomarker-based tests currently under discussion with FDA. EDRN investigators have published more than 700 collaborative papers, many of which have citation index of more than 300.

Openness and Inclusiveness: With a primary mission to discover and scrupulously validate biological markers that signal the earliest stages of cancer, including pre-malignant lesions and risk indicators, a number of outside investigators have joined the network through the EDRN Associate Membership Program. To date, more than 130 Associate Members, including more than 15 biotechnology companies, private foundations and international partners are participating and contributing to the EDRN. EDRN Steering Committee Meetings convened twice a year, are attended by more than 170 participants, more than the number of the EDRN PIs. EDRN Workshops convened every 18 months, attract more than 400 participants. More than 60% of the EDRN Headquarters Fund (also known as Core Fund) is used to support collaborative activities with outside investigators who are participating in EDRN-sponsored trials, Associate Membership program, and capacity-building activities. All of the above are the testimonies to the EDRN's openness and inclusiveness.

Biomarkers Pipeline at a Glance

- 1000s reviewed and evaluated
- More than 300 in Phase II validation (for details see chapters on respective Collaborative Groups)
- Eight Large validation studies completed
- More than nine Phase II and Phase III Studies in progress
- Three FDA approved tests
- Two In Vitro Diagnostic Devices (IVDs) pending FDA review
- More than five marker tests in CLIA Laboratories
- Two markers, GP73 and AFP L-3 for liver cancer in use in China

With continued attention, support and open cross-disciplinary, multi-institutional collaborations, the challenges of finding and developing accurate and useful biomarkers for early cancer detection and cancer risk will fade, and new, long-awaited, less invasive tools will be brought into clinical use.

Partnerships: The validation of biomarkers for early cancer detection is dependent on testing and validation using high quality specimens obtained many months or even years prior to clinical diagnosis of cancer. Hence, collaboration of the EDRN with screening trial cohorts from the U.S. and abroad is essential for securing sufficient numbers of relevant pre-clinical specimens. For example, EDRN is currently discussing with the directors of the PLCO, WHI, CARET and UKCTOCS cohorts an eventual collaboration for building a reference set for ovarian cancer, which can facilitate the discovery and rapid validation of new candidate biomarkers for the early detection of this lethal disease.

Over the past 10 years, EDRN has conducted multi-institutional trials for the clinical validation of biomarkers for several organ sites, including the bladder, liver, ovary and prostate. As other validation trials are ongoing or in planning, the need for trial participants will continue to grow. Such trials also need the participation of patients from diverse populations who are willing to contribute blood, urine, or tissue to help measure the incidence of early cancer, recurrence of cancer, diagnosis, and prognosis of cancer.

In summary, EDRN today combines numerous collaborative and multidisciplinary, investigator-initiated projects with a strong national administrative and data infrastructure. EDRN has created a rigorous peer-review system that ensures that preliminary data—analytical, clinical, and quantitative—are of excellent quality. The process begins with an internal review with clinical, biostatistical, and analytical expertise. The project then receives external peer-review and, finally, NCI program staff review resulting in an exceptionally robust and high-quality validation trial.

EDRN Advocacy Activities: Ways to Get Involved

The EDRN Advocates have fostered productive relationships with the advocacy community, legislative activists, educators, and fund raisers. They have disseminated information among these groups by coordinating monthly Webinars. These Webinars serve as a platform that improves communication between EDRN Principal Investigators, the advocacy community and other key constituents for cancer research. During the Webinar series, presenters and experts discuss current studies and offer information concerning the progression of the disease. The overall goal of these series is to improve awareness among potential users of the biomarker-based diagnostic tests by providing a knowledge environment for diverse cancer consumers.

To learn more about EDRN Advocacy Webinar, visit: www.http://edrn.nci.nih.gov/advocates

Appendix

Laboratory Listing

Biomarker Developmental Laboratories

Principal Investigator	Institution	Collaborative Group
Arul Chinnaiyan Mark Rubin	University of Michigan Weill Cornell Medical College	Prostate and Other Urological Cancers
Carlo Croce	Ohio State University	Breast and Gynecological Cancers and Lung and Upper Aerodigestive Cancers
Eleftherios Diamandis	Mount Sinai Hospital, Toronto, ON	Breast and Gynecological Cancers
Steven Dubinett David Elashoff Avrum Spira Marc Lenburg	University of California, Los Angeles University of California, Los Angeles Boston University School of Medicine Boston University School of Medicine	Lung and Upper Aerodigestive Cancers
Sanjiv Gambhir James Brooks	Stanford University	Prostate and Other Urological Cancers
Robert Getzenberg Robert Schoen	Johns Hopkins University School of Medicine University of Pittsburgh Cancer Institute	Colorectal and Other Gastrointestinal Cancers
William Grady Sanford Markowitz	Fred Hutchinson Cancer Research Center Case Western Reserve University	Colorectal and Other Gastrointestinal Cancers
Brian Haab Peter Allen Randall Brand	Van Andel Research Institute Memorial Sloan Kettering Cancer Center University of Pittsburgh Cancer Institute	Colorectal and Other Gastrointestinal Cancers
Michael Hollingsworth Surinder Batra	University of Nebraska	Colorectal and Other Gastrointestinal Cancers
Ann Killary Marsha Frazier Subrata Sen	University of Texas M. D. Anderson Cancer Center	Colorectal and Other Gastrointestinal Cancers
Joshua LaBaer Karen Anderson	Arizona State University	Breast and Gynecological Cancers
Paul Lampe Samir Hanash	Fred Hutchinson Cancer Research Center	Colorectal and Other Gastrointestinal Cancers
Daniel Liebler David Tabb	Vanderbilt Medical Center	Lung and Upper Aerodigestive Cancers Colorectal and Other Gastrointestinal Cancers
Alvin Liu	University of Washington	Prostate and Other Urological Cancers
Dan Mercola Chung Lee	University of California, Irvine	Prostate and Other Urological Cancers
Harvey Pass Margaret Huflejt	New York University	Lung and Upper Aerodigestive Cancers
Hemant Roy Vadim Backman	NorthShore University Health System Research Institute Northwestern University	Colorectal and Other Gastrointestinal Cancers
David Sidransky	Johns Hopkins University School of Medicine	Lung and Upper Aerodigestive Cancers
Steven Skates Michael Birrer Ronny Drapkin	Massachusetts General Hospital Massachusetts General Hospital Dana-Farber Cancer Institute	Breast and Gynecological Cancers
Hui Zhang	Johns Hopkins University School of Medicine	Prostate and Other Urological Cancers

Clinical Validation Centers

Principal Investigator	Institution	Collaborative Group
Dean Brenner	University of Michigan	Colorectal and Other Gastrointestinal Cancers
Paul Engstrom	Fox Chase Cancer Center	Breast and Gynecological Cancers
Christopher Li	Fred Hutchinson Cancer Research Center	Breast and Gynecological Cancers
Jeffrey Marks	Duke University	Breast and Gynecological Cancers
Pierre Massion	Vanderbilt Ingram Cancer Center	Lung and Upper Aerodigestive Cancers
William Rom	New York University School of Medicine	Lung and Upper Aerodigestive Cancers
Martin Sanda	Beth Israel Deaconess Medical Center	Prostate and Other Urological Cancers
Ian Thompson	University of Texas Health Science Center San Antonio	Prostate and Other Urological Cancers

Biomarker Reference Laboratories

Principal Investigator	Institution	Collaborative Group
Daniel Chan	Johns Hopkins University School of Medicine	Prostate and Other Urological Cancers
David Chia	University of California, Los Angeles	Colorectal and Other Gastrointestinal Cancers
Laurie Locascio	National Institute of Standards and Technology	Lung and Upper Aeorodigestive Cancers
Karin Rodland	Pacific Northwest National Laboratory	Breast and Gynecological Cancers
Sanford Stass	University of Maryland, School of Medicine	Colorectal and Other Gastrointestinal Cancers

Data Management Coordinating Center

Principal Investigator	Institution	Collaborative Group
Ziding Feng	Fred Hutchinson Cancer Research Center	Breast and Gynecological Cancers Colorectal and Other Gastrointestinal Cancers Lung and Upper Aerodigestive Cancers Prostate and Other Urological Cancers

Informatics Center

Principal Investigator	Institution	Collaborative Group
Daniel Crichton	NASA Jet Propulsion Laboratory	Breast and Gynecological Cancers Colorectal and Other Gastrointestinal Cancers Lung and Upper Aerodigestive Cancers Prostate and Other Urological Cancers

EDRN Network Consulting Team Members	
Members	**Affiliation**
Larry Norton, M.D., Chair	Memorial Sloan-Kettering Cancer Center
Robert Becker, Jr., M.D., Ph.D.	U.S. Food and Drug Administration
Jose Costa, M.D.	Yale University School of Medicine
Kenneth Cowan, M.D., Ph.D.	University of Nebraska Medical Center
Stanton L. Gerson, M.D.	Case Western Reserve University
Robert J. Gillies, Ph.D.	H. Lee Moffitt Cancer Center and Research Institute
Alberto Gutierrez, Ph.D.	U.S. Food and Drug Administration
Vijay Modur, M.D., Ph.D.	Novartis Institutes for BioMedical Research
Harold Moses, M.D.	Vanderbilt University School of Medicine
William G. Nelson, M.D., Ph.D.	Johns Hopkins University
Steve Shak, M.D.	Genomic Health, Inc.

Significant Activities (Last 5 years)*

August 2005	NIST-EDRN Workshop on Standards and Metrology for Cancer Diagnostics, Gaithersburg, MD
January 2006	EDRN Pancreatic Implementation Meeting, Denver, CO
February 2006	EDRN Lung Implementation Team, Rockville, MD
March 2006	12th EDRN Steering Committee Meeting and 4th Scientific Workshop, Philadelphia, PA
April 2006	Publication of the EDRN Prostate Cancer Risk Calculator
September 2006	13th EDRN Steering Committee Meeting, Pittsburgh, PA
October 2006	EDRN and Hepatitis B Foundation Workshop, Princeton, NJ
March 2007	14th EDRN Steering Committee Meeting, Denver, CO
September 2007	15th EDRN Steering Committee Meeting, Ann Arbor, MI
March 2008	16th EDRN Steering Committee Meeting, Bethesda, MD
March 2008	EDRN approved by NCI Board of Scientific Advisors for a third 5-year cycle
September 2008	17th EDRN Steering Committee Meeting, Seattle, WA
May 2008	NCI Partners with Canary Foundation on Prostate Cancer Study
March 2009	18th EDRN Steering Committee Meeting, Houston, TX
July 2009	Current Requests for Applications released for Biomarker Developmental Laboratories (U01), Clinical Validation Centers (U01), Biomarker Reference Laboratories (U24), and Data Management and Coordinating Center and Statistics and Biomarker Resource Center (U24)
August 2009	19th EDRN Steering Committee Meeting and 6th Scientific Workshop, Bethesda, MD
March 2010	20th EDRN Steering Committee Meeting, Tempe, AZ
November 2010	21st EDRN Steering Committee Meeting, Dallas-Ft. Worth, TX
February 2011	Think Tank meeting, "Companion Imaging and Molecular Diagnostics," Bethesda, MD
March 2011	22nd EDRN Steering Committee Meeting, Los Angeles, CA
September 2011	23rd EDRN Steering Committee meeting and 7th Scientific Workshop, Bethesda, MD

*See previous reports for earlier milestones.

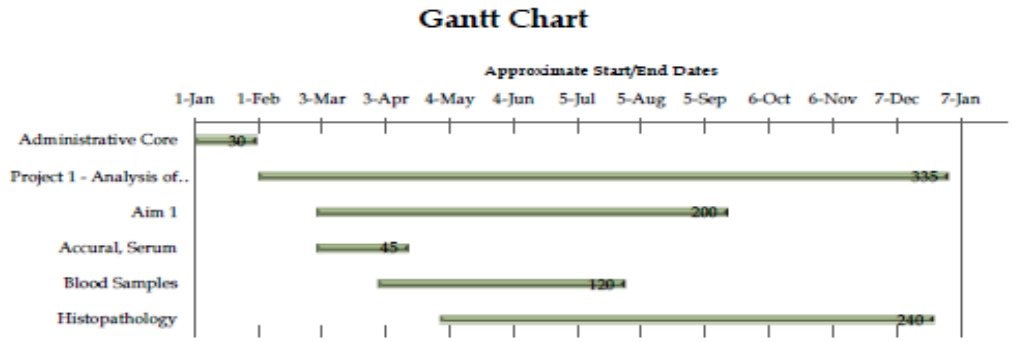
Project Management

A clear project management plan is a cornerstone of EDRN's successful complex, multi-institutional studies. The art of project control requires an interactive process to stay on-time and within budget to move each task along a predetermined schedule.

Activities center on three management aspects: the project and goals, the process of meeting the goals, and the performance of the individuals and organizations to accomplish these goals. Through the use of Gantt charts as a communication tool, shown in Figure A-1, the Program provides centralized coordination and progress monitoring. Gantt charts synchronize members in defining goals, scheduling specific aims, defining responsibilities, and determining timelines.

Figure A-1: Gantt charts used for synchronizing project milestones

Task	Responsible Party	Start	Days Duration	Start Date	End Date
Administrative Core	Principle Investigator	1	30	1-Jan	31-Jan
Project 1 - Analysis of Plasma	Team 1	32	335	1-Feb	31-Dec
Aim 1		60	200	3-Mar	10-Sep
Accural, Serum		60	45	1-Mar	
Blood Samples		90	120	1-Apr	
Histopathology		120	240	1-May	



The Data Management and Coordinating Center (DMCC) uses strong program management tools to coordinate stakeholders, and provides smooth communications and timely information sharing among EDRN members and NCI program directors. The EDRN Study Information System (eSIS) provides a set of interactive web-pages for project management, as shown in Figure A-2. Specific study information captured in a single website location allows users to quickly search and review EDRN studies via the start date, progress milestones, outcome, and project closure.

The Validation Study Information Management System (VSIMS), also shown in Figure 2, is a laboratory-based information management system that facilitates multi-center validation studies and trials requiring monitoring and accrual of specimens, and biomarker and assay related information. Both eSIS and VSIMS are powerful web-based tools that rely on a repository of common data elements. eSIS and VSIMS are valuable for the quick retrieval and review of EDRN studies and the ability to monitor, manage, coordinate and assess progress made in ongoing projects and studies

Figure A-2: EDRN Validation Study Information Management System (VSIMS) and Study Information System (eSIS)

Bioinformatics

The EDRN informatics infrastructure utilizes the network's Common Data Elements (CDEs) combined with the Object Oriented Data Technology (OODT) middleware software, developed by the NASA Jet Propulsion Laboratory, to enable interoperability among groups and their computing systems. Each participating institution within the EDRN knowledge system is able to use the Network CDEs to map the local data models to the knowledge system model in order to provide semantic consistency across the entire system.[37]

EDRN deployed the EDRN Resource Network Exchange (ERNE) knowledge system to more than 10 institutions to provide a common web-based client interface. ERNE unifies search and retrieval of biospecimen data from all participating institutions regardless of location, storage, or differences in the underlying data models. This helps scientists to locate, for example, tissue specimens for breast cancer by searching data catalogs at participating institutions across the country.

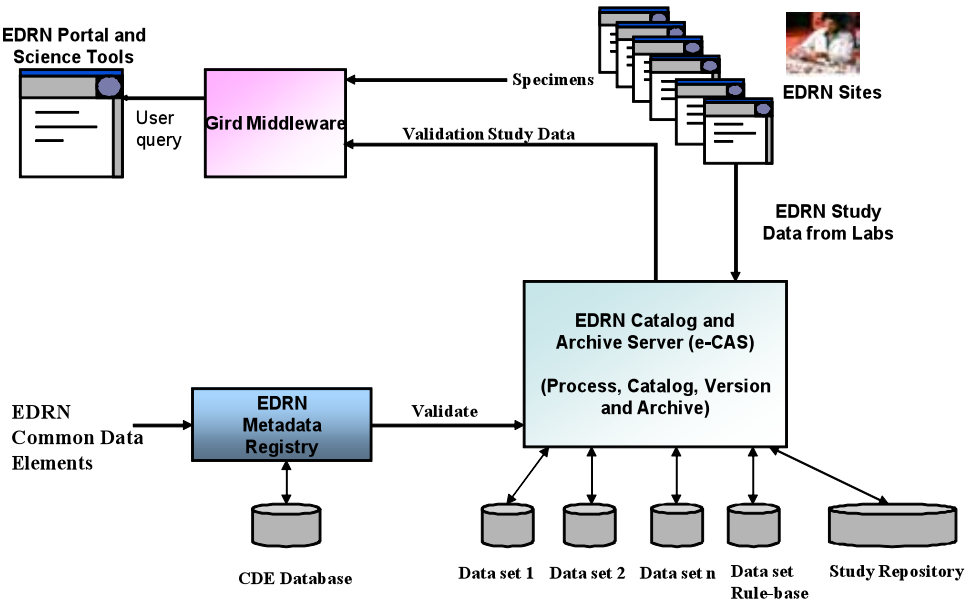
As the knowledge system evolves, the governing cancer CDE model and the use-cases derived in the working groups are used to drive the relationships between the data sets enabling discovery through data mining, as shown in Figure A-3. Scientists are able to query an assay result from a validation study and then find the associated specimens that were collected and included as part of that assay.

VSIMS is a major component of the EDRN knowledge system. Critical to any knowledge system is its ability to capture data as part of the science data processing and analysis infrastructure. Within the network, this occurs as part of the process to identify and validate cancer biomarkers.

The DMCC has designed this secure, web-based system for uniform data collection in multi-site studies that includes the main components needed for capturing and preserving the necessary metadata and data objects that integrate into the overall knowledge system architecture. These components include protocol management tools, communication tools, a data collection and processing system and a specimen tracking system. All are based on having a robust data architecture, which ensures that captured Information is securely maintained in the system and is stored separately for each multi-site study, thus allowing multiple protocols to be coordinated centrally through the same data management system.

[37] Tenenbaum D. Serving up specimens: NASA-NCI project links databases across the country. J Natl Cancer Inst. 2003;95(3)186-7.

Figure A-3: EDRN Informatics: High Level Component Infrastructure



A standard mechanism for monitoring the status of EDRN protocols is implemented in the EDRN Study Information System (eSIS). When a new EDRN protocol is initiated, the DMCC enters some basic information about the protocol and assigns it a protocol ID number. E-mail notifications are sent to both the lead investigator and all involved investigators requesting that they provide the required protocol information, track their milestones, and upload results as they occur. Each investigator enters their IRB number, Federal-Wide Assurance (FWA) number, and documentation that staff has been trained in human subject research. Sites that are using VSIMS are only able to access the system once IRB information is entered. DMCC also monitors IRB compliance of collaborative studies through the protocol database.

EDRN has also established a science data warehouse, the EDRN Catalog and Archive Service (eCAS), which represents a distributed metadata-driven system for the capture, tracking, processing and retrieval of scientific data from biomarker validation studies. eCAS is an invaluable tool that makes possible the sharing of results, correlating data, discovering of new biomarkers and much more.

eCAS is geared toward the capture and release of public data sets housed at NCI, as well as sharing specific science data from participating institutions. eCAS is also being used to establish a Biomarker Atlas as a means for discovering other related data, such as images that have been catalogued and stored according to organ-specific groups across EDRN institutions through advanced data searching and visualization mechanisms.

A biomarker data management database has been developed for tracking biomarker research, including collection of such biomarker-related data as phase of development, organ-site associations, performance, description of studies and related trials, and specific science data captured during the study of the biomarker.

Each phase of a biomarker is tracked throughout the development process from preclinical exploratory studies to cancer control studies. Data are captured consistently using the same set of CDEs and therefore, applications can interoperate and automatically correlate information. This forms the logical EDRN Knowledge Environment. For example, a biomarker tracked in the biomarker database can be associated to a specific organ site or cancer subtype, linked to a study protocol with a specific ID and related information captured in eSIS, including specific specimen records in ERNE or VSIMS; also, the results of an analysis of the biomarker can reference science data captured and stored in eCAS. Annotating, linking and accessing this information through a shared mechanism provide an integrated view of the information within the EDRN enterprise.

The EDRN public portal (http://www.cancer.gov/edrn) serves as a dynamic information dissemination service for the Network and the greater research community. This includes facts concerning investigators, on-going studies, meetings, funding opportunities, working groups, scientific discoveries and release of public data sets, publicly available informatics tools and news. The DMCC, NCI and NASA's JPL each play a critical role in developing and operating the informatics systems. Each partner, along with other EDRN institutions, requires the capability to share data, tools and information with both the Network and the broader scientific community.

EDRN's scalable infrastructure will advance the network's ability to expand its data and tools and provide a long-term platform for cancer research. New methods that make it possible for scientists to mine and correlate information across multiple data sets and studies will be created to aid the discovery process. This includes introducing data-understanding software and algorithms capable of developing the existing knowledge system infrastructure and constructing knowledge bases of metadata using automatic feature detection. These additional metadata will augment existing metadata used to describe EDRN data products, enhancing the informatics infrastructure overall, and enabling more sophisticated search and correlation capabilities.

NCI supports programs for emerging technologies, such as the NCI Alliance for Nanotechnology in Cancer (http://www.nano.cancer.gov) and developing standards for evaluating the performance of multiple platforms, such as the Clinical Proteomic Technologies Initiative for Cancer (http://www.proteomics.cancer.gov). EDRN works closely with these programs and stays abreast of maturing standards and technologies that are likely to accelerate biomarker analysis.

The cancer Biomedical Informatics Grid, or caBIG™, is helping NCI to lead in developing a research informatics infrastructure for scientists. It is expected that standardization of technologies such as high-throughput genotyping, genomics, proteomics, molecular imaging and nanotechnology will be necessary to generate data that are consistent and comparable. By leveraging resources and collaborations, EDRN will be able to develop interventions to identify individuals at risk for cancer, detect early stage disease, differentiate aggressive from indolent cancers and improve patient management.

Early Detection: Perspective from Research Advocates

Most people are affected in some way by cancer. The media report grim statistics of incidence and mortality; we lose loved ones to cancer; we become cancer patients or worry we will be. One half of men and one third of women will hear the words, "You have cancer." There is powerful motivation to work together to reduce the burden that accompanies a diagnosis of cancer. Patient and research advocacy provides an opportunity for those outside the scientific community to help reduce that burden and to support cancer research.

Early detection of cancer (at least of most solid tumors) is accepted as one of the critical factors in successful treatment. Improving early detection methods, whether perfecting current screening methods or developing new tests for discovering particularly difficult-to-treat cancers early in the cancer process has the appeal of giving patients the best possible outcomes. These discoveries and improvements in detection will reduce the burden of cancer in terms of both human suffering and the financial cost to society. Early detection represents hope.

There is a role for Patient Advocates within the EDRN:

- We can relay messages about the role and the goals of EDRN to the public.
- We can add the voices of the patient and their families, providing the human perspective, and communicating patient needs.
- We can provide mechanisms for a feedback loop between the research community and the public.
- We can help respond to the expectations of the public, such as letting the public know that researchers are working hard but "it won't happen tomorrow".
- We can help address barriers to research by speaking in lay language to patients about tissue collection, consent forms, and clinical trial accrual. Advocates can help educate patients and their families about clinical trials, overcoming some physician reluctance to do so.
- We can help support research by providing letters of endorsement from grass root organizations for funding proposals.
- We can help establish transparency and accountability between EDRN and funding organizations and, ultimately, the taxpayer.
- We can encourage principal investigators to provide public abstracts in lay terms describing EDRN research, while remaining compliant with non-disclosure agreements.

*This article was written by **Merel Grey Nissenberg, Esq.**, Patient Advocate, NASPCC/Mountain Foundation for Lung Cancer; **Elda Railey**, Patient Advocate, Co-Founder, Research Advocacy Network; and **Carole Seigel**, Patient Advocate, Consumer Advocates for Research and Related Activities (CARRA) at NCI.*

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